

The influence of endogenous testosterone and exogenous oxytocin on the response to baby schema in the female brain

Dissertation

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I. Abstract

The neuropeptide oxytocin is well known for its crucial role in childbirth and bonding. It seems to influence caretaking behaviors by modulating complex neuroendocrine reward systems. Contrary to oxytocin, high concentrations of the steroid hormone testosterone seem to antagonize caretaking behaviors. Whereas high testosterone concentrations were associated with competitive and defensive behaviors, low testosterone concentrations may support caretaking behaviors. For instance, the decrease of testosterone in new mothers and fathers is thought to be adaptive to promote parental care, while a decrease of oxytocin in new mothers was associated with postpartum depression risk. Interestingly, some behavioral effects of oxytocin may be steroid hormone dependent. Equally, oxytocin seems to modulate different steroid dependent reproductive functions like erection, ejaculation, orgasm, labor induction and lactation in mammals.

Reproductive success in mammals greatly depends on the caretaking effort of the parents or alloparents. A lack of caretaking behavior would probably result in neglect which could further cause the death of the vulnerable offspring. Therefore it may be adaptive that infants wear a key stimulus in the face that automatically elicits caretaking behavior in adults: the baby schema. Besides parental care, different kinds of mammals (especially primates) show alloparental care. Therefore it is likely that not only hormones involved in pregnancy and lactation influence maternal behavior, but that a neuroendocrine mechanism may be involved in the regulation of caretaking behavior in general. By reason of their important impact on reproduction and their inverse influences on caretaking behaviors it was the aim of this doctoral thesis to investigate the modulatory influence of testosterone and oxytocin on the processing of and the sensitivity for the baby schema and how both hormones interact in the modulation of a basic aspect of caretaking behavior, selective attention towards infants. For this, I used a combination of different established methods to investigate the neuroendocrine influences on selective attention towards infants: (1.) an implicit reaction time task to measure selective attention (the target detection paradigm), (2.) image manipulation (pictures of infants were manipulated in the degree of the baby schema), (3.) salivary hormone measurements, (4.) hormonal intervention and (5.) functional magnetic resonance imaging (fMRI). The results were published in two studies as part of this doctoral thesis: one behavioral study and one neuroimaging study.

As predicted, the results of the behavioral study [N=38; Study II of (Holtfrerich et al., 2016)] indicated that women with higher salivary testosterone were slower in orienting attention towards

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infant targets in the context of adult distractors, although the infant faces were not detected faster per se and although I could not find reaction time differences between the different degrees of baby schema.

Most interestingly, oxytocin administration seemed to diminish the negative effects of heightened testosterone concentrations, because only in women with high endogenous testosterone reaction times towards infant and adult stimuli decreased after oxytocin administration.

The following brain imaging study had the aim to investigate how the interaction of exogenous oxytocin and endogenous testosterone influences the processing of infant faces in the female brain [N=57 (Holtfrerich et al., 2018)]. The results supported the idea that oxytocin may counteract the negative effects of testosterone in the modulation of caretaking behavior, because activation of the putamen was positively correlated with selective attention towards infant faces in women that were treated with oxytocin, and even more importantly, this finding could be traced back to women with high testosterone concentrations and was not reflected in the low testosterone group.

Although missing any behavioral effect on the response to pictures with different degrees of baby schema, the second finding of the fMRI study, i.e., increased activation of the inferior frontal junction in response to an increased baby schema in women who received oxytocin, may leave room for speculations that oxytocin may enhance the salience of infants with stronger baby schema and thus increase the readiness to act by activating a brain region known to be involved in cognitive control, action perception and the detection of behaviorally salient cues – the inferior frontal junction.

Altogether, the results of the present thesis provide new evidence that oxytocin may counteract the adverse effects of testosterone on a central aspect of caretaking behavior – selective attention towards infants - and provide new indications for an adaptive hormonal mechanism that promotes caretaking behavior.

II. Zusammenfassung

Das Neuropeptidhormon Oxytocin ist insbesondere für seine wichtige Rolle bei der Geburt und dem Bindungsverhalten bekannt. Es scheint Fürsorgeverhalten zu beeinflussen, indem es komplexe neuroendokrinologische Belohnungssysteme im Gehirn aktiviert. Im Gegensatz zu Oxytocin scheinen hohe Konzentrationen des Steroidhormons Testosteron Fürsorgeverhalten zu antagonisieren. Während hohe Testosteronkonzentrationen eher mit kompetitiven und verteidigenden Verhaltensweisen in Verbindung gebracht werden, scheinen niedrige Konzentrationen des Steroidhormons Fürsorgeverhalten zu begünstigen. So wird zum Beispiel vermutet, dass der Abfall der Testosteronkonzentration bei neuen Müttern und Vätern adaptiv ist, um die elterliche Fürsorge zu erhöhen. Ein Abfall des Neuropeptids Oxytocin nach der Geburt wird dagegen mit der postpartum Depression assoziiert. Interessanterweise scheinen einige Oxytocin-induzierte Verhaltensweisen abhängig vom Steroidhormonlevel zu sein. Invers moduliert Oxytocin viele Steroidhormonabhängige reproduktive Funktionen wie die Erektion, Ejakulation, Wehen, den Orgasmus und Einschuss der Muttermilch bei Säugetieren.

Der Reproduktionserfolg von Säugetieren hängt maßgeblich mit dem parentalen oder alloparentalen Fürsorgeverhalten zusammen. Das Ausbleiben von Fürsorgeverhalten würde die Vernachlässigung und anschließend vielleicht sogar den Tod des hilflosen Nachwuchses bedeuten. Es könnte daher adaptiv sein, dass der Nachwuchs von Säugetieren (und anderen Fürsorge betreibenden Wirbeltierklassen) einen Schlüsselreiz im Gesicht trägt, der automatisch Fürsorgeverhalten auslöst: Das Kindchenschema. Neben der elterlichen Fürsorge zeigen viele Säugetierarten, aber auch Vögel, alloparentale Fürsorge. Es ist daher wahrscheinlich, dass nicht nur Schwangerschaftshormone Fürsorgeverhalten induzieren, sondern ein neuroendokrinologisches System an der Regulation von Fürsorgeverhalten im Allgemeinen beteiligt ist. Aufgrund der Bedeutung von Testosteron und Oxytocin für die Reproduktion und ihre gegensätzlichen Einflüsse auf das Fürsorgeverhalten, war es das Ziel dieser Doktorarbeit, die modulatorischen Effekte der beiden Hormone bei der Verarbeitung und der Sensibilität für das Kindchenschema zu untersuchen und im weiteren zu ergründen, inwiefern die beiden Hormone in der Ausprägung von Fürsorgeverhalten interagieren. Um diese modulatorischen Effekte zu untersuchen, wurde eine Kombination verschiedener etablierte Methoden verwendet: (1.) eine implizite Reaktionszeitaufgabe, um die selektive Aufmerksamkeit zu messen (*das Target Detection Paradigma*), (2.) Bildbearbeitung (das Kindchenschema im Gesicht von Säuglingen wurde in der Stärke manipuliert), (3.) Speichelhormonmessungen, (4.) Hormonelle Intervention und (5.) Funktionelle Magnetresonanztomographie (fMRT). Die Ergebnisse wurden in zwei Studien

publiziert, die Teil dieser Doktorarbeit sind: Eine Verhaltensstudie sowie eine bildgebende Studie (fMRT-Studie).

Wie erwartet, deuten die Ergebnisse der Verhaltensstudie [N=38; *Studie II aus* (Holtfrerich et al., 2016)] darauf hin, dass Frauen mit höheren Speicheltestosteronwerten eine geringere selektive Aufmerksamkeit für Baby-Bilder im Kontext von Adult-Bildern zeigten. Dennoch konnten keine reinen Präferenzen für Baby-Bilder und auch keine Reaktionszeitunterschiede für die unterschiedlichen Kindchenschema-Stärken ermittelt werden. Bemerkenswert war allerdings, dass nur Frauen mit hohen Testosteronkonzentrationen von der Verabreichung von Oxytocin profitieren und sich ihre Reaktionszeiten insgesamt verbesserten, während die Frauen mit niedrigen Testosteronkonzentrationen keinen solchen Effekt zeigten.

Die darauffolgende fMRT-Studie hatte das Ziel zu untersuchen, inwiefern die Interaktion von exogenem Oxytocin und endogenem Testosteron die Verarbeitung von Kinderbildern im Gehirn von Frauen beeinflusst [N=57 (Holtfrerich et al., 2018)]. Die Ergebnisse dieser Studie unterstützen die vorherigen Befunde, dass Oxytocin die negativen Effekte erhöhter Testosteronkonzentration in der Modulation von Fürsorgeverhalten kompensieren kann. Es wurde eine positive Korrelation zwischen einer erhöhten Aktivität im Putamen (ein Teil des Belohnungssystems des Gehirns) und der selektiven Aufmerksamkeit auf Baby-Bilder bei den Frauen gefunden, die mit Oxytocin behandelt wurden, nicht allerdings in der Placebogruppe. Dieses Ergebnis konnte wieder nur auf Frauen mit hohen Testosteronkonzentrationen zurückgeführt werden und war in der Gruppe der Frauen mit niedrigen Testosteronkonzentrationen nicht mehr reflektiert.

Obwohl ich keine Verhaltenseffekte bei der Antwort nulliparer Frauen auf verschiedene Kindchenschemastufen finden konnte, könnten die zweiten neurologischen Befunde einen Hinweis darauf geben, dass Oxytocin die Salienz von Kindern mit stärkerem Kindchenschema und so die Handlungsbereitschaft erhöhen könnte, indem es die Aktivierung im ‚inferior frontal junction‘ erhöht.

Zusammenfassend können die Ergebnisse dieser Doktorarbeit neue Hinweise auf einen kompensierenden Einfluss von Oxytocin gegenüber den negativen Effekten hoher Testosteronkonzentrationen in der Modulation von Fürsorgeverhalten zeigen und daher neue Anhaltspunkte für einen adaptiven neuroendokrinen Fürsorgemechanismus liefern.

III. Abbreviations

2D:4D	Second to fourth digit ratio
ANOVA	Analysis of variance
BOLD	Blood-oxygen-level dependent
fMRI	Functional magnetic resonance imaging
fMRT	Funktionelle Magnetresonanztomographie
GABA	Gamma-aminobutyric acid
G-protein	Guanine nucleotide-binding protein
IU	International units
Mg ²⁺	Magnesium ion
MRI	Magnetic resonance imaging
OXTR	Oxytocin receptor
PET	Positron emission tomography
Ph.D.	Doctor of Philosophy
RCF	Relative centrifugal force
RMET	Reading the mind in the eyes test
SPM	Statistical Parametric Mapping
x g	Times gravity

1. Introduction

1.1. The hormonal basis of reproduction and caretaking behavior

Hormonal transitions have a crucial impact in the development of reproduction and caretaking behavior. Different external and physiological factors may probably influence these hormonal transitions: This includes physiological adaptations during pregnancy (e.g. enlargement of the mammary glands) and external stimuli, like an offspring crying for the mother (Broad et al., 2006; Feldman, 2015; Keverne and Kendrick, 1992).

Caretaking behavior is not only observed after parturition but numerous species exhibit alloparental behavior in nulliparous individuals. Therefore it is likely that not only hormones involved in pregnancy and lactation influence maternal behavior, but that a complex neuroendocrine mechanism may be involved in the regulation of caretaking behavior (Numan and Insel, 2003). In this thesis, I further distinguish between ‘general nurturing behavior’, which I define as ‘care of the brood - behavior’ and thus includes actual caretaking behaviors like grooming as well as infant defense behaviors, and ‘caretaking behavior’, which solely includes positive infant-directed affectionate behaviors.

Caretaking behavior could be evolutionary adaptive to promote the mother’s and her offspring’s fitness (inclusive fitness), especially in species (e.g., mammals) in which reproduction is energetically costly (Kunz and Orrell, 2004). Provisioning (e.g. lactation), caretaking and protection of the offspring ultimately relate to the reproductive fitness (Klug and Bonsall, 2014). In vervet monkeys (*Chlorocebus pygerythrus*), for example, parental care enhances reproductive success of the female offspring (Klug and Bonsall, 2014). In humans, motherese (infant directed speech) may support infants health and may therefore be ultimately linked to infants growth (Monnot, 1999). Proximately, neuronal and hormonal systems could influence the motivation for caretaking and protection (Numan and Insel, 2003).

Hormones can be classified according to their functions. Thus ‘sexual hormones’ include all hormones that are involved in the regulation of reproduction. On the one hand, this includes hormones that are directly involved in the development of gender specific traits and the spermatogenesis and ovulation, like androgens and estrogens, on the other hand, this includes hormones that are involved in bonding behavior and attachment like, for example, oxytocin (Felberbaum et al., 2007; Löffler, 2008).

Reproduction in humans (and other mammals) begins with the gametogenesis in females and the spermatogenesis in males. The involved steroid hormones are regulated by negative or positive feedback mechanisms (Chedrese, 2009). Thereby the steroid hormone secreted by the target gland sends a signal to the producer gland to decrease or increase production. All steroid hormones derive from cholesterol. Besides its role as steroid hormone, progesterone is also a significant metabolic intermediate in the synthesis of testosterone and estradiol (Lüllmann et al., 2006). Since steroid hormones are primarily lipophilic and hydrophobic, they are transported via carrier proteins through the blood and can pass the blood-brain-barrier easily (Oren et al., 2004). Steroid hormones induce genomic and non-genomic actions. Genomic actions are characterized through a slower effect mechanism. In this case, the steroid hormone directly influences reproduction by the expression of steroid-dependent genes through specific intracellular receptors (Simoncini and Genazzani, 2003).

In contrast, signaling from the plasma membrane enables steroid hormones to enter the cell to cause rapid non-genomic actions (Norman et al., 2004; Rainville et al., 2015; Tian et al., 2000). Hormones that are highly associated with maternal behavior are prolactin, estrogen, oxytocin, dopamine and progesterone. The steroid hormones progesterone and estradiol are essential for reproduction because they initiate the maturation of the oocyte in the female menstrual cycle and prepare the uterus for pregnancy (Felberbaum et al., 2007). Besides, rats treated with estradiol and progesterone showed increased maternal behavior towards foster young when both hormones were administered simultaneously and when estradiol alone was administered in high amounts (Bridges, 1984). Progesterone also prepares the womb for nidation while prolactin is especially important during and after pregnancy by inducing the growth of the mammary gland and influencing lactation (Felberbaum et al., 2007). Besides the development of the follicles, estrogen induces the induction of oxytocin receptors [*here in rabbits* (Nissenson et al., 1978)].

The peptide hormone oxytocin modulates maternal behavior through rapid actions over guanine nucleotide-binding protein (G-protein) coupled receptors (Gimpl and Fahrenholz, 2001). Oxytocin and dopamine are essential neurotransmitters in the regulation of maternal bonding and attachment behavior (Strathearn, 2011). Particularly, limbic and hypothalamic structures are involved in parental behavior (Atzil et al., 2017, 2011; Numan and Insel, 2003; Strathearn, 2011). Further elaboration in the next chapters will provide deeper insights in the promoting role of the peptide hormone oxytocin and the inverse effects of the steroid hormone testosterone in caretaking behavior and its neuroendocrine mechanism.

1.1.1. Oxytocin

1.1.1.1. The role of oxytocin in general nurturing behavior

The neuropeptide oxytocin is well known for its major role in childbirth and bonding (Kendrick, 2000). Actions range from physical adjustments to complex neuroendocrine behavioral modulations that are related to reproduction and parental care. It is released in high quantities in the induction of labor and lactation and is highly relevant for mother-infant-bonding (Kendrick, 2000; Magon and Kalra, 2011). A lack of oxytocin is associated with maternal neglect and postpartum depression (Lara-Cinisomo et al., 2017; Strathearn, 2011). Administration of an oxytocin antagonist in the ventral tegmental area and in the medial preoptic area of mother rats was found to block maternal behavior (Pedersen et al., 1994). Then again, the injection of oxytocin in non-pregnant sheep could facilitate maternal behavior even towards alien lambs (Kendrick et al., 1987).

Research suggests that oxytocin boosts the motivation for maternal care through mesocorticolimbic dopamine pathways by improving the reward value of the offspring (Love, 2014). Mesocorticolimbic dopamine pathways are sets of dopaminergic neurons that are located in the substantia nigra and the ventral tegmental area. Their axons project to the striatum (which is part of the basal ganglia - e.g. caudate nucleus, putamen and nucleus accumbens) and the dorsal and ventral prefrontal cortex. Together these regions are core constituents of the reward system (Arias-Carrián et al., 2010). Further, the reward magnitude of both passive reward anticipation and the receipt of reward seems to be represented by the ventral striatum, whereas activations in the medial orbitofrontal cortex/ventromedial prefrontal cortex seem to appear with the physical presence of reward (Diekhof et al., 2012). These regions were also found to be activated in first-time mothers with secure mother-infant attachment in response to own infant cues. Further, activations in the ventral striatum were positively associated with the peripheral oxytocin response after episodes of mother–infant interaction (Strathearn et al., 2009).

Studies on the influence of oxytocin on different social and parental behaviors in humans and other mammals have gained importance in recent years. Rats, for example, showed higher oxytocin receptor densities when they received more liking and grooming as pups (Francis et al., 2002). Socially monogamous prairie voles (*Microtus ochrogaster*) made closer partnerships after oxytocin administration (Williams et al., 1994). Monogamous prairie voles are often used as a model for mating and parenting behavior in humans, because they also form monogamous pair-bonds and share parental care (Wang and Aragona, 2004). But since oxytocin receptor

distribution and parental care even seem to vary between different species of the voles (Genus *Microtus*), a direct comparison between primates and rodents needs to be considered with caution (Insel et al., 1993). In free-ranging rhesus macaques, a positive correlation between maternal behavior (including nursing and grooming) and high plasma oxytocin concentrations was also found (Maestripieri et al., 2009). Further, in pregnant and postpartum women plasma oxytocin levels were positively correlated with maternal behavior (Feldman et al., 2011).

Administered oxytocin may also promote empathy in humans (Domes et al., 2007b) and reduce stress in lactating women (Heinrichs et al., 2002). Especially in mothers increased empathy could be essential for understanding the nonverbal emotions of the offspring (Van Anders et al., 2011; Walker et al., 2007). The intranasal administration of oxytocin has further been found to decrease the amygdala response to auditory stimulation with crying babies in a placebo-controlled design and to increase activation in the insula and inferior frontal gyrus/ pars triangularis to auditory stimulation with crying babies compared to control sounds (Riem et al., 2011). In a following analysis the authors further found an increased functional connectivity between the amygdala, the orbito-frontal cortex, the hippocampus, the precuneus, the angular gyrus, and the middle temporal gyrus during auditory stimulation with infant laughter compared to control sounds (Riem et al., 2012). The authors interpreted that these results may indicate that oxytocin, on the one hand, facilitates the responsiveness to crying infants by reducing activations in a region involved in anxiety and aversion and by increasing activation in regions related to empathy and on the other hand, that an increased functional connectivity as a response to laughing infants may indicate an enhanced incentive salience of the infant laughter.

Further, administered oxytocin has been demonstrated to enhance arousal ratings of infant pictures in nulliparous women. In addition the ratings were positively correlated with amygdala activation in those women that were treated with oxytocin but not in placebo treated women (Rupp et al., 2013).

Taken together, oxytocin seems to direct attention and attraction towards salient infant stimuli and to increase sensitive parenting and caretaking behaviors [*for review see* (Feldman and Bakermans-Kranenburg, 2017; Luo et al., 2015; Van Anders et al., 2011)]. How oxytocin effects physiological and neurological processes will be further clarified in Chapter 1.1.1.2. Apart from its influence in caretaking behaviors, oxytocin is of great interest because it may assist the treatment of, for example, autism, postpartum depression and schizophrenia [*for review see* (Yamasue et al., 2012)]. Because of its possible interaction with sexual hormones and dopamine, as described in

Chapter 1.1.1.3, it is of large concern in the research for patients and other clinical participants (Wigton et al. 2014).

1.1.1.2. The physiology of oxytocin

Oxytocin is a neuropeptide whose effectiveness in physiological and neurological processes is extensive. In its primary structure, it has nine amino acids and a molecular weight of 1007.19 g/mol, which makes it difficult for oxytocin to cross the blood-brain barrier from blood circulation back to the central nervous system. The molecular formula of oxytocin is $C_{43}H_{66}N_{12}O_{12}S_2$ (Guastella et al., 2013; Young and Gainer, 2003). The neuropeptide is primarily produced in the cell bodies from the paraventricular nucleus and at a low level from the supraoptic nucleus in the magnocellular neurons in the hypothalamus and released by the pituitary gland (Bridges et al., 2008; Brownstein et al., 1980). Oxytocin (as well as vasopressin) appears to be synthesized from a precursor protein molecule and packaged in granules. The carrier protein neurophysin is included to the precursor protein. The granules then get transported down the posterior pituitary axon. Besides neurophysin, the granules probably contain enzymes for the posttranslational processing (Brownstein et al., 1980). In its synthesis the inactive precursor protein gets hydrolyzed in small active fragments (one of them is the active oxytocin) (Brownstein et al., 1980; Goldman, 1981). Until they are released, the granules can be stored in the posterior pituitary. When the nerve endings are depolarized, the granules release the content. Specific neuronal activation leads to a calcium-mediated neuronal exocytosis of oxytocin (Brownstein et al., 1980; Norman and Henry, 2015; Schulz et al., 2010). Figure 1 page - 11 - shows a schematic illustration of the biosynthesis of oxytocin up to the release of the neuropeptide.

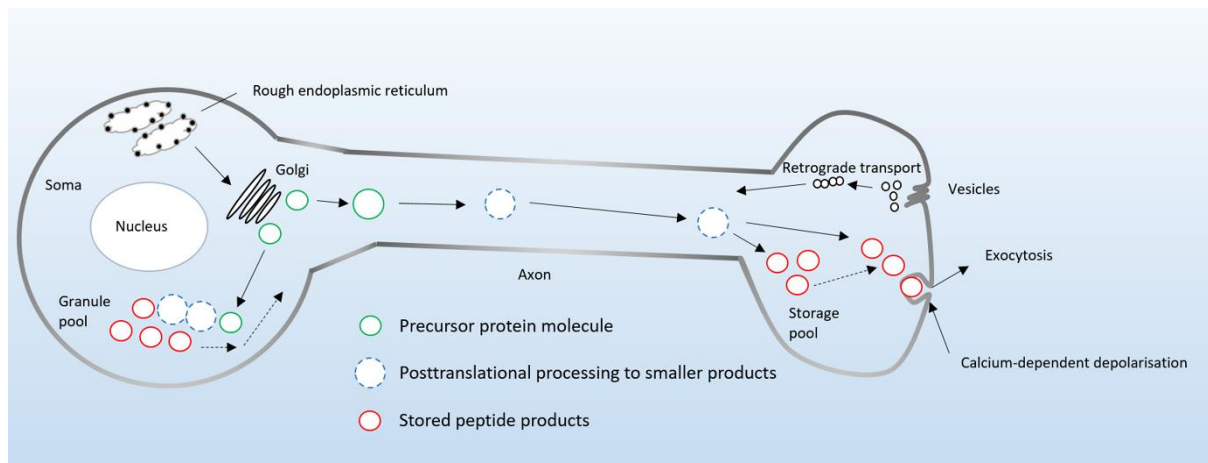


Figure 1: Schematic illustration of the biosynthesis and secretion of the neuropeptide oxytocin. Messenger ribonucleic acid gets translated on the rough endoplasmic reticulum yielding in a precursor protein molecule. Passing the Golgi body, the precursor protein molecule, together with its carrier protein neurophysin and probably with enzymes for the posttranslational processing, gets packaged in granules. The posttranslational processing of the precursor protein molecule occurs either in the cell body or during the axonal transport. The peptide products are stored in the granule until they are released in the nerve endings in the posterior pituitary. When the nerve endings get depolarized through calcium influx, the granular contents get released [adapted from (Brownstein et al., 1980)].

Oxytocin's signal transduction may be endocrine but also paracrine and autocrine. Beyond pregnancy oxytocin is degraded renal and hepatic (Dhuria et al., 2010).

The individual concentration of blood steroids determines oxytocin's half-life period in blood plasma (Rydén and Sjöholm, 1969; Sirotkin et al., 2014). For example, the impact of oxytocin in social recognition is estrogen-dependent – which means that the effect of oxytocin only occurs when plasma estradiol is high (Gabor et al., 2012). The half-life period of oxytocin in human blood is about three minutes and has a baseline concentration about 3.4 pg/ml in human females (Carmichael et al., 1987). This is significant for non-behavioral processes that underlie short term effects, like the stretching of the reproductive organs during birth process (Rydén and Sjöholm, 1969), whereas liquor concentrations of oxytocin may probably mediate long term behavioral effects in the brain like for example mother-infant bonding (Pedersen and Prange, 1979). Due to the invasiveness of liquor collection, data of human liquor oxytocin concentrations in healthy participants are typically limited. But in rhesus macaques (*Macaca mulatta*), for instance, liquor oxytocin concentrations were found to range from 36.02 to 134.41 pg/ml in adult female macaques (7–26 years old) and 35.94 to 77.3 pg/ml in infant macaques (38–134 days old) (Parker et al., 2010).

1.1.1.3. Oxytocin reception and pathways through the brain

The oxytocin receptor is a G-protein coupled receptor. The gene of the human oxytocin receptor is mapped to gene locus 3p25–3p26.2. It consist of three introns and four exons (Gimpl and Fahrenholz, 2001).

The human oxytocin receptor has three potential nitrogen-glycosylation sites (Gimpl and Fahrenholz, 2001). The regulation of the oxytocin receptor system seems to be highly dependent of steroid hormones (Gimpl et al., 2002). For high-affinity binding on the oxytocin receptor magnesium (Mg²⁺) and cholesterol function as allosteric modulators (Gimpl et al., 2002), while binding the antagonist only needs high concentrations of cholesterol (Gimpl et al., 2008). Progesterone apparently inhibits the signaling of the oxytocin receptor (and other G-protein coupled receptors) through rapid non-genomic actions and can thus stops the oxytocin-induced uterine muscle contraction (Gimpl et al., 2002). Contrary, estrogen-treatment in the myometrium of rabbits increased oxytocin-receptor density and thus increased the contractility of the myometrium, whereas treatment with progesterone inhibited the estrogen effect (Kelly and Wagner, 1999; Maggi et al., 1988).

In mammals the oxytocin receptor is primary expressed in gender-specific cells of the reproduction system (for example ovary, mammary gland and spermatic duct) and in the brain, but oxytocin receptors can also be found in fat cells, heart cells, thymus cells and pancreatic cells (Gimpl and Fahrenholz, 2001).

Cell culture studies showed that oxytocin receptors are located on hypothalamic neurons and on astrocytes. The location of oxytocin receptors in the brain is diverse in different mammals. In rats oxytocin receptors were already detected in regions of the olfactory system, cortical areas, basal ganglia, limbic system, thalamus, hypothalamus (e.g. medial preoptic area), brain stem and the pituitary gland. In humans and other primates, oxytocin receptors were so far found in the nucleus basalis of Meynert and the superior colliculus (Grinevich et al., 2015; Jirikowski et al., 2017). Oxytocin neurons were found to have widespread projections throughout the brain. For instance, projections of hypothalamic oxytocin neurons that are involved in the portal system of the anterior pituitary lobe were also found in the median eminence. Further, oxytocin contributes to the hypothalamo-adenohypophysial system by modulating corticotrophs (and thus stress-response). The release of prolactin in the anterior pituitary lobe seems also to be stimulated by oxytocin in the hypothalamo-adenohypophysial system. In addition, neuronal cell bodies that produce oxytocin were found in the periventricular nucleus. Projections of these neurons again

can access the brain ventricular lumen and are the source of oxytocin in the cerebrospinal fluid (Jirikowski et al., 2017).

In contrast to findings in other mammals, the human brain seems to exhibit oxytocin receptor binding sites in the pars compacta of the substantia nigra (Gimpl and Fahrenholz, 2001). The substantia nigra plays a key role in dopamine signaling and it influences activation of other reward-related brain areas (Wigton *et al.*, 2015). Therefore it is possible, that dopaminergic neurons could be target cells for oxytocin binding (Gimpl and Fahrenholz, 2001).

In fact, oxytocin treatment was found to modulate different reward-related brain responses [*for review see* (Wigton et al., 2015)]. Areas of the brain reward system were found to be activated through oxytocin administration in a number of studies. Consistent changes in brain activity of the reward system after oxytocin treatment were found in the basal ganglia, e.g.: putamen (Rilling et al., 2012), caudate and globus pallidus (Wittfoth-Schardt et al., 2012), as well as in the insula (Riem et al., 2011), thalamus (Domes et al., 2010), amygdala (Lischke et al., 2012) and the temporal lobe (Domes et al., 2010; Riem et al., 2011; Wittfoth-Schardt et al., 2012). Interestingly, the same brain areas have also been associated with the processing of cute infant faces in studies without an oxytocin intervention [(Glocker et al., 2009b) *for review see* (Luo et al., 2015)]. Therefore, a link between endogenous oxytocin and cuteness processing in the brain's reward system might be assumed.

1.1.2. Testosterone

1.1.2.1. The role of testosterone in general nurturing behavior

Contrary to oxytocin, the androgen testosterone seems to counteract caretaking behaviors and may increase antagonistic behaviors like competitive responses [*for review see* (Van Anders et al., 2011)]. Previous research found decreased testosterone concentrations in parents with young children (Alvergne et al., 2009; Gettler et al., 2011; Kuzawa et al., 2009; Weisman et al., 2014), which led to the inference that decreased testosterone concentration during parenthood could be adaptive to increase parental attributes and intensify caretaking behavior. According to Rilling (2013), low testosterone levels could promote caretaking behavior by increasing empathy, suppressing infant-directed aggression and/or suppressing sexual motivation to direct energy to caretaking behavior. In support of this claim, several previous studies found a negative

association between high testosterone concentrations and parental attributes like caring (Baucom et al., 1985), empathy (van Honk et al., 2011) and altruism (Harris et al., 1996).

Yet, raising the offspring also requires protective aggression and infant or mate defense. These rather antagonistic behaviors that are directed towards threat may enhance reproductive success, yet they might require an increased testosterone level [*for review see* (Van Anders et al., 2011)]. Van Anders and colleagues (2011) call this the ‘Offspring Defense Paradox’. In sense of the well-known ‘Challenge Hypothesis’ testosterone antagonizes infant-directed care, and fathers are required to balance the trade-off between infant or mate defense and infant-directed parental care [(Wingfield et al., 1990); *for review see* (Fernandez-Duque et al., 2009)]. An example for high infant-directed paternal care is provided by research in male marmosets. Male marmosets engage on different levels in parental care. Nunes *et al.*, (2001) found that high-caring male marmosets showed decreased testosterone concentrations, whereas low-caring males had much higher testosterone concentrations. Also in human females negative correlations between circulating testosterone levels and scores on measures of maternal personality were found (Deady et al., 2006). Further, in human fathers and non-fathers, lower testosterone levels were associated with a higher need to respond to and a higher sympathy towards auditory stimulation with crying babies than in fathers with high testosterone levels. But the authors also found an increase in testosterone in response to auditory stimulation with crying babies in new fathers (Fleming et al., 2002). These results were also supported by a testosterone administration study, in which testosterone increased neural responses to auditory stimulation with crying babies in women (Bos et al., 2010). Even though it remains unclear whether the administered testosterone or its metabolite estradiol were responsible for the findings of Bos et al., an increased response to auditory stimulation with crying babies through higher testosterone concentrations may also indicate enhanced protective aggression and infant or mate defense as a direct reaction to the cry of the infant, which would be compatible with the ‘Offspring Defense Paradox’ (Rilling, 2013; Van Anders et al., 2011).

1.1.2.2. The physiology of testosterone

Testosterone is an androgenic steroid hormone. Its molecular formula is C₁₉H₂₈O₂. In human males, more than 95% (6-7 mg per day) of the circulating testosterone is synthesized in the Leydig cells of testes (Rommerts, 2004). In women, testosterone is synthesized in much lower quantities in the theca cells of the ovaries, and during pregnancy in the placenta (Strauss and

Williams, 2014). In addition, small amounts are synthesized in the adrenal glands in both genders (Brooks, 1975). Men have seven to ten times higher testosterone concentrations than women (Liening and Josephs, 2010).

Steroid hormones - such as testosterone - derive from cholesterol. The oxidative cleavage of the side chain from cholesterol initiates the biosynthesis to pregnenolone in the mitochondria. Pregnenolone is the precursor hormone in the synthesis of testosterone and all other steroid hormones. Pregnenolone can be converted into progesterone and subsequently into 17 α -hydroxyprogesterone or directly by hydroxylation to 17 α -hydroxypregnenolone. With the cleavage of two carbon atoms 17 α -hydroxyprogesterone can be converted into androstenedione and 17 α -hydroxypregnenolone can be converted into dehydroepiandrosterone and subsequently into its direct metabolite androstenediol. Further oxidation leads to the formation of testosterone (Rommerts, 2004). Testosterone can then be converted into dihydrotestosterone by 5 α -reductase or into estrogen via aromatase (Rossetti et al., 2016). In addition, circulating steroids – such as progesterone and testosterone – can be converted into neuroactive steroids in the brain via 5 α -reductase and 3 α -hydroxysteroid dehydrogenase (Mellon and Griffin, 2002). See Figure 2 for a simplified schema of the steroid hormone synthesis.

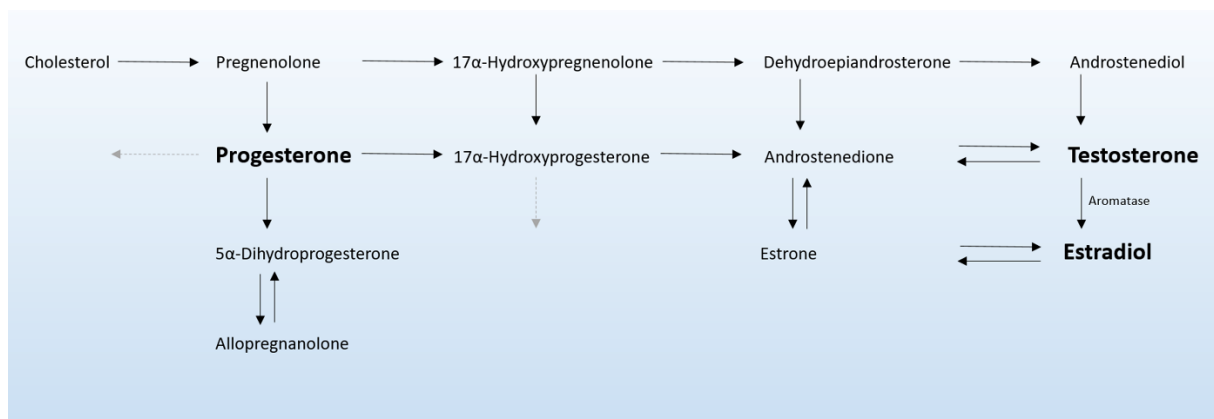


Figure 2: Simplified pathway of steroid hormone synthesis [adapted from (Felberbaum et al., 2007; Sun et al., 2016)].

In the body, free testosterone can rapidly pass a variety of organs and blood. Most of the testosterone in the body occurs to be protein-bounded to sex-hormone-binding-globulin or, in small amounts, to albumin (Rommerts, 2004). The active and unbounded form constitutes only 2-3 % of the testosterone concentration in the body. Only the free and unbounded testosterone

can pass the target cells and activate the receptor. This means, that only the unbounded bioactive form can enter the salivary glands and thus be measured out of saliva samples (Harris et al., 1996).

The production of the androgen testosterone is regulated by the hypothalamic-pituitary-gonadal axis via negative feedback. The hypothalamus releases gonadotropin-releasing hormones pulsatively which, then again, stimulate the pituitary gland to release the luteinizing hormone and the follicle-stimulating hormone. Henceforth, the pathways differ in men and women. In men, the luteinizing hormone stimulates the synthesis of testosterone in the Leydig cells of the testicle, whereas estradiol is synthesized out of androgens by aromatization locally in the hypothalamus and the pituitary gland. Further, the Sertoli cells secrete inhibin. Inhibin and testosterone inhibit the release of the follicle-stimulating hormone in the pituitary gland. Estradiol further reduces the amplitude of the luteinizing hormone pulses in the hypothalamus (Chedrese, 2009). In women, the secretion of testosterone is primarily proceeded in the ovaries (Davis and Tran, 2001). As described above, estradiol is secreted out of androgens. The production of testosterone in women is therefore regulated by the negative and positive feedback of estradiol and progesterone to the pituitary gland or directly to the hypothalamus and by negative feedback of inhibin to the pituitary gland (Chedrese, 2009). In this way testosterone and estradiol are able to control the secretion of other hormones in the brain by regulating the release and the synthesis of the hypothalamic hormones (Wilkinson and Brown, 2015).

Testosterone is responsible for a range of genomic and non-genomic actions. Besides the influence of testosterone on reproduction, proliferation, differentiation and homeostasis, organ systems, muscle and hair growth and bone mass, brain sexual differentiation, the development of sexual dimorphism and secondary sexual characteristics, testosterone also influences certain cognitive behaviors (Burger and P., 2004; Foradori et al., 2008; McEwen, 1988).

1.1.2.3. Testosterone reception and pathways through the brain

Testosterone and its 5α -reduced derivate dihydrotestosterone bind to androgen receptors. These high affinity intracellular nuclear receptors are located within the cells in a diverse range of tissues (Davey and Grossmann, 2016). Its receptor gene is located on the X-chromosome (Klocker et al., 2004).

The androgen receptor induces genomic actions of testosterone and dihydrotestosterone via ligand activated regulation of the gene transcription (Cunningham et al., 2012). Besides genomic actions, testosterone is also able to induce rapid and non-genomic actions through membrane-bound androgen receptors (Klocker et al., 2004). Non-genomic actions enable rapid behavioral modifications and are thus critically involved in social behavior (Balthazart et al., 2004).

Testosterone seems to modify neurotransmission using different mechanisms: e.g., changes in membrane flexibility, activation of second messenger pathways or rise of intracellular calcium concentrations. Non-genomic testosterone actions are defined through their speed – which implicates a time frame that does not allow gene transcription or translation and membrane mediation (Foradori et al., 2008). The androgen receptor exhibits a broad distribution in the brain, for instance, in the rat's brain, the androgen receptor could already be detected in the hypothalamus, amygdala, cortex and hippocampus. Irrespective of the receptor quantities, the distribution seems to be similar in males and females (Wilkinson and Brown, 2015).

In the literature testosterone is also described to influence behavioral modifications in regions of the mesocorticolimbic reward system – like oxytocin. For instance, evidence in humans demonstrated a positive effect of testosterone administration in the differentiation of reward versus non-reward in the ventral striatum – a major area of the mesocorticolimbic-dopamine-pathway (Hermans et al., 2010).

1.1.3. The interaction of oxytocin and testosterone in parental behavior

In macaques (*Simia inuus*) and humans oxytocin receptors were, inter alia, detected along the male reproductive tract, the testis and the uterus (Frayne and Nicholson, 1998). Oxytocin was found to increase the production of testosterone on a dose-dependent level in isolated Leydig cells of male rats, but had no influence on testosterone production stimulated by the luteinizing hormone (Frayne and Nicholson, 1995). Moreover, the release of oxytocin is highly dependent of steroid hormone levels. Thus oxytocin modulates different steroid dependent reproductive functions like, for instance, erection, ejaculation, orgasm, labor induction and lactation (Jirikowski et al., 2017). In the brain, hypothalamic oxytocin neurons seem to co-express aromatase. This finding may indicate that oxytocin is involved in the synthesis of estrogens and testosterone and consequently influences steroid hormone-dependent behavioral modifications (Jirikowski et al., 2017).

Viewed individually, testosterone is known to support behaviors that are associated with defense and sexuality, while it seems to counteract nurturing and bonding behaviors. Oxytocin instead is linked to nurturing and bonding behaviors and is known to promote caretaking (Crespi, 2016; Van Anders et al., 2011). Some reviews already listed the counteracting effects of testosterone and oxytocin in social contexts. For instance, it has been indicated, that trust, generosity, cognitive empathy, paternal care and sensitivity increased due to oxytocin administration or were positively influenced by serum oxytocin, whereas testosterone counteracted these social patterns. Contrary, attention to angry faces and non-defense aggression increased with higher testosterone levels and were negatively associated with oxytocin [reviewed in (Crespi, 2016)].

Although testosterone and oxytocin seem to modify contrasting characteristics of parental behavior, there has already been some evidence of an interaction in the modulation of parental care and general nurturing behavior. Research on the interplay of testosterone and oxytocin in status competition, parental investment and cooperation was investigated in the ethnic group of the Tsimane' people in Bolivian Amazon. The Tsimane' are an indigenous population of about 16 000 people. Besides fishing and horticulture, hunting offers a large proportion of food supply in Tsimane' people. Researchers determined the testosterone and the oxytocin concentrations out of saliva samples of Tsimane' men returning from a successful hunt and sharing their prey with their families. They found that, testosterone and oxytocin increased concurrently after a successful hunt. This result was surprising since both hormones were believed to counteract. The authors explained the result by viewing the behavioral cascades of hunting separately. They suggested that testosterone increased as subsistence strategy of hunting, while they explained oxytocin's increase through social salience enhancement due to sharing the food with the family which may represent status seeking of the hunter (Jaeggi et al., 2015). Nonetheless, positive associations of both hormones may also occur in other social behaviors like, for example, sexual intimacy (Van Anders et al., 2011).

In fathers, oxytocin administration was found to modulate testosterone production in a short-term pattern: After oxytocin administration testosterone increased in comparison to a placebo group. Yet, the oxytocin induced percent-change in father's testosterone was also associated with paternal behaviors, like touch and social gaze (Weisman et al., 2014). A second study published by the same research group found that a variation in the oxytocin receptor gene interacted with a proxy of the prenatal testosterone level experienced in the womb, i.e., by second to fourth digit ratio, in the performance of a common questionnaire to measure cognitive empathy ('reading the mind in the eyes test') – but only in male participants. Men with low fetal testosterone

concentrations, as indicated by a lower digit ratio, achieved higher scores in the test, when they carried the GG allelic variation of the oxytocin receptor gene, which is associated with a more efficient oxytocinergic system (Feng et al., 2015; Weisman et al., 2015). Empathy may be assigned to maternal behavior since it is crucial to understand the non-verbal needs of the offspring (Walker et al., 2007). Therefore, it may be speculated that testosterone may particularly enhance cognitive empathy in men with a more sensitive oxytocin system.

These are not the only results suggesting that the oxytocin-testosterone system may be sexually dimorphic. A recent study by Gordon and colleagues (2017) found differences in the oxytocin/testosterone-modulated response to infants between mothers and fathers. Oxytocin concentrations in fathers were negatively associated with paternal behavior – but only in fathers with high endogenous testosterone, whereas high testosterone concentrations in mothers were associated with a positive correlation between oxytocin and maternal touch (Gordon et al., 2017).

These results support the hypothesis of a complex interplay between oxytocin and testosterone in the modulation of general nurturing behaviors (please see Table 1 page - 20 - for an overview of studies that investigated the interaction of testosterone and oxytocin in a caretaking context).

Table 1: Overview of studies that investigated the interactive influence of testosterone and oxytocin on caretaking behaviors.

Authors	Year	Subjects (n)	Stimuli	Hormones	Design	Finding
Weisman et al.	2014	Fathers (35) + infants	Own infants	Oxytocin or placebo administration + salivary testosterone measurement	Recording of father–infant interaction	Oxytocin-induced changes in testosterone (higher in contrast to placebo) were associated with increased paternal behavior
Weisman et al.	2015	Chinese students (1463 - 55.6% ♀)	Pictures from the eye region of adult faces	2D:4D measurement + Genotyping of OXTR rs53576	Reading the mind in the eyes (RMET) test	Only men with low fetal testosterone achieved higher scores in the RMET, when they carried the GG allelic variation of the oxytocin receptor gene
Gordon et al.	2017	Mothers and fathers (80 couples) + infants	Own infants	Blood samples for oxytocin and testosterone measurement	Observing parent-infant interactions and micro-coding of parental behaviors	Mothers: positive interaction between increased testosterone levels and oxytocin on maternal touch - Fathers: negative interaction between increased testosterone levels and oxytocin on fathers affectionate touch

Abbreviations: second to fourth digit ratio (2D:4D); oxytocin receptor (OXTR); reading the mind in the eyes (RMET)

1.2. Baby schema as a key stimulus for caretaking behavior

The infant face constitutes a strong, socially salient stimulus, since it may be linked to reproductive success. Especially in mammals the offspring is highly dependent on parental care. The absence of general nurturing behavior would probably result in neglect which could cause the death of the vulnerable offspring - not only because newborns and infants depend on the maternal milk, but also because they are often incapable of acting (e.g. locomotion, independent digestion, preening). Therefore it may be adaptive, that infants of mammals carry typical child characteristic features that evoke feelings of protectiveness and care (Glocker et al., 2009b; Kringelbach et al., 2016; Luo et al., 2015). These childish features were originally defined by Konrad Lorenz as the baby schema (the German “*Kindchenschema*”) (Lorenz, 1943). The baby schema is defined as a combination of big eyes, a small mouth and nose, high eyebrows, a head ‘too large’ for the body, chubby cheeks and clumsy extremities (Lorenz, 1943; Glocker et al., 2009b; Glocker et al., 2009a; Kringelbach et al., 2016). Konrad Lorenz (1943) assumed that the

combination of these child characteristic traits triggers an innate releasing mechanism that elicits caretaking and thus decreases neglect. Several studies showed that infants wearing those characteristics were perceived as cuter (Sherman et al., 2009), initiated motherese (Spindler, 1961), were more smiled at (Hildebrandt and Fitzgerald, 1981), elicited feelings of protection (Alley, 1983) and evoked forgivingness (McCabe, 1988). It has been also reported, that increased cuteness may be an indicator of healthiness (Golle et al., 2015). Thus it is conceivable that a stronger baby schema in the infants face may signal increased healthiness of the infant and that an enhanced caretaking response in adults would therefore serve the protection of the community gene pool (Luo et al., 2015).

A number of previous studies investigated differences in the perception and the response to baby schema. Glocker and colleagues (2009a) showed, that children with a higher degree of baby schema (the facial aspects of the baby schema were artificially increased via Photoshop) were perceived as cuter in comparison to the normal (unmanipulated) and lower degree of baby schema (artificially decreased baby schema features via Photoshop) throughout the genders, but they also demonstrated that women seemed to be more sensitive to differences in cuteness. Due to the offspring's dependence on the maternal milk, females remain the primary caretakers, especially during the infant period, in most mammals so that high sensitivity to baby schema appears to be particularly adaptive for women. Another study showed pictures of infants together with pictures of adult women or men to female or male participants. Female participants fixated and looked longer at infant faces in contrast to adult faces of both sexes, whereas male participants showed contrary results when infant faces were shown with female adults, i.e., increased looking at female faces in comparison to infant faces (Cárdenas et al., 2013). Further evidence indicated gender differences in the accuracy and rapidity of the discrimination of facial expression of infants. In accordance with the 'primary caretaker hypothesis' female participants were significantly faster and more accurate in the discrimination of facial expression than male participants. Interestingly, the authors did not find any effect of childcare experience on gender differences, which indicates that nulliparous women may be equally sensitive to the baby schema as experienced female caretakers, thus also more sensitive than men (Babchuk et al., 1985).

The role of female sexual and reproductive hormones in maternal behavior has been investigated in a number of studies [*for a summary see* (Numan and Insel, 2003)], whereas the role of reproductive hormones (except from oxytocin) in the response to cuteness remains elusive. Sprengelmeyer *et al.* (2009) investigated the influence of reproductive hormones (by using the chronological age as indicator) on cuteness perception. They found that young women of

reproductive age (19-26 years) and premenopausal women (45-51 years) were more sensitive to differences in infant cuteness than young men (19-26 years) and older men (53-60 years) and menopausal or postmenopausal women (53-60 years). They further examined the influence of the intake of oral contraceptives and found that women taking oral contraceptives were more sensitive to variations of cuteness in infant faces. These findings lead to the assumption that female reproductive hormones probably take part in the modulation of cuteness perception.

To date, only a handful of studies examined the influence of variations of the baby schema on neural responses. Most research was concerned with the question of how perception of infant faces per se modulates neural activity. Indirect indications for hormonal influences on cuteness perception may be concluded from the results of Glocker and colleagues who found that children wearing a stronger baby schema were perceived as cuter and caused greater caretaking motivation and that those feelings were probably modulated through activations in regions of the mesocorticolimbic reward system (e.g. nucleus accumbens, basal ganglia) which probably show a high oxytocin and dopamine receptor density [(Glocker et al., 2009b) *see also* (Young and Wang, 2004)]

Nevertheless, infant stimuli per se were also found to activate brain systems involved in empathy, reward processing and attachment numerous times. That was to be expected, since the baby schema is a natural stimulus in the infant's face. In addition, regions widely recognized to be involved in motor control, attention and face perception were also shown to respond to infant stimuli [(Bartels and Zeki, 2004; Leibenluft et al., 2004; Ranote et al., 2004; Stoeckel et al., 2014) *for review see* (Luo et al., 2015)]. This could maybe be explained considering that one major aspect underlying caretaking behavior may be selective attention to the infant, since rapid actions may be essential to protect the infant's life. Therefore, besides rewarding emotions, fast processing and prioritized attention may also be adaptive to increase reproductive success (Kringelbach et al., 2016).

1.3. Design and measures of the present studies

It was the aim of this doctoral thesis to investigate the modulatory influence of testosterone and oxytocin on the processing of and the sensitivity for the baby schema and how both hormones interact in the modulation of selective attention towards infants. In the following chapters I will explain the objective of the present thesis and elaborate the design and measures that were used

to examine the influence of endogenous testosterone and exogenous oxytocin on human caretaking behavior.

1.3.1. Study objective

The aim of this cumulative thesis was to investigate the potential interactive influence of oxytocin and testosterone on the modulation of infant processing in nulliparous women. In the two studies as part of this thesis I invited nulliparous women to examine their selective attention to infant faces in dependence of their endogenous testosterone level after exogenous oxytocin or placebo administration and to investigate if their selective attention towards infant faces depended on the intensity of the baby schema in the infant's face.

The two studies were built on previous results on the influence of testosterone on attentional processing of infant faces (humans and other animals) that I investigated as a part of my master thesis. The findings of this study indicated that women with higher testosterone concentrations directed less attention towards infant faces in contrast to adult faces [*the results are published as Study I in* (Holtfrerich et al., 2016)].

To examine whether the negative effects of high testosterone concentrations on the processing of infant faces could be diminished through oxytocin administration as part of my thesis, another group of participants was invited to perform the target-detection-paradigm after administration of 24 international units (IU) oxytocin or the same amount of placebo (between-subjects-design; each participant self-administered 3 puffs of the nasal spray in each nostril). In addition, the endogenous testosterone concentration of the participants was analyzed out of saliva samples and the participants were divided into two groups: either women with high or with low testosterone concentrations [*see Study II of* (Holtfrerich et al., 2016)].

To further examine the neuronal basis of these findings, I performed a second study as part of my thesis. There, female participants performed the target-detection-paradigm after placebo or oxytocin administration while brain activation was measured by fMRI. Again endogenous testosterone concentration was determined.

The artificial short-term increase of oxytocin through intranasal inhalation may represent oxytocin's increase in lactating mothers (Weisman et al., 2012; White-Traut et al., 2009). Since a lack of postpartum oxytocin is associated with postpartum depression and maternal neglect

(Strathearn, 2011) and the decrease of testosterone may be adaptive to increase parental behavior (Gettler et al., 2011; Kuzawa et al., 2010), the findings of the present thesis may, prospectively, provide a basis for pharmacological research on hormonal transitions and resulting mental disorders.

1.3.2. Measuring selective attention – A means to operationalize the influence of the baby schema on cognitive and neural processing

Previous research on cuteness perception and parental behavior has primarily used methods like cuteness ratings (Glocker et al., 2009b, 2009a), pure view tasks [*e.g.* own versus acquainted child (Bartels and Zeki, 2004)], key-press tasks (Hahn et al., 2014) and one-back memory tasks (Leibluft et al., 2004). Especially self-reporting measures may be affected by reporting bias and responses in accordance to social expectancies. Accordingly, self-report questionnaires that use Likert-scales for evaluation are prone to distorted self-perceptions, with answers that might be given to meet expectations and hence represent untrue statements (Harrison, 1997). Similarly, observer biases might also pose a risk for data distortions because expectations may influence the observations of the observer (Risinger et al., 2002).

Therefore a common method to determine the extent to which a stimulus is perceived as salient is by measuring prioritized allocation of attention through implicit, more objective data, as for example reaction times (De Houwer, 2001; Linden et al., 1999). Implicit data show only weak correlations with data from self-reports (Greenwald et al., 1998), which appears plausible when assuming that implicit data should be less contaminated by the factors described above. A common method to determine implicit information about selective attention is the *odd-one-out* task. The fundamental assumption is, that biologically relevant stimuli (*e.g.*, socially salient stimuli) should automatically and rapidly recruit attention and should therefore be detected faster than less relevant stimuli, which would result in shorter reaction times (Brosch et al., 2007; Linden et al., 1999). In this context the performers provide information about themselves without awareness of the causation. He or she is only instructed to detect the one picture that does not fit to the others as fast and accurately as possible and has to confirm the detection via button press. The position of the target hereby corresponds to the position of the button (Greenwald et al., 1998; Teige-Mocigemba et al., 2010).

Selective visual attention seems to be modulated by frontoparietal networks for top-down and bottom up interactions (Corbetta and Shulman, 2002; Diekhof et al., 2009). In addition, target-detection was found to increase activation in regions like the supramarginal gyrus, frontal operculum, supplementary motor area, anterior cingulate gyrus, the insular cortex and in the primary and secondary visual cortex. (Linden et al., 1999).

As stimuli for *odd-one-out* tasks, for instance, emotional human faces are suitable. In this case, it would be the task of the participant to locate the one emotional expression (e.g. an angry face) out of a crowd of faces with another emotional expression (e.g. happy faces) (Shasteen et al., 2014).

Here I used four pictures: One picture was the target that had to be identified and confirmed via button press and the other three pictures represented the second target concept in form of the ‘distractors’. It was expected that if selective attention was directed towards the target picture, reaction times should decrease, whereas if attention was captured by the three distractor pictures, reaction times for the target should increase. Selective attention was therefore associated with the relative reaction time that the performer needed to select the one target in comparison to the other target concept (the distractors).

This specific version of the *odd-one-out* task was named the ‘*target detection paradigm*’. To the best of my knowledge, this thesis is the first that used the *target detection paradigm* in the context of infant stimuli. Figure 3 page - 26 - displays an example of one trial from the paradigm.

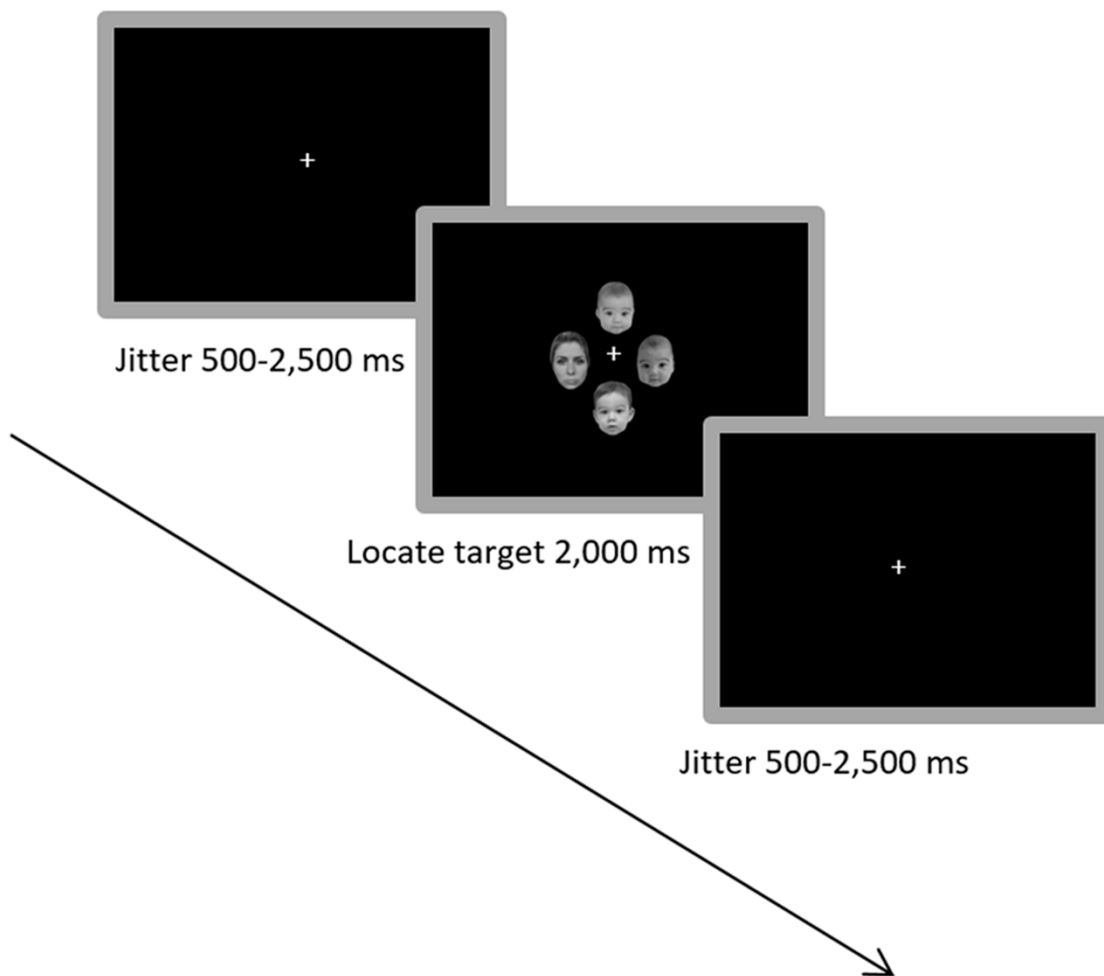


Figure 3: Procedure of the Target Detection Paradigm. The task was to select the one picture that did not fit with the category of the others and to confirm this via the associated button as fast and accurately as possible. The target was either an adult face shown in the context of three infant faces as shown here or an infant face among three adult faces [figure from (Holtfreich et al., 2018)]

1.3.3. Measuring brain activity with functional magnetic resonance imaging

Sectional images of the body can be displayed via magnetic resonance tomography (MRI). The procedure of the MRI is based on the spin property of hydrogen atomic nuclei (in most applications) and their inferential magnetic dipole moment. Under normal conditions the spins are disorganized. By using a static magnetic field the hydrogen atomic nuclei are stimulated to perform coordinated and phasesynchronous spinning movements. This induced signal can be

measured by MRI. However, the movements alone do not create an image. Radio frequency is needed to briefly switch the spins. The further organization of the spins after the 'switch' can be measured by the thermal conductivity of the tissue (T1-relaxation) or by the decline of the transversal magnetization (T2-relaxation) (Hashemi et al., 2012; Liang and Lauterbur, 2004).

The principal non-invasive method of mapping brain active processes is the functional MRI (fMRI) (Jezzard et al., 2003). For the display of real time brain active processes the fMRI makes use of the blood oxygenation level dependent (BOLD) signal. In case of a neural activation, an increase of the local energy demand is expected. The observation that the blood flow to the activated brain areas increased, led to the assumption, that measurable blood oxygenation and flow provides information about neural activation (Ullsperger and Debener, 2010). These changes in blood oxygenation and blood flow in the brain can consequently be measured with fMRI (Beisteiner, 2006; Ullsperger and Debener, 2010). The physical technique underlying the measuring of the BOLD signal is based on the diamagnetic properties of oxygenated haemoglobin and the paramagnetic properties of the desoxygenated haemoglobin (Ogawa et al., 1990). The paramagnetic properties of the desoxygenated haemoglobin generate a disruption in the homogeneity of the magnetic field and lead to a rapid spin dephasing and consequently to an accelerated drop of the signal. Consequently, the simultaneous measuring of the magnetic signal provides the possibility to measure the blood flow in the activated brain regions in contrast to non-active regions (Beisteiner, 2006). In comparison with BOLD signal changes (seconds), changes in neural activity are very short events (milliseconds). Computer algorithms must therefore be used to account for this time lack. For analysis of the brain imaging data sequences, the use of the *Statistical Parametric Mapping* (SPM) software has a frequent practice (Beisteiner, 2006; Friston et al., 2003). With this software fMRI data can be corrected, processed and analyzed. Using this technology enables to measure, for instance, visual, acoustic and olfactory as well as attentional and motoric cognitive status changes (Beisteiner, 2006) and reward processes (Gorka et al., 2015).

The SPM preprocessing consists of multiple steps. Preparing the data includes the coregistration & realignment. These steps imply the definition and positioning of the single images and the alignment on the basis of a reference picture. This process serves for reduction of an artifactual variance in the voxel time-series. Due to brain anatomical variations between the participants the images must be normalized onto a standard anatomical space thereafter. This step is followed by smoothing the data to improve the spatial resolution (Friston et al., 2003).

The preprocessing is then followed by the first level analysis. Here, a design matrix has to be prepared that contains information about the paradigm (e.g. number of trials & interscan interval). For first level analysis it has become standard practice to use a general linear model for a voxel-by-voxel analysis (Friston et al., 2003). In the following random-effect analyses the contrasts estimated from a first level are included in a second level analysis (Friston et al., 2003).

For the analysis of the fMRI data I made also use of a general linear model on the first level for event-related analysis [*see* (Holtfrerich et al., 2018)]. The predicted hemodynamic response to each experimental condition was defined in a vector with the temporal onset of the conditions ‘*target*’ (adult or infant), ‘*morph type*’ of the baby (higher, unmanipulated or lower baby schema), ‘*gender*’ (female or male) and the baseline task. To examine the specific effects of the baby schema, linear *t*-contrasts were defined. The individual contrast definitions can be seen in Holtfrerich et al. (2018).

A full factorial 3 by 2 repeated-measures general linear model analysis (Analysis of variance - ANOVA) was used for random effects analysis on the second level. The ANOVA included the factors ‘*morph type*’ (3 steps) and the between-subject factor ‘*treatment group*’ (2 steps). *T*-tests were used to assess specific differences between the conditions. Whole brain correlations were also used to analyze activations related to the attention to babies and the different treatment groups [*for details see* (Holtfrerich et al., 2018)].

1.3.4. Salivary hormone measurements

Neutral steroids can rapidly pass the salivary glands. Therefore salivary samples for steroid hormone measurements offer a precise and stress-free way and a non-invasive method in comparison to blood sampling. Another important benefit of salivary steroid hormone measurements is that the participant can collect the samples alone at home after a short instruction. As the bounded steroid hormones cannot pass the salivary glands, salivary steroid concentrations reflect the free and unbounded steroid hormones (Lewis, 2006). In this thesis, the free in saliva testosterone concentration of female participants was determined. Eppendorf tubes (2 mL) were used for collection at home at the day of testing. The participants brought the samples to the appointment, where the samples were subsequently frozen at -20°C until analysis. For analysis the samples were unfrozen and subsequently centrifuged at a relative centrifugal force (RCF) of 604 times gravity ($\times g$) for 5 minutes to separate the samples from mucus.

The analysis was done using a testosterone luminescence immunoassay from IBL International (TECAN group global; Hamburg, Germany). To control for accuracy, the samples, seven standards and two controls were pipetted twice on the plate. The analysis was done according to the manual of the testosterone luminescence immunoassay.

The technique is based on the competition principle. The unknown amount of antigens in the saliva sample competes with a defined and enzyme conjugated amount of alkaline phosphatase conjugated 3-carboxymethyl oxime-testosterone derivative for the binding sites of antibodies fixed on the wells. Over the analysis, the unbounded enzyme conjugated antigen gets eliminated by washing. The addition of the luminescence substrate than offers the opportunity to measure the inversely proportional amount of antigens in the saliva sample (Goncharov et al., 2006).

1.3.5. Hormonal intervention

Oxytocin poorly passes the blood to brain barrier after it has entered the periphery but it can pass the nervus olfactorius through intranasal administration and distribute to the central nervous system (Guastella et al., 2013). At the current state of research we do not know precisely how oxytocin administration exactly gets into the brain and thus influences neural activity. But it is likely that intranasal oxytocin can use different pathways to the body: through nasal vasculature into the systemic circulation, through swallowed mucosa into the gastrointestinal tract, through olfactory bulb routes in the cerebrospinal fluid and in the brain, over the trigeminus nerve in the brain and over paravascular spaces into the interstitial spaces of the brain (*for review see Guastella et al., 2013*). However, intranasal oxytocin was shown to increase the oxytocin concentrations in the cerebrospinal fluid and in plasma over twice as high 40 min after administration [*here in macaques (Simia inuus), (Dal Monte et al., 2014)*]. And it has been shown that vasopressin – which has a similar molecular structure as oxytocin (Jezova et al., 1995) - entered the human central nervous system, after intranasal inhalation (Born et al., 2002).

Further, 24 international units (IU) of intranasal administered oxytocin – such as used in the studies of this thesis - have been shown to increase oxytocin concentrations in saliva substantially and not to return to baseline within a four hours period. The study showed that 15 minutes after administration, the oxytocin level in saliva raised dramatically but reached a plateau at 45- 120 minutes after administration (Weisman et al., 2012). Therefore it is common practice to start the experiments after 45 minutes exposure time [*for review see (Bakermans-Kranenburg and van I*

Jzendoorn, 2013; Born et al., 2002)]. Another study found that the intranasal administration of 16 or 24 IU raised the oxytocin levels in saliva up to 6 to 10-fold higher than in the placebo condition up to seven hours after administration (van IJzendoorn et al., 2012).

In addition to the physiological parameters, it is important to note, that oxytocin administration has been shown to modulate behavioral changes in primates and other mammals several times [e.g. (Bertsch et al., 2013; Gamer et al., 2010); *for review see* (Luo et al., 2015; Wigton et al., 2015)].

To complete the information, a systematic review on safety and side effects of oxytocin administration showed that intranasal oxytocin did not cause any detectable subjective changes in the participants, did not cause any side effects specific to oxytocin and did not cause adverse outcomes in doses of 18-40 IU (MacDonald et al., 2011).

Collectively, oxytocin versus placebo administration seems to offer a reliable method to investigate behavioral influences of the neuropeptide.

To investigate the influence of endogenous testosterone and exogenous oxytocin on caretaking behavior, I made use of the methods reported above. Below, the main hypotheses of the behavioral and the fMRI study are briefly summarized.

1.3.6. Hypotheses of the behavioral study

Infant faces display a strong socially salient stimulus and should thus not only evoke positive feelings, but also rapidly recruit attention and motivate action. Oxytocin is known to promote caretaking and to modulate maternal behaviors (see Chapter 1.1.1.1). The behavioral study [(Holtfrerich et al., 2016); *Study II*] aimed to examine the interactive influences of endogenous testosterone and exogenous oxytocin on the attentional processing of infant faces in nulliparous women and to assess if infant faces with a varying degree of baby schema (pictures were manipulated towards or against the baby schema or remained unmanipulated) modulate attentional processing. Exogenous oxytocin was expected to promote attention towards infant faces and to diminish the negative effects of a high endogenous testosterone concentration on attention towards infant faces. Further, it was expected, that infant faces with a stronger baby schema may recruit selective attention to a greater extent than infant faces with a lower baby schema (see Chapter 1.2).

1.3.7. Hypotheses of the fMRI study

Functional brain imaging was used in order to further understand the neuronal basis of the influences of endogenous testosterone and exogenous oxytocin on the attentional processing of infant faces in nulliparous women (Holtfrederich et al., 2018). In the interest of comparability with the previous findings, the study design was similar to the behavioral study design. Building on the previous findings it was hypothesized that women with a high testosterone concentration would derive greater benefit from oxytocin administration and direct attention towards infants whereas women with low testosterone concentrations were predicted to remain less influenced or even unaffected. Further, it was expected, that infant faces with a stronger baby schema would increase attention in comparison to infant faces with lower baby schema. The neuronal basis of the interaction of oxytocin and testosterone in a caretaking context has been largely unexplored, but there already exists some evidence, that oxytocin and the degree of baby schema may modulate caretaking behaviors through mesocorticolimbic dopamine pathways (see Chapter 1.1.1.1 and 1.1.1.3.). It was therefore expected, that oxytocin administration may modulate caretaking behavior through activations in the reward system particularly through mesocorticolimbic dopamine pathways in the female brain that may influence processing of infant faces. Based on the behavioral findings it was further expected, that women with high endogenous testosterone concentrations may be more responsive to oxytocin administration and this responsiveness should be reflected in brain activations.

2. Publications

2.1. Publication I

Endogenous Testosterone and Exogenous Oxytocin Modulate Attentional Processing of Infant Faces.

Sarah K. C. Holtfrerich, Katharina A. Schwarz, Christian Sprenger, Luise Reimers, Esther K. Diekhof

Contribution to publication I

The studies of publication I were conceptualized by SH under supervision of ED. ED and SH designed the paradigm and the experimental procedure. Data were collected and curated by SH by communicating with KS in Study II. CS was the associated doctor of this study and provided medical supervision. The statistical analysis was performed by SH. SH wrote the first draft of the manuscript. All authors contributed to reviewing the manuscript.

Date and Place

Signature of the supervisor

Prof. Esther K. Diekhof

RESEARCH ARTICLE

Endogenous Testosterone and Exogenous Oxytocin Modulate Attentional Processing of Infant Faces

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Abstract

Evidence indicates that hormones modulate the intensity of maternal care. Oxytocin is known for its positive influence on maternal behavior and its important role for childbirth. In contrast, testosterone promotes egocentric choices and reduces empathy. Further, testosterone decreases during parenthood which could be an adaptation to increased parental investment. The present study investigated the interaction between testosterone and oxytocin in attentional control and their influence on attention to baby schema in women. Higher endogenous testosterone was expected to decrease selective attention to child portraits in a face-in-the-crowd-paradigm, while oxytocin was expected to counteract this effect. As predicted, women with higher salivary testosterone were slower in orienting attention to infant targets in the context of adult distractors. Interestingly, reaction times to infant and adult stimuli decreased after oxytocin administration, but only in women with high endogenous testosterone. These results suggest that oxytocin may counteract the adverse effects of testosterone on a central aspect of social behavior and maternal caretaking.

Introduction

Strong mother-infant interactions are vital for reproductive success [1]. They are modulated by different hormones, for instance the neuropeptide oxytocin, which is particularly known for influencing maternal behavior [2,3]. It not only plays a major role in the induction of labor and lactation (*here* in rats) [4], but also modulates behavior associated with caretaking (e.g., nestbuilding and nursing in mice) [5] and bonding [6]. Additionally, oxytocin administration improves empathy in humans [3,7]. Especially during the nursing period, improved empathy may enhance the mothers' capacity to understand the nonverbal needs of her offspring [8].

In contrast to oxytocin the androgen testosterone is mainly associated with aggressive and dominant behavior [9–11] and may decrease caretaking, helpfulness [12], and empathy [13–15]. Furthermore, testosterone promotes masculinization and correlates negatively with

prosocial behaviors that are important for childrearing (e.g., nurturance and empathy) and maternal attributes [3,9,12] (but also see [16,17] for a different role of testosterone in male-male cooperation during intergroup conflict). During parenthood testosterone levels decrease, which could be an adaptation to facilitate parental investment [18,19]. These previous findings raise the question considering a possible interaction and even opposing roles of testosterone and oxytocin in parental care. First evidence for an interaction between these two hormones in the modulation of behavior comes from a study by Winslow and Insel [20] on the hormonal modulation of sexual and aggressive behavior. The authors reported an increase of these behaviors in male squirrel monkeys after oxytocin administration, but only in dominant males with high plasma testosterone concentrations [20]. Further support for an interaction between testosterone and oxytocin is offered by research on variations in the oxytocin receptor gene that seem to be linked to a common biomarker (2D:4D ratio) to measure prenatal testosterone—but only in male participants [21]. Hence, these results also suggest that the interaction between testosterone and oxytocin is sexually dimorphic. Another study, which investigated hormonal shifts in Tsimané hunters, found that both, endogenous oxytocin and endogenous testosterone, increased after hunting [22]. The authors presumed that the elevated testosterone levels might have resulted from successful hunting while the increase of oxytocin could enhance social salience. Further suggesting an interaction between both hormones was the fact that the percentage of increase of both hormones was strongly correlated [22].

Most interestingly, there exists also initial evidence considering the interacting effects of oxytocin and testosterone on paternal behavior. Weisman et al. [23] found that the administration of 24 IU oxytocin nasal spray versus placebo led to a short-term alteration of salivary testosterone concentration in fathers. The authors observed that paternal behavior like positive arousal, social gaze, and vocal synchrony increased through oxytocin related changes in testosterone concentrations. In addition, lower baseline testosterone levels in those fathers were associated with more optimal paternal behavior [23]. Even though low testosterone levels as well as high oxytocin levels are usually linked to parental behavior [18,19,24–27] there might exist situations that lead to an increase of both hormones in order to modulate behavior (such as hunting-, protection-, or sexual-behavior) [22,23,26,27]. Nevertheless, a hormonal mechanism through interaction between testosterone and oxytocin that increases responsiveness to babies could be an adaptive feature that facilitates caretaking in adults and should thus enhance reproductive success.

One known mechanism, that automatically elicits parental care and thus enhances the survival rate of the offspring, is the baby schema [28–30]. Baby schema is a key stimulus (“Schlüsselreiz”) that automatically elicits caretaking in adults [28]. It is defined as a combination of different child characteristic features like chubby cheeks, a large forehead, and huge eyes [28]. Previous studies revealed that baby schema induces protection behavior [31], motivates adults to speak in “baby language” [32], and initiates caretaking behavior [33,34]. The impact of cuteness perception and baby schema was investigated by a number of studies [29,35–38]. Important to note is that baby schema seems to be a universal stimulus. On the one hand, because it appears in almost every mammal [28], and on the other hand, because the degree of baby schema in human adults or infants, cats and dogs modulates cuteness perception [37,39–41]. Especially for women of reproductive age, newborns are extremely relevant stimuli, because their survival is directly linked to reproductive success. This applies not only to one’s own, but also to other, associated offspring. Alloparental investment is common in humans, because human infants are very costly to raise. The assistance of alloparents could thus act as a benefit for reproductive success [42]. In this case, the stimulus baby schema should not only lead to a positive emotional reaction, but may also provoke prioritized attention as stimuli with high biological relevance are assumed to be processed preferably by the attentional system [43]. If

so, adult threatening stimuli (lions and polar bears in this case) should have a processing advantage in the attentional system too, because of their immediate relevance for survival [44].

Several studies suggest differences in the intensity of parental behavior between men and woman (for review see [3]). Cárdenas and colleagues [42] for example, were able to show that even young nulliparous women looked longer at infant faces and fixated the infant faces more often than female or male adult faces, whereas men were only interested in infant faces when these were presented with a male adult face and not with a female adult face. Nevertheless, the research field of the role of reproductive hormones, such as progesterone [45], estradiol [46] and testosterone [23] in parental behavior is still relatively young (for review see: [27] and [47]). Also the role of testosterone in processing baby schema and its interaction with oxytocin remains rather elusive to date (for review see: [3,24,26,27]).

The present project had the aim to investigate the effects of testosterone and oxytocin on behavior associated with parental care and to gain further insights regarding a possible interaction between these hormones. For one thing, we wanted to examine to what extent the endogenous testosterone level influences the processing of visual key stimuli (baby schema in different species) in women (Study I). Specifically, we wanted to assess how endogenous testosterone modulates selective attention to these stimuli and their evaluation. Based on previous evidence on the role of testosterone in social cognition, we predicted that in women the endogenous testosterone concentration should negatively correlate with the speed of attentional orienting to pictures of infants. For another thing, we were interested in the interaction between endogenous testosterone and exogenous oxytocin (i.e., intra-nasal administration), since these hormones seem to exert opposite influences on maternal caretaking behavior (Study II) [23,24,27]. For this purpose, we examined the effect of the “bonding hormone” oxytocin on attention to baby schema in women with high versus low endogenous testosterone concentrations.

We expected that oxytocin administration would promote maternal behavior by diverting prioritized attention towards baby faces especially in women with high testosterone concentrations.

Methods

The present project was divided into two parts. In both studies, participants performed a computer based implicit *face in the crowd* paradigm (i.e., the ‘*target detection task*’). The target detection task was based on the *odd one out* principle. The participant was instructed to select the one stimulus out of four pictures that did not fit in the crowd (e.g., the infant among three adults, or the adult among three infants). Our aim was to gain information about selective attention as represented by the reaction time (RT). As implied by the specific demand of the target detection task, RT should decrease if the attention is focused on the target (an infant or an adult individual): However, if the participant’s attention is focused on the three pictures that were used as distractors (three infant or three adult individuals), RT should increase.

In the first study we examined the universality of the baby schema by using different human and non-human animal species as stimuli. For each species we had two conditions: the high distracting condition (with the adult target and the infants as distractors) and the low distracting condition (with the infant target among three adults).

In a second study we investigated the relevance of the amount of baby schema for attention processes with another target detection paradigm. To this end, we manipulated different pictures of human babies with respect to the degree of their baby schema. We had three different high distracting conditions in this task (adult among three babies with high, neutral or low amount of baby schema) and three different low distracting conditions (baby with high, neutral or low amount of baby schema among three adults).

All participants were women of reproductive age who took hormonal contraception. Informed and written consent was given by each subject and ethical approval was obtained from the ethics committee of the *Ärztammer Hamburg*.

Methods Study I

Participants of Study I. Twenty healthy women (mean age \pm SD = 23.95 \pm 2.67 years) with normal or corrected to normal vision were recruited to participate in this study. They received 15 Euros for their participation.

Stimuli and procedure of Study I. In the first study, we used pictures of humans (female and male), two species of predators (lion and polar bear), two domestic animals (dog and goat) and a non-human primate (orang-utan). Pictures from different species were used as stimuli in order to test for the universality of baby schema (see [43] for a similar procedure). We took the pictures of adult humans out of the FACES database of the Max-Planck-Institute Institute for Human Development, Center for Lifespan Psychology, Berlin, Germany [48]. All other pictures were obtained from a google picture search.

Using Adobe Photoshop CS5 the faces were cropped from the background and presented on a black square sized 300 pixels. In each trial (duration = 2000 ms), four pictures of the same species were arranged in a cross pattern and presented on a computer monitor. The presented pictures of adult humans and lions were from the same gender. The gender of the other animals was not obvious. The order of the trials was randomized. Subjects were asked to identify the *odd-one-out* on a display of four faces (either an adult target out of three infant distractors or an infant target out of three adult distractors; see Fig 1) via button press, which had to be executed as fast and as accurate as possible. The button positions conformed with the picture positions. During this procedure, the participant's response time to the targets was measured. The duration between the appearance of the stimulus and the button press was associated with the selective attention the stimulus attracted.

Fig 1 illustrates an example of two trials of the *target detection paradigm*. The paradigm had 392 trials. Each combination was shown 56 times (28 times with the baby target and 28 times with the adult target).

Sampling procedure of Study I. Eppendorf tubes (2 ml) for saliva collection were handed to the participants in advance. To determine a proxy of the bioactive testosterone (T) concentration, subjects were instructed to collect five saliva samples in the morning at the day of testing. This was done to control for fluctuating secretion patterns of T. Subjects were asked to provide a saliva sample every 30 minutes starting at normal wake up time. During the sampling procedure (two hours in total) they were not allowed to eat or to consume any other animal source products like, for instance, milk. Furthermore, the time lag between the last consumption of animal source products and saliva sampling had to be at least 12 hours. Smoking was forbidden during the whole sampling procedure. Subjects were allowed to drink water

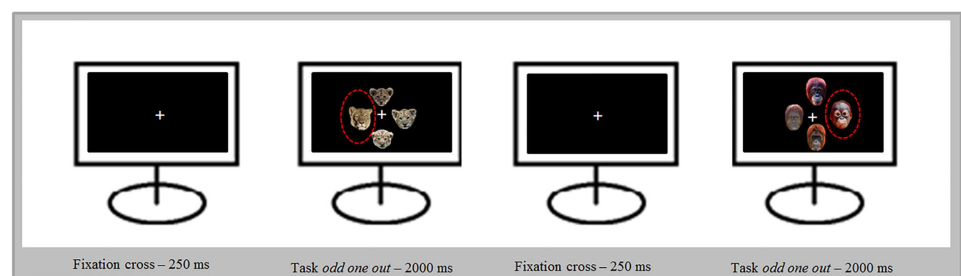


Fig 1. Illustration of two trials out of the *target detection paradigm*.

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between collection intervals as well as to brush teeth directly after the first sample collection (to avoid blood contamination), but only until five minutes before the sample collection.

Until analysis, saliva samples were frozen at -20°C . Before assaying, saliva samples were thawed and then centrifuged at RCF 604 \times g for 5 minutes (i.e., 3000 rpm in a common Eppendorf Minispin centrifuge) to separate saliva from mucus. Following this, an aliquot out of the five samples was prepared by extracting and combining equal volumes (depending on the filling level of the tubes) of the clear and colorless supernatant from each sample. Subsequently, saliva samples were analyzed with a T luminescence immunoassay from IBL (Hamburg, Germany). Each sample was assayed twice and two standards as well as one high and one low control sample were also analyzed on the same assay. Intra- and inter-assay coefficient variances were denoted to range between 1.47–3.01% and 4.04–6.96%, and the formal sensitivity of the assay kit between 1.8 pg/ml and 500 pg/ml T in saliva. Samples were pipetted into the assay plate. The plate was coated with mouse anti-testosterone antibody. Standards and one high and one low control sample were also analyzed on the same assay. Analysis was performed according to the description in the manual and took place in our in-house laboratory.

Salivary measurements provide a reliable and precise method to quantify the bioactive and unbounded T concentration, because the bounded T cannot pass the salivary glands [9]. Moreover, salivary measurements offer a decisive advantage compared to serum measurements in behavioral studies: They are non-invasive and therefore stressless for the participant [49].

Data analysis Study I. Data were analyzed with IBM SPSS statistics 19. All data were tested for a significant deviation from normal distribution with the Kolmogorov Smirnov Test. Response times were analyzed with a 2×7 (target (adult or infant) \times species) repeated measures ANOVA with T concentration as covariate. We used the Greenhouse-Geisser corrected values, if the sphericity assumption was not met. Paired *t*-tests were used for follow-up comparisons.

The mean T concentrations from the aliquot were correlated with response time differences representing the distraction by baby schema in human babies using Pearson correlations. For this purpose, relative RTs were calculated by subtracting the low distracting condition (i.e., infant target and adult distractors) from the high distracting condition with an adult target and three baby faces as distractors. The higher the relative RT the more the participant was distracted by the baby schema. Three participants were excluded from data analysis because of missing T data and one participant was excluded because of problems during data recording. Significances are reported two-tailed if not otherwise indicated and one-tailed in case of clear a priori assumptions.

Methods Study II

Participants Study II. Thirty-eight healthy and nulliparous women were recruited to participate. They had normal or corrected to normal vision. Mean age (\pm SD) averaged 24.12 ± 2.44 years. They received 25 Euros for their participation.

Stimuli and procedure of Study II. To investigate the effects of high habitual T concentrations on selective attentions towards baby faces after oxytocin administration versus placebo administration, we employed a replication of study I, but with a changed stimulus set. On basis of the results of study I, we used only human pictures to investigate the relevance of the amount of baby schema for attention processes. The presented pictures of adult humans were from the same gender. To this end, we parametrically manipulated the pictures of human infants with respect to the amount of baby schema (BS) using Adobe Photoshop CS5. We altered the size of the eyes, mouth, nose and the shape of face, which are the typical features that determine the baby schema after Konrad Lorenz (see [33] for a similar procedure). For an example of the manipulation stimuli see Fig 2.



Fig 2. Example for a parametrically manipulated baby. From left to right: low BS condition, unmanipulated baby picture and high BS condition.

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Prior to our study the pictures of the babies were evaluated by another 36 female participants using a scale from zero to nine (0 = “not cute at all”, 9 = “extra cute”).

For the target detection task we only used pictures, for which the manipulated version with a high amount of baby schema was rated as significantly cuter than the corresponding low baby schema version as shown by Wilcoxon-tests (**Baby A**: $M_{minus} = 6.28$, $SD = 2.26$; $M_{plus} = 7.19$, $SD = 2.12$; $Z = -3.334$, $p = 0.001$; **Baby B**: $M_{minus} = 4.97$, $SD = 2.26$; $M_{plus} = 7.25$, $SD = 2.32$; $Z = -4.711$, $p < 0.001$; **Baby C**: $M_{minus} = 5.03$, $SD = 2.47$; $M_{plus} = 7.06$, $SD = 1.90$; $Z = -4.460$, $p < 0.001$; **Baby D**: $M_{minus} = 5.50$, $SD = 2.51$; $M_{plus} = 6.50$, $SD = 2.57$; $Z = -3.105$, $p = 0.002$).

The faces were cropped from their original background and presented on a black square.

The pictures of adult humans were taken from the data base FACES from the Max-Planck-Institute for Human Development, Center for Lifespan Psychology, Berlin, Germany [48]. Infant pictures were obtained from a google picture research.

As in study I, each trial consisted of four pictures that were arranged in a cross pattern around a fixation cross. In each trial either three baby pictures with the same amount of BS (i.e., high BS, low BS, or unmanipulated BS) and one adult target were shown, or three adults in combination with one baby target (high BS, low BS, or unmanipulated BS) were presented (The experimental procedure conformed to study I but the stimuli in this task consisted of human faces only; the infant faces were manipulated in their amount of baby schema). The order of the trials was randomized.

Each trial lasted 2000 ms. Altogether, this paradigm consisted of 240 trials with each condition shown 40 times.

Oxytocin administration in Study II. The experiment was double-blind placebo controlled and used a between subjects design. 19 of the female participants were included in the oxytocin group (experimental group) and 19 were included in the placebo group (control group). In the presence of an assistant, participants self-administered a nasal spray. The nasal spray contained either oxytocin or the placebo-control consisting of chlorobutanol-hemihydrate (0.5%) with no active treatment. In total, 24 IU of oxytocin nasal spray (Syntocinon) were administered per participant in the experimental group. It is known from previous studies that 24 IU of intranasal oxytocin are sufficient to increase the oxytocin levels in saliva up to 6 to 10-fold higher than oxytocin levels in the placebo condition 7 hours after administration (for a review see [50]). After administration we waited 45 minutes to continue with the experimental procedure to ensure high treatment efficacy [51].

Former research has shown that the beliefs about the hormonal administration can also influence the behavior of the participant [52]. To control for the participants' treatment expectations, we pseudo-instructed the participants about the content of the nasal spray. For this purpose the assistant handed an envelope, which included either an 'oxytocin' or an 'empty compound' label, to the participant. Half of the participants of both the experimental and the placebo group received the information that their nasal spray contained oxytocin. The other half was instructed that their nasal spray contained an inactive substance. The participants were instructed by the assistant not to inform the experimenter about the content of the nasal spray. Hence, the experimenter neither knew which treatment nor which instruction a participant was given. The assistant left the room after the nasal sprays were administered, so that the following questionnaires and experiments were conducted only under the supervision of the experimenter in a double-blind fashion. After the experimental sessions were completed, the participants were asked if they had believed the previous instruction.

Sampling procedure of Study II. In this study three saliva samples were collected over one hour directly after waking up. This was done for reason of time economy, since the whole testing procedure (i.e., oxytocin administration and behavioral testing) required another two hours on the same day. However, three samples are sufficient for a representative daily T measure.

Data analysis of Study II. Data were analyzed with IBM SPSS statistics 19. The general effects were assessed in a 2 (drug group) x 2 (median split of the testosterone concentration) x 2 (target: adult or infant) x 3 (condition: target or distractor with low BS, unmanipulated BS, or high BS) repeated measures ANOVA.

For exploratory research on the basis of the results of study I, the sample was then divided into two parts via a median split to separate women with high from women with low T concentrations. Response times were then analyzed with a 2 (drug group) x 2 (target: adult or infant) x 3 (condition: target or distractor with low BS, unmanipulated BS, or high BS) repeated measures ANOVA. Greenhouse-Geisser corrected values are reported in cases where the sphericity assumption was not met. Post hoc *t*-tests were used to compare the mean RTs between the control and the experimental group in women with high and low T concentrations and to compare the RTs of low T and high T subjects directly.

For replication of study I, T concentrations were correlated with response time differences representing prioritized attention to baby schema using Pearson correlations. For this purpose, relative RTs were calculated by subtracting the low distracting condition (i.e., infant target and adult distractors) from the high distracting condition (i.e., adult target and three baby faces as distractors). Additionally, we analyzed the relative RTs (representing prioritized attention to baby schema) for the three baby schema conditions separately. Finally, to test for a possible belief effect through pseudo-instruction, the response time differences were analyzed with a one-way ANOVA (for a similar analysis procedure see [52]) including the independent variable pseudo-instruction (i.e. if the participants were instructed accurately or incorrectly). Significances are reported two-tailed if not otherwise indicated and one-tailed in case of clear a priori assumptions.

Results

Results of Study I

The RTs were significantly influenced by the species of the presented pictures ($F_{6,102} = 68.23$, $p < 0.001$, $n_p^2 = 0.801$), and the age of the target (adult vs. infant) ($F_{1,17} = 6.14$, $p = 0.024$, $n_p^2 = 0.265$). Furthermore there was a significant interaction between the age and the species of the target ($F_{6,102} = 3.32$, $p = 0.005$, $n_p^2 = 0.163$). We also found a statistical trend for an interaction between target age and T concentration ($F_{1,17} = 3.77$, $p = 0.069$, $n_p^2 = 0.182$).

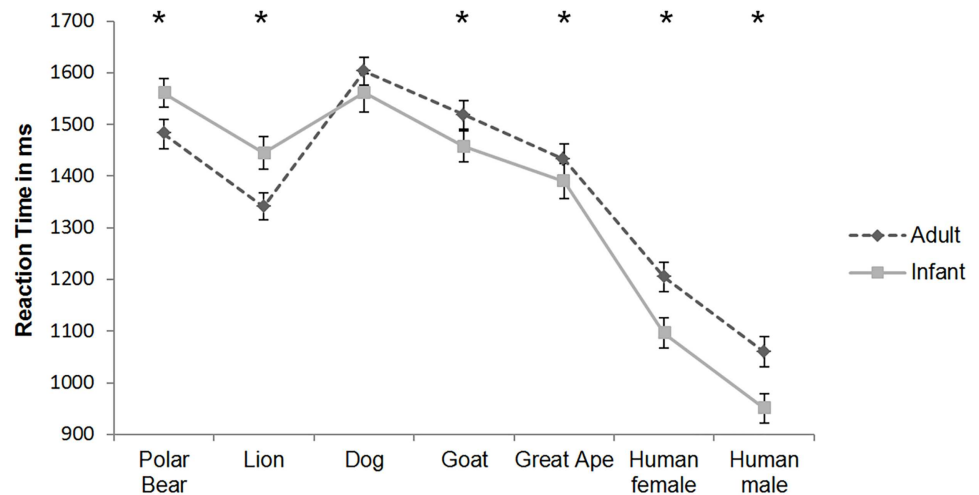


Fig 3. Mean reaction times in ms to select the infant or the adult target (± SEM). * $p < 0.05$, paired t -test, one-tailed.

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Post hoc t -tests showed that participants required significantly more time to select an adult stimulus when primates (including humans) and goats were presented (all p values < 0.05). We also observed a trend in the same direction in the dog condition ($p = .0092$, one-tailed).

In contrast, participants required significantly more time to locate the infant predator (lion or polar bear) among three adult predators. Results of post hoc t -tests are presented in Fig 3.

The mean salivary T concentration of the participants was $M = 15.77$ pg/ml \pm $SD = 9.3$ pg/ml. T levels were negatively associated with the distraction by baby schema in human pictures as represented by the relative reaction times (i.e., RTs for adult target minus RTs for adult distractors: $r = -0.402$, $p = 0.04$; one-tailed; see Fig 4). Thus, T concentration in women seems to influence orientation and responding to infant targets.

Results of Study II

The mean T concentration of the participants was $M = 18.84 \pm SD = 16.97$ pg/ml. T concentration did not differ between the medication groups ($M_{oxytocin\ group} = 15.8$ pg/ml \pm $SD 16.42$; $M_{placebo\ group} = 21.88$ pg/ml \pm $SD 17.41$; $t_{36} = -1.108$, $p = 0.275$). To control for so called 'belief effects' the participants were pseudo-instructed about the content of the nasal spray, whereby half of the participants were instructed accurately and half of the participants were instructed incorrectly. This was done in light of previous research suggesting that the "folk wisdom" about the effects of the androgen T is partly responsible for behavior of the participant [52]. Interestingly, if the participants thought they got oxytocin, they were significantly slower in orienting attention towards adult targets and the minus baby target condition ($p < 0.05$). When participants really got oxytocin participants were faster in identifying any target in the minus condition ($p < 0.05$) and still, with a statistical trend, in the plus condition ($p < 0.1$). However, in the present study, the pseudo-instruction (if the participant was instructed accurately or incorrectly) had no effect on the distraction by baby schema (e.g. Delta adult target—baby target) ($F_{1,37} = 0.716$, $p = 0.403$), only on the reaction times in general, which is important for the following analysis. Therefore this will not be considered in the following analyses, but is a very interesting research topic for further studies.

The general effects were analyzed in a 2 (drug group) \times 2 (median split of the testosterone concentration) \times 2 (target) \times 3 (condition) ANOVA. We found an interaction between target

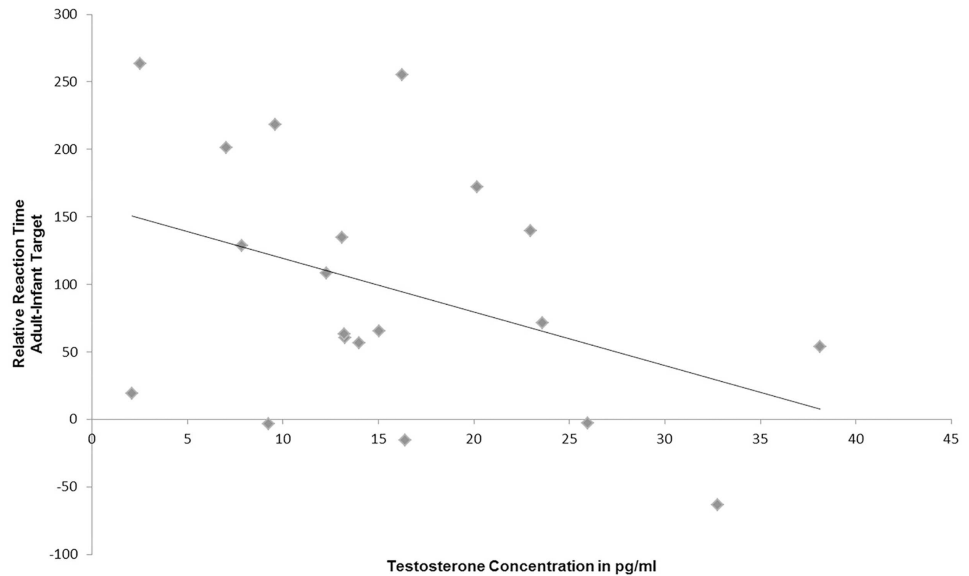


Fig 4. Negative correlation between salivary testosterone concentration and selective attention to human infant portraits ($r = -0.402$, $p = 0.04$, one-tailed).

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age (adult or baby) and condition ($F_{2,68} = 6.277$, $p = 0.003$, $n_p^2 = 0.156$). There also was a significant effect of the condition ($F_{2,68} = 5.685$, $p = 0.005$, $n_p^2 = 0.143$) and of the target age (adult or infant) (target age: $F_{1,34} = 25.404$, $p < 0.001$, $n_p^2 = 0.428$). The effect of the drug group ($F_{1,34} = 2.347$, $p = 0.135$, $n_p^2 = 0.065$) and the effect of the median split of the testosterone concentration did not reach significance ($F_{1,34} = 2.270$, $p = 0.141$, $n_p^2 = 0.063$). We could not find any further interaction between the drug group or the median split of the testosterone concentration and the other factors.

As T concentrations decrease in parents during parenthood [18,19] and oxytocin leads to increased bonding behavior during parenthood [53], it seems viable that particularly individuals with high endogenous T levels might be affected by oxytocin administration. For exploratory reason and on the basis of the results of study I we tested this hypothesis, by dividing the participants into a high and a low T group ($n = 19$ per group). A $2 \times 2 \times 3$ ANOVA was run to test for effects of target age and the condition of the target or the distractor (low BS, high BS or unmanipulated BS) on the attention behavior in women with low and women with high T concentrations separately. In women with low T concentrations the condition of the target or the distractor affected the participants' RT to the target stimuli ($F_{2,34} = 4.59$, $p = 0.017$, $n_p^2 = 0.213$). We also found a significant effect of target age ($F_{1,17} = 6.487$, $p = 0.021$, $n_p^2 = 0.276$). We also found a significant main effect of the interaction between target age and condition ($F_{2,34} = 4.092$, $p = 0.026$, $n_p^2 = 0.194$). There was no significant effect or interaction with the other factors of medication in the low T condition.

Interestingly, in women with high T concentrations, there was a significant main effect of medication ($F_{1,17} = 5.096$, $p = 0.037$, $n_p^2 = 0.231$). However, the condition of the target or the distractor did not reach significance anymore ($F_{1,34} = 1.969$, $p = 0.15$, $n_p^2 = 0.104$) and the interaction between target age and condition only reached statistical trend level ($F_{1,34} = 2.461$, $p = 0.1$, $n_p^2 = 0.126$). Nonetheless, there was an effect of the factor target age in the high T condition ($F_{1,17} = 24.067$, $p < 0.001$, $n_p^2 = 0.586$).

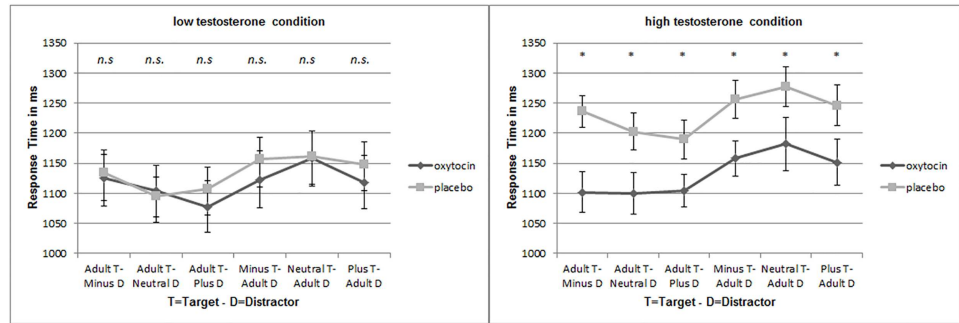


Fig 5. In both figures, the mean reaction times to select an adult or an infant target are shown for both administration conditions (oxytocin or placebo) (\pm SEM). The reaction times to select an infant target are shown for the three baby schema conditions: minus = low BS, neutral = unmanipulated BS, plus = high BS. On the left side you can see the results for women with low T concentrations ($n_{lowT} = 19$) and on the right side you can see the results for women with high T concentrations ($n_{highT} = 19$). Not significant = n.s., * = $p < 0.05$, t-test, one-tailed.

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T-tests were used to compare the mean RTs between the experimental and control group (i.e., oxytocin vs. placebo). We were especially interested in the effects of oxytocin on attention to baby schema in women with high versus low endogenous T concentrations. The participants' mean RTs to adult or infant targets did not differ between the oxytocin and placebo treatment groups in women with low T concentrations (for low T women see Fig 5 left side). However, women with high T concentrations needed more time to locate the target when they were in the placebo treated group (irrespective of target age and amount of baby schema). Interestingly, RTs of high T women that were treated with oxytocin aligned with the low T group (there was no significant difference between response times of high T women that were treated with oxytocin and response times of the low T women in the oxytocin or placebo condition) (see Fig 5 right side). The RTs of the low and the high T subjects differed significantly in all three baby target conditions (high BS: $t_{36} = -1.994$, $p = 0.026$; neutral BS: $t_{36} = -1.952$, $p = 0.029$; low BS: $t_{36} = -2.086$, $p = 0.022$, t-test; one-tailed) and in the adult target condition with the plus morphed distractor ($t_{36} = -1.804$, $p = 0.039$; t-test; one-tailed). In the condition with the adult target and the minus morphed distractor ($t_{36} = -1.454$, $p = 0.077$, t-test; one-tailed) and in the condition with the adult target and the unmanipulated distractor (neutral baby) the differences in the RTs between low T and high T subjects reached statistical trend level ($t_{36} = -1.673$, $p = 0.051$; t-test; one-tailed).

As in study I, we were interested in the effects of high T concentrations on the distraction by baby schema in particular. So, we correlated T concentrations of the participants with the relative RTs (RT for adult target minus RT for baby target). Therefore we calculated the mean RT to select an adult target (i.e., the high distracting condition) and subtracted the mean RT to select an infant target (i.e. the low distracting condition). Again, we found a negative correlation between T concentration in women and the relative RTs ($r = -0.394$, $p = 0.007$, $N = 38$) (see Fig 6).

We also calculated the correlation between the T concentration of the participants and the distraction by baby schema for all three baby schema conditions separately. We found negative correlations between the habitual T concentration of the participants and the relative RTs in all three conditions (RT for adult target out of three minus, out of three neutral or out of three plus morphed babies minus RT for minus, neutral or plus morphed baby out of three adults) (high baby schema condition $r = -0.288$, $p = 0.04$, unmanipulated baby schema condition

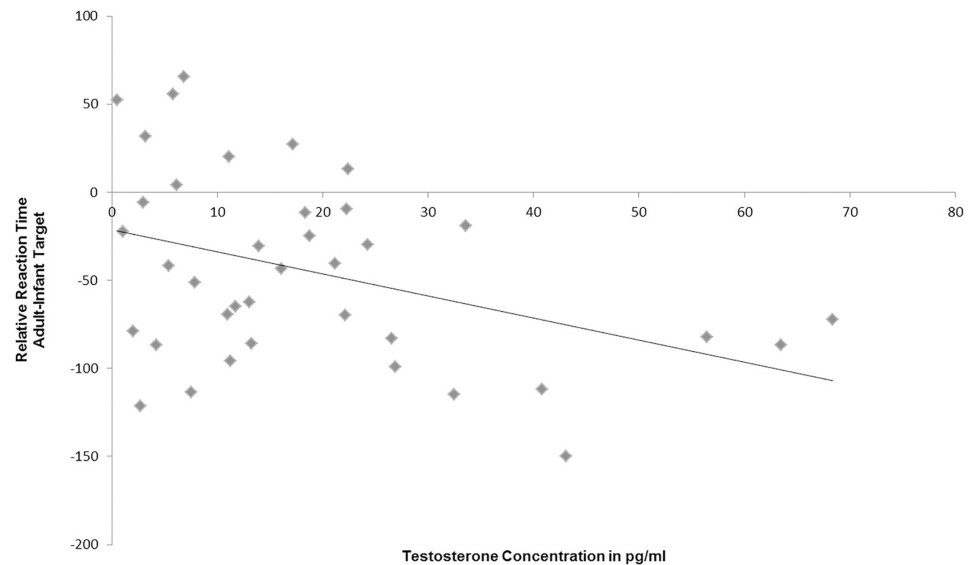


Fig 6. Negative correlation between salivary testosterone concentration and selective attention to human infant portraits ($r = -0.394$, $p = 0.007$; one-tailed).

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$r = -0.345$, $p = 0.017$ and low baby schema condition $r = -0.295$, $p = 0.036$) (all p values are reported for one-tailed tests).

Discussion

The current study investigated the connection between endogenous testosterone and selective attention to infant faces in women. Additionally, we assessed influences of exogenous oxytocin on attentional processes regarding baby schema. Most importantly, high testosterone concentrations in female participants were negatively associated with attention towards infant faces. Moreover, our preliminary findings indicate that oxytocin might diminish the negative effects of high testosterone levels in women. Selective attention in women with high testosterone concentrations aligned to the low testosterone group after oxytocin treatment.

In our first study women located infant targets of non-threatening stimuli more quickly than the adult targets of the same species. Both, target age (adult or infant) and target species, had an effect on reaction time with participants locating infant targets faster than adult targets when pictures of humans, other primates or of other non-threatening species were presented (see Fig 3). Our results are consistent with those reported by Golle and colleagues [40] who compared the perceptual properties of human infant faces and puppy dogs in a visual adaption paradigm. They found that facial cuteness adaption transferred across both species.

Interestingly, adult predators were identified faster than infant predators (lion and polar bear, see Fig 3). This is consistent with the view that threatening stimuli have a processing advantage in the attentional system, because of their immediate relevance for survival [44]. Lions and polar bears still belong to the most dangerous terrestrial predators and represent danger in the wild [54,55]. However, the attentional benefit is remarkable because Europeans normally won't get in contact with these predators. In conclusion, it could be a case of an inherent attention benefit, as in the case of snakes [44]. According to theory, key stimuli should trigger behavior that is beneficial for reproduction and survival [28,56]. The survival of

the offspring of humans and other non-human mammals fully depends on the caregiving of their parents [30]. Since a parent's inattentiveness could cost the offspring's life (for example through predators or accidents), prioritized attention to newborns and infants could be adaptive to increase genetic success and thus indirectly increase parental fitness. In 95% of mammals, females are essentially responsible for rearing the offspring [57]. Cárdenas and colleagues [42] found that women took longer time and looked more often at unknown infant faces, than at unknown adults. The authors explained this as an adaptation of human cognition to infant care as a result of alloparental care in humans. Our findings thus support the notion that highly relevant stimuli should automatically and rapidly capture prioritized attention [44]. In addition, they may also conform to the view that typical child characteristic features, which elicit maternal care, could enhance survival rate of the offspring and thereby increase reproduction success, supposedly through the prioritization of attention. Furthermore, the present results are consistent with several previous studies that reported preferences for cute infant faces independent of species or ethnicity [37,39,40].

To investigate the influences of the different factors on the RTs we performed a 2 x 2 x 2 x 3 ANOVA with the factors drug group, median split of the testosterone concentration, target age and condition of the distractor. We found significant effects in the factor target age and condition of the distractor on the RTs. As the influence of the factor condition alone is hard to interpret (because it remains unclear whether the distraction or the attention is responsible for the RT differences) the significant interaction between the target age and the condition may be more meaningful.

For further exploration, we performed two separate ANOVAs for the high and the low T groups with the drug group as between subject factor. We found a main effect of the drug group only in the high T group. This might indicate that oxytocin opposes the negative effects of testosterone in the context of face processing. The neuropeptide oxytocin supports behavior that is advantageous for parental care (for example grooming and attachment; [58,59] (for review see [24]), while testosterone tends to be associated with antisocial behavior [9] (for review see [27]). In the present project, we show for the first time that endogenous testosterone in women may reduce selective attention to pictures of infants (see Figs 4 and 6), while oxytocin administration might reverse the negative effects of high testosterone levels and might probably improve face discrimination (see Fig 5). Several authors have already shown that testosterone is associated with aggressive and egocentric behavior [9,60]. This effect may be useful in the context of competition for food and territory [61], but it is not very beneficial for caretaking behavior in parent-infant interactions. It has been repeatedly demonstrated that testosterone concentration decreased during parenthood, possibly as a consequence of increased parental investment [18,19,23,62]. Only two studies have so far assessed the effect of testosterone on behaviors in the context of maternal caretaking. An administration study presented results suggesting that testosterone might upregulate neural responses to infant crying in young women. However, it remains unclear whether testosterone or its metabolite estradiol is responsible for these results [63]. Hahn and colleagues [36] investigated the effects of high salivary testosterone levels on cuteness perception. High habitual testosterone concentrations seemed to increase the time participants voluntarily decided to view cute baby faces. In this cuteness perception task, women could decide to extend the time to look at a cute baby via key press. This could explain divergent findings, because we investigated selective attention towards baby schema in a rapid attentional reaction time task. So our task was more sensitive for aspects of automatic attentional orienting, while the task used by Hahn et al. [36] was rather characterized by evaluative components of cuteness perception, which could explain the divergent findings in both studies.

Hormones and attention towards infant faces

Salivary testosterone was analyzed to test for a correlation between the habitual testosterone status and women's selective attention towards pictures of infants. As expected, women with higher salivary testosterone concentrations were significantly slower in orienting their attention and responding to infant targets when these were presented in the context of adult distractors. In study I, a negative correlation between salivary testosterone and selective attention to babies was observed. Women with a higher testosterone concentration exhibited longer reaction times to locate the infant (see Fig 4). This finding provides strong evidence for the hypothesis that testosterone may negatively influence attention towards infants and baby schema in women. The effects of testosterone mainly manifest in behaviors, which stand antagonistic to parental care and prosocial attributes that are important for childrearing [9,24,60]. So far, several studies found negative correlations between salivary testosterone concentration and prosocial behaviors such as caretaking and helpfulness ([9,11,12] (for review see [26])). Assuming that testosterone reduces parental care [18,19,62] it also seems plausible that it may affect the attentional system by modulating responses to baby schema in infants.

In contrast to testosterone, the „birth hormone” oxytocin has been demonstrated to promote parental care [7,46] (for review see [3,26]). To investigate the interaction between testosterone and oxytocin, we designed a second paradigm in which only human pictures were shown. In this paradigm, we manipulated the cuteness of the baby by increasing or decreasing the amount of baby schema (see [33] for a similar procedure) to investigate processing assets and drawbacks in the attentional system for babies with high or low baby schema. We expected that an increased baby schema, as a key stimulus, should automatically draw selective attention while decreased baby schema should reduce attention to these stimuli because of a greater similarity to adult individuals and less cuteness. Our data revealed that women with higher testosterone concentrations needed significantly more time to select the infant target out of three adults relative to the adult target out of three infants (see Fig 6). Previous comparison studies have shown that infants with a high baby schema were perceived to be cuter than infants with a lower baby schema [33,64]. Besides that, Glocker et al. [29] investigated the associated neural correlates. They observed an increase of activation in the nucleus accumbens with increasing baby schema (i.e., low < neutral < high BS). This area belongs to the reward system and probably has a high oxytocin receptor density in social mammals [65]. Yet, the nucleus accumbens may also be responsive to salient stimuli per se (e.g. [66]), which could lead to prioritized processing of these stimuli. One may therefore speculate that in the present study oxytocin administration diminished the negative effects of high testosterone concentrations through modulation of responses in the nucleus accumbens.

Reaction times did not differ between the oxytocin or placebo treatment groups in low testosterone women, but reaction times selectively decreased in high testosterone women in the oxytocin treatment group (see Fig 5). Although little is known about the precise interplay of testosterone and oxytocin in the brain, previous evidence indicates that they may have contrasting effects on behavior and are both associated with a change of parental investment [18,19,23,53,59,62]. Decreased testosterone concentrations during fatherhood are thought to promote caretaking behavior like affectionate touch, duration of parental vocalization and gaze to the infant's body [18,23]. In contrast, enhanced oxytocin is associated with parenting and bonding behavior in humans and different non-human mammals [53]. Interestingly, Weisman and colleagues [23] found that oxytocin administration induced short-term increase of testosterone levels in fathers but also increased the quality of playing with the toddlers. This observation is in accordance with Frayne and Nicholson [67], who demonstrated that testosterone production increased in isolated Leydig cells from male Wistar rats that were incubated with oxytocin.

Since testosterone has different effects on behavior in men and women (for review see [26]), it is possible that oxytocin modulates testosterone concentration in a different way in women. A recently published study indicates that oxytocin receptor genotype interacts with 2D:4D ratio (a biomarker to measure prenatal testosterone) in the performance of a common empathy test, but only in men, which could be another hint for a sexually dimorphic endocrine system [21].

Additionally, in females the majority of the testosterone is converted into estradiol via aromatase, whereas males have far lower estradiol concentrations [68].

However, as could be demonstrated by Weisman and colleagues [23] one does not exclude the other: Although testosterone decreased in fathers that spend more time with their children, as shown in several studies [18,23], oxytocin related increase of testosterone levels of these fathers positively correlated with parental behavior. The authors presume a more sensitive and responsive testosterone system in fathers with low testosterone concentrations. In comparison to the habitual testosterone concentration of men in general, fathers have a testosterone deficiency. This contrasts with our study, since the female participants in the present study were all nulliparous.

Furthermore, oxytocin is known to improve face processing [69–71], which might explain the decreased reaction times in the oxytocin treatment group. Please note, however, that this effect was found only in the high testosterone group. Thus, our results provide evidence for an interaction between both hormones regarding face discrimination. Previous research in the context of autism and Asperger Syndrome fits well to these data. The “Reading the Mind in the Eyes” test (RTME-test) helps to measure perspective taking abilities in humans. Testosterone administration impaired the performance in the RTME-test depending on the prenatal androgen marker (2D:4D ratio) indicating that testosterone administration led to significant impairment of cognitive empathy in women with high prenatal level of androgens, but not in women with low prenatal androgen levels [15]. Hence, it is possible that androgens have a negative influence on face reading and processing. In contrast, oxytocin administration not only improved the cognitive empathy in the RTME-Test in men without autism or Asperger Syndrome [7] but also increased activation in brain areas during social information processing in children with autism [72]. According to our results it is highly likely that both hormones modulate social cognition in an opposing fashion.

Beyond expectation, in the second study the reaction time differences between the low, neutral (unmanipulated) and high baby schema and the adult target condition did not correspond to our assumption that baby faces should be faster identified (see Fig 5). A possible explanation for this could be that the student group that we tested had only little experience with babies, so that the participants were more familiar or even attracted by same age stimuli. This could also be one reason for the low relative RTs in the second study (RTs for infant targets were longer than RTs for adult targets with infant distractors (Fig 6)). We suppose that the young female participants were also interested in same age stimuli. But nonetheless, the relative RTs of the participants decreased with higher testosterone concentrations. The attention towards infant faces was reduced in women with higher T concentrations—as in Study I.

Still, examining the present research questions with participants potentially more sensitive to baby schema (e.g., mothers compared with nulliparous) and to realize the target detection with older adult stimuli (that do not represent a potential partner or competitor) could be one of several possible avenues for future studies in this regard.

Conclusion

Given the findings of the present project, there is strong evidence that endogenous testosterone negatively modulates selective attention to infant faces. Repeatedly, we found a negative

correlation between the distraction by infant faces and the habitual testosterone concentration of female participants. In contrast to the negative effects of endogenous testosterone, exogenous oxytocin apparently seems to promote face discrimination ability, but only in women with high endogenous testosterone levels. These results were not limited to infant faces but also to adults. A possible explanation could be, that the oxytocin administration also promoted the attraction to the same age adult stimuli as described in the literature [73]. On the whole, the results of the present project provide initial support for the idea of a complex interplay of testosterone and oxytocin in the modulation of social behavior and maternal care. Based on the small sample size further research is needed.

Supporting Information

S1 Table. Behavioral Data Study 1.

(PDF)

S2 Table. Behavioral Data Study 2.

(PDF)

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Formal analysis: SH.

Funding acquisition: ED KS.

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Methodology: ED SH.

Project administration: SH KS.

Resources: KS CS ED SH.

Software: ED SH.

Supervision: ED.

Validation: SH.

Visualization: SH.

Writing – original draft: SH ED LR.

Writing – review & editing: SH ED LR.

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2.2. Publication II

Endogenous testosterone and exogenous oxytocin influence the response to baby schema in the female brain

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Contribution to publication II

The study of publication II was conceptualized by SH under supervision of ED. ED and SH designed the paradigm and the experimental procedure. SH performed the research and the statistical analysis. AEG and EB supervised the administration of the treatment. RP programmed the analysis batches. SH wrote the first version of the manuscript. All authors have given approval to the final version of the manuscript.

Date and Place

Signature of the supervisor

Prof. Esther K. Diekhof

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Endogenous testosterone and exogenous oxytocin influence the response to baby schema in the female brain

Sarah K. C. Holtfrerich¹, Roland Pfister², Alexander T. El Gammal³, Eugen Bellon³ & Esther K. Diekhof¹

Nurturing behavior may be critically influenced by the interplay of different hormones. The neuropeptide oxytocin is known to promote maternal behavior and its reduction has been associated with postpartum depression risk and child neglect. Contrariwise, the observed decrease in testosterone level during early parenthood may benefit caretaking behavior, whereas increased testosterone may reduce attention to infants. Here we used functional magnetic resonance imaging to investigate the interactive influence of testosterone and oxytocin on selective attention to and neural processing of the baby schema (BS). 57 nulliparous women performed a target detection task with human faces with varying degree of BS following double-blinded placebo-controlled oxytocin administration in a between-subjects design. Our results support the idea that oxytocin enhances attention to the BS. Oxytocin had a positive effect on activation of the inferior frontal junction during identification of infant targets with a high degree of BS that were presented among adult distractors. Further, activation of the putamen was positively correlated with selective attention to the BS, but only in women with high endogenous testosterone who received oxytocin. These findings provide initial evidence for the neural mechanism by which oxytocin may counteract the negative effects of testosterone in the modulation of nurturing behavior.

The survival of the offspring is crucial for one's reproductive success. Newborns may therefore carry specific features that act as key stimulus, which automatically captures attention and motivates actions such as caretaking. The baby schema (BS) is such a key stimulus. In the infant face it is defined as a combination of child characteristic features like big eyes, small nose, chubby cheeks, and higher forehead¹. Humans bear high costs in raising their offspring, because pregnancy and production of maternal milk are energetically expensive², which should make newborns highly relevant, at least for mothers. But since humans practice alloparental care³ and the BS is further known to be a universally relevant stimulus⁴ the BS should be highly significant for women in general. The increased cuteness of infant faces in comparison to adults may therefore prioritize selective attention^{5,6} and may enhance evaluation of these stimuli as socially relevant for actions like caretaking⁷.

There is already evidence for an adaptive neural mechanism through which the perception of cuteness as a feature of the BS is most likely promoted in humans. Accordingly, cute baby faces have repeatedly yielded activations of regions of the mesolimbic reward system^{8,9}. For example, Glocker *et al.*¹⁰ observed an increase of activation in the nucleus accumbens in women while evaluating pictures of babies with increasing BS (i.e., low BS < unmanipulated BS < high BS). Interestingly, the mesolimbic reward system may also be highly responsive to the influence of two hormones that have opposing roles in human nurturing behaviors. Firstly, a high oxytocin (OT) receptor density can be found in the mesolimbic reward system of social mammals¹¹ and increased activation of the ventral tegmental area to images of crying infants after OT treatment suggested that OT influences the reward value of stimuli carrying the BS¹². Further, OT administration increased the preference for pictures of young children.

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This preference was determined through rs53576G homozygote participants (a polymorphism in the OT receptor gene)¹³. Finally, OT has been consistently associated with higher maternal attachment and increased caretaking in humans^{14,15}. The androgen testosterone (T) may also have reinforcing effects in the mesolimbic reward system¹⁶. Yet, in contrast to OT it may compromise behaviors associated with maternal care (e.g. nurturing)¹⁷. Previous studies have noted a reduced T level during early parenthood and this decline appeared to be associated with the quality of childcare^{18–22} (but see²³).

However, the interactive influence of T and OT on neural processing of the BS currently remains elusive. Only a few behavioral studies have so far assessed their combined influence on aspects of nurturing behaviors. In the context of paternal caretaking behavior Weisman and colleagues²⁴ found that OT administration led to an unexpected short-term alteration of endogenous T, whereby nurturing behaviors like social gaze also increased. Given that women are the main caretakers during the first months after childbirth when infants depend on maternal milk, and yet sometimes show impairments like postpartum depression or child neglect²⁵, it is of crucial importance to take a closer look on these interactive influences in women as well. In nulliparous women a higher endogenous T reduced selective attention to infant targets in the context of adult distractors⁶. Importantly, attention to infant stimuli increased after OT administration, but only in women with high endogenous T. This suggests that OT may oppose the negative effects of T on nurturing behavior in women⁶. There are already some indications on the behavioral level that T and OT may have gender-specific impacts on parental behavior. Gordon and colleagues²⁶ found that high T levels in fathers negatively influenced the association between OT and paternal behavior, whereas in mothers high T levels evoked positive associations. Nevertheless, the neural mechanisms underlying the effect of the OT by T interaction on selective attention to infants in general and the highly relevant key stimulus BS in particular remains elusive.

In order to further our understanding of the nature of hormonal interactions in nurturing behavior, the present study used functional magnetic resonance imaging (fMRI) and a double-blind placebo-controlled OT administration between-subjects protocol to examine the influence of exogenous OT and endogenous T on selective attention to the BS in nulliparous women. To assess selective attention to the BS we used an implicit association task. Implicit association tasks have the advantage that the degree of attentional capture by an infant as opposed to an adult face can be determined implicitly by the mean reaction time (RT) of the participant, with a shorter RT indicating faster attentional processing of the baby schema and increased action. Previous neuroimaging studies combined passive viewing and explicit evaluation tasks (e.g., cuteness perception on a Likert-Scale), which are more vulnerable to experimenter demand effect. Further, these studies do not allow the assessment of the mechanism that promotes faster reactions to infants. Building on previous findings⁶ we hypothesized that OT administration would compensate the negative effects of high endogenous T on attentional processing of the BS, probably through the modulation of activation in key nodes of the mesolimbic reward system.

Results

Behavioral data. The influence of the endogenous T concentration (measured out of one saliva sample that was collected directly before administration) on attention to babies was analyzed with a 3 (morph type: low BS, natural BS and high BS) \times 2 (treatment: OT vs. placebo) \times 2 (T: median split of the sample that was collected directly before administration) ANOVA. Of particular interest was the significant three-way interaction between the factors “morph type”, “treatment” and “T” ($F_{2,104} = 4.359$; $p = 0.015$; see Table S1 for details). Building on previous research⁶ we assumed that women with high endogenous T derive greater benefit from OT treatment than women with low endogenous T and hence will become more sensitive for the BS. We found that OT-treated women with high endogenous T exhibited an attentional preference for the natural BS (positive Delta-RT: $M_{\text{highT}} = 6.4 \pm \text{SEM } 24.2$ ms) compared to OT-treated women with low endogenous T (positive Delta-RT: $M_{\text{lowT}} = -50.6 \pm \text{SEM } 16$ ms) but these results remained on statistical trend level [Bonferroni-corrected statistical threshold was $p \leq 0.0166$ ($t_{27} = 1.86$, $p = 0.03$; one-tailed, a priori hypothesis based on⁶)]. In the placebo group women with low endogenous T directed less attention to the plus morphed babies ($M_{\text{lowT}} = -39.1 \pm \text{SEM } 21.3$ ms) than women with high T concentrations ($M_{\text{highT}} = 23.4 \pm \text{SEM } 14.6$ ms; $t_{25} = 2.29$, $p = 0.015$, one-tailed, a priori hypothesis based on⁶). The placebo-treated participants with high or low T did not show any differences in the preference for the natural BS.

Drug treatment did not influence the participants RTs to geometric figures. Yet, the type of target ($F_{2,110} = 39.485$; $p < 0.001$; $\eta_p^2 = 0.418$) and the interaction between target and distractor ($F_{2,110} = 25.871$; $p < 0.001$; $\eta_p^2 = 0.32$) influenced the participants’ RTs in the control task (see also Table S2). These results are consistent with the assumption that simple geometric forms could have emotional meanings and may even trigger an attentional bias²⁷.

Neuroimaging results. When contrasting “baby vs. adult target” for the different morph types, we found an effect of treatment in the direct comparison of the OT and placebo group in the left frontolateral cortex (MNI-coordinates [t -value; p_{FWEcorr}] $-33, 5, 49$ [$4.62; 0.023$]), but only for the high BS condition (Fig. 1). The identified region was located at the intersection of the precentral sulcus and inferior frontal sulcus, which corresponds to the inferior frontal junction (IFJ)²⁸. Additional t -tests on the parameter estimates extracted from the left IFJ, confirmed the difference in activation between the OT and the placebo group in the high BS condition ($t_{55} = 4.43$; $p < 0.001$) (Fig. 1). We could not find any difference in the direct comparison of OT > placebo in the unmanipulated or the low BS conditions.

The SPM multiple regression analysis was used to analyze the influence of the degree of selective attention to babies on brain activation. For this, we used the mean Delta RT of all baby conditions from each subject and correlated them with the individual activation in the contrast of “baby vs. adult target”, separately for OT- and for placebo-treated participants. We found increased activation in the right putamen of OT-treated women (MNI-coordinates [t -value; p_{FWEcorr}] $30, -1, 13$ [$5.65; 0.052$]; Fig. 2a) that was positively related to selective attention to babies, but barely missed the FWE-corrected threshold in the whole-brain analysis. Yet, when extracting

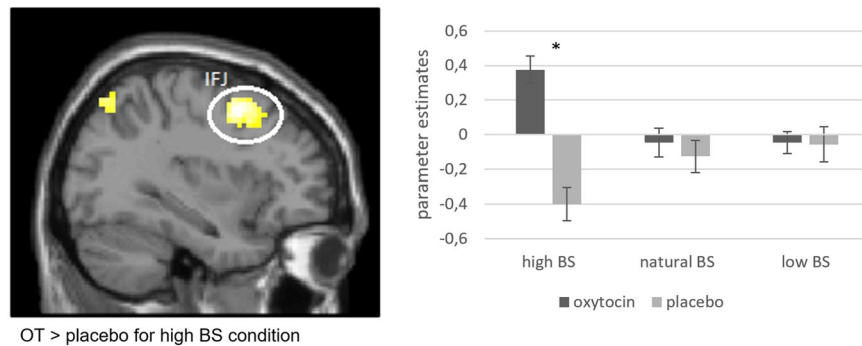


Figure 1. Increased activation of the inferior frontal junction (IFJ) for baby targets that were morphed towards higher BS > adult targets in the comparison of the OT and placebo group (MNI coordinates X, Y, Z [*t*-value]: -33, 5, 49 [4.62]). Activation is displayed in MNI space on an axial slice. Parameter estimates were extracted from spheres at the local maximum with a radius of 3 mm. They were significantly different between the OT and the placebo group for the high BS condition.

the parameter estimates from the right putamen, a significant positive correlation with selective attention to babies emerged (Fig. 2a). We also found a positive correlation with activation in the left putamen at the statistical threshold of $p < 0.001$, uncorrected (MNI-coordinates [*t*-value] -30, 14, 10 [4.85]; Fig. 2a). In the placebo group, two homologous clusters emerged in the posterior temporal cortex of which one survived the FWE correction on cluster level and one barely missed the statistical threshold (MNI-coordinates [*t*-value; p_{FWEcorr}] 48, -22, -2 [5.69; 0.067]; MNI-coordinates [*t*-value; p_{FWEcorr}] -66, -49, -2 [4.99; < 0.001]).

Following the behavioral results, we subdivided the sample in women with either low or high endogenous T concentrations. OT-treated women with high endogenous T showed significantly enhanced activation in the left and right putamen in the contrast of “baby vs. adult target” in association with the individual Delta-RTs as an indicator of enhanced selective attention to babies (i.e., a positive correlation). The positive correlation with the left putamen also surpassed the statistical criterion (MNI-coordinates [*t*-value; p_{FWEcorr}] -21, 14, -11 [5.97; 0.005]; Fig. 2b), while the one with the right putamen was significant at $p < 0.001$, uncorrected (MNI-coordinates [*t*-value] 30, -1, 13 [5.33]; Fig. 2b). OT-treated participants with low endogenous T and participants of the placebo group did not show this positive correlation between activation in the putamen and selective attention to babies. The brain-behavior correlations based on the parameter estimates extracted from the left and the right putamen supported these results (Fig. 2c; Table S3).

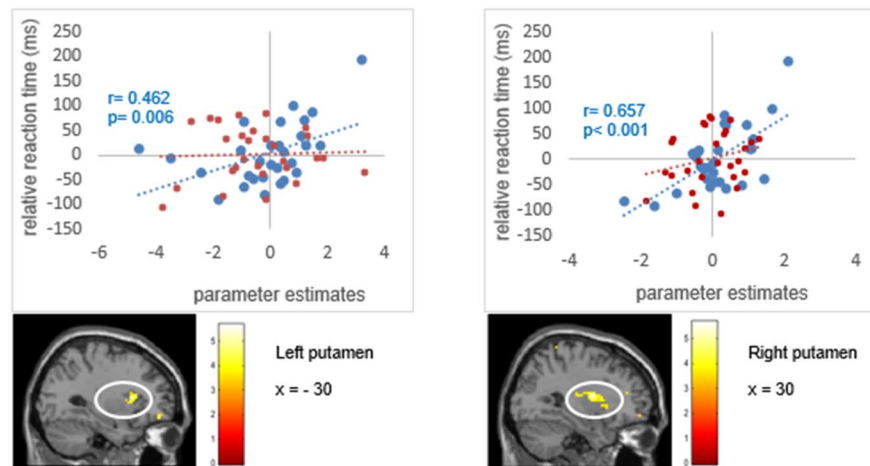
Discussion

The current study investigated the influence of exogenous OT and its interaction with endogenous T on brain activity during processing of infant faces, which varied in the intensity of BS. We found a positive effect of drug treatment in the direct comparison of OT versus placebo using the contrast of “baby versus adult target” in the IFJ, but only in the high BS condition. This finding supports the idea that OT may enhance attention to increased BS and may further promote one’s motivation to act. Additionally, we found a positive correlation between selective attention to babies, as indicated by increasing Delta-RTs, and increased activation in the left putamen following OT treatment. Interestingly, this correlation was specific for women with high endogenous T. Collectively, these findings may provide a hint to the neural mechanism by which OT may support the sensitivity for the BS and may counteract the negative effects of T in the modulation of nurturing behavior.

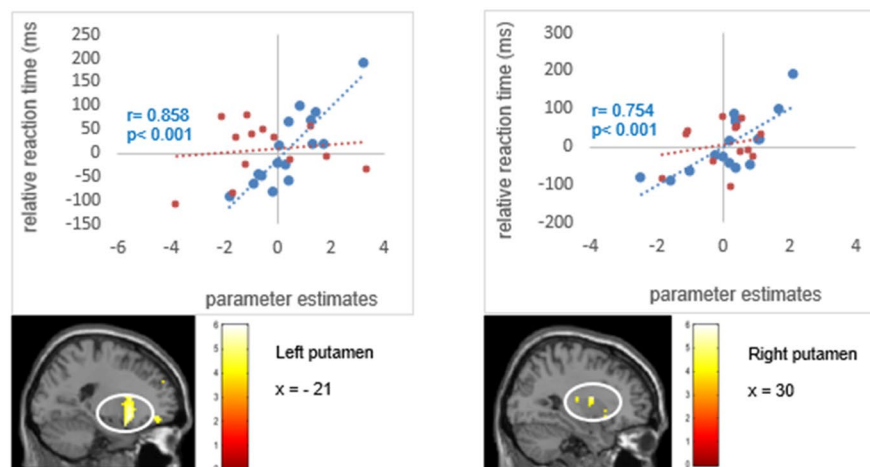
Based on our previous study⁶ we expected that OT administration would modulate attention to infant faces especially in women with high endogenous T. In line with this hypothesis, we found an interaction between the degree of BS, the treatment group and endogenous T level. OT-treated women with high endogenous T showed an attentional bias towards babies (positive Delta-RTs) compared to OT-treated women with low endogenous T (negative Delta-RTs), but only for the natural BS and this result remained on statistical trend level. We could not find further differences in the attention to babies between high and low endogenous T concentrations in the OT group, but the Delta RTs of the conditions with lower and higher BS were positive in both T groups. We suppose that adult pictures could also represent salient stimuli for nulliparous women in reproductive age, which may have rendered any behavioral effects rather small (see⁸ for review). Yet, the presence of a statistical trend in the OT-treated women with high endogenous T may conform to the idea that a system influenced by high levels of T may be more receptive to OT treatment, because in the female brain T could be directly converted into estradiol and thus modulate OT receptor density, which has been demonstrated in rats²⁹.

We found a positive effect of treatment (OT > placebo) on processing of targets with higher BS in the left IFJ. The IFJ is located in the frontolateral cortex between the premotor and the prefrontal cortex and has been implicated in cognitive control^{30,31}. Recent research further demonstrated its specific involvement in the control of selective visual attention³², action perception³³ and the detection of behaviorally salient cues³⁴. In addition, the IFJ has been associated with the understanding of action³⁵, which may be considered as one basis of social cognition and empathy. Previous studies also detected increased activation during processing of infant faces in areas near our IFJ cluster. Caria and colleagues³⁶ located activation in the precentral cortex (BA 6; MNI coordinates: 36–6 45) in the contrast of “unknown infant > adult faces”. A study on synchrony and specificity between the maternal and paternal brain found activation in the inferior frontal gyrus (IFG) for both sexes in response to

a) OT treated participants (in blue) and placebo treated participants (in red)



b) OT treated participants (in blue) and placebo treated participants (in red) with high T concentration before administration



c) OT treated participants (in blue) and placebo treated participants (in red) with low T concentration before administration

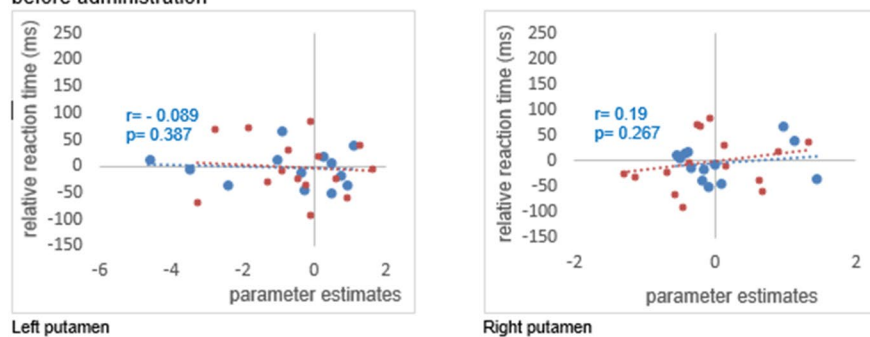


Figure 2. Degree of selective attention to babies for OT treated participants (in blue) or placebo treated participants (in red) with high or low T concentrations. (a) Activation in the left and the right putamen for baby relative to adult targets positively correlated with selective attention to babies after OT treatment ($N = 29$). Parameter estimates from local activation maxima (L: -21 14 – 11 ; R: 30 – 1 13 ; spheres with 6 mm radius) for the right and the left putamen are also displayed for illustration purposes. (b) Positive correlation of activation in the left and right putamen with selective attention to babies for OT treated women with high endogenous T concentrations ($N = 16$). (c) OT treated women with low endogenous T concentrations ($N = 13$) did not show activation of the putamen in relation to the relative RTs (i.e., selective attention to babies).

own-infant-parent-interaction videos³⁷. Finally, the IFG has also been related to OT-induced emotional empathy³⁸. In our study, the activation of the IFJ was limited to babies with a high BS. Previous observations indicating that babies with stronger BS were treated preferentially³⁹ and perceived as cuter^{40,41} provide some space for speculation, that the stronger BS could provoke an increased motivation to act. The findings of Glocker *et al.*¹⁰ who also found activation in the precentral gyrus (MNI coordinates: $-42\ 0\ 29$; $-46\ 6\ 33$; $-51\ 8\ 36$) across the three BS conditions, which was located near our cluster, could also provide indications for this speculation. Yet, it is difficult to distinguish between participants who searched for the target or avoided the distractors, which makes it difficult to interpret the difference between the OT group (positive parameter estimates) and the placebo group (negative parameter estimates). This would be of great interest and should be taken into account in a future study.

Additionally, we observed a positive correlation between activity in the left putamen and increased selective attention to babies in OT-treated women. The putamen belongs to the mesolimbic reward system and is part of the striatum. Equivalent to many other studies the detected activation appeared only in participants that were treated with OT¹⁵. We suppose that this finding supports the idea that OT increases the reward value of babies, probably as an adaptation to motivate caretaking behavior through mesolimbic-reward pathways³⁷. This assessment is also supported by previous reports that have demonstrated OT-dependent activations in areas of the reward system as consequence of listening to crying babies (amygdala and insula⁴²), to laughing babies (amygdala⁴³) and processing of child pictures (globus pallidus⁴⁴). Yet, the OT-related response in the putamen did not parametrically change with the amount of BS inherent to the presented pictures. This indicates a rather general mechanism that may ensure reflexive attention to babies regardless of their cuteness.

Even more importantly, the positive correlation between increased selective attention to babies and activation in the left putamen only remained significant in the high endogenous T group after OT administration. Only a few behavioral studies have so far examined the interactions between OT and T in women, and, to the best of our knowledge, our study is the first that used functional neuroimaging to assess the underlying neural mechanism. Here we used the T sample that was collected directly before administration to capture the current T state of the participant and not the habitual concentration, because we could not control for daytime fluctuations. This method also allows the comparability with previous research^{26,45,46}. The single T sample collection before administration provided the opportunity to analyze the real-time T concentration at the measuring time. But, for the same reasons, we cannot exclude further T fluctuations that might have occurred during scanning, which may have weakened the stability of this single measurement and might therefore constitute a potential limitation of this study. However, the T concentration before administration and post-test concentration after measurement were highly correlated ($r = 0.447$; $p < 0.001$). This may suggest that T concentration may have been relatively stable during the actual scanning period.

The present study shows that the reward system may be involved in this attentional bias towards babies. Little is known about the precise interaction of OT and T in the brain. In the female brain the majority of T may be converted to estradiol via aromatase⁴⁷, and estradiol can increase the number of OT receptors in the brain²⁹. Yet, the observation of higher systemic T concentrations in women may indicate an impaired ability to convert it to estradiol, thus either leaving a higher amount of T in the circuitry that may counteract maternal behavior through an unknown mechanism or by leaving the number of OT receptors unaffected by reduced influence of estradiol. In a similar vein one may speculate that high OT concentrations may serve as a compensatory mechanism for high T concentrations, we presume that women with low T concentrations were less sensitive to OT administration, because low T concentrations should support nurturing behavior *per se*⁶. This would indeed explain why we could not find any activation in the brain reward system in women with low endogenous T after OT administration. But since babies nevertheless remain a strong social cue, this does not explain why neither in the low T nor in the placebo group the baby pictures were rewarding by themselves. We suppose that the young adult stimuli may also be socially salient for young participants, for instance as potential mates, and therefore could be equally rewarding and evoke similar activation in the brain reward system⁴⁸. Our experimental design does not allow the isolation of the response to adult or infant faces, since pictures were always presented in combination, but only enables the identification of activation differences during processing of these stimuli. So we cannot answer this question. However, for reasons discussed before we presume that the brain of women with a high endogenous T concentration may be more sensitive to OT administration than women with a low endogenous T. One could therefore speculate that same age adult stimuli as well as infant pictures both constitute a strong social stimulus, but OT could function as an enhancer of reproductive behavior and direct attention towards infant stimuli in women with high T level, who may be more sensitive to OT. In this study, we focused on nulliparous women that reacted to unfamiliar infant or adult stimuli to investigate the neuroendocrine basics of the key stimulus in general. As oxytocin is especially known to influence attachment behavior¹⁵ it could be of great interest to use the same paradigm with familiar infants in further research. The results of this study are therefore limited to the general mechanism of the key stimulus BS and are not necessarily transferable to attachment behavior in mothers or other caretakers. Gordon *et al.*²⁶ found a positive interaction between increased T levels and OT on maternal touch in mothers, while the effect in fathers was in the opposite direction. Their results may indicate that the interaction between T and OT may be sexually dimorphic. Therefore, our results on nulliparous women may resemble those in mothers but it is possible that they deviate to the results in fathers and men who have never sired a child. Although the findings of Gordon and colleagues²⁶ were focused on parents, we assume that the results in mothers support our previous findings that OT seemed to promote adult-infant discrimination and increased attention to baby pictures, but only in women with high endogenous T⁶. OT is a peptide hormone that is critically involved in mother-infant attachment⁴⁹. Especially lactating mothers show increased OT levels⁵⁰. The artificial increase through intranasal administration could be closely related to the concentration found in new mothers (see Weisman *et al.*⁵¹ for the active drug effects reflected in saliva). Although there already exists excellent research about the active drug effects and reflection in saliva^{49–51}, a direct comparison of mothers and non-mothers salivary OT concentration with and without OT administration would be of great interest. But yet,

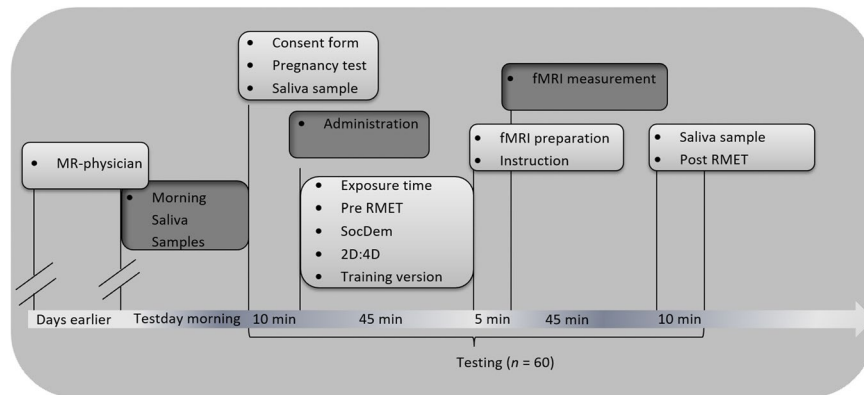


Figure 3. Time schedule of the experimental procedure. 60 participants were invited for testing. 57 participants could be included into the analysis (29 of the OT group; 28 of the placebo group). 20 of the participants performed the RMET test before the fMRI measurement and 37 participants performed the RMET test after the fMRI measurement.

aside from oxytocin, postpartum hormonal changes have large transitions which makes the results of nulliparous women not necessarily comparable with mothers^{52,53}.

The detected activations in the putamen correspond well with the literature and accentuate the previous research on oxytocin and maternal behavior^{8,9,54}. Since we know very little about the interactions of T and OT in the female brain, we abstained from using an *a priori* region-of-interest approach and analyzed our data on whole brain level. However, findings in the IFJ are not common in the context of OT administration studies and in an *a priori* region-of-interest approach the IFJ would have probably not been included. This might limit the interpretation of this finding, although the activation of the IFJ survived the correction for multiple testing on a whole-brain basis. Future studies will therefore be necessary to show if this finding can be replicated in this context.

All of the participating women used hormonal contraceptives and were tested in the pill-phase. This was done to control for a potential pregnancy and to prevent cyclic changes in steroid hormone levels. But it's important to note that previous research showed that the intake of oral contraceptives increased performance in affective responsiveness and that affective responsiveness was positively influenced by oral contraceptives (pill-intake phase versus pill-free week)⁵⁵. Further, testosterone in females on oral contraceptives may be downregulated⁵⁶. Therefore, using participants on hormonal contraceptives for an OT-intervention study may not represent the ideal model to detect OT sensitivity, that may be modulated by T. Yet, ethical concerns (increased pregnancy risk in women with a natural cycle), the fact that T level fluctuates across the natural cycle (e.g., rises during the first half), as well as our previous results in women on oral contraceptives⁶, led to our decision to test only women who received hormonal contraception in this between-subjects fMRI design. As a future direction, it would be of great interest to further examine normal cycling women and the influence of the intake of oral contraceptives on the recent results.

Collectively, the present findings support the idea of an adaptive hormonal mechanism that promotes selective attention to babies. Here, we demonstrate that an increased BS was associated with enhanced activation of the IFJ after OT treatment, a brain region implicated in cognitive control and the motivation to act. We also found that OT may modulate selective attention to babies through the recruitment of the reward system. We found a positive correlation between activity in the putamen and attention to baby faces. However, after accounting for endogenous T level, this correlation only remained significant in women with a high T concentration. These results are consistent with our previous findings⁶ and may indicate that OT possibly compensates high T concentrations and the reduced attention to infant stimuli through an enhancement of the reward value of babies.

Material and Methods

Participants. Sixty nulliparous female university students (mean age \pm SD = 24.63 \pm 3.08 years) participated in the study. All participants were right handed, healthy, and were not taking any medication except from hormonal contraceptives to prevent cyclic changes in T level. They also performed a pregnancy test on the test day and met the criteria to participate in an fMRI study.

Subjects provided written- informed consent and were paid for participation. The study was approved by the local ethics committee of the *Ärztchamber Hamburg*. All methods were performed in accordance with the relevant guidelines and regulations.

Experimental Procedure and Paradigm. Administration of OT was placebo-controlled and double-blind (see Fig. 3 for experimental schedule). Participants brought three saliva samples from home sampling and provided two further saliva samples at the test place (one before administration and one after measurement). In the scanner participants performed two versions of a target detection paradigm (see also Supplementary Material and Methods).

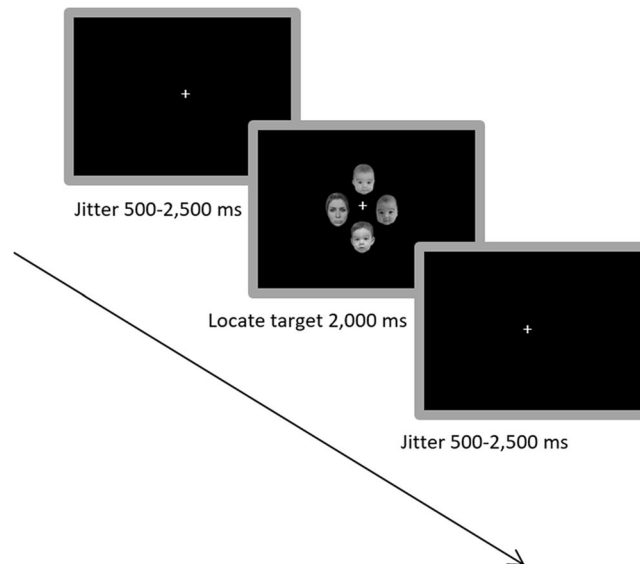


Figure 4. Illustration of the target detection paradigm with example images. The three picture of babies stand for the distractors *here* (photographs of either 3 adult faces or 3 infant faces were shown; the 3 infant faces had the same amount of BS [higher, unmanipulated or lower BS]). The adult picture on the left stand for target *here*, which could be either an adult or infant face of higher, unmanipulated or lower BS. The task was to identify the target that did not fit to the other pictures via button press.

The target detection paradigm was based on the odd-one-out principle (Fig. 4 for schematic illustration). In the first task subjects were asked to identify the odd-one-out on a display of four human faces, which was either an adult face (target) out of three infant faces (distractors) or an infant target out of three adult distractors, as fast and accurate as possible. The BS of the infant faces was parametrically manipulated (see also⁶ for the procedure). The same odd-one-out procedure was repeated in the baseline task, in which task geometric shapes (triangles, squares, or circles) were used (Supplementary Material and Methods for detailed information). The first paradigm was 16.4 minutes long and the baseline task was 6.4 minutes long.

Hormone Samples. The salivary T samples were collected on the test day and analyzed in our in-house laboratory with a T luminescence immunoassay from IBL International (TECAN group global; Hamburg, Germany) (see Supplementary Material and Methods for detailed information).

Hormone administration. OT was administered double-blind and placebo controlled in a between-subjects design. The participants self-administered 3 puffs in each nostril alternately of the unlabeled nasal spray. The amount of OT corresponded to 24 IU. The placebo spray consisted of chlorobutanol-hemihydrat (0.5%) with no active treatment. To ensure treatment efficiency⁵⁷, exposure time was 45 min until the fMRI measurement began.

Behavioral data analysis. 57 participants were considered for analysis (29 were in the OT group and 28 were in the placebo group; for further details see Supplementary Material and Methods).

IBM SPSS statistics 19 was used to analyze the behavioral data. To represent selective attention to babies of different BS conditions, the relative reaction times (Delta-RTs) were calculated by subtracting the low distracting condition (i.e., infant target and three adult distractors) from the high distracting condition (i.e., adult target and three baby faces as distractors) (see also⁶). For a subject with an increased sensitivity for the BS, we predicted higher Delta-RTs, resulting from the combined effect of increased distraction by infant faces in the adult target condition and more rapid selection of the infant target in the first condition.

Following the procedures used previously^{26,46,58}, our analysis of T content focused on the one saliva sample that was collected directly before treatment. Based on the endogenous T concentration, we calculated the median to separate the participants in two groups of either high or low endogenous T concentration (see⁶ for a similar procedure).

We used a repeated measures ANOVA and post hoc *t*-tests to assess the effects of the factors ‘*morph type*’ (low BS, unmanipulated BS, high BS), ‘*treatment group*’ (OT or placebo) and ‘*endogenous T concentration*’ (high or low T) on the Delta-RTs. Bonferroni correction yielded a corrected statistical threshold of $p \leq 0.0166$ and a corresponding statistical trend level of $p \leq 0.033$.

For analysis of the baseline task with geometrical shapes we performed a repeated measures ANOVA with the factors ‘*target figure*’ (triangles, squares, or circles), ‘*distractor figures*’ (triangles, squares, or circles) and ‘*treatment group*’ (OT or placebo) on the RTs.

Statistical effects are considered significant at $p < 0.05$ (two-tailed), if not otherwise indicated. If the sphericity assumption was not met in the ANOVA, we report the Greenhouse–Geisser corrected values. Since all data followed the assumed normal distribution, we used post-hoc *t*-tests.

fMRI data analysis. Participants performed the paradigm in a 3 Tesla Siemens fMRI Scanner at the University Medical Center Hamburg-Eppendorf (see Supplementary Material and Methods for details).

On the first level, we used a general linear model (GLM) for statistical analysis of event-related activity. A vector with the temporal onsets of the experimental conditions was convolved with a canonical hemodynamic response function (hrf) to produce the predicted hemodynamic response to each experimental condition. The conditions ‘*target*’ (adult or infant), ‘*morph type*’ of the baby (higher, unmanipulated or lower BS), ‘*gender*’ (female or male) and the baseline task with the geometric figures were modeled as regressors. Linear t-contrasts were defined for assessing the specific effects of the varying amount of BS.

On the second level, a random effects analysis was performed. For this we used a full factorial 3 by 2 repeated-measures analysis of variance (ANOVA) that included the within-subject factor ‘*morph type*’ (3 steps) and the between-subject factor ‘*treatment group*’ (2 steps). *T*-tests tested for specific differences between conditions.

We also assessed whole-brain correlations for analysis of the association between treatment-related activations and selective attention to babies (Delta RTs). For this purpose we used the multiple regression routine implemented in SPM8. For the display of these behavior-brain correlations individual parameter estimates were extracted with marsbar-0.44.

A whole-brain correction for multiple testing using the family-wise error (FWE) on the cluster level was applied to all analyses, if not indicated otherwise. For display purposes we used a threshold of $p < 0.001$, uncorrected.

Data availability. All data analyzed and generated during this study are available from the corresponding author on reasonable request.

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Author Contributions

S.C.K.H. and E.K.D. designed the study. S.C.K.H. performed the research. E.K.D. supervised the research. A.T.E.G. and E.B. supervised the administration of the treatment and helped revising the manuscript. R.P. programmed the analysis batches and helped in the analysis of the data and revising the manuscript. S.C.K.H. and E.K.D. analyzed the data and wrote the original manuscript. All authors have given approval to the final version of the manuscript.

Additional Information

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2.2.1. Supplementary material and methods of Publication II

for

Endogenous testosterone and exogenous oxytocin influence the response to baby schema in the female brain

Sarah K. C. Holtfreich, Roland Pfister, Alexander T. El Gammal, Eugen Bellon, Esther K. Diekhof

Supplementary Material

for

Endogenous testosterone and exogenous oxytocin influence the response to baby schema in the female brain

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Supplementary Tables

Table S1: Results of repeated measures ANOVA.

<i>ANOVA</i>	<i>factor</i>	<i>F</i>	<i>df</i>	<i>error</i>	<i>p</i>	<i>n_p²</i>
ANOVA with T	Morph	1.874	2	104	0.159	0.035
	Morph*treatment	2.606	2	104	0.079	0.048
	Morph*T	0.764	2	104	0.469	0.014
	Morph*treatment*T	4.359	2	104	0.015*	0.077
	Treatment	0.085	1	52	0.772	0.002
	T	1.135	1	52	0.292	0.021
	Treatment*T	0.042	1	52	0.838	0.001

Significant effects at $p < 0.05$ are marked with *

Table S2: Post hoc t-tests of the baseline task ($n = 57$). In this task geometric figures were used as stimuli (triangles, squares, or circles).

<i>Paired t-tests</i>	<i>target</i>	<i>distractor</i>	<i>mean RT (ms)</i>	<i>SEM</i>	<i>t</i>	<i>df</i>	<i>p</i>
<i>pair 1</i>	triangles	circles	825	19	-5.558	56	< 0.001
	triangles	squares	900	20			
<i>pair 2</i>	triangles	circles	825	19	2.109	56	0.039
	circles	triangles	799	16			
<i>pair 3</i>	triangles	squares	900	20	2.606	56	0.012
	squares	triangles	871	20			
<i>pair 4</i>	circles	triangles	799	16	1.052	56	0.297
	circles	squares	788	19			
<i>pair 5</i>	squares	triangles	871	20	4.280	56	< 0.001
	squares	circles	824	17			
<i>pair 6</i>	squares	circles	824	17	3.461	56	0.001
	circles	squares	788	19			

Table S3: Results of significant whole-brain activation in OT-treated women when processing babies relative to adult targets in general and separated by endogenous T.

Oxytocin group (n= 29)	Oxytocin group with high T (n= 16)	Oxytocin group with low T (n= 13)
<i>Positive correlation with attention to babies (Delta- RT across all morph types)</i>	<i>Positive correlation with attention to babies (Delta- RT across all morph types)</i>	<i>Positive correlation with attention to babies (Delta- RT across all morph types)</i>

<i>Brain region</i>	MNI- coordinates	t-value; cluster size	MNI- coordinates	t-value; cluster size	MNI- coordinates	t-value; cluster size
L/R putamen	30 -1 13	5.65 ¹ ; 102	30 -1 13	5.33 ² ; 34	-	-
	-30 14 10	4.85 ² ; 49	-21 14 -11	5.97; 148	-	-

Activations are FWE-corrected at cluster level with a threshold of $p < 0.05$, if not otherwise indicated.

¹ $p < 0.10$, FWE-corrected

² $p < 0.001$, uncorrected

Table S4: Mean values of the four different T measurements, age of the participants and empathy score for the OT and for the placebo group. None of the measured values differed between the OT and the placebo group (*oneway ANOVA, $p < 0.05$, two-tailed*).

Measurement	Oxytocin			Placebo		
	N	mean	SD	N	mean	SD
<i>Morning testosterone samples (pg/ml)</i>	29	25.48	13.1	27	26.2	13.7
<i>Proxy of prenatal testosterone (ratio of 2nd digit to 4th digit)</i>	29	0.98	0.02	27	0.98	0.03
<i>Testosterone sample before nasal spray (pg/ml)</i>	29	25.02	20.9	28	18.74	9.9
<i>Testosterone sample after fMRI measurement (pg/ml)</i>	29	23.28	12.7	28	24.2	17.3
<i>Age of participants</i>	29	24.93	3.6	28	24.32	2.4
<i>Empathy score (RMET)</i>	29	26.1	3.5	28	25.07	4.9

Supplementary Material and Methods

Experimental Procedure and Paradigm

Consent forms, instructions, and Eppendorf tubes for saliva sampling were handed to the participant in advance. Participants were instructed to collect three samples of morning saliva on the day of measurement.

Measurement started with a clarification of open questions, followed by a pregnancy test of the participant. After a negative test the participants provided another saliva sample followed by the self-administration of the nasal spray (24 IE, three puffs per nostril). Administration was placebo-controlled and double-blind. After 45 min exposure time, fMRI measurement began ¹. During exposure time, participants performed a short training version of the paradigm (with pictures of adult and infant animal faces) and completed a social demographic questionnaire (SocDem). In order to ensure comparability of the groups, participants performed a reading the mind in the eye test (RMET – after ²). Furthermore, we measured the 2nd to 4th digit ratio as a proxy of endogenous androgen reception ³. For this purpose, we measured the length of the index and ring finger from the fingertip (most distal point) to the lowest fold of the finger (most proximal point) of the right hand with a common caliper (accuracy $\pm 0,02/ \pm 0,001$). Subsequently we divided the length of the index finger through the length of the ring finger (2nd:4th digit) (for similar procedure see ³)

In the scanner participants performed two versions of the target detection paradigm (for a similar procedure see ⁴) The first target detection paradigm had 240 trials with each condition shown 40 times (i.e. 3 adult conditions, with an adult target and three either higher, unmanipulated or lower BS faces as distractors, and 3 baby conditions, with a baby target of either higher, unmanipulated or lower BS and three adult faces of the same gender as distractors). Each trial lasted 2,000 ms. A fixation cross was shown between trials. Its duration was jittered for 500-2,500 ms and randomized between trials. The first paradigm was 16.4 minutes long.

The same odd-one-out procedure was repeated in the baseline task. In this task geometric figures were used as stimuli (triangles, squares, or circles). Each condition was shown 30 times (i.e., triangle target, square target, circle target with three distractors of the other shapes), so that the paradigm was 90 trials long. The baseline task was intended to control for the influence of the OT treatment on participants' RT in general. The baseline task was 6.4 minutes long.

Subjects were asked to identify the target (the one picture that didn't fit the other three pictures) as fast and as accurate as possible via button press. The button positions matched the picture positions. The time of trial appearance was not influenced by button press and hits or false responses were not displayed for the participant (see Figure 4 for schematic illustration of the target detection paradigm).

The fMRI measurement was followed by another saliva sample of the participant.

Hormone Samples

The salivary T concentrations of the participants were analyzed in our in-house laboratory. Eppendorf tubes (2 ml) and an instruction for saliva sampling were handed over in advance at the first meeting with the MR-physician. The participants were asked to collect three saliva samples over the course of 1 hour at home. They started directly after waking up on the day of testing. During the collection of morning saliva the participants were instructed to refrain from eating and consuming cigarettes or any products that could influence the hormone measurement (for example milk, chewing gum or lip balm). They could drink water between sampling intervals and were allowed to brush teeth directly after the first saliva sample, leaving a gap of at least 15 minutes between tooth brushing and the second saliva sample to avoid a potential blood contamination. One additional saliva sample was collected right before administration of OT or placebo and a final one was obtained after the fMRI measurement.

The samples were frozen at -20°C until analysis. For preparing an aliquot out of the three morning samples and to separate these and the other two samples from mucus, all samples were unfrozen and centrifuged at RCF 604 x g for 5 minutes (i.e., 3,000 rpm) in a common Eppendorf Minispin centrifuge. After further refreeze and defrost of the samples, they were analyzed with a T luminescence immunoassay from IBL International (TECAN group global; Hamburg, Germany). Each sample, seven standards and two controls were pipetted twice on the assay plate. The analysis was performed according to the IBL manual. Formal sensitivity of the assay kit lies between 1.8 pg/ml and 500 pg/ml T in saliva. The intra- and inter-assay coefficient variances were declared to range between 1.47 - 3.01 % and 4.04 – 6.96 %.

To control for a potential pregnancy and to prevent cyclic changes in T level, we exclusively examined participants that used hormonal contraceptives. But it's important to note that previous research showed that the intake of oral contraceptives increased performance in affective responsiveness and that affective responsiveness was positively influenced by exogenous hormones through intake of oral contraceptives (pill-intake phase versus pill-free week ⁵). Further, the intake of combined oral contraceptives may decrease T concentrations in women ⁶. Therefore, it will be of great interest to examine the influence of the intake of oral contraceptives as opposed to a natural menstrual cycle in a future study.

Behavioral data analysis

Two participants had to be excluded because they missed more than 30% of the trials. Another participant felt unwell during the fMRI measurement and did not finish the task.

One participant of the placebo group failed to bring the morning saliva samples and another participant missed a fingertip on the ring finger of the right hand, so we could not measure her 2D:4D ratio. These participants were nevertheless included.

We performed one-way ANOVAs to evaluate the differences between OT treated participants versus placebo treated participants in the RMET score, digit ratio, age and T measurements. A potential T

modulation through OT administration was also analyzed with a one-way ANOVA. Mean T values (T_{morning} , T concentration before fMRI measurement, T concentration after fMRI measurement and digit ratio), age and reading the mind in the eye test score (RMET – after ²) did not differ between the OT and the placebo group (see Table S4). The RMET score was not influenced by the OT treatment (M_{11} pre administration = $25.18 \pm SD 4.35$; M_{18} post administration = $26.66 \pm SD 2.91$; $F_{1,55} = 0.823$; $p = 0.368$).

The T change (Delta of pre minus post measurement concentration) over the experiment did not differ in the OT or placebo group (OT: $M_{\text{DeltaT}} = 1.74 \pm SD$; placebo: $M_{\text{DeltaT}} = -5.45 \pm SD 12.82$; $F_{1,55} = 2.724$; $p = 0.105$).

Based on these analyses, the treatment and the placebo group were considered as comparable.

For analysis of the interaction between treatment and T concentration we calculated the median of the T concentration out of the sample before nasal spray administration. The median of the T concentration was 17.96 ± 16.6 pg/ml ($n = 57$).

fMRI data analysis

The whole brain measurement was set to thirty-nine axial slices with a voxel size of $3 \times 3 \times 3$ mm³ (distance factor 25 %). A total of 696 image volumes were obtained parallel to the anterior commissure–posterior commissure plane adjusted in descending direction in two sessions (target detection paradigm – 498 image volumes; baseline task – 198 image volumes). The field of view was set to 216 mm. The interscan interval was 2 s and the echo time was 25 ms. Participants viewed the paradigm through a head-coil mounted mirror.

Imaging data were preprocessed and analyzed with SPM8 (Wellcome Department of Cognitive Neurology, University College London, London, UK). Coregistration, correction of movement-related artifacts (realignment and unwarping), corrections for slice-time acquisition differences and low-frequency fluctuations, normalization and spatial smoothing were included in preprocessing process.

Parameter estimates were extracted from spheres at the local maxima (*IFJ*: -33, 5, 49 with a radius of 3 mm and *putamen*: L: -21 14 -11; R: 30 -1 13; spheres with 6 mm radius).

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3. Discussion

In humans, nurturing and caretaking mechanisms underlie a variety of factors and widespread influences. Proximately hormones have a major impact on the development of parental behaviors. In the following chapters, I will discuss the neuroendocrine influences of caretaking behavior and explain the opposing roles of oxytocin and testosterone on the expression of caretaking behavior based on the findings of the present thesis. Further, I will elucidate the influence of infants' cuteness on selective attention and discuss the baby schema as a possible neurological releasing mechanism to motivate action in adults and get into possible hormonal influences. Finally, I will set the findings in an evolutionary context and debate the medical and pharmacological relevance of the findings.

Especially oxytocin is well known to promote caretaking behaviors [*for review see* (Wigton et al., 2015)] After parturition, a lack of oxytocin is associated with postpartum depression and maternal neglect (Strathearn, 2011). Studies showed that children that experienced maternal neglect by themselves exhibited lower oxytocin receptor densities and were prone to show caretaking deficits as well (Strathearn, 2011). Contrary to oxytocin, the postpartum decrease of testosterone was repeatedly described as adaptation on the increase of parental care (Gettler et al., 2011; Kuzawa et al., 2009).

This neuroendocrinological thesis had the aim to investigate the modulatory influence of testosterone and oxytocin on the processing and sensitivity for the baby schema and how both hormones interact in the modulation of a basic caretaking behavior, selective attention towards infants. Caretaking behavior has been associated with prioritized attention towards infants based on the assumption that biologically salient stimuli should be processed preferentially. In the literature, infants were found to elicit rapid neural responses in adults (Brosch et al., 2007; Kringelbach et al., 2016).

The first behavioral study investigated how endogenous testosterone in women may influence selective attention towards infants and if the negative effects of heightened testosterone concentrations in women could be diminished by oxytocin administration [*The behavioral study is published in* (Holtfrerich et al., 2016)]. The following brain imaging study had the aim to investigate how the interaction of exogenous oxytocin and endogenous testosterone influences the processing of infant faces in the female brain [*The fMRI study is published in* (Holtfrerich et al., 2018)]. Based on the literature it was hypothesized that exogenous oxytocin may compensate for the negative effects of high endogenous testosterone concentrations on the attention towards

infants, and it was expected that this process may be modulated by activations in key nodes of the mesocorticolimbic reward system.

In the following, I will discuss the results of this thesis in more detail and put them in a greater context.

3.1. The endocrinology of caretaking behavior

Numan and Insel (2003) explained the importance of three different approaches regarding the neuroendocrinology of caretaking motivation: descriptive studies, pharmacological interventions and lesion studies. Here, I will present descriptive studies on hormonal influences of general nurturing behaviors and the modulatory effects of hormones in pharmacological interventions, implemented in a behavioral and in an fMRI study. In this chapter, I will concentrate on the endocrinological influences on selective attention towards infant faces.

In the first study (Holtfrerich et al., 2016), women with high habitual testosterone were found to increase attention to socially salient stimuli (measured by reaction times) after oxytocin administration, whereas reaction times of the low testosterone group stayed unaffected. To the best of my knowledge this was the first empirical finding about a possible interaction of oxytocin and testosterone in the modulation of caretaking behavior.

The experimental data of the behavioral study [*Study II in* (Holtfrerich et al., 2016)] replicated the initial results of my previous research on the effect of endogenous testosterone alone that was part of my master thesis, which demonstrated that high endogenous testosterone concentrations in women presumably had a negative influence on selective attention towards infant faces. These findings represented the basis for my following Ph.D. research. It was of particular interest that the administration of a single dose of 24 IU oxytocin obviously diminished the negative effects of high endogenous testosterone concentrations on attentional processing and apparently restored selective attention towards infant, but also adult faces in women with habitually high testosterone. Moreover, the attention of women with a low endogenous testosterone concentration remained unaffected by oxytocin administration (Holtfrerich *et al.*, 2016). Finally, these findings were mirrored by the subsequent fMRI study. In that Study I observed increased activation in the putamen that scaled with increased attention towards infants, yet only in case women with high endogenous testosterone received oxytocin (Holtfrerich et al., 2018). The

findings of both studies included in this thesis provide indications for a complex hormonal interplay in the expression of caretaking behavior. Although testosterone and oxytocin seem to exert opposing behavioral effects, simultaneous hormonal transitions (e.g. following parturition) may modify social behaviors like caretaking. This will be discussed in more detail in the following chapters.

3.1.1. Influences of testosterone on caretaking behavior

Testosterone has widespread influences on reproduction. In men it is, inter alia, responsible for the expression of the male sexual characteristics and the production of sperm (Nieschlag et al., 2004). Testosterone can further be converted into estradiol via aromatase and thus indirectly influence estradiol-induced behavioral modifications [*e.g.*, libido in men; *for review see* (Schulster et al., 2016)]. In the context of general nurturing behavior testosterone has been described to antagonize behaviors that were associated with caretaking and to promote protection and defense behaviors (Van Anders et al., 2011).

In this thesis, the influence of endogenous testosterone concentrations in women was investigated regarding one major aspect of caretaking behaviors: selective attention towards infants. In *Study I* and *Study II* of Holtfrederich et al. (2016) higher levels of endogenous testosterone were found to be associated with decreased relative reaction times, whereby higher relative reaction times represented increased selective attention for infants. The findings were in line with the hypothesis that high testosterone concentrations in women may antagonize this important aspect of caretaking behavior by decreasing selective attention towards infants. The results further indicated that women with high testosterone concentrations were more distracted by same age stimuli and showed increased selective attention towards pictures of young adults. In sense of the ‘Challenge Hypothesis’ (Wingfield et al., 1990) and the ‘Offspring Defense Paradox’ (Van Anders et al., 2011) high testosterone concentrations promote infant-protection and defense instead of caretaking behaviors (see Chapter 1.1.2.1). One could therefore speculate that reflexive attention towards unknown adults in the surrounding area of infants may display infant-defense behavior in women with high testosterone concentrations. But it may also be likely, that the nulliparous participants of reproductive age were partly attracted by same age adult stimuli. Testosterone concentrations may be probably highly correlated to estradiol levels, because estradiol biosynthesis in the brain can be catalyzed out of testosterone via aromatase. The resulting estradiol may then bind to estradiol receptors and consequently modify brain function

(Wilkinson and Brown, 2015). Higher testosterone concentrations in the female participants may therefore also implicate that the participants had higher estradiol levels. High attention towards adult stimuli may therefore be traced back to mate choice behavior [e.g. (Danel and Pawlowski, 2006)], which should be further investigated. In the present Study I did not directly control for the estradiol level of the participants, but women used oral contraceptives and should thus show a relatively low concentration of estradiol (Fleischman et al., 2010).

However, in line with the hypothesis, high testosterone concentrations counteracted attention towards infants [see *Study I and II of* (Holtfrerich et al., 2016)]. These findings are in line with the assumption that for example lower postpartum testosterone concentrations may be adaptive to promote caretaking behaviors (Gettler et al., 2011; Kuzawa et al., 2009), yet my studies showed that this may also be the case in nulliparous women, who have no immediate interest to engage in caretaking behaviors. One may therefore speculate that this finding could reflect the reduced readiness of high testosterone women to engage in caretaking behaviors oriented towards unknown babies, which needs to be tested by future studies.

The behavioral results of the fMRI study showed a significant interaction between the oxytocin treatment and the endogenous testosterone level in the modulation of attentional processing of infant faces (i.e., relative reaction times). Post hoc t-tests only showed very small effects for an attentional bias towards infant faces, i.e. only for the natural baby schema in the oxytocin treated group with high testosterone levels compared to low testosterone levels [page 2 of (Holtfrerich et al., 2018), Chapter 2.2). Only small behavioral effects could have been expected because all stimuli (i.e. the infant faces per se and same age adults) represent strong social salient stimuli and may thus be equally captured by the attentional system, probably influenced by individual preferences of the participants (Luo et al., 2015). The pure and unmanipulated baby schema may thereby cause the strongest behavioral responses, since it represents the natural baby schema to which our participants may have been generally used to in their everyday live. The manipulated infant faces may further have looked a little strange to the observer and therefore caused an alien effect (which may have led to avoidance behavior of some observers towards the stimulus). It may be reasonable to replace the same-age adult stimuli of reproductive age by pictures of older adult people that may not trigger mate choice behavior in the participants, in future studies, to diminish unwanted side effects through mate choice behavior.

The interaction found in the behavioral study was also represented in the BOLD signal analysis of the fMRI study that showed an increased activation in the putamen after oxytocin

administration, which could be traced back to women with high endogenous testosterone concentrations (after separating participants into two groups by median split of testosterone concentration). An influence of endogenous testosterone on mesocorticolimbic activation may therefore be likely, but the findings may further indicate a complex interplay of different endocrine factors in the modulation of caretaking behavior (e.g. a more sensitive oxytocin system in these subjects). This will be discussed in the following chapters.

3.1.2. Influences of oxytocin on caretaking behavior

Promoting influences of oxytocin on maternal behavior in rats have already been described in 1979 by Pedersen and Prange. The authors injected oxytocin in the brain of virgin female rats and found that 42% of the rats showed maternal behavior towards foster pups, whereas none of the control group displayed this behavior. Consequently, immense research on oxytocin-induced maternal behavior continued in the last decades across a wide range of model organisms [*for an overview see* (Insel et al., 1998; Insel and Young, 2001; Kendrick, 2000; Numan and Insel, 2003; Van Anders et al., 2011)]. The following chapter will focus on the influences of exogenous oxytocin on selective attention towards infant faces and discuss how increased oxytocin levels may influence the motivation to show caretaking behaviors.

Without regard of the endogenous testosterone concentrations of the participants, fMRI data analysis revealed two possible ways through which oxytocin may influence caretaking behavior and cuteness perception. Firstly, correlative results showed that oxytocin administration supposedly increased activity in the putamen which in turn might have increased selective attention towards infant faces. These results may provide information on how oxytocin motivates caretaking behavior. The putamen is part of the basal ganglia which belong to the mesocorticolimbic reward system (Haber, 2011; Haber and Knutson, 2009). Regions of the mesocorticolimbic reward system are not only rich in dopamine receptors, but also have a high number of oxytocin receptors (Bartels and Zeki, 2004; Gimpl and Fahrenholz, 2001; Wigton et al., 2015). The assumption, that the influence of oxytocin on reward areas of the brain may explain the motivation to show caretaking behaviors has already been discussed in some basic articles and in the introduction in Chapter 1.1.1.1 (Bartels and Zeki, 2004; Kringelbach et al., 2016; Luo et al., 2015). Bartels and Zeki (2004), for instance, assumed that human attachment behavior (which includes parental behavior) may be traced back to a push-pull mechanism that

deactivates networks that influence social distance behaviors, but enhances activation in the reward circuitry and thus motivates attachment behaviors like maternal love.

Evidence for a direct influence of oxytocin on dopamine release was delivered by a study of Shahrokh et al. (2010) who found an increased dopamine signal in the nucleus accumbens after direct infusion of oxytocin into the ventral tegmental area of maternal rats. Interestingly, the processing of cuteness also seems to be modulated through regions of the mesocorticolimbic reward system. Glocker and colleagues (2009b) could not only demonstrate that looking at cute infant faces activated regions of the mesocorticolimbic reward system, but also found increased activity in the nucleus accumbens (which is a core region of the reward system) with rising cuteness of the infant faces. In accordance with the results of the present fMRI study, increased activation in the putamen was also detected by Glocker *et al.* (2009b) as a response to infant faces versus viewing of a crosshair across the three baby schema conditions. In contrast to the present fMRI study, the study by Glocker et al. (2009b) did not consider oxytocin levels nor did they collect a behavioral measure of selective attention, but combined a passive viewing with a conscious cuteness evaluation task. The present fMRI results may therefore constitute further insight into mechanism through which caretaking behavior may be motivated by oxytocin and its effect in mesocorticolimbic dopamine pathways.

Secondly, *t*-tests revealed that oxytocin versus placebo administration increased the BOLD signal in the inferior frontal junction as response to infant faces that were manipulated towards a stronger baby schema (using the contrast 'baby versus adult target') (Holtfrerich et al., 2018). The inferior frontal junction is located at the transition of the premotor and the inferior prefrontal cortex (Adolphs, 2003; Brass et al., 2005a). Activation in the inferior frontal junction were further found to be associated with increased cognitive control (Adolphs, 2003; Brass et al., 2005b), the control of selective visual attention (Baldauf and Desimone, 2014), action perception (Avenanti and Urgesi, 2011) and the detection of behaviorally salient cues (Muhle-Karbe, Andres and Brass, 2014). As far as I know, an involvement of the inferior frontal junction in cuteness perception or the processing of key stimuli has not been described in the human literature so far. An interpretation of this finding must therefore be handled with care. Yet, there already exists some limited evidence, that oxytocin may modulate maternal behaviors in regions of the prefrontal cortex: For instance, the blockade of oxytocin receptors in the medial prefrontal cortex of postpartum rats was found to decrease maternal care and to increase maternal aggression (Sabihi et al., 2014). The results of the present fMRI study may provide new indications about an influence of oxytocin in the response towards cute infants and maybe constitute a first careful

explanation on how the key stimulus baby schema provokes action by engaging a region crucial for attentional selection in the prefrontal cortex. Although still far from a significant behavioral finding, relative reaction times in oxytocin treated participants resulted in a positive Delta (N= 29; $M \pm SEM = 17.5 \pm 17.1$ ms), whereas reaction times of the placebo treated participants resulted in a negative Delta (N= 28; $M \pm SEM = -15.3 \pm 14.6$ ms) in response to the trial-conditions with the higher degree of baby schema ($T_{55} = 1.45$; $p = 0.15$). The relative reaction time represented the selective attention towards infants (see Chapter 1.3.2 for detailed information). In case of a significant result, this finding could have stand for an increased attention towards infant pictures with a higher degree of baby schema after oxytocin treatment which may consequently have influenced the activation in the inferior frontal junction, but, due to the lack of significant behavioral findings and comparable literature, this assumption remains speculative and future studies will be necessary for further interpretations. One disadvantage of a between-subjects design might be a loss of statistical power through intersubject variability (Charness et al., 2012). For future studies I would therefore highly recommend a within-subjects design to counteract a loss of effect through inter-individual variations.

3.1.3. Interaction of oxytocin and testosterone in the modulation of caretaking behavior

As described in the last two chapters, there is consistent evidence for a promoting influence of oxytocin on caretaking behavior and also for an antagonistic effect of testosterone. In both studies of this thesis further interactions between both hormones in the modulation of caretaking behavior were investigated. Collectively, the results may provide insights in a potential counteractive hormone interaction in the modulation of caretaking behavior.

In both studies, oxytocin administration was found to influence behavior of nulliparous participants with high endogenous testosterone, but not the behavior of nulliparous participants with low testosterone concentrations. In the behavioral study, women with higher endogenous testosterone concentrations were found to be slower in orienting attention towards infants. Oxytocin administration seemed to diminish these negative testosterone effects and decreased reaction times towards both of the displayed stimuli (infant and adult targets). In the literature, neural responses to adult stimuli were described to overlap with the responses to infant stimuli (Luo et al., 2015). Considering that both same-age-adult and infant stimuli, may constitute socially salient stimuli for nulliparous women it may be plausible that both attracted attention to the same

extent. However, a study in Japanese macaques (*Macaca fuscata*) could not find any evidences for an attentional prioritization of infant faces in contrast to adult faces either. The authors assumed that the context of the infants' face (*e.g.* laughing or crying) may especially capture the attention in adults and may therefore constitute a more reliable stimulus in contrast to a neutral facial expression (Koda et al., 2013). A further explanation why testosterone was rather selectively negatively associated with the attention towards infants, whereas oxytocin administration decreased reaction times towards both adult and infant targets -may be related to the observation that oxytocin may improve face processing in general and may reduce uncertainty about the valence of a social stimulus [*e.g.* (Bate et al., 2014; Domes et al., 2007a)]. Nevertheless, the finding that only women with high testosterone concentrations were affected by oxytocin administration strongly indicates that the increase in oxytocin concentration may have counteracted heightened testosterone concentrations in this particular social behavioral context.

The fMRI study delivered additional insight in the adaptive neuroendocrinological system that may influence caretaking behavior through testosterone and oxytocin interactions. Here I would like to emphasize that the behavioral findings from the first study were widely reflected in the fMRI study. In accordance with the behavioral results, that indicated that oxytocin administration significantly influenced behavior of women with high testosterone concentrations only, the findings of the fMRI study suggested that oxytocin administration increased activation in the putamen concordantly with the attention towards babies and that this finding could be traced back to women with high endogenous testosterone concentrations only, after separating the participants into two groups (one group with high, above-the-median and one group with low, below-the-median testosterone concentrations). Both findings corresponded to the assumption that oxytocin and testosterone may constitute a complex interactive mechanism in the modulation of caretaking behavior (Van Anders et al., 2011).


Investigations on the interactive influences of testosterone and oxytocin in the modulation of caretaking behaviors currently remain elusive. Studies in male California mice found paternal caretaking behaviors to be testosterone dependent, but that this effect could be traced back to the conversion of testosterone into estradiol (Trainor and Marler, 2002). Considering the influence of estradiol on the oxytocin receptor expression (Chapter 1.1.1.3.), one may speculate about a possible interaction of testosterone and oxytocin in this context (Lee et al., 2009). It may therefore be possible that the findings of the present thesis may be partly traced back to a direct conversion of testosterone into estradiol in the brain, which could have consequently influenced oxytocin receptor (de Kloet et al., 2008). However, free testosterone was measured from saliva

samples in the two studies, which indeed doesn't exclude a rapid conversion into estradiol in the brain, but further makes it likely that the sample represented the neurological active, unconverted part of testosterone (see Chapter 1.3.4 for detailed information).

Further, the presence of an effect in oxytocin treated women that was only evident in those with high endogenous testosterone in both studies implicates, that the female hormonal system involved in caretaking behavior may be more receptive to oxytocin administration in a state of high testosterone. Since studies of this thesis focused on women and because the transferability of the findings to men will be discussed in Chapter 3.2.4, I will concentrate on the results in women here. In the literature, testosterone has been described to increase during pregnancy, coincidentally with progesterone and estradiol, and to rapidly decline in postpartum period (Fleming et al., 1997). Further, it has been repeatedly demonstrated that testosterone decreases in both, men and women, following parturition (Barrett et al., 2013; Gettler et al., 2011; Kuzawa et al., 2010, 2009; Saxbe et al., 2017). The rapid decrease of testosterone after parturition may further be adaptive to increase parental investment and to promote caretaking behaviors. Concurrently with the decline of testosterone, oxytocin raises in the course of pregnancy and parturition. An interrelated mechanism that compensates for high testosterone levels may be likely, considering the conflicting influences of both hormones on social bonds (Van Anders et al., 2011). Quite in line with this assumption, Gordon and colleagues (2017) found that high testosterone levels in new mothers were positively associated with a more positive influence of high endogenous oxytocin concentrations on maternal behavior, whereas the results in fathers pointed towards a contrary direction. In light of previous evidence, the findings of the present studies may therefore suggest that high endogenous testosterone not only inversely influences caretaking behavior, but also seems to interact with heightened oxytocin levels, which could supposedly be by enhancing the effect of exogenous oxytocin on selective attention.

Figure 4 page - 82 - shows a framework of the influences of oxytocin and testosterone on general nurturing behavior.

A

Oxytocin	High concentrations	Testosterone
nurturing empathy (Domes et al. 2007) caretaking motivation (Finkenwirth et al. 2016) bonding (Kendrick 2000) attachment (Insel & Young 2001)	 Low concentrations	defense infant defense (Fleming et al. 2002) protective responses (van Anders et al. 2011) decreased attention towards infant faces (Holtfreich et al. 2016)
withdrawl postpartum depression (Skrundz et al. 2011) emotional neglect (Strathearn 2011)		maternal care increased attention towards infant faces (Holtfreich et al. 2016)

B

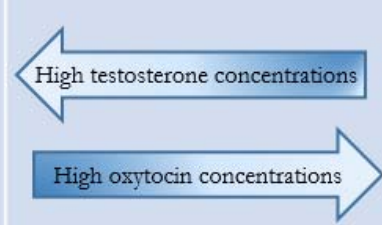
Oxytocin		Testosterone
Increased oxytocin counteracts high testosterone concentrations and directs attention towards infants (Holtfreich et al. 2016) and increases the reward value of the infant (Holtfreich et al. 2018)		Only higher testosterone concentrations are responsive to increased oxytocin levels [displayed in selective attention (Holtfreich et al. 2016) and the brain reward system (Holtfreich et al. 2018)]

Figure 4: Framework of the influences of oxytocin and testosterone on general nurturing behavior in women. **A** Summarized findings of behavioral effects of oxytocin and testosterone in the context of general nurturing behavior. **B** Summary of findings of this thesis on the interactive influences of increased oxytocin and testosterone in nulliparous women, which finally lead to the question if higher postpartum oxytocin levels could further be adaptive to compensate high testosterone levels and thus support the increase in caretaking behaviors which necessary to ensure offspring survival during the newborn and infant period.

3.2. The neuroendocrinology of the processing of infant faces

Child characteristic features in the infants face are perceived as cute and elicit caretaking behavior, as already been described in 1943 by Konrad Lorenz. Processing unfamiliar infant faces per se was found to activate neural face processing regions and regions associated with attention, emotion and empathy but also motor control and cognition. Interestingly, these findings seem to overlap with those found with adult stimuli. The main difference seems to be, that the responses towards infant stimuli were found to be stronger and faster [(Kringelbach et al., 2016) *for review see* (Luo et al., 2015)]. More rapid reactions towards infants in contrast to adults were also detected in Study I of Holtfrerich et al. (2016), as long as the stimuli were non-threatening. Besides evoking positive emotions, rapid and intuitive responses to infants may be adaptive to enhance the survival rate of the offspring and thus increase reproductive success (Kringelbach et al., 2016, 2008). Kringelbach and colleagues (2008) demonstrated that both, parents and non-parents, showed rapid selective neural responses in the orbitofrontal cortex to cute infant faces, but not to adult faces or infant faces with craniofacial abnormality. Further, an increased baby schema in the infants face was found to increase reward related activations in the nulliparous female brain (Glocker et al., 2009b). The following chapters will therefore focus on the neural responses towards infant faces and will put the endocrine findings in a neurological context.

3.2.1. Baby schema as a key stimulus motivates action in the female brain

Hormonal changes in the end of pregnancy modulate a number of intuitively shown maternal behaviors. This could be evolutionary adaptive to help to ensure caretaking and thus reproductive success (Numan and Insel, 2003). Yet, it is important to point out, that hormonal changes during late pregnancy are probably not the only reason for caretaking behaviors. Both men and women show caretaking behaviors, even when they never had a baby [*for review see* (Luo et al., 2015)]. Even small children show caretaking behaviors, for instance towards dolls and pets. This may be partly traced back to social development (Gourdon et al., 1994), but may also be modulated by an adaptive caretaking releasing mechanism triggered through typical child characteristic features. This adaptive key stimulus has been described as ‘the baby schema’ (see Chapter 1.2). It has been shown that infant faces are not only perceived as cute, but also elicit caretaking behaviors, recruit prioritized attention and motivate action [*for review see* (Luo et al., 2015)]. If these behaviors are triggered through the innate releasing mechanism ‘baby schema’ they may increase with

increasing cuteness of the baby – especially in species in which the offspring is dependent on parental care. As an innate releasing mechanism, the baby schema may automatically trigger caretaking behaviors (Alley, 1983; Lorenz, 1943). A stronger baby schema in the infant's face could therefore equally constitute a stronger attraction and a stronger motivation in the initiation of caretaking behaviors.

Beyond expectation, the behavioral results did not correspond to the hypothesis that infant faces with a stronger baby schema recruited selective attention to a greater extent than infant faces with a lower baby schema [*Study II of* (Holtfrerich et al., 2016)]. Here it may also be traced back to inter-individual differences in the stimulus-preferences of the participants caused by a between-subjects-design. But based on the findings, that testosterone concentrations of the participants negatively correlated with all of the three baby schema conditions (analyzed separately), one may assume, that the habitual testosterone concentration of the participant may exert an influence in the individual preferences for adult or infant stimuli (see Chapter 3.1.1 for more detailed information).

However, although missing an behavioral effect as well, the results of the fMRI study showed an activation in the inferior frontal junction when contrasting “baby versus adult target” for the high baby schema condition in oxytocin treated participants in contrast to placebo treated participants (Holtfrerich et al., 2018). This observation is somewhat unusual since most studies report finding in regions of the reward system (Luo et al., 2015).

The fact that findings in the inferior frontal junction in the context of caretaking remain elusive may be explained by its location in the brain. To be precise, the inferior frontal junction is anatomically defined as the junction between inferior prefrontal sulcus, the middle frontal gyrus and the precentral gyrus (Brass et al., 2005). As it is a small region (but it can well be recognized by its anatomical location) it has been widely neglected in brain research. Brass et al. (2005) suggested that the lack of research in the inferior frontal junction may be caused by a „self-fulfilling prophecy-error“, whereby the inferior frontal junctions has been consistently excluded in region of interest analyses or been ignored in general. However, rather consistent associations between the inferior frontal junction and cognitive control have been noted (Brass et al., 2005) (see Chapter 3.1.2 for more information).

In the context of caretaking behaviors I am only aware of findings in the inferior frontal gyrus, which is at least a region related to the inferior frontal junction [*see* (Paulsen and Waschke, 2013)

for an anatomical atlas of the brain]. For instance it has been found, that oxytocin increased activation in the inferior frontal junction in women during emotion recognition of infant faces compared to gender identification (Voorthuis et al., 2014). Furthermore, viewing the own infant's face seems to increase activity in the inferior frontal junction of fathers [(Kuo et al., 2012; Mascaroa et al., 2014), *for review see* (Luo et al., 2015)].

Yet, to the best of my knowledge, there is only one group of researchers that consciously investigated the neuronal basis of the innate releasing mechanism 'baby schema' (Kringelbach et al., 2016, 2008). Kringelbach et al. (2016, 2008) described a rapid attentional bias towards cute infant faces in the orbitofrontal cortex. The authors interpreted their finding as a neural signature for a parental instinct and supposed that infants may constitute a "potent positive stimulus" which releases fast attentional biasing (0-250 ms, 100-350 ms, 200-450 ms) and slow appraisal processes and thus elicits caregiving behaviors (Kringelbach et al., 2016). The authors found that even minor interruptions of the baby schema diminished the "parental instinct" and decreased the cuteness perception (Kringelbach et al., 2016). They further found increased activation in the inferior frontal gyrus/ pars triangularis, a region related to the inferior frontal junction (Brass et al., 2005; Paulsen and Waschke, 2013), in an implicit task for both, infant and adult faces, that were shown for 300 ms each (Kringelbach et al., 2008). As the *target detection paradigm* that was used for the studies of this thesis simultaneously shows adult and infant faces (four pictures with either an adult or an infant target, inversely surrounded by infant or adult distractors) and the increased activation in the inferior frontal junction was only found for the contrast 'baby (target) versus adult (target)' (and notably only for the high baby schema condition), this finding may be more differentiated because here we can directly compare the salience of the stimuli (direct comparison between adult and infant targets per trial). Then again, due to a missing behavioral effect, these results cannot provide a clear statement whether the infant target or the adult distractors effected the neuronal response.

At the end of this chapter, I would finally like to mention that all of the infant faces used to investigate the impact of baby schema in this thesis were evaluated by independent subjects as significantly cuter in the 'high' baby schema condition than the 'low' baby schema version [*see* (Holtfrerich et al., 2016) *for the procedure*]. Already very early studies showed that infants with more 'babyish' characteristics in the face received more attention in form of smiling and looking at than less cute infants (Hildebrandt and Fitzgerald, 1983, 1981).

Based on the involvement of the inferior frontal junction in the control of visual selective attention, the detection of salient stimuli (Baldauf and Desimone, 2014; Muhle-Karbe et al., 2014) and action perception (Avenanti and Urgesi, 2011), one may suggest that an increased activation in the inferior frontal junction might indicate an enhanced readiness to act to infants with stronger baby schema, because only the trials with the infant targets with a high baby schema increased influenced this activation. This may further imply a selection advantage for infants wearing a stronger baby schema. In addition, this mechanism may be supported by higher oxytocin levels, because the activation in the inferior frontal junction was found in women that were treated with oxytocin in contrast to women that were treated with placebo. These results may further be in keeping with findings in the inferior frontal gyrus that has been associated with oxytocin induced emotional empathy (Shamay-Tsoory, 2011), an attribute that is also closely linked to maternal care (Van Anders et al., 2011; Walker et al., 2007).

3.2.2. The interaction of testosterone and oxytocin in the processing of infant faces in the female brain

Comparisons with the literature show that activations in the putamen are typical in the context of caretaking behaviors and have been described in oxytocin administration studies [*for review see* (Luo et al., 2015; Wigton et al., 2015)]. Interestingly, the putamen is also known to be involved in cuteness perception (Glocker et al., 2009b) and maternal love (Stoekel et al., 2014). Therefore, the finding that oxytocin administration led to increased activation in the putamen along with increased attention towards infant faces corresponds well with the literature. Particularly remarkable in this context was, that the results could be traced back to women with high testosterone concentrations, which has not been described in brain research so far. In this chapter, I will therefore discuss the results presented in the literature of interactions between testosterone and oxytocin in a caregiving context and how previous findings in the brain concord with these results.

To adjust the system for potential caretaking behaviors, a compensative mechanism, that would counteract high testosterone concentrations (and the resulting caretaking deficits) in women, and would stimulate the mesocorticolimbic reward system to motivate caretaking behaviors, could be adaptive to enhance reproductive success. Only a few studies revealed interactions between testosterone and oxytocin concentrations in the brain so far. Besides that, most of the few studies that investigated an interaction between both hormones used male subjects. In male rats it has

been shown, for instance, that the reduction of oxytocin receptor binding in the brain annulled after testosterone treatment (but not in the putamen) (Arsenijevic and Tribollet, 1998). In contrast, results in castrated mice showed that the oxytocin receptor binding in the ventromedial nucleus of the hypothalamus doubled in comparison to intact male mice or testosterone treated castrates, but oxytocin receptor binding the intermediate zone of the lateral septum increased with testosterone treatment (Insel et al., 1993). However, comparisons between mouse and rat give evidence that the oxytocin receptor distribution in the brain varies across species. Further, brain regional regulation by gonadal steroids seems to vary across brain regions in the mouse and to differ across species (Insel et al., 1993). These indications in mice and rats leave some doubt, if results found in rodents or other non-primate mammals are transferable to humans at all, but need to be considered due to the lack of research.

Not only oxytocin receptors, also androgen receptors have a widespread distribution in the brain and are located in regions of the mesocorticolimbic reward system (Nieschlag et al., 2004; Sheridan, 1983; Wilkinson and Brown, 2015). Moreover, testosterone was also described to increase reward sensitivity (Hermans et al., 2010b). Op de Macks et al. (2011) could further demonstrate a positive correlation between reward-related activation in the putamen and high testosterone levels in boys and girls. Testosterone administration in women was further found to increase activation in the ventral striatum during reward anticipation (Hermans et al., 2010b), which is also referred as 'head' of the caudate nucleus and the putamen (Redouté et al., 2000).

However, there already exists some evidence that the interaction between testosterone and oxytocin may be sexually dimorphic. I must therefore assume that results in male subjects may not necessarily be transferable to those in females (please see Chapter 3.2.4 for further details).

Finally, testosterone may be converted into estradiol which may then have an impact on the oxytocin receptor density (please see Chapter 1.1.2.2 and 1.1.1.3 for further details). Given this evidence it follows that female participants who have higher testosterone concentrations compared to the group, may also have a higher oxytocin receptor density and an increased oxytocin receptor binding, for instance in regions of the mesocorticolimbic reward system, like the putamen (Van Anders et al., 2011; Young et al., 2001). Indeed, the present findings in the putamen could be traced back to oxytocin treated women with high endogenous testosterone concentrations and were not reflected in the low testosterone group, which allows me to suggest that the caretaking system of the female participants may be especially sensitive to oxytocin when testosterone concentrations (possibly along with estradiol concentrations) were higher. Yet, in the

absence of oxytocin stimulation a heightened testosterone concentration might rather reduce the interest in infant faces as described above.

3.2.3. Other possible neuroendocrine interactions in the modulation of general nurturing behavior

Postpartum maternal behavior seems to be modulated by a variety of neuroendocrine mechanisms. The reaction to motivationally relevant stimuli seems to be strongly dependent of dopamine and its mesocorticolimbic dopamine pathways (Strathearn, 2011). Infants in mammals display such motivationally highly relevant stimuli (Luo et al., 2015). Recent research discussed the question how dopamine is involved in the motivation of caretaking behavior and how oxytocin makes use of dopaminergic neurons and dopamine pathways. However, not only oxytocin but also dopamine plays a key role in attachment and maternal behavior. Pair-bonded male prairie voles, for example, displayed increased paternal behavior (in contrast to single prairie voles) that was also associated with increased dopamine receptor expression in the nucleus accumbens (Lei et al., 2017). In situ experiments in rats indicated that facilitatory allosteric receptor–receptor interaction in oxytocin-dopamine-receptor heteromers in the nucleus accumbens may be part of the molecular mechanism of social behavior (Romero-Fernandez et al., 2013). Further, lesions in reward regions, which are rich in dopamine neurons (Arias-Carrián et al., 2010), were repeatedly found to disrupt maternal behavior (Numan and Insel, 2003). For instance, lesions of the medial preoptic area were shown to interfere maternal behavior in wistar rats (Numan, 1974).

The putamen is involved in reward sensitivity by innervation of dopaminergic neurons and is part of the mesolimbic reward system (Mizuno et al., 2016). The present results showed evidence that oxytocin-induced caretaking behaviors may be initialized through activation in the putamen (Holtfrerich et al., 2018). Since oxytocin apparently uses the same pathways as dopamine, it is conceivable that dopamine and oxytocin interact in the motivation and expression of parental behavior. Actually, there already exists some evidence that dopamine and oxytocin may interact and influence reward related behaviors through mesocorticolimbic dopamine pathways. Gregory and colleagues (2015) demonstrated that oxytocin treatment increased the response to infant stimuli in the ventral tegmental area. The ventral tegmental area is considered as the origin of dopaminergic neurons (Oades and Halliday, 1987). More evidence was delivered by a study in lactating rats which showed that mother rats that expressed high maternal behavior showed

increased projections of oxytocin-positive cells to the ventral tegmental area. Further, infusion of oxytocin into the ventral tegmental area of the female rats enhanced the dopamine signal in the nucleus accumbens. In the direct comparison of high maternal behavior expressing rats with low maternal behavior expressing rats, the authors could show, that the high expressing rats had a greater increase of dopamine signals during maternal behavior than the other group. This increase was abolished by an infusion of an oxytocin receptor antagonist (Shahrokh et al., 2010).

The nucleus accumbens is located in the striatum. It sends gamma-aminobutyric acid (GABA) -ergic projections to the ventral tegmental area, the substantia nigra and the ventral pallidum and receives projections from the amygdala, the hippocampus and the prefrontal cortex (Haber and Knutson, 2009). The dopamine activity in the nucleus accumbens seems to constitute a major part in grading social salience (Love, 2014). A study in humans by Atzil *et al.*, (2017) investigated mothers dopamine response to own infants, the connectivity of the nucleus accumbens, the amygdala, and the medial prefrontal cortex (synchronous firing of these regions) and mothers plasma oxytocin level using combined positron emission tomography (PET) and fMRI measuring. The researchers found that the connectivity of the defined brain regions was positively correlated with an increase of dopamine but a decrease of oxytocin as response to own infant videos. However, an additional finding showed a trend for a positive association between oxytocin and dopamine in the left ventral striatum.

The BOLD response in the ventral striatum was then again repeatedly found to correspond to testosterone levels in reward processing (Hermans et al., 2010; Op De MacKs et al., 2011). Here, it is important to note that, like dopamine and oxytocin, testosterone also has a crucial impact in the modulation of social behaviors. Say, testosterone is indispensably involved in the modulation of reproductive behavior and sexual responses (Rommerts, 2004; Schultz, 2015; Van Anders et al., 2011). In castrated rats, for instance, testosterone administration was found to return sexual responses in the mesocorticolimbic reward system (Everitt and Stacey, 1987).

However, there are many questions left about hormonal interactions and the receptor distribution in the human brain. Especially studies examining the influence of a possible hormonal interaction in human maternal behavior remain elusive. But the existing literature and the present findings indicate that caretaking behavior is likely to be modulated by the mesocorticolimbic reward system, which is crucial for reward, motivation and attachment (Love, 2014).

3.2.4. Transferability of the results to other subjects

There already exists some evidence that a possible interaction between oxytocin and testosterone may be sexually dimorph (Gordon et al., 2017). In concordance with the results of the present thesis, Gordon et al. (2017) found a positive interaction between increased oxytocin concentrations and increased testosterone concentrations on the expression of maternal behavior in new mothers. In contrast, the result in fathers pointed in the opposite direction: When testosterone was high in fathers, the researchers determined a negative association between oxytocin and paternal behavior. Previous findings in fathers further showed that especially new fathers with low testosterone concentrations responded more strongly to oxytocin administration than fathers with higher testosterone concentrations. The authors interpreted that the decreased testosterone levels in new fathers were part of a more sensible androgen system that probably was more responsive to oxytocin administration (Weisman et al., 2014).

The different and partly contradictory findings between female and male subjects may imply that the results of this thesis may not be transferable to men.

New mothers, then again, may represent a comparable group of participants to nulliparous women with artificially increased oxytocin levels because studies showed that lactating women may probably have comparable oxytocin levels to nulliparous women with artificially increased oxytocin concentrations by intranasal administration of 24 IU oxytocin (Weisman et al., 2012; White-Traut et al., 2009). Therefore, the effects of artificially increased oxytocin concentrations may not necessarily be applicable to those in nulliparous women. But since lactating women further underlie a number of other strong hormonal transitions (Gammie et al., 2016; Hendrick et al., 1998), nulliparous participants, that use hormonal contraceptives, may probably offer a physiologically plausible model to examine how testosterone and oxytocin influence and interact in the modulation of caretaking behavior.

In contrast to the increase of oxytocin during pregnancy and after parturition, testosterone was found to decrease rapidly in the postpartum period [after making an increase during pregnancy (Fleming et al., 1997)]. This rapid decline of testosterone, as well as the increase of oxytocin, may be related and may play an important role in the development of maternal caretaking behaviors (Holtfrerich et al., 2018, 2016; Van Anders et al., 2011). An essential difference between mothers and nulliparous participants may be that the habitual testosterone concentrations of nulliparous participants are probably relatively stable in contrast to the large steroid hormonal transitions

experienced by new mothers (Gammie et al., 2016; Hendrick et al., 1998). This may be addressed by future research.

The female participants of this study used hormonal contraceptives to prevent cyclic fluctuations and to inhibit the probability of a potential pregnancy. However, the literature showed that the intake of oral contraceptives influenced affective responsiveness in female participants (Radke and Derntl, 2016) and that the intake of oral contraceptives may suppress free testosterone concentration and increase sex hormone binding globulin (Pastor et al., 2013; Zimmerman et al., 2014). Further, the intake of oral contraceptives was found to influence cuteness perception. In contrast to the comparison groups, premenopausal women and younger women using oral contraceptives were found to be more sensitive to the cuteness perception of infant faces (Sprengelmeyer et al., 2009).

Finally, the distribution of oxytocin receptors in the brain seems to be diverse across mammalian species (see Chapter 1.1.1.3). And so it also appears that different mammalian species show entirely different maternal behaviors. But they all have at least two things in common: the influence of steroid hormones in reproduction and the dependence on the maternal milk which is strongly influenced by oxytocin (Bridges, 2015). Of course, the results of this thesis are not transferable one to one to other mammalian species – not even to all primate species – but the presence of oxytocin or oxytocin equivalent hormones (e.g., vasopressin) in almost all vertebrates (Knobloch and Grinevich, 2014) and the widespread association of oxytocin and the expression of maternal behaviors across species provides a fundament for further investigations on the interaction of testosterone and oxytocin in the induction of maternal behavior in other species.

3.3. Evidence for hormonal interactions in the response to infant faces

Most of the existing studies concentrated on the influence of steroid or peptide hormones and classical neurotransmitters on the expression of parental behavior, but studies on steroid-peptide interactions are seldom. ‘The Steroid/Peptide Framework of Social Bonds’ after Van Anders et al. (2011) created a basis for research on the counteracting roles of oxytocin and testosterone in the context of social bonds, like, for example, parent-offspring-bonds. The authors developed a theory, that testosterone and oxytocin influence different behavioral contexts of social bonds in opposite directions. Namely, high testosterone concentrations were found to influence competitive, sexual and defensive behaviors whereas high oxytocin concentrations were

associated with bonding and intimacy. To the same extent, low concentrations of oxytocin and testosterone also have antagonistic roles in the expression of social behaviors. Whereby low testosterone concentrations were assigned to nurturing and warm behaviors and low oxytocin concentrations were related to social withdrawal and a lack of interest. The findings of the present thesis may expand the framework of Van Anders et al. (2011). High testosterone levels appeared to counteract on an important aspect of caretaking behavior and rather seemed to be associated with the direction of attention away from infants towards adults which could, speculatively, implicate infant-defense behavior or sexual interest towards same-age-adults (Holtfrerich et al., 2016). High oxytocin levels, in contrast, seemed to promote social behavior by diverting attention towards socially salient stimuli (Holtfrerich et al., 2016), by increasing the motivation to act in response to cute infant faces and by enhancing the reward value of infants in contrast to adult faces (Holtfrerich et al., 2018). Low testosterone levels, on the other hand, were found to be positively associated with this specific aspect of caretaking behavior by raising the attention and the reward value of infant faces and, in addition, the motivation to act did not vary between a high and low oxytocin state in the comparison of the placebo and oxytocin group when considering only women with habitually low testosterone (Holtfrerich et al., 2018).

Regulatory effects of steroid hormones, neuropeptides and classic neurotransmitters, such as testosterone, estradiol, prolactin, vasopressin, oxytocin and dopamine, on the expression of caretaking behavior and bonding have been demonstrated several times (Bos et al., 2012; Luo et al., 2015; Van Anders et al., 2011; Ziegler, 2000). Each of these hormones plays a role in reproduction and in the preparation for birth and breeding. For instance, rising estrogen levels prime oxytocin synthesis in the brain and provide oxytocin receptors which prepares the uterus for parturition. Dopamine and vasopressin than rise concurrently with oxytocin and initiate further adaptations to breeding behavior (Bos et al., 2012). Collectively, a whole cascade of hormonal adaptations and hormonal transitions seems to be involved in reproduction, parturition, breeding and parental as well as alloparental care. An interplay of those hormones therefore seems most likely but has been investigated in by only a handful of studies (Atzil et al., 2017; Gordon et al., 2017; Holtfrerich et al., 2018, 2016; Weisman and Feldman, 2013). Evidence that the same brain regions (e.g. reward system) seem to be involved in both reproduction and cuteness perception (e.g. the response to the key stimulus baby schema) and can be mediated by endogenous and exogenous concentrations of the involved hormones, makes it likely that a complex neuroendocrine system is involved in the regulation of caretaking and gives a basis for many more studies on hormonal interactions in the context of caretaking behaviors and maternal

love. For instance, in pharmacological studies on mental disorders like postpartum depression and maternal neglect these neuroendocrine aspects may provide new research avenues (Luo et al., 2015; Strathearn, 2011; Van Anders et al., 2011).

3.4. Evolutionary relevance of the findings

Evidence highly suggests that parental care is conserved across mammalian species (not at least because the offspring depends on the maternal milk) and birds. The evolution of environmental, social, hormonal and experiential adaptations may probably have had critical relevance in the evolution of caretaking behaviors (Numan and Insel, 2003; Riedman, 1982).

Apart from kin selection, alloparental care could serve to gather parental experiences. It has been shown, for instance, that prairie voles that gained practice as alloparents were more successful in raising the future genetic offspring (Stone et al., 2010). Alloparental care might have further evolved (e.g. in human hunter-gatherer societies) to enhance reputation and consequently increase the social rank of the alloparent in the group. In non-human primates, for instance, it could be further demonstrated, that subdominant females received social privileges when they cared for the offspring of a dominant female (Bell et al., 2013; Blaffer Hrdy, 2008; Kenkel et al., 2017).

Proximately, complex neuroendocrinological systems may modulate the motivation to show caretaking behaviors. Interestingly, in alloparental marmosets, oxytocin was found to be positively associated with the expression of particularly intrinsic caretaking behaviors in parents and alloparents. The researchers suggested that there exists a positive link between oxytocin and the motivation of caretaking (Finkenwirth et al., 2016). Since testosterone was found to antagonize parental caretaking behaviors it consequently seems conceivable that testosterone may also be negatively associated with alloparental care [*e.g.* (Vleck and Dobrott, 1993)].

Testosterone and oxytocin are both critically involved in social behaviors and social cognition. They have been implicated in several reproductive processes, like pair-bonding, sexuality and procreation. Oxytocin thereby seems to take over a bigger part in bonding and affiliation, whereas testosterone seems to take a greater proportion in sexuality (Van Anders et al., 2011). An evolutionary adaptive opposing hormonal interaction could therefore serve bonding and reproduction to the right time (Van Anders et al., 2011). Furthermore, alloparental care has been

reported in hundreds of mammalian and avian species which makes a sole influence of pregnancy hormones in the modulation of caretaking behaviors unlikely (Riedman, 1982). It therefore seems reasonable that other hormones involved in social behaviors also play an important role in the evolution of alloparental care. If the expression of caretaking behaviors is adaptive, an innate releasing mechanism in the infant's face that automatically triggers this system and elicits caretaking behaviors in adults may further be adaptive. Kringelbach *et al.* (2016; page 546) stated that: „Cute infants attract our attention, and they also capture it quickly”. This statement coincides with the results of Study I in Holtfrerich *et al.* (2016) that showed a faster response to infant faces of non-threatening species in contrast to adult faces. Study I and II of Holtfrerich *et al.* (2016) further demonstrated that the relative reaction time to human infant stimuli was negatively influenced by high testosterone concentrations and results of Study II showed that oxytocin administration diminished the negative effects of high testosterone concentrations and decreased reaction times (to the level of the low testosterone group). The results of the fMRI study also pointed towards an interrelated neuroendocrine system. The oxytocin-induced increase in activation of the putamen along with increased attention towards infants could be traced back to participants with high testosterone concentrations. In contrast to placebo administration oxytocin administration led to increased activation in the inferior frontal junction when women directed attention towards high baby schema infants. This may indicate an increased readiness to act in response to infants with a higher baby schema and may thus indicate a neuronal mechanism through which the key stimulus motivates action (Holtfrerich *et al.*, 2018). The findings of the present thesis may therefore provide further predictions for an evolutionary adaptive and interrelated neuroendocrine releasing mechanism that modulates caretaking behavior and promotes the infants' evolutionary aim of survival.

3.5. Clinical relevance of the findings

A widespread impact of different neurotransmitters in mental or neurological disorders is very considerable (Campeau *et al.*, 2007). Since this thesis focused on the interactive influences of testosterone and oxytocin in caretaking behaviors, I will primarily discuss the potential relevance of the findings in the context of postpartum depression and maternal neglect here, but will also mention briefly how research on the interaction of both hormones may have an impact in other mental disorders. A lack of oxytocin in postpartum period seems to be centrally involved in postpartum depression and maternal neglect (Skrundz *et al.*, 2011) and, as a consequence, may

further influence the neuroendocrine development of the infant (Strathearn, 2011). In contrast, a number of studies showed that high oxytocin levels seem to activate caretaking behaviors, motivate reaction to infant cues and decrease activity in brain regions associated with aversion and fear [*for review see* (Luo et al., 2015)]. An interdependence between oxytocin levels and caretaking is therefore highly likely.

Prioritized attention towards infants makes up an extremely significant part of caretaking behavior. Attention deficiency is further associated with emotional maternal neglect (Strathearn, 2011). Hence, the present finding together with the literature may provide a basis on pharmacological research in postpartum depression and maternal neglect.

A more innovative approach may be delivered by the findings, that testosterone was found to antagonize selective attention towards infant stimuli and that this negative effect could be diminished through oxytocin administration. To the best of my knowledge a possible (antagonistic) interplay of testosterone and oxytocin has not been considered in research on postpartum depression and maternal neglect yet. The present results may therefore initiate an impulse for further research on this topic.

Further, to control for the estradiol-induced oxytocin receptor expression in a within subject design in pregnant and postpartum women and to investigate the possible interaction with salivary steroid hormone transitions [like the rapid decline of testosterone after birth (Fleming et al., 1997)] and their interactive influences on selective attention towards infants may offer further insights in the expression of caretaking behavior and the development of postpartum depression. Here, it would be of interest to examine the effects of selective attention with own versus unfamiliar infant stimuli.

Autism spectrum disorders have further been found to be associated with lower plasma oxytocin levels (Green et al., 2001) and higher fetal testosterone levels (Knickmeyer and Baron-cohen, 2006) and have been linked to an oxytocin-receptor-gene-polymorphism (Wu et al., 2005). Autistic people further show a significant deficit in cognitive empathy (Baron-Cohen et al., 2001) whereas oxytocin administration was found to increase cognitive empathy in men (Domes et al., 2010). Interestingly, autism also seems to be associated with testosterone. A hint for a possible link between testosterone and autism is resulting from findings that men have lower second to fourth digit ratios than women (an index of the fetal testosterone) and autistic people have even lower values. Moreover, autism spectrum disorders seem to be more common in human males

than in females (Knickmeyer and Baron-cohen, 2006). Testosterone administration was further found to counteract cognitive empathy in women and this finding could be traced back to women with high fetal testosterone [low second to fourth digit ratio (van Honk et al., 2011)]. Untreated healthy women (placebo group), and especially mothers, in turn may have higher cognitive empathic abilities than men - which could be a consequence of the increased need to understand the nonverbal signals of the offspring (Walker et al., 2007). The findings of a possible interaction between testosterone and oxytocin in the brain may therefore constitute a future approach in the field of pharmacological autism research, but since the interaction between testosterone and oxytocin seems to be sexually dimorphic, future research in male participants would be necessary to develop a clear hypothesis.

Collectively, the potential relevance of research on interactions between testosterone and oxytocin in the treatment of mental disorders may be wide-ranging and could provide further insights in the development and expression of the mentioned psychiatric disorders.

3.6. Conclusion

The findings of the present thesis provide initial evidence for interactive influences of endogenous testosterone and exogenous oxytocin on caretaking behavior in women. In line with the results of my master thesis, higher testosterone concentrations were found to negatively influence selective attention towards infants in nulliparous women (Holtfrerich et al., 2016). According to theory, the rapidly decreasing testosterone concentration in the postpartum period may be adaptive to increase caretaking behavior [*e.g.* (Gettler et al., 2011; Kuzawa et al., 2010)]. If the simultaneous increase of oxytocin in pregnant or postpartum women would further be adaptive to compensate higher testosterone concentrations and to initiate caretaking behavior, the artificial increase of oxytocin (to concentrations comparable with lactating women) may be especially effective in women with higher testosterone concentrations (Holtfrerich et al., 2018, 2016). Exogenous oxytocin was therefore expected to direct attention towards infant faces and to diminish the negative effects of high endogenous testosterone concentrations on attention towards infant faces. Along with the expectation, the administration of 24IU oxytocin diminished the negative effects of higher endogenous testosterone concentrations. Yet, this result was not limited to infant stimuli. Therefore I suggest an interactive influence of both hormones in the processing of social salient stimuli in general (Holtfrerich et al., 2016).

In line with the behavioral findings, the following fMRI study showed that artificially increased oxytocin concentrations positively influenced the reward processing of infant faces and that this result could be traced back to women with higher endogenous testosterone concentrations. The related findings from the behavioral and the fMRI study support the idea of a complex interplay between testosterone and oxytocin in the modulation of caretaking behavior in nulliparous women. Prospectively, these findings may promote pharmacological research on mental disorders like postpartum depression and extend basic research on the evolution of maternal and alloparental care.

In addition to the interactive hormonal influences, the releasing mechanism ‘baby schema’ was further investigated. Oxytocin administration versus placebo administration was found to increase activations in the inferior frontal junction as a response to infant faces with higher baby schema (Holtfrerich et al., 2018). As a key stimulus, the baby schema should not only induce caretaking behaviors but also increase the readiness to act. One could therefore speculate, that the finding in the inferior frontal junction may provide indications for a neurological mechanism on how the key stimulus baby schema initiates reaction in the observer. To the best of my knowledge this is the first finding in this context, therefore and in order to gain more insights in this mechanism, further research would be needed.

Overall, the results of the present thesis provide new indications in research on the development and the neuroendocrinological mechanisms underlying caretaking behaviors.

3.7. Outlook

As described above, the results of the present thesis may provide a fundament for further neuroendocrinological and pharmacological research on the development of caretaking behaviors and mental disorders like postpartum depression and maternal neglect. In the literature, oxytocin was described to play an important role in the development of mental disorders (please see Chapter 3.5 for a detailed description of the influences of oxytocin in different mental disorders). As far as I know, a possible interaction of oxytocin with the steroid hormone testosterone have not been considered in the literature yet. The present results provide the first indication for a neuroendocrine interplay between testosterone and oxytocin in the expression of caretaking behavior and may provide an important starting point for future research.

Nevertheless, more research is necessary to gain a progressive understanding of this neuroendocrinological mechanism. Firstly, I would suggest to expand the collective of subjects. Nulliparous women on oral contraceptives, like the participants in this studies, certainly represent a valid group for basic research to avoid cyclic fluctuations and to control for parental experiences. But, considering the influences of hormonal contraceptives on cuteness perception (Sprengelmeyer et al., 2009), affective responsiveness (Radke and Derntl, 2016) and testosterone level depression [(Liening et al., 2010) *but see* (Tremblay and Dube, 1974)] it would be of great interest to investigate normal cycling female participants and to compare female subjects using androgen versus antiandrogen effective hormonal contraceptives. Considering the influence of estradiol on oxytocin and its receptor density (discussed in Chapter 3.2.2), it would be of special interest to examine the influence of estradiol in the late follicular phase on the oxytocin-induced expression of caretaking behaviors. There is one other study to date, that investigated interactions of testosterone and oxytocin in mothers, but in this study blood plasma was used for hormonal analysis (Gordon et al., 2017). Since oxytocin cannot pass the blood-brain barrier from blood to brain (Guastella et al., 2013), it is not clear if plasma oxytocin levels can deliver reliable information about the neuroendocrine activity. Future research with salivary oxytocin measurements would therefore be of keener concern.

Secondly, although the interaction of oxytocin and testosterone seems to be sexually dimorph (see Chapter 3.2.4), it may be of great interest to investigate male subjects, because they may give a deeper insight in the interactive mechanisms of oxytocin and testosterone, considering that men's estradiol concentrations are comparably low in contrast to women which may influence the oxytocin receptor density (de Kloet et al., 2008; Gabor et al., 2012) Further it would be of interest to compare groups of man that have normal testosterone concentrations with androgen deprived men and to examine differences in the expression of caretaking behavior and salivary oxytocin concentrations.

Finally, polymorphisms of the oxytocin receptor gene may influence the oxytocin administration uptake and may also be involved in caretaking dispositions (Marsh et al., 2012). In future research I would therefore strongly recommend to control for oxytocin receptor gene polymorphisms. Altogether, the present findings indicate a significant interaction of testosterone and oxytocin in the modulation of caretaking behavior, which should be considered in future research.

4. References

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5. Figure Index

Figure 1: Schematic illustration of the biosynthesis and secretion of the neuropeptide oxytocin. Messenger ribonucleic acid gets translated on the rough endoplasmic reticulum yielding in a precursor protein molecule. Passing the Golgi body, the precursor protein molecule, together with its carrier protein neurophysin and probably with enzymes for the posttranslational processing, gets packaged in granules. The posttranslational processing of the precursor protein molecule occurs either in the cell body or during the axonal transport. The peptide products are stored in the granule until they are released in the nerve endings in the posterior pituitary. When the nerve endings get depolarized through calcium influx, the granular contents get released [adapted from (Brownstein et al., 1980)]..... - 11 -

Figure 2: Simplified pathway of steroid hormone synthesis [adapted from (Felberbaum et al., 2007; Sun et al., 2016)]. - 15 -

Figure 3: Procedure of the Target Detection Paradigm. The task was to select the one picture that did not fit with the category of the others and to confirm this via the associated button as fast and accurately as possible. The target was either an adult face shown in the context of three infant faces as shown here or an infant face among three adult faces [figure from (Holtfrerich et al., 2018)] - 26 -

Figure 4: Framework of the influences of oxytocin and testosterone on general nurturing behavior in women. **A** Summarized findings of behavioral effects of oxytocin and testosterone in the context of general nurturing behavior. **B** Summary of findings of this thesis on the interactive influences of increased oxytocin and testosterone in nulliparous women, which finally lead to the question if higher postpartum oxytocin levels could further be adaptive to compensate high testosterone levels and thus support the increase in caretaking behaviors which necessary to ensure offspring survival during the newborn and infant period. - 82 -

6. Table Index

Table 1: Overview of studies that investigated the interactive influence of testosterone and oxytocin on caretaking behaviors. - 20 -

7. Publications

Diekhof, E.K., Reimers, L., Holtfrerich, S.K.C., n.d. Hormonal Modulation of Reinforcement Learning and Reward-Related Processes – A Role for 17 β -Estradiol, Progesterone and Testosterone, in: Schultheiss, O., Mehta, P. (Eds.), *Routledge International Handbook of Social Neuroendocrinology*. Routledge.

Holtfrerich, S.K.C., Pfister, R., El Gammal, A.T., Bellon, E., Diekhof, E.K., 2018. Endogenous testosterone and exogenous oxytocin influence the response to baby schema in the female brain. *Sci. Rep.* 8. <https://doi.org/10.1038/s41598-018-26020-4>

Jakob, K., Ehrentreich, H., Holtfrerich, S.K.C., Reimers, L., Diekhof, E.K., 2018. DAT1-genotype and menstrual cycle, but not hormonal contraception, modulate reinforcement learning: Preliminary evidence. *Front. Endocrinol. (Lausanne)*. 9. <https://doi.org/10.3389/fendo.2018.00060>

Holtfrerich, S.K.C., Schwarz, K.A., Sprenger, C., Reimers, L., Diekhof, E.K., 2016. Endogenous testosterone and exogenous oxytocin modulate attentional processing of infant faces. *PLoS One* 11, 1–19. <https://doi.org/10.1371/journal.pone.0166617>

8. Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

_____ Ort, Datum Unterschrift

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