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PhD Thesis

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Salivary oxytocin in pediatric obsessive-compulsive disorder

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Content

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2. List of publications

Study I:

The association between salivary oxytocin, age, and puberty in children with and without OCD[1]

Anna-Rosa Cecilie Mora-Jensen, Line Katrine Harder Clemmensen, Manja Gersholm Grønberg, Eli R. Lebowitz, Daniel S. Quintana, Niklas Rye Jørgensen, Charlotte Sewerin Larsen, Lasse Kristoffer Bak, Gitte Lund Christensen, Linea Pretzmann, Valdemar Uhre, Sofie Heidenheim Christensen, Camilla Uhre, Nicoline Løcke Jepsen Korsbjerg, Christine Lykke Thoustrup, Julie Hagstrøm, Melanie Ritter, Kerstin Jessica Plessen, Anne Katrine Pagsberg, Nicole Nadine Lønfeldt, Scientific Reports 14, 28693 (2024).

Note 1:
Study I was preprinted during the review process prior to publication on the preprint platform "Research square". In the review process the title of the paper was changed.
Preprint: Anna-Rosa Cecilie Mora-Jensen, Line Katrine Harder Clemmensen, Manja Gersholm Grønberg et al. Salivary oxytocin concentrations in children and adolescents with and without OCD, 26 August 2024, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-4654936/v1]
Note 2: In the final and printed version of Study I, in Scientific Reports, the figures had been truncated at the top, which resulted in the removal of some observations from the figures. We are in contact with Scientific reports on this issue. The correct figures have been included in the thesis and is also available in the pre-printed version.

Study II:

The role of oxytocin in family processes in the presence and absence of pediatric OCD[2]

Anna-Rosa Cecilie Mora-Jensen, Line Katrine Harder Clemmensen, Eli R. Lebowitz, Niklas Rye Jørgensen, Lasse Kristoffer Bak, Linea Pretzmann, Nicoline Løcke Jepsen Korsbjerg, Camilla Uhre, Christine Lykke Thoustrup, Valdemar Funch Uhre, Sofie Heidenheim Christensen, Melanie Ritter, Kerstin Jessica Plessen, Anne Katrine Pagsberg, Nicole Nadine Lønfeldt, (Manuscript, not submitted for publication).

Study III:

Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD[3]

Anna-Rosa Cecilie Mora-Jensen* and Christine Lykke Thoustrup*, Eli R. Lebowitz, Julie Hagstrøm, Linea Pretzmann, Nicoline Løcke Jepsen Korsbjerg, Emilie Damløv Thorsen, Valdemar Funch Uhre, Sofie Heidenheim Christensen, Camilla Uhre, Melanie Ritter, Kerstin J. Plessen, Anne Katrine Pagsberg, Line Katrine Harder Clemmensen** and Nicole Nadine Lønfeldt**, (Manuscript, in review).

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3. List of abbreviations

ANCOVA	Analysis of covariance
APA	American Psychiatric Association (APA)
BF	Bayes Factors
CAMHC	Child and Adolescent Mental Health Center
CBT	Cognitive-behavioral therapy
CGAS	Children's Global Assessment Scale
CPR number	Civil registration number civil registration number
CSF	Cerebrospinal fluid
CY-BOCS	Children's Yale Brown Obsessive Compulsive Scale
DSM	Diagnostic and Statistical Manual of Mental Disorders (DSM)
DSM-X	Diagnostic and Statistical Manual of Mental Disorders, X^{th} version
ELISA	Enzyme-linked immunosorbent assay
ELPD-LOO	Expected log-pointwise predictive density estimated by leave-one-out cross validation
Emreg	Emotion regulation abilities
ERP	Exposure and response prevention
FAS-SR	Family Accommodation Scale for OCD-Self Rated Version
FASA	Family Accommodation Scale Anxiety
FES	The Family Environment Scale
H_0	The null hypothesis
H_1	The alternative hypothesis
ICD	International Classification of Diseases
ICD-X	International Classification of Diseases, X^{th}
JZS prior	Jeffrey's prior
K-SADS-PL	Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version
Ln	Natural logarithm
LS	Lack of satisfaction
MSE	Mean squared error
NA	Non applicable
NHST	Null hypothesis significance testing
OCD	Obsessive-compulsive disorder
OOB	Out-of-bag
OXTR	Oxytocin receptors
RCT	Randomized clinical trial
PC	Principal components
PCx	Principal Component X
PCA	Principal component analysis
PRT	Psychoeducation and Relaxation Training
PS	Parental stress subscale
PSS	Parental Stress Scale
PVN	Paraventricular nucleus
q-q plots	Quantile–quantile plot
rpm	Revolutions per minute
SDs	Standard deviations
SE	Standard error
SNP	Single-nucleotide polymorphism
SON	Supraoptic nucleus
SRS	Social Responsiveness Scale questionnaire
TECTO	Treatment Effects of Family Based Cognitive Therapy in Children and Adolescents with Obsessive-Compulsive Disorder
TEC-M	The Tangram Emotion Coding Manual
VIF	Variance Inflation Factor
WAIS-IV	Wechsler Adult Intelligence Scales
WHO	World Health Organization
WISC-V	Wechsler Intelligence Scales for children

4. Introduction

There has been extensive interest in the possible role of the neurohormone oxytocin in various psychiatric disorders with the hope that oxytocin could provide ways to improve the understanding of disorders and their treatments[4]. Oxytocin's possible role in obsessive-compulsive disorder (OCD) has gained interest since the 1980s[5], but whether oxytocin is implicated remains a question. The aim of this PhD was to explore the relevance of salivary oxytocin in pediatric OCD. The long-term goal is to find ways to improve the treatment of children with OCD. However, oxytocin is a neuropeptide with various physiological and psychological functions and OCD is a heterogenous disorder[6,7]. Therefore, research in these two areas combined is complex and initial steps with exploratory studies could help guide our understanding and directions of research. In this PhD, we conducted three studies that explored the potential relevance of oxytocin in pediatric OCD, in three different ways, with the hope that they could increase the understanding and point to potential areas for future research.

The background section will first provide a short description of OCD with a focus on families in OCD and especially family accommodation. It will then provide a short description of the history of oxytocin research, the previous research in oxytocin's possible role in OCD, oxytocin's suggested role in parenting and in family accommodation. Finally, some of the challenges in oxytocin research will be described, as these have influenced some of our methodological choices.

4.1. Obsessive-compulsive Disorder

OCD is a common psychiatric disorder that often onsets in childhood[8] and is associated with a significant burden on children and their families[9]. The lifetime prevalence has been described in adults as 2.3%[10] and in adolescents between 1.9% and 3%[8]. Estimating the average age of onset of OCD is challenging, as the age of onset is frequently reported many years after the onset, and some cases of childhood OCD remit before adulthood[8,11]. However, large studies have found a mean age of onset of 16.9 years and 60-70% debuting before 25 years of age[8]. The first-line treatment for pediatric OCD is cognitive behavioral therapy (CBT) with exposure and response prevention (ERP)[12]. Meta-analyses have shown that CBT with ERP is effective in treating OCD[13–15]. However, one of the meta-analysis examined 10 trials of children and adolescents receiving CBT (8 studies using CBT with ERP and two studies of CBT without ERP), and found an average response rate of 68% to CBT and a remission rate of 57%, indicating that many children do not respond to the treatment[14]. The course of OCD in unrecognized youth is unknown, but the lack of treatment has been suggested to increase the risk of chronicity[8]. The large number of children that fail to sufficiently respond to treatment and the possible risk of chronicity in the disease, has led to multiple calls for increased understanding of factors that could affect the course and treatment of the disorder[13,16,17].

4.1.1. Diagnostic criteria of OCD

OCD is characterized by impairing obsessions and/or compulsions[8]. It is diagnosed using the diagnostic manuals the "International Classification of Diseases" (ICD) from the World Health Organization (WHO)[18] or the "Diagnostic and Statistical Manual of Mental Disorders" (DSM) from the American Psychiatric Association (APA)[19]. The diagnostic manuals have been used and further developed in various versions since the 1940s[20,21], with the most current versions being the ICD-11[18] and DSM-5[19]. Both manuals describe obsessions as repetitive/recurrent and persistent thoughts, images, and urges (for ICD-11 also "impulses") and describe obsessions as intrusive, unwanted, and related to distress or anxiety[18,19]. In addition, both manuals describe that individuals with OCD often try to ignore, suppress, or neutralize the obsessions[18,19]. Compulsions are described as repetitive behaviors and mental acts that a person feels a need to perform as a response to obsessions and following rigid rules (in ICD-11 also be to achieve a sense of "completeness" and DSM-5 also to "reduce distress")[18,19]. Besides having obsessions and/or compulsions the manuals state that the symptoms are time consuming, and cause significant distress or impairment[18,19]. In both manuals it is possible to add a "specifier" describing the insight of the patient and in DSM-5 also a specifier describing whether the OCD is tics-related[18,19]. In our study, we used the diagnostic criteria from ICD and as our study started in 2018, we used the previous version – the ICD-10[22]. Important differences between the ICD-10 and the currently used definitions in DSM-5 and ICD-11 are, that the ICD-10 required a minimum of 14 days of symptoms whereas the DSM-5 and ICD-11 have no requirements regarding durations of symptoms[23]. In addition, the ICD-10 required that patient had insight into the symptoms, and the three manuals include different specifiers[23].

4.1.2. OCD related impairment in children and adolescents

Functional impairment is a part of the diagnostic criterion in OCD[18,19] and compared to individuals without OCD, patients with OCD have been described with lower overall quality of life[8]. Children with OCD are often impaired in multiple areas, such as their interactions with peers, disruption of family routines, and frequent being late or absent from school[13,24]. In OCD, the impairment and burden of the disease is not limited to the child but frequently affects the entire family, causing distress for the family members and affecting family functioning[9,13,24–26].

4.1.3. Families in the context of pediatric OCD

Families have been a focus of research in pediatric OCD as family impairment is often high and the families are frequently involved in the disorder and in the treatment[16]. As a normal part of the parent role and child development, children are dependent on their parents for help and protection, especially when they experience distress or danger[27]. In pediatric OCD, child distress and/or anxiety are a part of diagnosis and naturally, the parents often become involved in handling the OCD related distress and anxiety to help their child[27,28]. The involvement of the family in OCD can cause emotional and practical burden for the family[9]. However, involvement of the family and especially the parents also means that characteristics of the family,

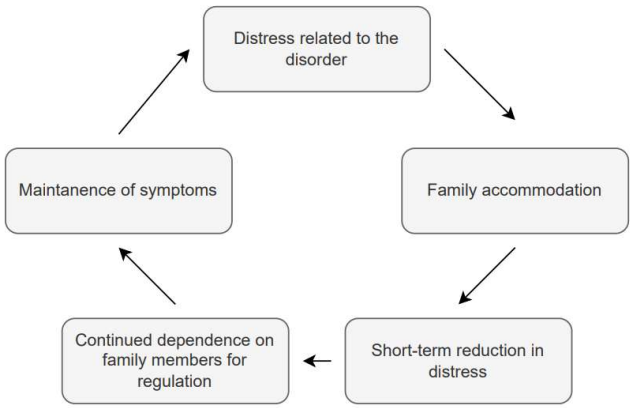
for example how the family handles problems, may affect the child and the course of symptoms[26]. Multiple studies have found aspects of the family that are important in pediatric OCD[9,16,17,26]. Families of children with OCD have for example been described with higher levels of parental psychopathology, family strain and stress[17], and with higher levels of family conflict and lower levels of family cohesion, expressiveness and organization[26] in comparisons to families of children without OCD. In addition, aspects of the family have been found related to symptom severity and treatment outcomes[16,29].

In the studies of this thesis, we investigated some of the family aspects that have been suggested to differ in families of children with and without OCD and that have been suggested to impact the effect of treatment[13,16]: family accommodation, parental stress, family conflict and family cohesion.

4.1.3.1. Family accommodation

Family accommodation describes a process where family members change their behavior to help another family member with distress related to a psychiatric disorder[25]. Family accommodation was initially studied in the context of OCD, but the understanding of the concept has been broadened and accommodation has been found relevant across multiple psychiatric disorders e.g. OCD, anxiety, and autism spectrum disorders[25,30]. In a conceptual model by Shimshoni et al., family accommodation has been described as a cycle, where distress related to a specific psychiatric disorder elicit accommodation by family members, that are often highly motivated to alleviate the distress[30] (see Figure 1). The accommodation can take various forms both participation in symptoms, facilitating symptoms or avoidance or modifying family routines[30]. The accommodation can reduce the short-term distress, but the long-term consequences are increased reliance on family members to reduce the distress[30]. These long-term consequences can contribute to maintaining the disorder thereby increasing the distress and further increase the family accommodation[30].

Figure 1. Conceptual model of family accommodation. (Shimshoni et al., 2019[30])



Note: Figure from the study "Family accommodation in psychopathology: A synthesized review"; Shimshoni, Yaara; Shrinivasa, Basavaraj; Cherian, Anish V.; Lebowitz, Eli R.; DOI: 10.4103/psychiatry.IndianJPsychiatry_530_18; with permission under the Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA) license (https://creativecommons.org/licenses/by-nc-sa/4.0/). The figure has been adapted by a change in color.

Family accommodation is common in pediatric OCD and has gained attention in research and treatment strategies, as it has been shown to impact the severity of OCD and outcome of therapy[25,31]. In pediatric OCD, accommodation often involves the parents that participate or facilitate compulsions, modify family routines or facilitate avoidance to relieve the child's OCD related negative emotions[25]. Parents may accommodate when the child shows distress or when they anticipate the child will feel distressed[32]. As described in the model by Shimshoni et al., accommodation can reduce the OCD-related distress shortly, but accommodation has been found related to symptom severity of OCD and to predict poor treatment outcome[30]. Accommodation can be time consuming and stressful for the parents[27]. The high levels of distress in the children can mean, that children can react with coercive and disruptive behavior if the parent does not engage in accommodation, which can lead to high levels of conflict and parental burden and distress[25,27].

Whether accommodation occurs in non-clinical populations is less studied. One study investigated youth aged 7-17 years with either OCD, anxiety, or without a psychiatric disorder[33]. The children with OCD and anxiety reported higher levels of accommodation than the youth without a psychiatric disorder[33]. In addition, severity of anxiety was positively correlated with the level of accommodation in the clinical groups, but not in the group of youth without a psychiatric disorder[33]. This made the authors speculate that parents of the children without a psychiatric disorder were perhaps less responsive to expressed anxiety[33]. However, the questionnaires on family accommodation has been developed in relation to OCD and anxiety but not for non-clinical samples with lower levels of distress and anxiety[34,35], which can complicate the use of these scales in and interpretation of the results from non-clinical samples.

4.1.3.2. Parental stress

Parental stress describes the stress that parents can experience in association with parenting a child[36]. It has been described as "the aversive psychological reactions to the demands of being a parent"[37]. Parents are important in children's daily lives - both in fulfilling basic and practical needs, but also in the child's cognitive, social and emotional development[38,39]. Therefore, being a parent is a complex role with many and various demands which can be stressful[38]. In a review of parental stress in relation to child behavioral and emotional problems, parental stress was suggested as both an exposure of child behavioral and emotional problems, but that child problems could also predict parental stress[40]. Families of children with OCD have been described with high levels of family distress[16]. As for parental stress and child emotional and behavioral problems[16], child OCD could also both affect the level of parental stress and be affected by the parental distress. For example, accommodation has been suggested to cause burden for parents[25] and coercive behavior in children with OCD has been found associated with parental distress[41]. High levels of parental stress have been found associated with more harsh and less effective parenting strategies, which could affect the child's or families handling of distress[42]. Thus, parental stress and related parent behaviors and child OCD and related child behaviors, may affect each other in a dynamic relationship. The impact of parental

stress is indicated by a study showing that children with OCD from families with higher levels of parental stress are more resistant to treatment[43].

4.1.3.3. Family conflict

Family conflict has been described as "the amount of openly expressed anger and conflict among family members"[44]. Family conflict can be a part of all family systems, but the level of family conflict has been found higher in families of youth with OCD compared to families of youth without a psychiatric history[26]. Family conflict can arise from the symptoms related to OCD (e.g. strong emotional reactions when accommodation is not provided)[28]. However, family conflict has also been suggested to affect the child OCD[26]. For example, high levels of family conflict could be related to high levels of affective arousal in the family, which could make it difficult for the family to manage the OCD related distress[26]. The impact on family conflict on child OCD is also suggested by a study showing a correlation between baseline family conflict and OCD symptom severity after treatment[16].

4.1.3.4. Family cohesion

Family cohesion is described as "the degree of commitment, help, and support family members provide for one another"[44]. The level of family cohesion has been found lower in families of children with OCD compared to families of children without OCD (or other psychiatric disorders)[26]. The level of family cohesion has been suggested to affect how the family handles problem-solving, which may affect management of the OCD[26]. In addition, higher levels of family cohesion has been found associated with better response to treatment[16].

4.2. Oxytocin

4.2.1. The oxytocinergic system

Oxytocin is a neuropeptide that has received enormous attention due to its various physiological and psychological effects[45,46]. Oxytocin is primarily produced in the magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, but is also produced in some peripheral tissues e.g. in the uterus, heart, and thymus[45]. Oxytocin has peripheral functions as a neurohormone and central functions as a neuropeptide[6]. It binds to oxytocin receptors (OXTR) that are distributed in both the central nervous system, and in peripheral organs, e.g., the mammary gland, uterus, kidneys and thymus[47]. Oxytocin's peripheral functions are carried out by projections from the hypothalamus to the pituitary gland, which can release oxytocin to the bloodstream that then transport oxytocin to target areas in the periphery of the body[45]. Centrally, oxytocin acts via transport from the SON and PVN to other areas of the brain via axonal and dendritic release[45,46].

Initially, oxytocin was most studied and used in obstetrics[48]. In the early 1900s, it was discovered that administering an extract from the pituitary gland could cause uterine contraction in various animals[48]. Later,

researchers discovered the structure of oxytocin and in the 1950s it was synthesized[48]. This facilitated research in oxytocin's effects and the clinical use to stimulate uterine contractions, which is important to induce birth and to prevent or treat postpartum hemorrhage[48]. The initial focus was largely on the peripheral effects of oxytocin as a neurohormone, but in the 1970s, D. de Wied and colleges returned the focus of neuropeptides to the brain[46]. The first research into oxytocin's central effects came from animal studies, where the central effects of oxytocin can be studied via intracerebroventricular infusions and by measuring the concentration of oxytocin in the cerebrospinal fluid (CSF)[49]. Several animal studies have for example highlighted the role of oxytocin in behaviors such as maternal caregiving and pair bonding[49]. In humans, the investigation of oxytocin is more difficult since central administration or measuring of oxytocin is invasive and since the by the blood-brain-barrier is not being permeable to oxytocin, administration or measuring oxytocin in the blood stream is not ideal[46,49]. In the 1980s, it was discovered that intranasally administered oxytocin could reach the brain and that it could influence cognitive function and behavior, which created the opportunity of studying the effects of oxytocin administration in humans[49]. The number of human studies increased in the early 2000s after studies showed prosocial effects, such as increased trust, mentalization and recognition of facial expressions, after intranasal oxytocin administration[50]. The huge interest led to multiple studies focusing on the treatment implications of disorders with social impairments[49,51]. However, problems with replications and findings of oxytocin having anti-social effects, led to decreased enthusiasm and a change in the overall perception of oxytocin functions[6,50,51].

Thus, studies in oxytocin have undergone a change over the past decades, which Alvares et al. described as resembling a "hype cycle"[51]. A hype cycle is a framework usually used to understand how new technologies progress and to find the optimal times for investments[51]. Alvares et al. used this to describe changes in oxytocin research with a focus on oxytocin and autism[51]. Initially, there was a huge interest in oxytocin, a "hype", particularly following a study finding that intranasal oxytocin enhanced trust behavior (described as the individuals willingness to accept social risks) in a trust experiment[51,52]. During this hype, oxytocin was mainly described as a neurohormone with multiple prosocial effects[51]. After a series of studies were unable to replicate the early findings and studies demonstrating anti-social effects, the field entered a period of "disillusionment" with decreased enthusiasm of oxytocin's potential[51]. The following phase describes a "period of enlightenment", where improvement of methodological steps are important to help the field forward[51]. Alvares et al. suggested that we are now in this period of enlightenment[51]. Methodological problems in oxytocin research have been a focus of multiple papers, calling for improvements of methods to measure endogenous oxytocin, improved methods to administer oxytocin intranasally, and improvement of statistical methods and overarching theories that can describe oxytocin function across various contexts[6,45,50,53–56].

One overarching theory that has been proposed to describe oxytocin's various function is "The allostatic theory of oxytocin" proposed by Quintana and Guastella[6]. Allostasis is described as "the process of maintaining stability through change by anticipating future changes in environmental conditions and

adjusting response setpoints"[6]. Allostasis is important for maintaining many different physical, psychological and behavioral process, that must be adapted to the environment to ensure survival[6]. The authors argue that the oxytocin system is important for allostasis and has been adapted to be a flexible system that can adapt through effects on learning, prediction, and response to the environment[6]. They note that the ability, through oxytocin, to adapt to different conditions may "come at the cost of psychiatric disorders", if the oxytocin systems is impaired[6]. Due to its effects on interpersonal behaviors, parent-child interactions, anxiety, and cognition, oxytocin has been suggested of importance in various psychiatric disorders[4,57].

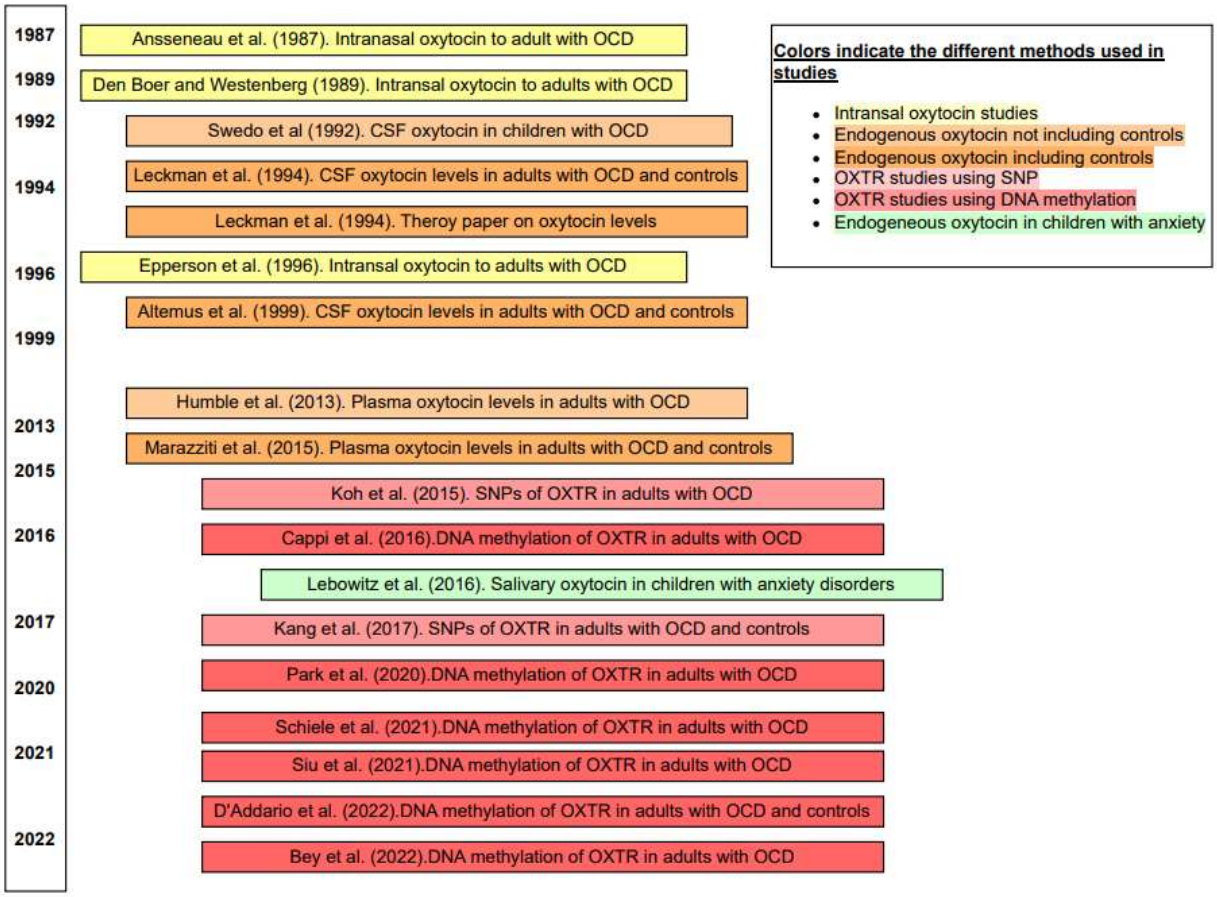
4.2.2. Oxytocin's possible role in OCD

Oxytocin's role in OCD was first explored in the 1980s and 1990s[5] (see Figure 2). The initial studies focused on intranasal administration, suggesting that oxytocin could improve OCD symptoms[58–60]. This was based on research showing that oxytocin could have anamnestic properties and thereby perhaps reduce avoidance behavior and conditioned responses in OCD[5,58–60]. The studies were limited in size, employed different methods, and did not provide evidence of an effect of intranasal oxytocin on OCD symptoms[58–60].

In the 1990s the focus seemed to shift to endogenous oxytocin levels in OCD (see Figure 2). First, Swedo et al. examined CSF levels of various neurohormones, including oxytocin, in 43 children with OCD[61]. They did not find a relationship between oxytocin levels in CSF and OCD symptom severity[61]. In 1994, James Leckman and colleagues formulated a theory proposing a potential maintaining role of oxytocin in OCD, hypothesizing that some patients with OCD may have elevated oxytocin levels[5]. This was followed by three studies that examined level differences in adults – two in the 1990s[62,63] and one later in 2015[64]. The first two studies found no significant differences between patients with OCD and controls[62,63], however one of the studies found higher levels in a subgroup of patients with OCD without a family history of tics[62]. The latest study in 2015 took up the interest in oxytocin level differences and found significant higher oxytocin levels in adults with OCD compared to controls[64]. Oxytocin levels have also been examined in one study that did not compare oxytocin levels with controls, but explored changes in oxytocin levels across treatment with serotonin reuptake inhibitors[65]. They found positive associations between baseline oxytocin and OCD severity, and differences in oxytocin changes during treatment between responders and non-responders[65].

In 2015, the focus on oxytocin in OCD shifted to the oxytocin receptor (OXTR) and implication of genetic variations and DNA methylations of the OXTR gene. Whereas studies on single nucleotide polymorphisms of the OXTR have not showed differences between patient with OCD and controls[66,67], studies on DNA methylations have shown both increased areas of hypermethylation[68–71] and hypomethylation[72,73] in patients with OCD compared to controls. The interpretation of methylations in relation to oxytocin levels is complex, and studies have emphasized that the connection remains poorly understood with DNA methylations both discussed as a compensatory reaction to high oxytocin levels, but also as factor that can lead to high oxytocin levels[69,71].

Figure 2. Overview of studies on oxytocin and OCD in the methods they used.



Note: OCD, Obsessive-compulsive disorder; OXTR, oxytocin receptor; SNP, single nucleotide polymorphisms. The colors show the different methods used in the studies.

In summary, the studies on oxytocin's possible relevance in OCD have used different methods and suggested different hypothesis, but the way oxytocin could play a role in OCD is not yet clearly understood. In 2013, Lebowitz et al., suggested that oxytocin could be related to family accommodation, thus, related to specific interpersonal behaviors in distress and anxiety regulation[57]. They investigated this in relation to pediatric anxiety[57], but also discussed how this could be relevant in pediatric OCD, were family accommodation is also common[25]. Below, the aspects of oxytocin in OCD, that were addressed in this PhD will be described in more details: the difference in oxytocin levels in patients with OCD compared to controls, oxytocin's role in parenting, and the suggested role of oxytocin in family accommodation.

4.2.2.1. Differences in oxytocin levels

Leckman et al. based their hypothesis of the role of oxytocin in OCD on the neuroanatomic distribution of oxytocin receptors in brain areas, that have been suggested as important in OCD, the periodic pulsatile release of oxytocin with positive feedback mechanisms (e.g. during lactation and birth) and animal and

human behavior in conditions with high levels of oxytocin[5]. As an example, they observed how administering oxytocin to the central nervous system of animals induced for example grooming-, sexual- and aggressive behaviors, which was suggested to resemble cleaning compulsions and sexual and aggressive obsessions[5]. Thus, they speculated that in some individuals, an obsession can be associated with a pulse of oxytocin that stimulates the limbic system and autonomic center, and that this can start a positive feedback loop of oxytocin, thereby increasing oxytocin further[5]. The increased oxytocin levels was suggested to prolong and intensify the obsessions and to create an urge for completing compulsions[5]. This was hypothesized to be important in specific individuals e.g., after steroid priming based on the observation that oxytocin increase after puberty and during pregnancy, or that there may be changes in oxytocin projections and receptor systems at different ages[5].

In a study from 1994, Leckman and colleagues tested whether oxytocin levels in CSF were elevated in 29 adult patients with OCD, 31 controls and 23 patients with Tourettes syndrome[62]. When comparing the groups, they found no overall group differences[62]. However, Leckman et al. found a positive correlation between OCD symptoms severity and oxytocin levels, and in a subgroup analysis, patients with OCD without a family history of tics had higher levels than the controls[62]. These findings were interpreted as supporting the hypothesis of higher oxytocin levels in OCD[5]; however, Leckman et al. also noted that oxytocin is perhaps not linked to all forms of OCD and that the findings should be replicated[62]. In 1999, Altemus et al. tried to replicate the findings by Leckman[62], in a study of oxytocin in CSF of 14 adult patients with OCD and 26 controls[63]. They did not find a difference between the groups and no difference in the same subgroups as studied by Leckman in the 1994 study[63]. The question about oxytocin level differences in OCD was then not studied for many years until 2013, where a study of oxytocin in plasma of 44 adults with OCD and 44 controls found higher levels of oxytocin in patients with OCD compared to controls, but a negative correlation with OCD symptom severity[64]. There are no studies of oxytocin level differences in children or adolescents with OCD compared to controls.

4.2.2.2. A role of oxytocin in parenting

Before we consider oxytocin's role in parenting in the context of OCD, it is interesting to look at the role of oxytocin in parenting in general. Although there are multiple studies addressing oxytocin and parenting, their findings are difficult to comprehend due to some methodological challenges[74]. For example, studies often use cross-sectional designs and multiple studies have been conducted in the same labs decreasing the reliability of the findings[74].

Some studies have explored the relationship between parental oxytocin and parenting behaviors using administration of intranasal oxytocin, or by measuring oxytocin levels before a parent-child interaction[75]. For example, intranasal oxytocin administered to fathers has been shown to be associated with more stimulatory and less hostile behavior[76]. Intranasal oxytocin administered to women (that are not parents themselves), has been shown to increase responsiveness to infant crying by reducing activation in brain regions involved in

anxiety and increasing activity in brain regions involved in empathy[77]. This findings has been suggested as important, as reducing anxiety and arousal may increase parent's ability to sense child cues and respond appropriately to a child in distress[78].

Studies exploring oxytocin levels before a parent-child interaction have, for example, found a positive association between maternal oxytocin levels and frequency of affective touch, and higher paternal oxytocin levels associated with more stimulatory touch[79]. In addition, a study found higher maternal oxytocin levels were associated with more affectionate parenting ("motherese" vocalizations, positive affect, and affectionate touch) and paternal oxytocin levels with more stimulatory parenting (proprioceptive contact, tactile stimulation and object presentation)[80]. One study explored oxytocin levels during pregnancy and found a positive association with "maternal behavior" postpartum (defined as maternal gaze at infant, motherese vocalizations, positive affect and affectionate touch)[81]. In summary, some studies have found a relationship between parent's oxytocin levels and their parenting behavior.

Studies have also examined the relationship between children and parent's oxytocin levels before and after parent child interactions. One study found, that oxytocin levels of children between 7-16 years of age increase more after mother-child interactions, with high levels of affectionate touch, and higher levels of engagement, compared to low levels of affectionate touch and engagement[82]. Another study showed that the oxytocin levels of infants (4-6 months of age) before and after an infant-parent interaction, were positively associated with levels of affect synchrony and levels of infant social engagement[83]. The same study found that parents oxytocin levels after an interaction were positively associated with infant social engagement, and that parent oxytocin both before and after an interaction, was associated with affect synchrony[83]. Thus, studies have suggested that children and parent's oxytocin levels can be affected by the behaviors during a parent-child interaction.

The associations between parent oxytocin and parent behaviors, and child oxytocin and child behaviors have led researchers to suggest, that the oxytocin systems of children and parents are interconnected and reactive to the social interaction between children and parents[83]. A connection between parents and children's oxytocin systems has been examined three studies[83–85]. One study found a positive correlation between oxytocin in mothers and 4-month-old children (not examining correlations between single point measurements but exploring measured as "Area under the Ground" describing total hormonal output across multiple measurements)[84]. Another study found a positive correlation in urinary oxytocin levels between 6-year old children and their mothers[85]. A third study of fifty-five 4- to 6-month-old children and their mothers and fathers, showed a correlation between both mother and infant and father and infant oxytocin[83]. This study found that the correlation was stronger in dyads with higher levels of affect synchrony[83] and suggested that the parent-child interaction was important for the connection between then child and parent oxytocin systems.

The role of oxytocin in parenting was initially mostly studies on maternal parenting, however paternal oxytocin have gained increased attention[74]. This is important as oxytocin seem to be differently related to mothers and fathers behaviors[79,80]. Overall, studies have not found differences between mothers and fathers oxytocin levels[74]. However, differences in oxytocin levels before parenthood have been described, suggesting that the oxytocin levels of pregnant women increase during pregnancy compared to non-pregnant women, whereas men's oxytocin levels increase after birth[74]. This has led to the suggestion that the father oxytocin system is adapted to parenthood after the birth, when the father engagement with the child[74].

Oxytocin has been suggested to, through evolution, be adapted to regulate more complex social functions such as parenting and attachment[6]. Sensitive parenting, described as the parent's ability to sense, interpret and react appropriately to a child's signals, is an important predictor for attachment between children and parents[78]. Appropriate reactions have been described as flexible, adaptive behaviors tailored to a given situation[78]. As oxytocin has been related to parents ability to sense child signals and parental behaviors – it is interesting in the context of sensitive parenting[78]. The appropriateness of behaviors are situational and dependent on a variety of factors thus highlighting the importance of flexibility[78]. Affectionate behaviors can be appropriate in a situation where a child needs comfort or security, however, perhaps not if the child is in acute danger. Similarly, stimulatory parenting can be appropriate in some situations e.g., during play, but not if the child is in distress and needs comfort. Other parental behaviors are perhaps never appropriate, such as hostile or aggressive behavior towards the child. However, parental aggression towards strangers has been investigated in animal and human studies suggesting that parental stranger aggression can provide situationally appropriate protection their children[78].
Protective parenting behavior can be relevant, especially, when the child is in danger or experience fear or anxiety[78]. The role of oxytocin in both attachment and fear/anxiety has led to suggestion, that oxytocin is an evolutionary conserved system that help regulate both attachment and fear/anxiety, and that oxytocin is important in the interaction between these two system[86]. The systems for attachment and anxiety are closely related through shared social/attachment stimuli and stimuli that signal danger, shared reactions involved in social interaction and anxiety, and shared brain regions implicated in social processing and anxiety[86]. In a paper by MacDonald and Feiffel, it was suggested that these stimuli, processing, and brain areas all affect each other and is regulated by complex mechanisms, including oxytocin, but also interactions with other hormones and neuropeptides (e.g., gonadal steroids and vasopressin)[86]. By creating a link that affects and modifies attachment and fear/anxiety, oxytocin becomes central in the handling of an environment with many inputs and a need for flexible outputs to adjust to both attachment and fear/anxiety[86].

4.2.2.3. Oxytocin's possible role in family accommodation

The possible role of in oxytocin in the interplay between attachment and fear/anxiety has created the suggestion, that oxytocin could play a role in family accommodation[27,57]. The normal process of parenting, in

which a parent helps a child in distress, is interesting in the context of pediatric OCD, as it resembles the process of family accommodation, where a parent helps a child in OCD-related distress[27]. But in family accommodation, the behaviors are perhaps appropriate for short term relief of distress, but not appropriate over time, where the symptoms are often reinforced[27]. Therefore, it is interesting to ask: what role does the oxytocin system play when child OCD enters the family system, accompanied by excessive child distress and interaction in family accommodation?

Although no studies have investigated child oxytocin in relation to family accommodation in pediatric OCD, one research group has explored this connection in the context of pediatric anxiety[57]. They found that lower child oxytocin levels were negatively associated with mother-reported overall family accommodation and a factor describing "maternal participation" in the symptoms[57]. Additionally, they found that youth oxytocin was negatively associated with child consequences when parents did not accommodate (with "child consequences" based on three items describing child distress/anxiety and angry/abusive behavior when the parent does not provide assistance)[57]. In another study, the same research group examined youth oxytocin in relation to mother and child behaviors during an interaction[82]. They found a significant association between youth oxytocin and various behaviors, including a positive correlation between touch and youth oxytocin[82]. This led them to suggest that children with anxiety disorders and low oxytocin levels may seek parents to increase oxytocin through proximity in family accommodation, which, in turn, reinforces their dependence on the parents and reinforce the anxiety[82]. A clinical implication of this findings could be that parent could be guided in how to manage attention and physical contact with the child and thereby guided to the help the child learn how to independently cope with distress[82].

4.2.3. Oxytocin research – state of the art

As noted previously, there has been significant interest in oxytocin for several decades. However, issues with replication and the interpretation of results have led to criticism of the methods used in oxytocin research and recommendations of how these could be improved[45,53–55]. Of particular importance to this PhD, is the state of the art in sampling and analyzing endogenous oxytocin concentrations, the importance of covariates and statistical considerations in the oxytocin field.

4.2.3.1. Oxytocin sampling and analyses

The interest in oxytocin within psychology and psychiatry is primarily based on oxytocin's central effects on, for example, regulation of anxiety and interpersonal behavior[87]. Central oxytocin levels can be measured in the CSF, but this requires invasive procedures such as lumbar punctur[87]. Therefore, research on CSF oxytocin levels is limited to animal studies and conditions involving patients who have already undergone lumbar puncture to access CSF (e.g. patients in intensive care)[88]. The limited feasibility of measuring CSF oxytocin has created an interest in peripheral measures of oxytocin in e.g., plasma, urine, and saliva. However, whether oxytocin measured in these matrices can be used as a proxy for central levels has been

debated[55,87,89]. Problems with peripherally measured oxytocin include varying correlations with central oxytocin levels, interference of proteins and proteolytic enzymes that can degrade oxytocin[88].
A meta-analysis found a positive association between central concentrations (CSF) and peripheral concentrations (plasma) after experimental stress induction, such as a child being separated from a mother, but no association under baseline conditions (without stimulation)[87].

Blood samples have been used in many studies as a less invasive method than lumbar puncture[55]. Some studies have suggested that release of oxytocin centrally (in CSF), and peripherally to the blood, is coordinated in some conditions[87,88]. However, one study described a small correlation between plasma and CSF oxytocin levels[90]. Differences between oxytocin concentrations in plasma and CSF, can perhaps be explained by a sometimes pulsatile release of oxytocin to the blood stream, a different half-life for oxytocin in blood and CSF, and high protein content of oxytocin in the blood[88,91]. Oxytocin is excreted via the kidneys and can be measured in the urine[88]. Urinary oxytocin have been used in some studies, but the correlation between the oxytocin in urine and plasma appears to be small[88]. Salivary oxytocin has been widely used in studies, particularly in children, due to the non-invasive and feasible nature[88]. The exact mechanism by which oxytocin enters saliva is not fully understood, but oxytocin levels in saliva have been found to increase in response to stimuli that are known to increase central oxytocin levels, e.g. breastfeeding and exercise[55,88]. The correlation between oxytocin levels in saliva and CSF has been found to be stronger than the correlation between oxytocin levels in plasma and CSF[90]. The protein content in saliva is lower than in plasma which lowers the binding of oxytocin; however, the measurement of oxytocin in saliva can be complicated by secretion rate and the content of proteolytic enzymes[55,88].

One topic that has been highly discussed is the use of extraction during sample preparation for oxytocin analysis, which describes procedures to remove potential molecules that can interfere with the analysis[88]. Studies have shown large differences in oxytocin levels between studies using extraction and those not using extraction, with high variation in between extraction/no extraction in studies using plasma, smaller variations in studies using saliva, and smallest differences in studies in CSF[55,88]. The different methods, varying results, and poor associations have led to calls for a universal measurement method, so studies can be more easily compared[54,88]. However, one study described the measurement issue as a "blind people and the elephant" problem, where people investigate different aspects and thus uncover different findings[55]. The authors state, that these differing methods and results should be used to create a complete picture, rather than criticizing or dismissing contradictory findings[55]. They also note that their thoughts on using different methods is not “a free pass on methodological rigor” and also calls for new rigorously developed methods that are accurate and reproducible[55]. Regarding reproducibility, not only extraction but various pre-analytical steps can influence the results, and thorough description of the methods is important to enhance reproducibility[55,88].

4.2.3.2. The importance of covariates

Oxytocin levels often show large intra-individual variations, inter-individual variations, and variation between studies, which has led to a focus on covariates[55,89,91]. A covariate is a variable that, while not the primary focus of the study, is included in statistical analysis, because it may influence the dependent variable[92]. By adjusting for covariates, it is possible to account for variability in the dependent variable that is not explained by the independent variable of interest, thereby enhancing the accuracy of the analysis[89,92]. Due to oxytocin's various central and peripheral functions, several covariates have been suggested[89]. These include both variables related to the individual such as age, sex, menstrual phase, and menarche, but also variables related to the context of the sampling such as time of day, and food and liquid intake before the sampling[88,89]. In this PhD, we focused especially on the variables age, pubertal stage, and sex.

Studies have suggested that age is important for oxytocin concentrations in humans since physiological and social demands change with age, e.g., parent-child interaction, pair-bonding, pregnancy and menarche[93,94]. Some studies briefly state their findings on oxytocin levels in relation to age; however, the number of studies is limited and much knowledge on oxytocin and age is based on animal studies[95]. A meta-analysis in adults, found that studies with a higher age in the population reported higher oxytocin levels[89]. In general, few studies report associations between oxytocin and age in children and adolescents[57,96] or their findings are challenging to locate (e.g., not a part of title, abstract or key words), which creates a knowledge gap in the field.

Oxytocin was initially considered a "female hormone" with functions in reproduction and lactation, but with time oxytocin has been linked to more general functions relevant for both females and males[97,98]. Sex-specific factors affecting oxytocin in females such as phase of menstrual cycle and hormonal contraception have led some researchers to include only males[97], whereas studies in parenting has included mostly females[57]. However, this is problematic since studies including both sexes have suggested sex specific differences in e.g. endogenous oxytocin and behaviors[79,80] and in different effects of intra-nasal oxytocin between females and males[89], highlighting that findings can often not always be generalized across genders. Some studies have reported sex differences in oxytocin levels with higher levels in females[98,99], while others have found higher levels in males[100]. In children and adolescents, sex and age differences could be especially important as there are many changes in social and emotional demands in addition to sex specific hormonal changes across puberty[97].

4.2.3.3. Statistics in oxytocin research

The problem with replication is not specific to the oxytocin field but has been a problem in multiple areas, leading to recommendations of increasing replication of findings, to increase reproducibility and to increase robustness of findings[101,102]. Replication refers to the test of reliability of results with different data[101], and has been recommended to ensure that findings are not prematurely used to change clinical guidelines or politicians' positions[103]. However, replication is not straight forward, as creating the exact same study

conditions is difficult[101]. Reproducibility refers to testing the reliability of results using the same data and the same analyses[101]. To increase reproducibility, the use pre-registration and shared data and code have been recommended[101]. Robustness describes how fragile findings are to variations in methods, e.g., whether results differ depending on which observations and covariates are included[101]. Important in relation to findings in previous studies, is to consider the often high emphasis on "significant findings", *p*-values and the significance level below 0.05[104]. The tendency that significant findings are more likely to be published, make some researchers use methods such a *p*-hacking in the search of significant findings[102], and the value of correct interpreted null-findings is often forgotten[104]. Consideration of power is important, as studies are often underpowered with the risk of false positive discoveries, inflated effect sizes and incorrect interpretation of null findings[53,102,104]. Meta-analyses have been suggested as an important tool to increase the emphasis on effect sizes and cumulative evidence where it is also possible to measure the effect heterogeneity between studies[105].

In oxytocin research, power calculation and interpretation of null findings have been highlighted as problematic[53]. To address this, thorough power calculations and statistical methods to qualify the interpretation of null findings, e.g. Bayesian hypothesis testing or frequentist equivalence testing, have been recommended[53]. In addition, the use of pre-registration of hypotheses, statical methods, and power calculations have been recommended to ensure transparency of research[53]. To increase the reproducibility and improve understanding, it has been suggested to include female and male participants, clinical and non-clinical samples and to focus on theory development[53]. In addition, replication has been recommended-preferably in separate labs[53]. All of these recommendations have been mentioned as important in the oxytocin field that has perhaps moved too quickly to ensure a qualified development of the research[53]. However, the importance of exploratory studies with more "researcher degrees of freedom" has also been highlighted as a way to discover findings that can help generate hypothesis[103].

5. Aims of the thesis

The aim of this thesis was to explore the relevance of oxytocin in pediatric OCD. Improved understanding of oxytocin, as a possible neurobiological mechanism involved in pediatric OCD, could enhance our understanding of this disorders and especially understanding of the family involvement in OCD. With an awareness of the complexity and history of oxytocin research in mind, the aim of the three studies in the thesis was to explore and increase our understanding of oxytocin in pediatric OCD. In this way we hoped to reveal possible important areas for hypothesis generation in future research with the long-term goal of improving treatments.

5.1.Study I – objectives

Study I: The association between salivary oxytocin, age, and puberty in children with and without OCD

The objective of study I, was to examine differences in salivary oxytocin levels between children with OCD and children without a psychiatric disorder. In addition, we attempted to reduce the large variation in oxytocin in our analysis by exploring the role of the covariates age, pubertal stage, and sex.

5.2.Study II – objectives

Study II: The role of oxytocin in family processes in the presence and absence of pediatric OCD

In Study II, we examined whether the findings on child oxytocin and family accommodation in pediatric anxiety could be extended to our population of children with OCD. The primary objective was to examine whether child salivary oxytocin is related to mother- and father-reported family accommodation. We tried to increase our understanding by also examining a non-clinical sample. The secondary objective was to explore the associations between child oxytocin and family conflict and cohesion, parental stress, and parental oxytocin, in children with OCD and children without a psychiatric disorder.

5.3. Study III – objectives

Study III: Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

In Study III, we extended our exploration of oxytocin and family factors from study II by focusing of parent-child interactions. The objective of study III was to explore association between child and parent oxytocin and parent-child interactions in parent-child dyads of children with pediatric OCD and dyads of children without a psychiatric disorder.

6. Methods

6.1.The TECTO trial

This Ph.D. project is a sub-study in the larger TECTO trial (Treatment Effects of Family Based Cognitive Therapy in Children and Adolescents with Obsessive-Compulsive Disorder) which consist of a randomized clinical trial (RCT) and a combined case-control study[106]. The TECTO RCT compared 16 weeks of family-based Cognitive Behavioral Therapy (CBT) to 16 weeks of family-based Psychoeducation and Relaxation Training (PRT) in 130 children and adolescents with OCD[106]. In the combined case-control study, 90 children and adolescents without a current or previous diagnosis of psychiatric disorders (in the rest of the thesis referred to as "controls")[106] were included. The controls were age- and sex-matched to the children with OCD who participated in the RCT.

The children with OCD and controls were examined on various time points on clinical, neural, cognitive, emotional, and neuroendocrine parameters with the aim to investigate markers of pediatric OCD and to investigate how these parameters may predict, moderate or mediate treatment response[106]. The flow of the TECTO RCT and case control study can be seen in Figure 3. Data collection started July 2018 and finished September 2022. This project concentrates on the neuroendocrine component of the TECTO trial, specifically examining the neuropeptide oxytocin. In the three studies, baseline data from the children with OCD and the controls and the children's parents were used. Many of procedures on the TECTO trial, described below, have also been described in the TECTO protocol paper[106].

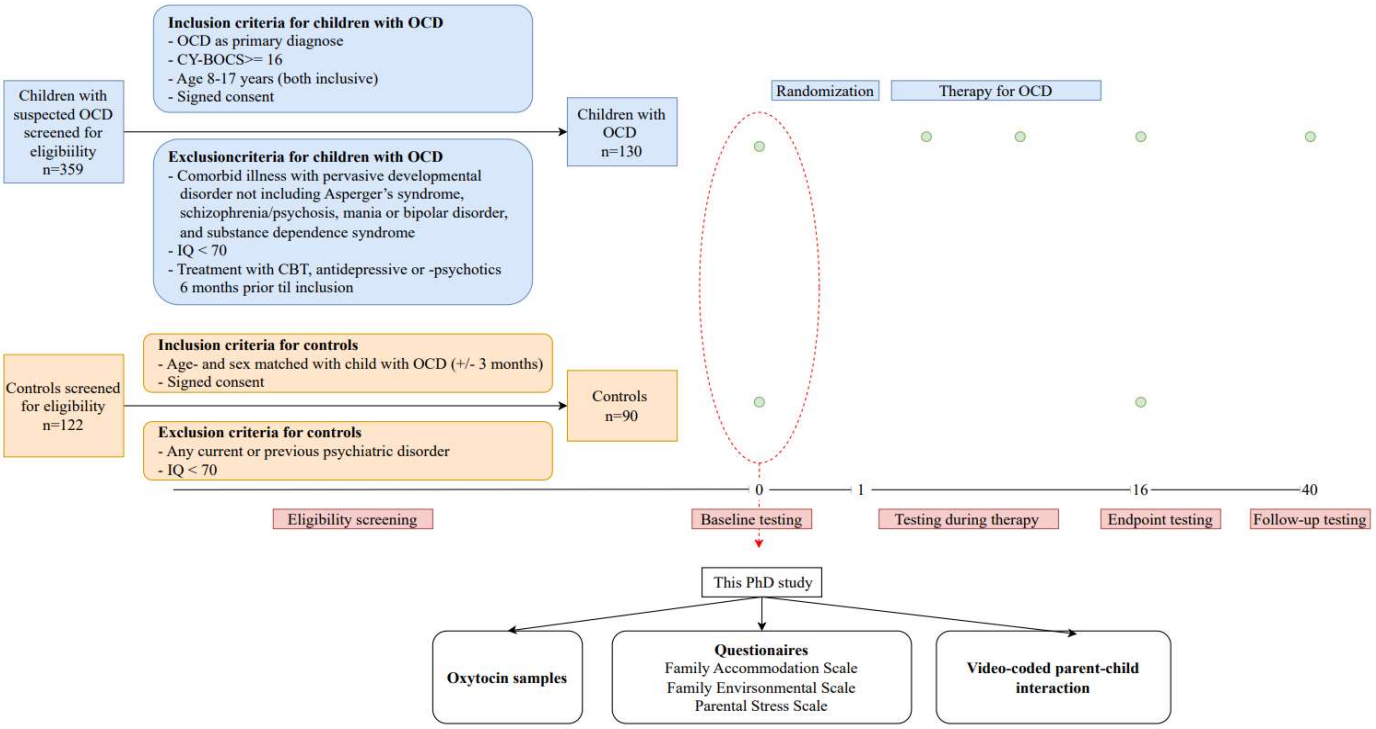
6.2. Participants – recruitment and assessment for eligibility

In the TECTO trial we included patients from the Child and Adolescent Mental Health Center (CAMHC), Copenhagen University Hospital – Mental Health Services CPH, Denmark[106]. Children and adolescents living in the capital region, between 8-17 years of age and referred with suspicion of OCD were clinically assessed by an OCD team in CAMHC. If the children met the inclusion and exclusion criteria after their clinical assessment, they were offered participation in the TECTO trial.

Controls were recruited from the population of children in the Capital Region through a random sample of children and adolescents between 8 and 17 years of age, drawn from the Danish Central Person Registry[107]. The controls were sex- and aged matched to the patients in the trial. Information was acquired from the registries on ten random children or adolescents for each included patient. Parents of children or adolescents were contacted and invited to eligibility screening. The children were offered participation if they met the inclusion and none of the exclusion criteria for controls.

The inclusion and exclusion criteria for children with OCD and controls can be seen in Figure 3. We included parents who had a child participating in the study and who gave informed consent to participation[106].

Figure 3. Overview of the TECTO trial study design and the data used in this PhD.



Notes: OCD, Obsessive-compulsive disorder. CBT, cognitive behavioral therapy, CY-BOCS, Children's Yale Brown Obsessive Compulsive Scale

6.2.1. Assessment of psychiatric diagnoses

All children were assessed for the presence or absence of psychiatric diagnoses as defined by the ICD-10[108] with the Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL)[109]. The K-SADS-PL is a semi-structured interview screening for the most common psychiatric disorders in youth. Patients were first assessed with a screening interview and if reaching threshold ratings, then also with additional relevant supplements. Children and parents were interviewed, and their answers scored separately with the interviewer synthesizing the answers in a final score. The K-SADS-PL is based on the DSM-IV criateria[109]; however, we used the items from the K-SADS-PL to diagnose according to the ICD-10 criteria[108]. As part of the TECTO project, we worked as an OCD-team consisting of medical doctors, psychologists or master-level psychology students who assessed the children and adolescents. All assessors in the OCD-team were trained in using the K-SADS-PL and received continuous supervision in the method[106]. The diagnostic status of the patients was confirmed by a psychiatrist or psychologist specialized in child and adolescent psychiatry. In any case of doubt of previous or current diagnoses in the controls, the results from the K-SADS-PL were discussed with a psychiatrist specialized in child and adolescent psychiatry and the principal investigator of the trial[106].

6.2.2. Assessment of OCD severity

OCD severity was assessed with CY-BOCS[110]. The CY-BOCS is a semi-structured interview assessing the symptom severity over the previous week. The CY-BOCS consist of a checklist of common obsessions and compulsions. The severity of the obsessions and compulsions are rated separately on five items rated on a 5-point scale; time, interference, distress, resistance and control[110]. The score for the 5 ratings on obsessions and the 5 ratings on compulsions a summed creating a CY-BOCS total score with a range from 0 to 40 with higher ratings indicating more severe symptomatology. The CY-BOCS were completed with the children and/or the parents by the OCD-team[106]. All assessors were trained in the methods in using the CY-BOCS.

6.2.3. Assessment of cognitive level

Intelligence was tested with the full-scale Wechsler Intelligence Scales for children (WISC-V) for children aged 16.9 years or below, or the Wechsler Adult Intelligence Scales (WAIS-IV) for adolescents above age 16.9)[111,112]. These were completed by psychologists or psychologist students trained in the assessments.

6.3. Ethical considerations

The TECTO trial complies to the Helsinki Declaration[113] and was approved by the ethics committee (Scientific Ethics Committees for the Capital Region of Denmark) (H-18010607) and the Knowledge Centre on Data Protection Compliance in The Capital Region of Denmark and was registered with clinical trials.gov (NCT03595098)[106]. Children and parents/legal guardians were given verbal information and children above 15 years and parents/legal guardians were also given written information about the child's participation in the trial. Parents or legal guardians signed informed consent regarding the child's participation. Parents or legal guardians received verbal and written information and gave consent on their own participation.

6.4. Methods used in the three PhD studies

Some methods are used across all three studies whereas others are used only in specific studies. Table 1 shows the methods used in the three PhD studies.

Table 1. Overview of methods in the three PhD studies.

			Paper		
	Procedure/measure	**Self-, clinician- or parent-reported**	**I**	**II**	**III**
Oxytocin levels					
Salivary oxytocin	Saliva sampling, enzyme-linked immunosorbent assay (ELISA)	NA	x	x	x
Clinical OCD diagnose					
OCD status	Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL)	Clinician-rated	x	x	x
Possible covariates for child oxytocin					
Pubertal stage	Self-reported from drawn pictures inspired by Tanner staging	Child-reported	x	x	x
Age	From CPR number	NA	x		
Sex	From CPR number	NA	x		
Menarche (girls)	-	Child-reported	x		
Menstrual phase (girls and mothers)	Calculated from self-reported first day of last menses	Child- and mother reported	x		
Menopause (mothers)	-	Mother-reported	x		
Time of saliva sample	-	Clinician-rated	x		
Functional level	Children's Global Assessment Scale (CGAS)	Clinician-rated	x		
OCD severity	The Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)	Clinician-rated	x		
Social impairment	Social Responsiveness Scale questionnaire (SRS-2)	Parent-reported	x		
Family aspects and parent-child interaction					
Degree of family accommodation	Family Accommodation Scale for OCD-Self Rated Version (FAS-SR)	Parent-reported		x	
Stress associated with parenting	The Parental Stress Scale (PSS) (parental stress subscale (PS))	Parent-reported		x	
Family conflict/cohesion	The Family Environment Scale (FES) (conflict and cohesion)	Child-reported		x	
Parent- and child behaviors	The Tangram Emotion Coding Manual (TEC-M)	Observation/coded			x

Notes: NA, not applicable; CPR number: Civil registration number civil registration number

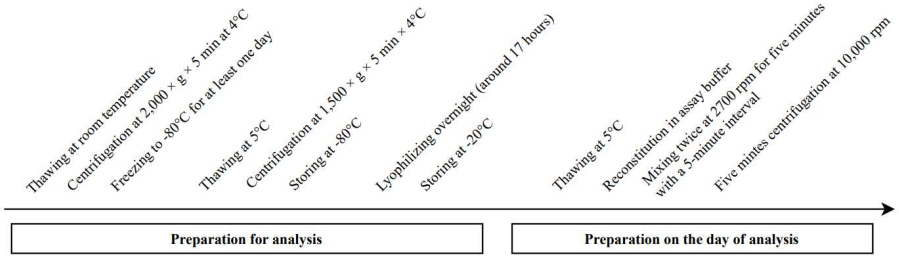
6.4.1. Methods used in all three studies

6.4.1.1. Oxytocin levels

The method for collecting and analyzing the saliva samples to measure oxytocin was developed and carried out in collaboration with the Department of Clinical Biochemistry, Rigshopitalet/Glostrup University Hospital. We collected saliva with Salivettes® (Sarstedt, Rommelsdorf, Germany). The participants were instructed to avoid eating two hours and drinking liquids 30 minutes before sampling. The samples were

collected in relation to research visits between 8AM and 4PM. The samples were stored in a -20 °C freezer until the preparation for analysis. The preparation for analysis was done with the steps shown in Figure 4.

Figure 4. Procedure for preparation of oxytocin samples for analysis.



Notes: rpm, revolutions per minute

We did not use procedures for extraction in the preparation of the samples. We analyzed using enzyme-linked immunosorbent assay (ELISA) with Enzo® oxytocin kits (NY, USA) and calculated the oxytocin concentrations using WorkOut 2.5 (Dazdaq Solutions Ltd, Brighton, UK). We found an intra-assay coefficient of variation on 13% and an inter-assay coefficient of variation on 20%.

6.4.1.2. Pubertal stage

Children and adolescents reported their pubertal status after instruction from a trained investigator. We used drawn pictures inspired by Tanner staging[114,115]. The children and adolescents were asked to choose the pubertal stage they considered most fitting from pictorial presentations of secondary sex characteristics on a 5-point scale ranging from 1 (pre-pubertal development) to 5 (full adult development)[114,115]. We thus used the most fitting "overall" pubertal stage and we did not use classification of each of the specific sex characteristics (which is used in the original tanner staging[114,115]), but asked the children and adolescents to report the best fitting pubertal stage based on consideration of all the pictorial presentations of secondary sex characteristics.

6.4.2. Study I - methods

Study I: The association between salivary oxytocin, age, and puberty in children with and without OCD

6.4.2.1. Age and sex

Age at sample were calculated from the children's civil registration number (CPR number)[107] and the date of saliva sampling. Information on biological sex were obtained from the last cipher of the CPR number (equal number for girls, unequal number for boys)[107].

6.4.2.2. Phase of menstrual cycle, menarche, pregnancy, breast feeding and menopause

The phase in the menstrual cycle of the female participants (both female children and mothers) was estimated based on the self-reported first day of their last menstrual period (onset of blood flow). The normal menstrual cycle is considered 28 days, with the onset of menses marking "the menstrual phase" (describing the beginning of the follicular phase) and ovulation approximately half-way through the menstrual cycle[116].

If the day of saliva sampling was on the first day of last onset of menstrual period, or two days before or after, the participant was considered in "menstrual phase". The participant was estimated in "ovulatory phase" if day of saliva sampling was performed between the 10th and the 14th day after the first day of last menstruation. This method of estimating menstrual phase holds considerable risk of misclassification which will be discussed in section 8.3.5. Information on menarche, pregnancy and breast feeding was available from questionnaires answered by both children and mothers, and from mothers also information on menopause.

6.4.2.3. Social Responsiveness Scale questionnaire

The children's social responsiveness was assessed for the children with OCD with the Social Responsiveness Scale questionnaire (SRS-2)[117]. The scale consists of 65 questions that are answered on a 4-point scale by the parents. We used a total score that can be calculated from the questionnaire with a range between 0 and 195 and higher values indicating problems with social responsiveness.

6.4.2.4. Children's Global Assessment Scale

Child functional level was measured with the Children's Global Assessment Scale (CGAS)[118]. The scale provides a global score of level of psychosocial functioning[118]. Trained investigators scored the CGAS based on all available relevant information following the initial clinical assessment of the. The CGAS range from 0 to 100. A higher score indicates better function[118].

6.4.2.5. Time of day

Time of the saliva sample was registered by investigators. Time of sample was categorized in four time-groups 8am-09:59am, 10am-11:59am, 12am-01:59pm, and 2pm-03:59pm.

6.4.3. Study II - methods

Study II: The role of oxytocin in family processes in the presence and absence of pediatric OCD

6.4.3.1. Family Accommodation Scale for OCD-Self Rated Version (FAS-SR)

The FAS-SR measures the types and frequencies of accommodation behaviors performed by family members in the context of OCD, and thereby provides a measure of the level of family accommodation[35]. The scale consists of an OCD symptom checklist followed by a list of 19 different accommodating behaviors where family members are asked to answer how often they have engaged in 19 different accommodation behaviors in the preceding week on a 5-point scale ("never", "1 day", "2-3 days", "4-5 days", "every day"). The 19 answers can be summed to a total score with a range from 0-76 with higher levels indicating more family accommodation[35]. In the development of the FAS-SR by Pinto et al., it showed acceptable internal reliability with a Cronbach-$\alpha = 0.925$[35] and the internal reliability in our study was similar (Cronbach-$\alpha = 0.9$)[35]. We chose the FAS-SR as it has been developed to OCD specifically and developed for self-reporting. Various measures of family accommodation exist. Some scales, developed for interview, have been used either in their original form or modified to self-reporting[35]. Other scales have been developed for other disorders than OCD e.g., the FASA for family accommodation in pediatric anxiety[34]. The scales share many similarities but

also important differences such as the number of items and formulations of questions, which makes comparing scores across studies challenging.

6.4.3.2. Parental Stress Scale (PSS) – the Parental Stress subscale (PS)

The Parental Stress Scale (PSS) is a self-reported measure of stress associated with parenting[36]. The Parent Stress scale consists of responses on nine items on a 5-point scale creating a total score with a range from 9 to 45[42]. Research in the psychometric properties of the PSS has suggested that the scale consist of two subscales – parental stress (PS) and lack of satisfaction (LS)[38,42]. We chose to use the parental stress subscale, as parental distress has previously been showed related to child oxytocin in children with anxiety disorders[57] The Parental Stress Subscale has demonstrated acceptable reliability for both parents of children from a community sample and parents of children with behavioral problems[42]. We used the parental stress subscale and found a good internal reliability with a Cronbach-$\alpha = 0.82$[2].

6.4.3.3. The Family Environment Scale (FES) – family conflict and cohesion

The Family Environment Scale (FES) measures aspects of the family environment[44]. The FES consists of ten subscales describing the family environment[44]. Each of the ten subscales consists of nine true/false responses which can be summarized in a score with a range from 0 to 9. We used the Family conflict and Family cohesion subscales in study II, as cohesion and conflict have been found important in child OCD. These two subscales have showed acceptable internal reliability (family conflict Cronbach-α= 0.75 and family cohesion – Cronbach-$\alpha = 0.78$)[44]. The family conflict scale and family cohesion scale showed acceptable internal reliabilities in our study (family conflict Cronbach- $\alpha = 0.70$ and family cohesion Cronbach- $\alpha = 0.63$[2]).

6.4.4. Study III - methods

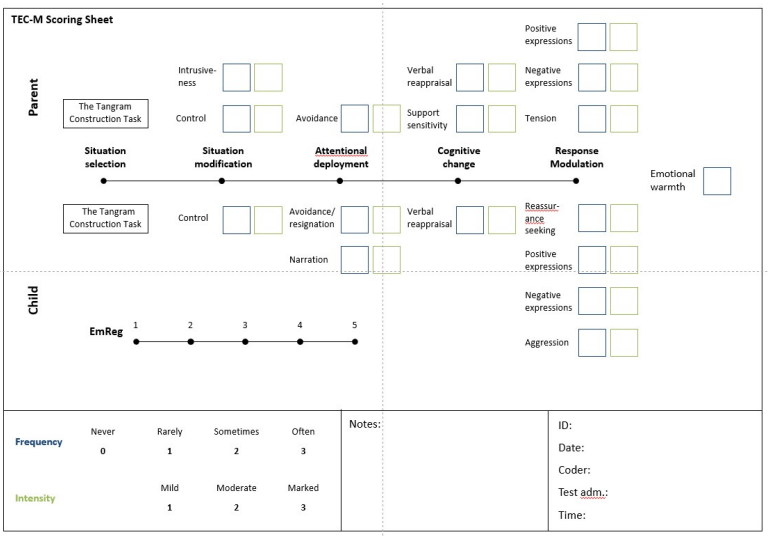
Study III: Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

6.4.4.1. Coded parent-child behaviors of child-parent interactions

We used information on parent-child behavior from parent-child interactions in a Tangram Construction Task coded with The Tangram Emotion Coding Manual (TEC-M)[119,120]. In The Tangram Construction Task, a child and their parent are alone in a room for five minutes, during which the child is asked to solve as many of six puzzles as possible[119]. The parent receives a solution booklet and is instructed to only help the child with the solutions if is absolutely necessary[119]. The completion of the task and the interaction is recorded on video for later coding of parent- and child behaviors. The TEC-M coding scheme is developed to measure profiles of emotion regulation in the context of parent-child interactions[120]. The coding scheme consist of eight parental behaviors (intrusiveness, control, avoidance, verbal reappraisal, support/sensitivity, positive expressions, negative expressions, and tension), eight items child behaviors (control, avoidance/resignation, narration, verbal reappraisal, reassurance-seeking behavior, aggression, positive expressions, and negative expressions), one item for the dyadic relationship (emotional warmth), and a global score on the child's

emotion regulation abilities (EmReg)[119]. All items are scored on frequency (never, rarely, sometimes, often) and intensity (mild, moderate, marked)[119]. The TEC-M coding scheme is illustrated in Figure 5.

Figure 5. The TEC-M scoring scheme (Hagstrøm and Spang(2020)[119]



Note: Figure used with permission from author of the TEC-M, Julie Hagstrøm.

The TEC-M has been validated in both a clinical and non-clinical group if children[120]. Videos were coded by trained medical doctors and psychologists (graduates and non-graduates), and consensus coding was performed to ensure consistency. We used all behavioral codes on both frequency and intensity, but not the global EmReg score.

6.5.Statistical methods

6.5.1. Study I – statistical methods

Study I: The association between salivary oxytocin, age, and puberty in children with and without OCD[1]

6.5.1.1. Overall statistical method

In Study I, we pre-registered the analysis plan (https://osf.io/hmc43). We used frequentist statistics to test the association between child OCD status and oxytocin levels. Given the limited number of studies in the field, we were unsure of the power calculation, and we chose to also use Bayesian statistics, which have been recommended as a supplement to the reporting of p-values[104]. We used post hoc tests to explore non-linearity in data - both using quadratic value of variables and a random forest approach. Analyses were performed using R (version 4.2.0 and 4.3.0 for the random forest analyses).

We chose not to use correction for multiple comparisons as Study I was exploratory. However, we reported all comparisons we made and discussed the risk of false discoveries (see section 8.3.2). We excluded outliers in oxytocin concentrations (values ±3 standard deviations (SDs) from the mean) which have also been used in other studies of salivary oxytocin[80]. We checked assumptions using quantile–quantile plot (q-q plots) and applied a natural logarithmic transformation of the oxytocin values.

6.5.1.2. Consideration of sample size and power

During the study planning (and in our pre-registration), we assumed a sample size of n = 220 (children and adolescents with OCD: 130, healthy controls: 90), set α = 0.05 (2-sided) and β = 0.80, and calculated that we were able to reliably detect a Cohen's d of at least 0.39. Missing information reduced our sample size, but it had a minimal impact on the effect size we were able to detect (n = 201 (children and adolescents with OCD: 113, children and adolescents without OCD: 88), α = 0.05 (2-sided), and β = 0.80, Cohens d of 0.40).

6.5.1.3. NSHT and Bayesian statistics

Null hypothesis significance testing (NHST) is a commonly used approach where probabilities described with *p*-values are often reported[104]. *P*-values can provide information on the probability of observing a specific value, or a more extreme value assuming a given null hypothesis is true. This can be useful if it is interpreted as a measure that can reject the null hypothesis; however, it cannot provide evidence of the null hypothesis being true[104]. Important in this context is that *p*-values and especially null findings should always be considered after careful a priori power analysis to ensure that the methods used are sensitive enough to detect the investigated effect sizes[104]. Bayesian statistics has been recommended as a valuable supplement to NHST as a way to quantify the degree to which the data favor the alternative hypothesis (H_1) or the null hypothesis (H_0)[104]. Bayesian statistics can be used as a way to describe the probability of observing data by updating prior information about the data with observed knowledge of the data[104]. It is based on the Bayes rule also called Bayes theorem[121]. This Bayes rule can be presented as done by Lee and Wagenmaker as (Equation 1)[121]: (1)

$$p(\theta|D) = \frac{p(D|\theta)p(\theta)}{p(D)}$$

θ is a given parameter that is investigated. p(θ) describes the prior distribution which quantify our prior information about the parameter before the data is observed. D describes the data we observe. p(D|θ) describes the probability of observing our data given our prior beliefs of the parameter is true.

p(θ|D) describes the posterior distribution which is the probability of the parameter being true given our data. As a result, we get a measure of the likely hood of our data[121]. A commonly used approach to compare models in Bayesian statistics is using Bayes Factors, where the likelihood of two hypotheses given the same data are compared[104]. Lee and Wagenmaker have presented this as shown below (Equation 2)[104].: (2)

$$\frac{p(H_1|data)}{p(H_0|data)} = \frac{p(H_1)}{p(H_1)} \; x \; \frac{p(data|(H_1)}{p(data|(H_0)}$$

Posterior odds Prior odds Bayes factor BF_{10}

The Bayes factor can be used to describe the probability of observing our data given that the alternative hypothesis (H_1) is true compared to the probability of observing our data given the null hypothesis (H_0) is true[121]. This is calculated by using the prior odds (the prior belief in the H_1 compared to the prior belief in H_0) and the posterior odds (the probability of the H_1 given our data compared to the probability of H_0 given our data) [121].

Bayes factors can be classified in various ways[104]. We used a classification for the Bayes factors that was described by Lee and Wagenmaker[121]. This can be used to interpret the Bayes Factors, BF_{10}, which describes the evidence of H_1 compared to the H_0[121]. In this classifications scheme, values above 1 describes evidence for H_1 and values below 1 describes evidence for H_0. Increasing or decreasing values (thus further distance from 1) describes increased degree of evidence[121]. The level of support is divided in categories with 1-3 describing anecdotal evidence for H_1, 3-10 moderate evidence for H_1, 10-30 strong evidence for H_1, 30-100 very strong evidence for H_1, >100 extreme evidence for H_1[121]. The values below 1 describes evidence of H_0 and can similarly be divided into categories with decreasing values describing increased level of evidence with 1- 1/3 describing anecdotal evidence, 1/3-1/10 moderate evidence, 1/10-1/30 strong evidence, 1/30-1/100 very strong evidence, <1/100 extreme evidence[121].

Bayes factors provide an easy way for interpreting model comparisons; however, their use has been criticized for being highly sensitive to the choice of prior[104]. Some researchers have advised against the use of Bayes factors to compare models[122] and especially advise not to use Bayes Factor for model comparison, when hypotheses that are not truly discrete[122]. Following these recommendations, we used Bayes factor, when we tested a discrete parameter such as in the models including the binary variable OCD status (describing children with OCD/controls). For model comparison of variables that were not truly discrete, we used the expected log-pointwise predictive density estimated by leave-one-out cross validation (ELPD-LOO). In addition, we supplemented the calculations of Bayes factors with calculation of ELPD-LOO.
To address the sensitivity to priors, it has been recommended to pre-register the priors and use sensitivity analyses to explore how sensitive data is to the priors[104]. We pre-registered the use of Cauchy priors on fixed effects and Jeffrey's prior on the variance (JZS priors) for model comparison using Bayes Factors. The use of these priors have been suggested by these references[104,123]. We explored prior sensitivity by examining different scale factors. For ELPD-LOO estimates, we used default priors provided in the Rstudio brms package[124]. Bayesian statistics was performed with R studio (version 4.2.0) and the packages brms[124] (version 2.20.4) and rstan[125] (version 2.32.3).
We examined four models in Study I to examine oxytocin difference in children with and without OCD both without correction for covariates (Model 1), and with correction for covariates including age and sex in the analysis (Model 2) and including pubertal stage and sex (Model 3). In addition, we examined age and sex in a sample of both children and parents (Model 4). All models were examined with NSHT and Model 1-3 also using Bayesian statistics (see Table 2).

6.5.1.4. Exploring possible non-linearity in data with NSHT, Bayesian and Random Forest analysis

Our results from Model 2 and 4 suggested a possible non-linear relationship between oxytocin and age, and Model 3 suggested a non-linear relationship with pubertal stage. We explored non-linearity further in the data with several post hoc tests.

We examined non-linearity in the child and adolescent data by re-running Model 2 with a quadratic age variable (age^2). We then used Bayesian analysis to determine whether a model with quadratic age or a model with pubertal stage better explained the data.

We explored possible non-linearity in the data of all participants by repeating Model 4, but instead of age, we used a pubertal stage for all participants. Here, we assigned the parents an adult "pubertal stage 6". Model comparison was performed using Bayesian analysis, comparing the model with all participants, and modified pubertal stage, to the model with all participants and age. In addition, we investigated the relationship between oxytocin and age in adults alone. To get a better understanding of the possible non-linearity, we also used random forest analyses to investigate associations between the response (oxytocin) and relevant covariates/predictors. Random forest analyses can be used to measure predictions based on a collection of decision trees[126]. We explored variables concerning sex, age, and hormonal status (pubertal stage, and information on menarche status, menopause status and use of hormonal contraception). We explored random forest models on the data set only including children and on the data set, including children and parents. The random forest models were fitted and optimized using the R-package randomForest (version 4.7-1.1)[127]. We have provided a detailed description of the Random Forest method in the Appendix of Study I (See Section 13).

Table 2. Description of Model 1-4 in Study I

Model 1	**NHST method**	
	Students t-test	Dependent variable: Child oxytocin
		Independent variable: OCD status
	Bayesian statistic	
	Bayes factor	H_1: an effect of OCD status
		H_0: no effect of OCD status
Model 2	**NHST method**	
	Backwards stepwise regression	Dependent variable: Child oxytocin
		Independent variable: OCD status, age, sex, age-sex interaction
	Bayesian statistic	
	Bayes factor	H_1: the reduced model + OCD status
		H_0: the reduced model
	ELPD-LOO	H_1: the reduced model
		H_0: the intercept model
Model 3	**NHST method**	
	Backwards stepwise regression	Dependent variable: Child oxytocin
		Independent variable: OCD status, pubertal stage, sex, pubertal stage-sex interaction
	Bayesian statistic	
	Bayes factor	H_1: the reduced model + OCD status
		H0: the reduced model
	ELPD-LOO	H1: the reduced model
		H0: the intercept model
Model 4	**NHST method**	
	Backward stepwise regression of a mixed effects model	Dependent variable: Participant oxytocin
		Fixed independent variable: age, sex, age-sex interaction Random independent variable: family-variable

Note: "Family-variable" (a nominal variable denoting family membership); OCD, obsessive-compulsive disorder; H_0, the null hypothesis; H_1, the alternative hypothesis

6.5.2. Study II – statistical methods

Study II: The role of oxytocin in family processes in the presence and absence of pediatric OCD

6.5.2.1. Overall statistical method

In study II, we used regressions, including analyses of covariance (ANCOVAs) to address our main aim (the association between child oxytocin and family accommodation), and an ANCOVA, with backwards stepwise regression to address our secondary aim (association between child oxytocin and family conflict, cohesion, parental stress, and parent oxytocin). Analyses were completed using R studio (version 4.2.0).

6.5.2.2. Consideration of sample size, power, and the risk of false discoveries

Given the limited prior evidence supporting the aims of study II and the sample size, that was determined by the power calculation for the larger TECTO trial, we considered the study to be exploratory, despite addressing the aims through statistical hypothesis testing. We did not pre-register the analysis plan and did not adjust for multiple comparisons. We conducted a power calculation for the sample size required to address the primary aim, to estimate the effect size, we could detect. The power analysis was conducted using the r package "pwrss"[128]. We had information on 83 children on both saliva, mother-reported family accommodation, and child pubertal stage. Therefore, assuming a sample size of n=83, setting α = 0.05 (2-sided) and β = 0.80, and pubertal stage with five steps as covariate, we expected to be able to detect a large effect size (eta squared =0.135). For the models with information on fathers, we had data from 72 children available for analysis (with no missing data), therefore, assuming n=72, setting α = 0.05 (2-sided) and β = 0.80, and pubertal stage as a covariate, we anticipated to be able to detect a large effect size (eta squared =0.152). We did not perform power calculation for our analyses answering the secondary aim, but also used a significance level threshold of p<0.05.

6.5.2.3. Regressions

The primary aim was answered using two ANCOVAs where we analyzed how variation in child oxytocin is associated with mother-/father-reported family accommodation with child pubertal stage as a covariate (see

Table 3). The secondary aim was answered using two backwards stepwise regressions analyzing how variation child oxytocin is associated with family conflict and cohesion, mother-/father-reported parental stress, and mother/father oxytocin levels and if associations were different between children with OCD and controls (see

Table 3). The stepwise analyses were performed with stepwise exclusion of the variable with the highest p-value based on F-statistics, until only variables with a significance level below p = 0.05 remained. The analyses were performed using the using the 'drop1' function in R studio. Pubertal stage was retained, independent of significance, as a possible covariate.

6.5.2.4. Outliers, transformation of variables, missing data, and assumption check

We again excluded oxytocin outliers defined as oxytocin levels three SDs from the mean. In addition, we applied a natural logarithmic transformation (ln) of oxytocin levels. Scores on the questionnaires were only calculated for participants with responses on all items on the scales we used. We analyzed models for participants with no missing information. Assumptions were explored using correlation matrices of all variables (check of co-linearity of the independent variables), residual vs. fitted plots (check linearity of the models and autocorrelation among residuals), q-q plots (check normality of residuals), scale-location plots (check for homoscedasticity), residuals vs leverage plots (check for influential cases). In addition, we investigated assumptions for all models and the Variance Inflation Factor (VIF) of the final models (details of the assumption check were described in appendix of Study II, see Section 14).

Table 3. Description of models testing primary and secondary aims in study II (Model 1-4)

Primary aim (child oxytocin and family accommodation)		
Model 1	ANCOVA	Dependent variable: Child oxytocin
		Independent variable: Mother reported family accommodation Covariate: Pubertal stage
Model 2	ANCOVA	Dependent variable: Child oxytocin
		Independent variable: father reported family accommodation Covariate: Pubertal stage
Secondary aim (child oxytocin and family conflict, cohesion, parental stress and parent oxytocin)		
Model 3	Backwards stepwise regression	Dependent variable: Child oxytocin
		Independent variable: Family conflict, family cohesion, mother reported parental stress, mother oxytocin, OCD status Covariate: Pubertal stage Interactions: Family conflict-OCD status, family cohesion-OCD status, mother reported parental stress-OCD status, mother oxytocin-OCD status, mother reported parental stress-mother oxytocin
Model 4	Backwards stepwise regression	Dependent variable: Child oxytocin
		Independent variable: Family conflict, family cohesion, father reported parental stress, father oxytocin, OCD status Covariate: Pubertal stage Interactions: Family conflict-OCD status, family cohesion-OCD status, mother reported parental stress-OCD status, father oxytocin-OCD status, father reported parental stress- father oxytocin

Notes: OCD, obsessive-compulsive disorder

6.5.3. Study III – statistical methods

Study III: Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

6.5.3.1. Overall methods

In the last study, we used two data-driven methods to create latent constructs or interaction patterns - Principal component analysis (PCA) and Archetypal analysis. We used the interaction patterns generated with these methods as variables in regression models with child, mother, and father oxytocin as outcomes in separate models. To increase the understanding of how associations were different, depending on whether the

child had OCD (OCD status), we also ran the models testing the interaction effect of OCD status. All interaction patterns were created using datasets from only mother-child pairs or only father-child pairs to investigate maternal oxytocin in relation to only maternal behavior, and paternal oxytocin only in relation to paternal behavior. We included data sets with complete data on the variables of interest. All analyses were performed with R (version 4.2.0).

6.5.3.2. Consideration of sample size, power, and the risk of false discoveries

Study III was exploratory, and we performed the study without power calculation for each model. We chose to correct for multiple testing, as we did not know how many interactions patterns the PCA and archetype analyses would generate, and we were therefore unsure of how big the risk of false discoveries would be. We used the Benjamin Hochberg method to adjust for multiple testing with a false discovery rate of 20%.

6.5.3.3. Principal Components Analyses and archetype analysis.

We used two different machine learning methods to compute interactive parent-child patters. Both methods can be used to reduce dimensions and creating new variables that explain a new structure of the data. In PCA, dimensionality reduction is used to simplify large data, consisting of multiple variables, into a fewer number of variables, while still maintaining significant patterns and trends[129]. New variables, called principal components (PC), are developed as orthogonal linear functions of the original variables[129]. The PCs are developed to successively account for as much of the variance in the data as possible[129]. The PCs can be interpreted by looking at how the original variables load to each PC. The participant data receives a score for a given PC, indicating how much their data align with or deviate from the variance explained by given PC[129]. Whereas PCA creates variables that describe most of the variance in data, archetype analyses use the periphery of the data or extreme values to create new data structures[130]. The score a participant receives for a given archetype reflects how close their characteristics align with those of the given archetype[130]. Using these two methods, we were able to create variables that described interactions patterns in the data set. We conducted the PCA and the archetype analysis on both frequency and intensity measures of the 17 behavioral codes from the parent-child interaction task (see Figure 6). The PCA was based on the covariance matrix as all behaviors are measured on the same scale[129] and performed with the R package 'FactoMineR'[131]. Archetype analyses was performed with the R package 'archetypes'[130,132].

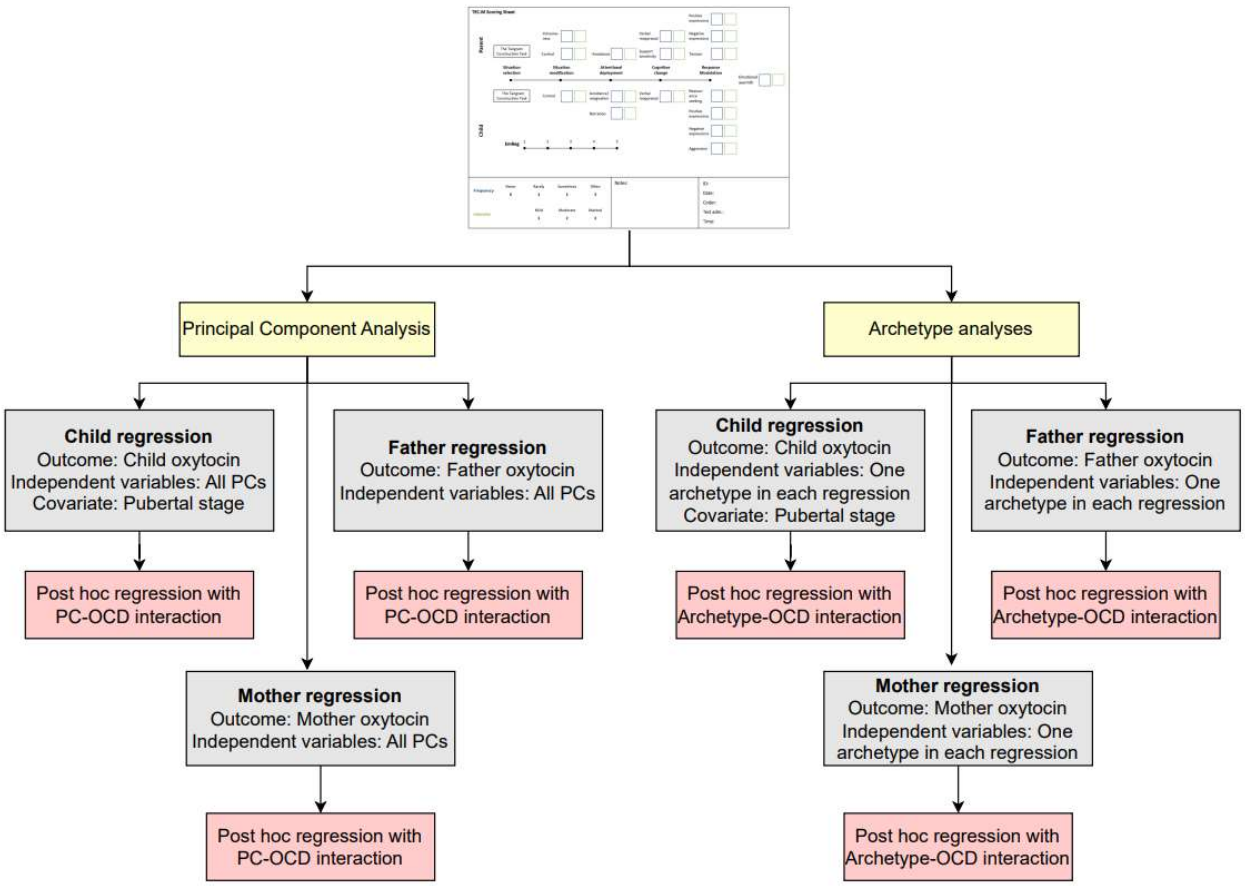
6.5.3.4. Regression analyses

The created variables were used in regression analyses with child, mother, and father oxytocin as the outcome in different analyses (see Figure 6). Again, child pubertal stage was included as covariate in the models with child oxytocin as outcome. The PCs were entered in the same model as these are orthogonal, but the archetypes were entered in separate models as these could be correlated. In *post hoc* analyses, we used analyses of interactions to explore if the relationships between oxytocin and parent-child interactive patterns were moderated by the presence of child OCD.

6.5.3.5. Outliers, transformation of variables, missing data, and assumption check

We excluded outliers of oxytocin, defined as mean values +/- 3 SDs, and applied a natural logarithmic transformation to the oxytocin values. We included observations with complete data on the variables of interest and checked for assumptions of each model using the same methods as described in study II (see Section 6.5.2.4).

Figure 6. Illustration of statistical models used for study III.



Notes: PC, principal component, OCD, obsessive-compulsive disorder

7. Results

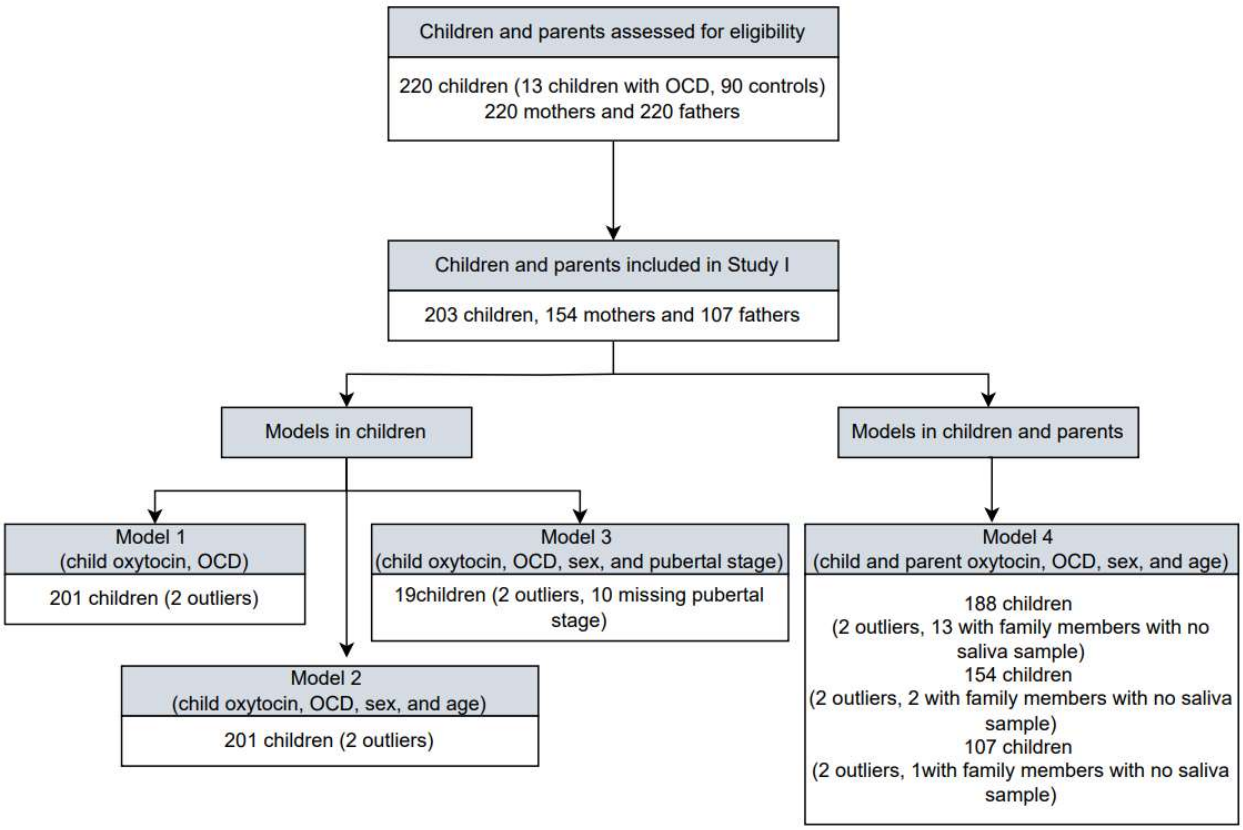
7.1.Study I – results

Study I: The association between salivary oxytocin, age, and puberty in children with and without OCD

7.1.1. Sample characteristics

In Study I, we included 203 children (see flowchart in Figure 7). Two outliers in the child oxytocin data were excluded from all analyses. The two outliers were one child with OCD and one control (oxytocin values 414.3 and 198.1 pg/ml respectively). We did not find an explanation for the outliers (outliers further described in the Appendix of Study I, see Section 13). The participant characteristics are described in Table 4 and Table 5. We found no statistically significant difference between the children with OCD and controls on age, sex, or pubertal stage. We did not have information on medication or somatic disorders for the control group. We included 150 mothers and 104 fathers (see flowchart in Figure 7). Some families provided information on only one family member (child, mother, or father) and some provided information on two or more family members.

Figure 7. Participant flowchart Study I, showing the four primary model but not post hoc models.



Note: OCD, obsessive-compulsive disorder

Table 4. Descriptive characteristics of the children included in Model 1 (child oxytocin and OCD) and Model 2 (child oxytocin, OCD and age). Children in Model 3 and Model 4 are a subsample of the children in this table. Table from Mora-Jensen et al. (2024)[1].

	Children and adolescents with OCD	Children and adolescents without OCD	Test (*p*-value)
Child salivary oxytocin (pg/ml)			
Mean (*SD*)	26.61 (14.28)	25.61 (13.95)	
Missing	0	0	
n	113	88	
Age (years)			0.26_A
Mean (*SD*)	13.37 (2.83)	12.92 (2.78)	
Median (min, max)	13.81 (8.08, 17.99)	12.37 (8.09, 17.83)	
Missing (*n*)	0	0	
Sex (*n* (%))			0.98_B
Female	58 (51.3%)	45 (51.1%)	
Male	55 (48.7%)	43 (48.9%)	
Missing	0	0	
Pubertal stage (*n* (%))			0.39_B
Pubertal stage 1	32 (28.3%)	23 (26.1%)	
Pubertal stage 2	13 (11.5%)	20 (22.7%)	
Pubertal stage 3	20 (17.7%)	14 (15.9%)	
Pubertal stage 4	25 (22.1%)	23 (26.1%)	
Pubertal stage 5	13 (11.5%)	8 (9.1%)	
Missing	10 (8.8%)	0 (0%)	
Nationality (*n* (%))			-
Danish	95 (84,1%)	63 (71.6%)	
Other	3 (2.7%)	2 (2.3%)	
Missing	15 (13.3%)	23 (26.1%)	
Comorbid psychiatric disorders (*n* (%))		*na*	-
Anxiety disorders ICD-10 F40-41, F93	12 (10.6%)		
Stress and adjustment ICD-10 F43	12 (10.6%)		
Asperger's Syndrome ICD-10 F84.5	14 (12.4%)		
Hyperkinetic disorders ICD-10 F90.0	13 (11.5%)		
Tic disorders ICD-10 F95	13 (11.5%)		
Other*	20 (17.7%)		
Medication (*n* (%))		*na*	-
Children receiving medication*	24 (21.2%)		
Somatic disorders (*n* (%))		*na*	-
Children with a somatic disorder*	32 (28.3%)		

*Note: n, number; SD, standard deviation; OCD, obsessive-compulsive disorder; ICD-10, International Classification of Diseases-10,"na" refers to the information not being available,"-" refers to statistical testing not being relevant or possible marked by. * Detailed information on patient medication and comorbid and somatic disorders is available in Appendix of study I, Section 13.* $_A$*t-test,* $_B$*Chi-squared test. The table is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).*

Table 5. Descriptive characteristics of parents included in Model 4. Table from Mora-Jensen et al. (2024)[1].

	Children and adolescents with OCD	Children and adolescents without OCD
n		
Mothers	92	58
Fathers	72	32
Mother age (years)		
Mean (*SD*)	45.72 (5.03)	45.97 (5.10)
Median (min, max)	45.61 (32.76, 56.24)	46.45 (34.97, 57.19)
Missing (*n*)	0	0
Father age (years)		
Mean (*SD*)	48.17 (5.67)	47.47 (6.78)
Median (min, max)	48.91 (33.1, 73.51)	47.08 (34.45, 62.73)
Missing (n)	0	0
Nationality Mother (*n* (%))		
Danish	77 (83.7%)	46 (79.3%)
Other	5 (5.4%)	3 (5.2%)
Missing	10 (10.9%)	9 (15.5%)
Nationality Father (*n* (%))		
Danish	57 (79.2%)	24 (75%)
Other	5 (6.9%)	1 (3.1%)
Missing (*n*)	10 (13.9%)	7 (21.9%)
Parental education mothers (*n* (%))		
Upper secondary education or below	8 (8.70%)	1 (1.7%)
Short-cycle tertiary education	14 (15.2%)	3 (3.2%)
Bachelor's degree or equivalent	38 (41.3%)	22 (37.9%)
Master's degree or above	22 (23.9%)	23 (39.7%)
Missing (*n*)	10 (10.9%)	9 (15.5%)
Parental education fathers (*n* (%))		
Upper secondary education or below	12 (16.7%)	3 (9.4%)
Short-cycle tertiary education	8 (11.1%)	3 (9.4%)
Bachelor's degree or equivalent	16 (22.2%)	6 (18.8%)
Master's degree or above	25 (34.7%)	13 (40.6%)
Missing (*n*)	11 (15.3%)	7 (21.9%)

Note: n, number; SD, standard deviation; OCD, obsessive-compulsive disorder. The table is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).

7.1.2. Difference in oxytocin levels between children with OCD and controls

We found no significant difference between salivary oxytocin levels in children with OCD and controls using a crude *t*-test, nor using regression analyses correcting for age and pubertal stage (see Table 6). The Bayes factors for the comparison of children with OCD and controls were below 1 in all analyses, indicating support for the null hypothesis, "no difference in oxytocin levels between children with OCD and controls", (BF from 0.37 to 0.52, see Table 6). However, the size of the Bayes factors was only in the range of anecdotal to moderate support. All Bayesian analyses showed high prior sensitivity, indicating that results influenced be the chosen methods (results of sensitivity analyses using different priors see Appendix of Study I, see Section 13).

Table 6. Result of student's t-test (Model 1) and stepwise regressions (Model 2 and 3) and Bayesian statistics. Table from Mora-Jensen et al. (2024)[1].

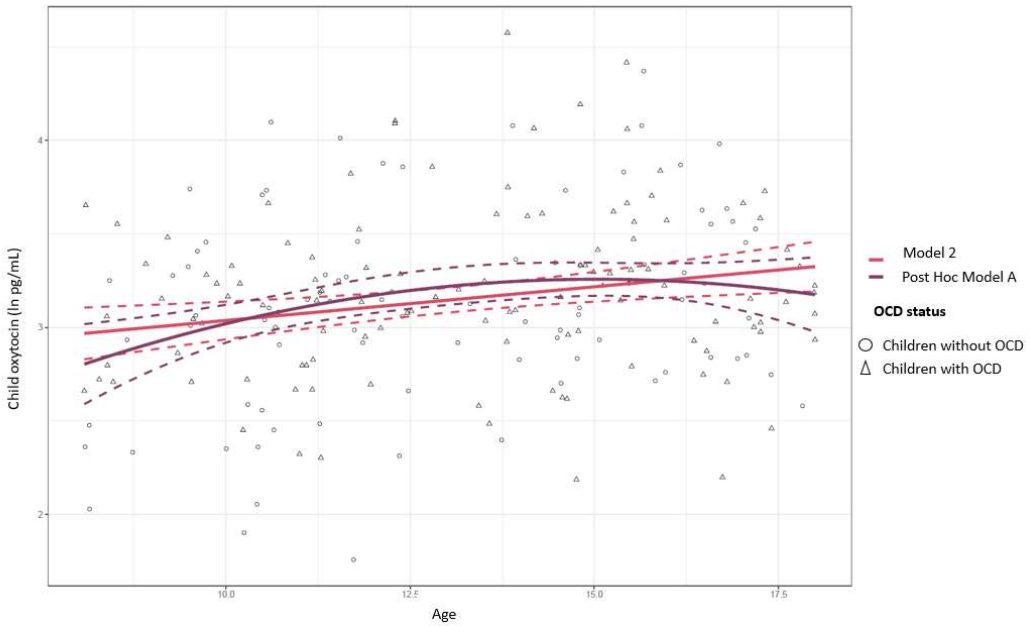
			t/F-statistics	*p*-value	Bayesian analyses
Model 1					
1. Test	H_1	OCD-status	*t*=1.111	0.2682	BF_{10}=0.37
	H_0	Intercept			
Model 2					
1. Test	H_1	Reduced model (age)	*F*=8.913	0.0031	H_1- H_0 ELPD-LOO (*SE*) = 3.5(2.7)
	H_0	Intercept			
2. Test	H_1	Reduced model (age) and OCD-status	*F*=0.840	0.3605	BF_{10} = 0.31
	H_0	Reduced model (age)			
Model 3					
1. Test	H_1	Reduced model (pubertal stage)	*F*=5.384	0.0004	H_1- H_0 ELPD-LOO (*SE*) = 6.6(4.1)
	H_0	Intercept			
2. Test	H_1	Reduced model (pubertal stage) and OCD-status	*F*=2.113	0.1477	BF_{10} = 0.52
	H_0	Reduced model (pubertal stage)			

Note: BF, Bayes factor; H_1, alternative hypothesis; H_0, null hypothesis; ELPD-LOO, expected log-pointwise predictive density estimated by leave-one-out cross validation; SE, standard error. The table is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).

7.1.3. The association between oxytocin and age, sex, and pubertal stage in children

The results showed that age and pubertal stage were significantly associated with child oxytocin, but none of the models showed an association with sex or an age-sex interaction (See Table 6). Child age showed a positive significant linear relationship with child oxytocin (Estimate: 0.036, for detailed results, see Appendix Study I Section 13, and pink line Figure 8).

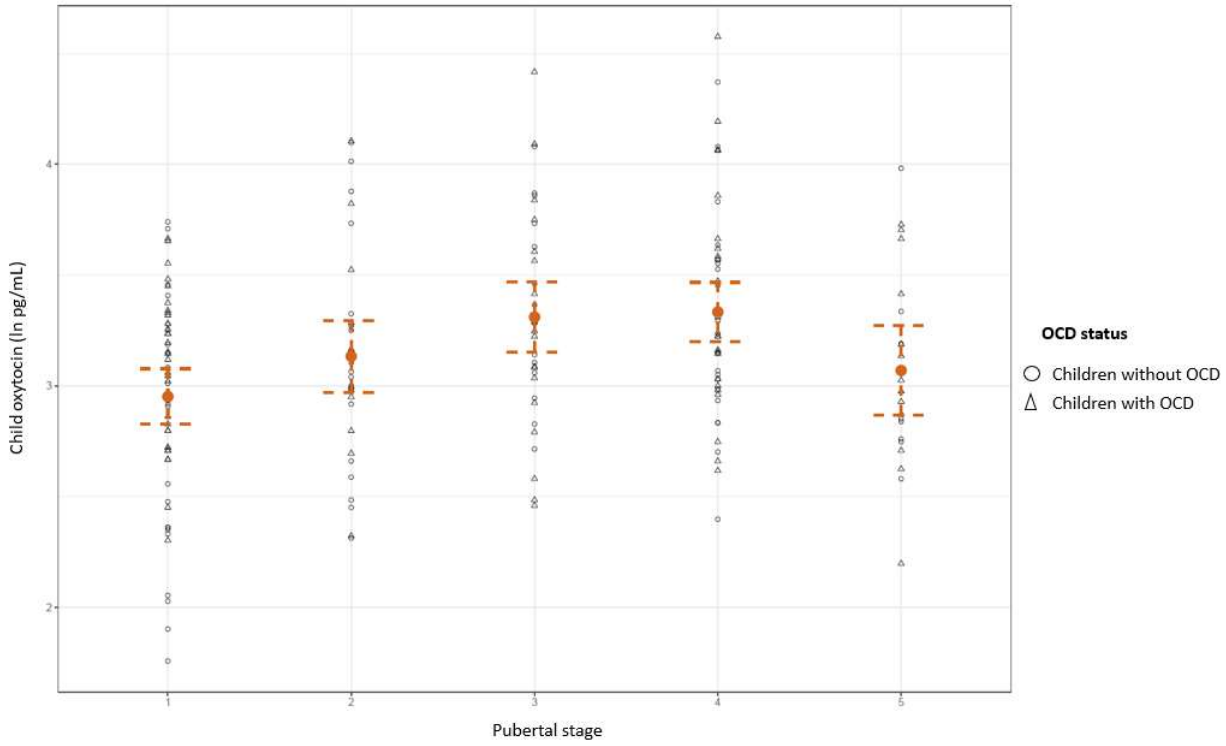
Figure 8. The relationship between oxytocin and age in children with and without OCD predicted by the reduced Model 2 (OCD and age (pink line) and the reduced Post Hoc Model A (OCD, age and age^2)(purple line). Figure from Mora-Jensen et al. (2024)[1].



Note: The dashed lines represent the 95% confidence interval of the mean. Abbreviations: Ln, natural logarithm. The figure is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).

Pubertal stage showed increasing values of child oxytocin levels from pubertal stage 1 to 4 and then a lower value at pubertal stage 5 (see Figure 9). Visual inspection of the data and the drop in pubertal stage 5 made us suspect a non-linear relationship between oxytocin and age and pubertal stage.

Figure 9. The relationship between oxytocin and pubertal stage in children with and without OCD predicted by the reduced Model 3 with pubertal stage. Figure from Mora-Jensen et al. (2024)[1].



Note: The dashed lines represent the 95% confidence interval of the mean. Abbreviations: Ln, natural logarithm. The figure is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).

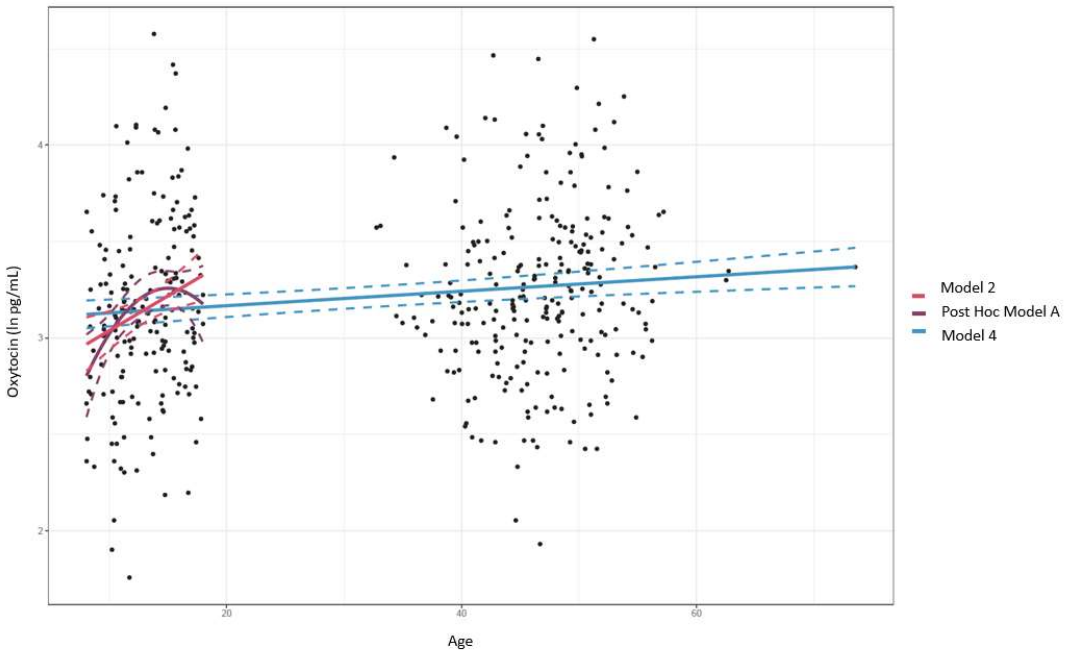
A possible non-linear relationship between child oxytocin and age was explored in a *post hoc* model using quadratic child age. We found a significant ELPD-LOO, suggesting that the model including pubertal stage was better than the model with quadratic age (difference in ELPD-LOO= 2.9 (*SE* 3.0) in favor of the model with pubertal stage, see Appendix Study I, Section 13). However, the large standard variation of the ELPD-LOO difference shows that the results are uncertain and should be interpreted carefully.

To further explore the non-linearity of age and pubertal stage in the children, we used random forest approach. Visual presentation of the predictions and the mean squared error (MSE) of the linear models (Model 2 and Model 3, and Post Hoc Model A) and the random forest model with age indicated that the random forest approach captured the same signals as the linear models (Appendix Study I, Section 13).

7.1.4. The association between oxytocin and age, sex, and pubertal stage in children and adults

We also explored the relationship between age and sex across ages in children and adults. We found a positive linear relationship between participant age and participant oxytocin (p=0.0003, see blue line Figure 10 and the Appendix Study I, Section 13, for detailed results).

Figure 10. The relationship between oxytocin and age predicted by the reduced Model 4 with age of all participants (blue line), the reduced Model 2 with age in children (pink line) and the reduced Post Hoc Model A with age and quadratic age in children (purple line).



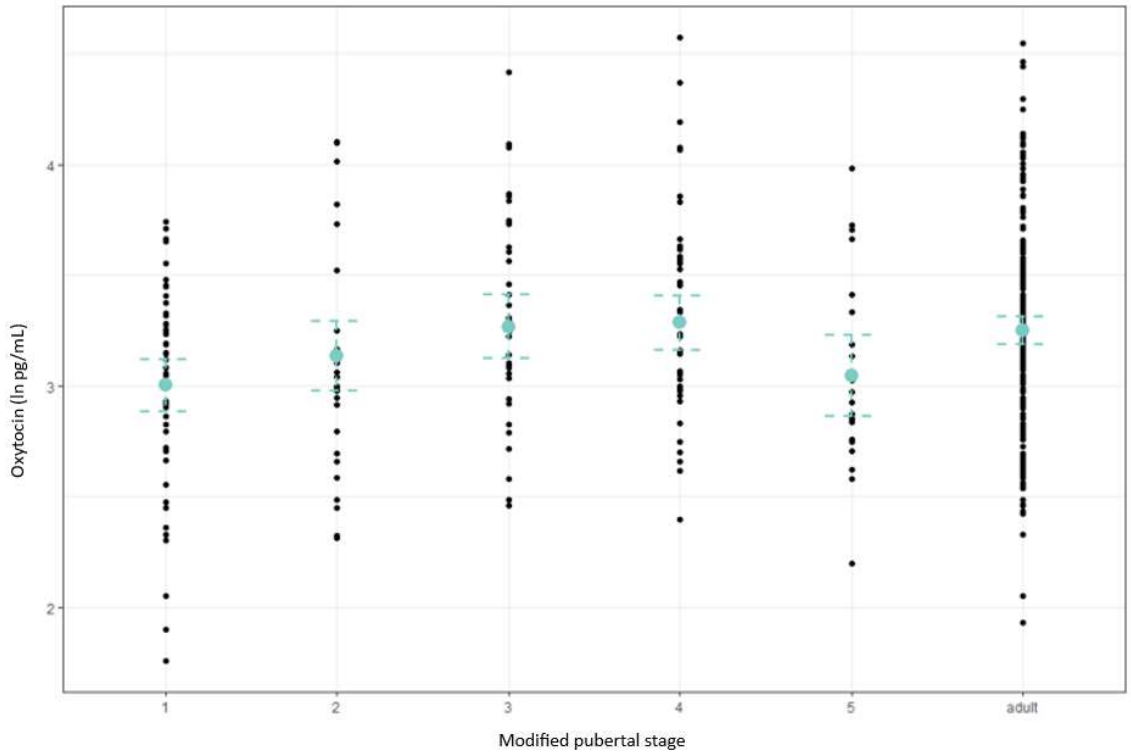
Note: The dashed lines represent the 95 % confidence interval. Abbreviations: Ln, natural logarithm. The figure is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).

When we inspected the illustration of the model with age for all participants, it was clear, that it did not capture the same signals at the models for child age (comparing the blue and pink line in Figure 10). Looking at the illustrations for the models in the children, it is also obvious that these could not be used to predict the adult values. The model of child age seemed to overestimate values in adults – extrapolation using the pink line would predict oxytocin values around 4 pg/ml ln oxytocin for an adult of 40 years of age, which from the data points in adult seems unlikely and too high. However, examining the model of quadratic child age, it would underestimate the values – extrapolation using the purple line would estimate values around 2.2 pg/ml ln oxytocin for an adult of 40 years of age, which seems too low.

Since pubertal stage in the children seemed to fit the child data the best (with a higher ELPD-LOO), we further explored the data using a "modified pubertal stage" in all participants - where children had their reported pubertal stage and parents were assigned a "pubertal stage 6" (indicating "adult"). We found a significant relationship (Figure E.1 in Appendix Study I, Section 13 between salivary oxytocin and the

modified pubertal stage. This model captured the non-linearity in the children, followed by an adult value a bit higher than child pubertal stage 5, but the adult value showed a large variation (see Figure 11).

Figure 11. The relationship between oxytocin and modified pubertal stage predicted by the reduced Post Hoc Model C.



Note: The dashed lines represent the 95 % confidence interval of the mean. The figure is used under the terms of the Creative Commons Attribution 4.0 International License. (https://creativecommons.org/licenses/by/4.0/).

The large variation in adults made us explore non-linearity in all participants using random forest, to see if we could capture the relationship across ages more accurately. Looking at the MSE of the Random Forest of all participants we saw that the Random Forest did not capture signals in the data, that were not captured Model 4 (with age in all participants) and Post Hoc Model C (with modified pubertal stage in all participants) (illustrated in the Appendix Study I, Section 13).

7.1.5. Supplementary analysis of oxytocin and additional possible covariates

Our results indicated that adult females had higher oxytocin levels when they were considered in ovulation compared to when they were considered in the menstrual phase (estimate=0.53, p=0.0008 and see the Appendix Study I, Section 13). We found no significant association with time of day for the oxytocin sample, CGAS, CY-BOCS, SRS or menarche status (see the Appendix Study I, Section 13).

7.1.6. Summary of findings in study I

In summary, children with OCD do not seem to have different oxytocin levels than children without OCD. Oxytocin levels seem to change across ages in a complex manner that is not simply linear. In children, the changes in oxytocin in our data was best described with child pubertal stages.

7.2. Study II - results

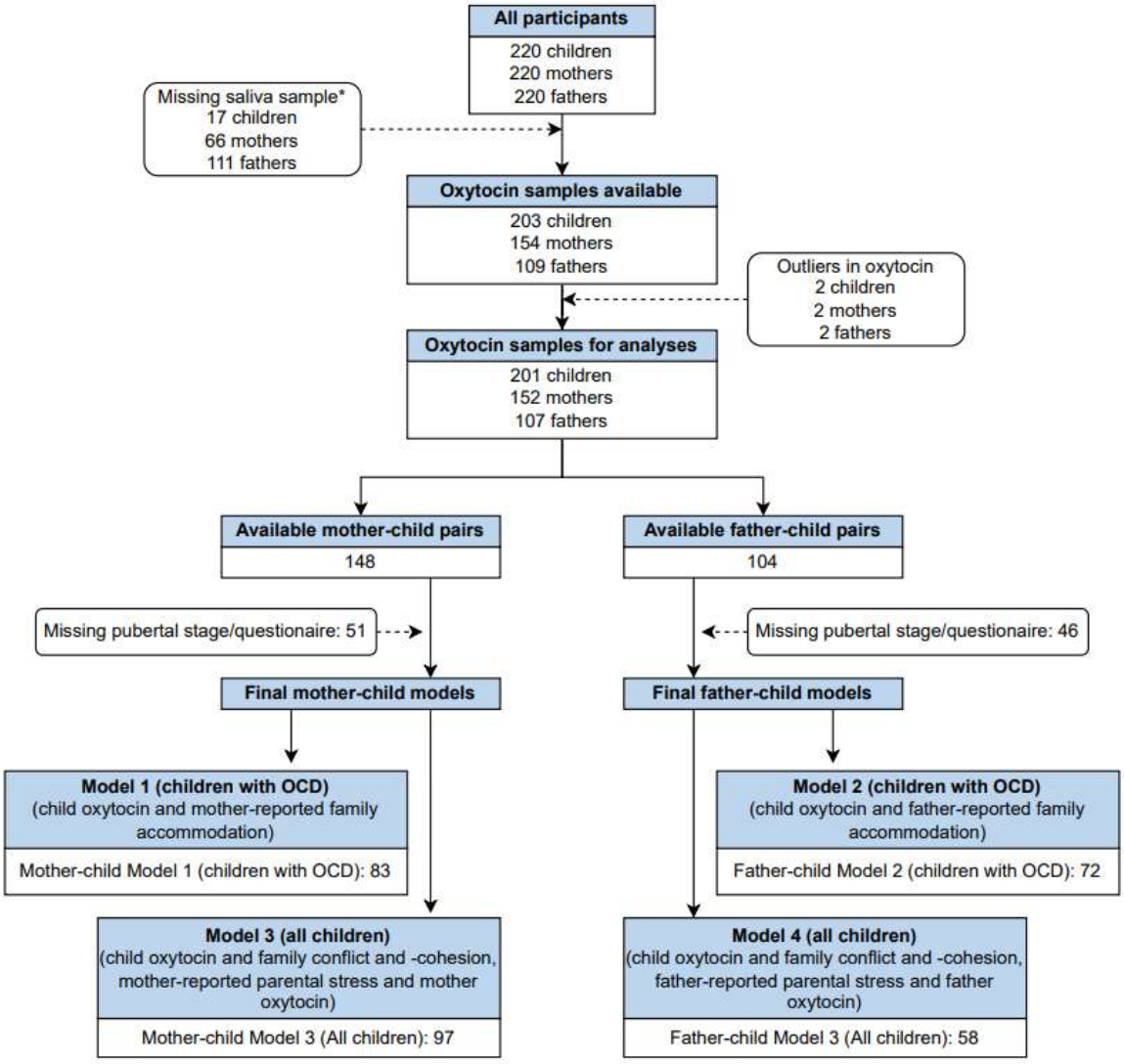
Study II: The role of oxytocin in family processes in the presence and absence of pediatric OCD

7.2.1. Sample and sample characteristics

We included 120 children in the analyses of Study II. All observations had information on child oxytocin and child pubertal stage. For some children, information was available from both from mothers and fathers, and the observations from these children were therefore included in both analyses with mothers and fathers. Some children had only information from either mother or father and were therefore only included in the respective analysis with mothers or fathers. The participant flowchart is illustrated in Figure 12. In the two models investigating child oxytocin and family accommodation in children with OCD, we included children with OCD, available data on child oxytocin and pubertal stage, and available mother- and/or father-reported family accommodation. In the two models in all children, we included mother-child pairs and father-child pairs with no missing data on the relevant questionnaires and available child and parent oxytocin and child pubertal stage.

Demographic and clinical characteristics for the 120 children, 97 mothers and 58 fathers are included in Table 7. We found no difference between children with OCD and controls on age, pubertal stage and sex, The control children reported higher family cohesion, than the children with OCD; however, this finding was of borderline significance. The mothers and fathers of controls reported lower parental stress than the mothers and fathers of children with OCD ($p<0.01$). We found no significant difference in salivary oxytocin between mothers of children with OCD and mothers of controls. However, a comparison of oxytocin levels between fathers of children with OCD and fathers of controls revealed higher levels in the control group, with *p*-value indicating a borderline significant difference.

Figure 12. Participant flowchart Study II, showing the four models.



*Note: *Reasons for missing saliva sample for children were the same as described in Study I (see 39).*

Table 7. Participant characteristics of children with OCD and controls study II. Table from Mora-Jensen et al. (manuscript)[2]

	Children with OCD	Controls	OCD vs Controls
Number of children *n* (% of sample)	71 (59%)	49 (41%)	
Child age, years (Mean (SD))	13.24 (2.73)	13.55 (2.84)	*p*=0.57[a]
Missing (*n*)	0	0	
Child sex (*n* (%))			*p*=0.59[b]
Female	37 (52%)	21 (57%)	
Male	34 (48%)	28 (43%)	
Missing (*n*)	0	0	
Child pubertal stage (*n* (%))			*p*=0.92[b]
Pubertal stage 1	22 (31%)	13 (27%)	
Pubertal stage 2	9 (13%)	8 (16%)	
Pubertal stage 3	130 (18%)	7 (14%)	
Pubertal stage 4	18 (25%)	14 (29%)	
Pubertal stage 5	9 (13%)	7 (14%)	
OCD severity			-
CY-BOCS total score (Mean (SD)	25.78 (4.94)	-	
Child psychiatric comorbidity (*n* (%))			-
Asperger's syndrome F84·5	10 (14%)	-	
Hyperkinetic disorders F90.0	9 (13%)	-	
Anxiety disorders F40-41, F93	8 (11%)	-	
Stress and adjustment disorders F43	8 (11%)	-	
Tic disorders F95	8 (11%)	-	
Other disorders (*n*)	9	-	
Child Nationality (*n* (%))			-
Danish	69 (97%)	46 (94%)	
Other	1 (1.5%)	2 (4%)	
Missing (*n*)	1 (1.5%)	1 (2%)	
Parental education mothers (*n* (%))			
Upper secondary education or below	7 (10%)	0	
Short-cycle tertiary education	11 (15%)	2 (4%)	
Bachelor's degree or equivalent	24 (34%)	20 (41%)	
Master's degree or above	26 (37%)	24 (49%)	
Missing (*n*)	3 (4%)	3 (6%)	
Parental education fathers (*n* (%))			
Upper secondary education or below	13 (16%)	2 (4%)	
Short-cycle tertiary education	8 (10%)	3 (6%)	
Bachelor's degree or equivalent	16 (23%)	12 (24%)	
Master's degree or above	22 (31%)	16 (33%)	
Missing (*n*)	14 (20%)	16 (33%)	
Salivary oxytocin in pg/ml* (Mean (SD))			
Child	26.21 (14.45)	27.01 (12.92)	*p*= 0.94[a]
Mother	28.66 (13.5)	28.71 (16.2)	*p*= 0.76[a]
Father	27.72 (9.3)	22.48 (6.78)	*p*= 0.05[a]
Family accommodation[1] (Mean (SD))			
Mother reported	21.99 (16.5)		
Father reported	15.46 (14.4)		
Family cohesion[2] (Mean (SD))			
Child reported	6.81 (1.8)	7.47 (1.7)	*p*=0.05[a]
Family conflict[2] (Mean (SD))			
Child reported	2.32 (2.2)	1.65 (1.7)	*p*= 0.07[a]
Parental stress[3] (Mean (SD))			
Mother reported	23.03 (6.54)	19.68 (5.43)	<0.01[a]
Father reported	23.36 (5.50)	18.76 (4.70)	<0.01[a]

Note: OCD, obsessive-compulsive disorder, SD, standard deviation. The t-tests on oxytocin levels were performed on the natural logarithm of oxytocin levels to meet the assumptions of the t-test; however, the summary statistics show the values without natural logarithm. [1]Family Accommodation Scale-SR (FAS-SR), [2]Family Environmental Scale (FES), [3]Parental Stress Scale (PSS). [a] t-test, [b] chi-square test.

7.2.2. Child oxytocin and family accommodation in children with OCD

Neither mother-reported nor father-reported family accommodation was associated with variation in child oxytocin in our sample.

Table 8. Associations between the natural logarithm of child oxytocin and mother- and father-reported family accommodation. Table from Mora-Jensen et al. (manuscript)[2]

Final model	Intercept (μ (*SE*))	Parameter (β (*SE*))	F-statistics	*p*-value	Adj. R^2
Model 1*	2.970 (0.11)			0.239	0.02
Mother-reported family accommodation	-	0.003 (0.003)	0.866	0.355	-
Model 2*	3.037 (0.12)			0.421	0.002
Father-reported family accommodation	-	0.0005 (0.005)	0.009	0.926	-

*Note:*Adjusted for pubertal stage (detailed results on pubertal stage for these models in Appendix study II, Section 14). SE, standard error.*

7.2.3. The association between child oxytocin and family conflict, family cohesion, parental stress, and parent oxytocin in children with OCD and controls

When examining the association between child oxytocin levels and information from the children and mothers (Model 3), we entered mother oxytocin levels, mother-reported parental stress, family conflict and family cohesion, and the interactions (described in Section 6.5.2), into a backwards stepwise regression. In this analysis, all variables were dropped in the stepwise procedure except mother oxytocin (pubertal stage was retained as a covariate) (Table 9, details in Appendix study II, Section 14).

When running the same analysis with information on children and fathers (thus father oxytocin levels and father-reported parental stress instead of the information from mothers) (Model 4), multiple variables remained significant. The variables that were kept in the final model were: father oxytocin levels, the interactions between child OCD status and child reported family conflict, and the interaction between child OCD status and father reported parental stress (and pubertal stage retained as a covariate) (see Table 9).

Table 9. Results from final step in backwards multiple regression Model 3 and 4 predicting the natural logarithm of child oxytocin from family conflict, cohesion, parental stress, and oxytocin levels in children with OCD and controls. Table from Mora-Jensen et al. (manuscript)[2]

Final model	Intercept (μ (*SE*))	Parameter (β (*SE*))	F-statistics	*p*-value	Adj. R^2
Model 3*	1.38 (0.32)			<0.001	0.25
Ln mother oxytocin	-	0.53 (0.10)	29.32	<0.001	-
Model 4*	0.44 (0.67)			<0.05	0.20
Ln father oxytocin	-	0.54 (0.17)	10.57	<0.01	-
Father reported parental stress		0.06 (0.03)	5.43	0.02	
Child reported family conflict		-0.18 (0.08)	4.75	0.03	
Child OCD status		0.99 (0.58)	2.94	0.09	
Father reported parental stress x child OCD status		-0.07 (0.03)	5.76	0.02	
Child reported family conflict x child OCD status		0.20 (0.09)	5.50	0.02	

*Note: Ln, natural logarithm, SE, standard error. OCD; obsessive-compulsive disorder *Adjusted for pubertal stage (detailed results on pubertal stage for these models in Appendix study II, Section 14).*

7.2.4. Model check and sensitivity analyses

We inspected the correlation matrices to explore associations between the variables in the models. All correlations were below r= 0.517 in the mother-child (Model 3) and r=0.440 in the father-child model (Model 4). Both models had acceptable VIFs, which indicate that there was no problematic multicollinearity.
In the mother-child model, we found correlations between child-reported family cohesion and conflict (r= -0.44), between mother perceived parental stress and child-rated family conflict (r= 0.32) and between mother perceived parental stress and child OCD status (r= 0.26).
In the father child model, we calculated correlations between child-reported family cohesion and conflict (r= -0.26), father reported parental stress and child pubertal stage (r= -0.38), father reported parental stress and child OCD status (r= 0.37), and father oxytocin level and child OCD status (r= 0.26).
The sensitivity analyses using a forward approach showed no difference in the final model for the models including mothers and children, but in the model of father and child pairs in all children, only father oxytocin was retained which indicates that this model was sensitive to the model selection strategy. The adjusted R^2 was higher for the backwards regression (R^2=0.20 compared to R^2=0.15), and we chose to conclude on the backwards approach for the father-child model.

7.2.5. Summary of findings in study II

In summary, child oxytocin does not seem related to how much the mothers and fathers accommodate to the child's OCD. Child oxytocin was related to how much family conflict the child reported and how much parental stress the father reported, but how child oxytocin was related to conflict and parental stress was different in children with OCD and controls. For all children, child oxytocin levels were related to mothers and fathers oxytocin levels.

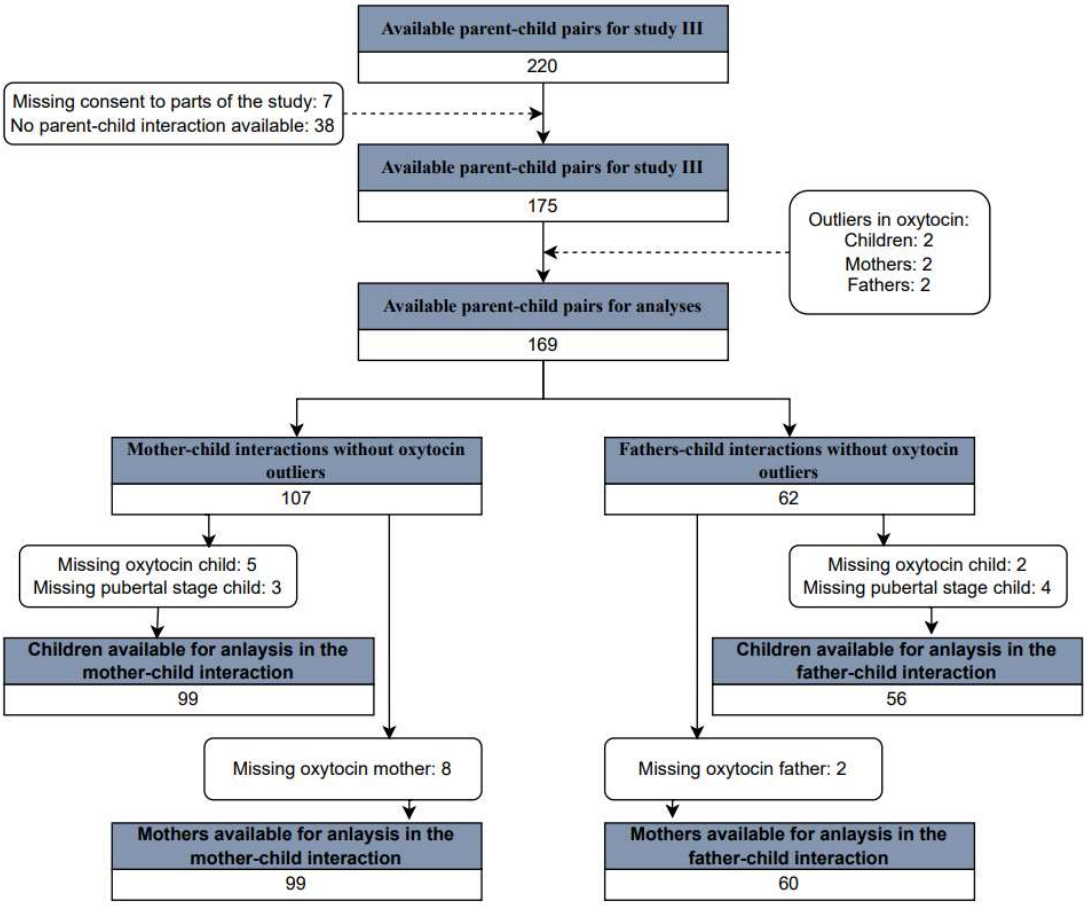
7.3.Study III – results

Study III: Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

7.3.1. Sample characteristics

Study III was based on a subset of the sample from Study I. In Study III, we only included children for whom we had a video-recorded and coded parent-child interaction available. For the analysis of child oxytocin as outcome, we included children with available results on oxytocin and pubertal stage. For the parents, we included mothers and fathers with available information on oxytocin. The resulting samples available for analyses are illustrated in Figure 13.

Figure 13. Participant flowchart Study I, showing the four primary model but not post hoc models.



Demographic and clinical variables for the children and the parents are included in Table 10. The counts of the various behaviors included is available in the Appendix of Study III, Section 15.

Table 10. Descriptive characteristics of children, mothers, and fathers in Study III. Table from Mora-Jensen et al. (2024)(in review)[3].

	Children with OCD	**Children without OCD**
Children		
Number of children (*n*)	74	81
Age (years) Mean (*SD*)	12.96 (2.82)	12.95 (2.74)
Sex (*n* (%))		
Female	37 (50%)	42 (52%)
Male	37 (50%)	39 (48%)
Pubertal stage (*n* (%))		
Pubertal stage 1	26 (35%)	21 (26%)
Pubertal stage 2	8 (11%)	18 (22%)
Pubertal stage 3	16 (22%)	14 (17%)
Pubertal stage 4	18 (24%)	20 (25%)
Pubertal stage 5	6 (8%)	8 (10%)
Nationality (*n* (%))		
Danish	64 (86%)	59 (73%)
Other	2 (11%)	1 (26%)
Missing	8 (3%)	21 (1%)
Comorbid psychiatric disorders (*n* (%))		
Anxiety disorders, ICD-10 F40-41, F93	8	-
Stress and adjustment disorders, ICD-10 F43	7	-
Asperger's Syndrome, ICD-10 F84.5	13	-
Hyperkinetic disorders ICD-10 F90.0	10	-
Tic disorders, ICD-10 F95	9	-
Other*	11	-
Oxytocin concentration (log) Mean (*SD*)	27.17 (15.26)	25.96 (14.31)
Mothers		
Number of mothers (n)	55	52
Age (years) Mean (*SD*)	44.81 (5.09)	45.55 (4.87)
Nationality (*n* (%))		
Danish	41 (75%)	41 (79%
Other	2 (3%)	2 (4%)
Missing	12 22%)	9 (17%)
Oxytocin (log) Mean (*SD*)	28.91 (15.01)	31.13 (17.90)
Fathers		
Number of fathers	33	29
Age (years) Mean (*SD*)	47.30 (4.23)	47.24 (7.01)
Nationality (*n* (%))		
Danish	29 (88%)	21 (72%)
Other	1 (3%)	1 (4%)
Missing	3 (9%)	7 (24%)
Oxytocin (log) Mean (*SD*)	28.79 (8.90)	24.99 (11.81)

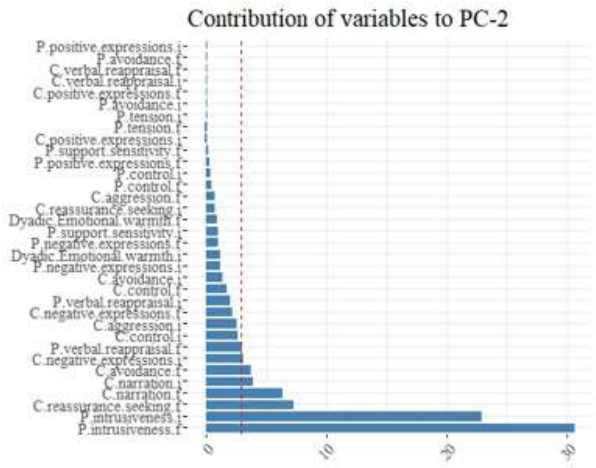
*Note: *Other describes all other diagnoses (for information on these see Appendix study III, Section 15, Table A.2.3). Note: n, number; SD, standard deviation; OCD, obsessive-compulsive disorder; ICD-10, International Classification of Diseases-10.*

7.3.2. Computationally derived parent-child interaction patterns from principal component analysis (PCA)

We inspected scree plots to determine the number of PCs to include in further analyses. We chose to use the first six components for further analyses, as these explained around 60% of the variance in the mother-child and the father-child data sets. The principal components were labelled after inspection of contribution plots of the PCs and the table of Eigenvectors (both shown in Appendix study III, Section 15).

To provide an example of how we labeled the principal components, the interpretation and labelling of PC2 from the mother-child interaction will be described. We first used the contribution plot of PC2 (Figure 14) and found that six of the original variables had a loading that exceeded the red line, which indicates that these loadings are of a significant magnitude and should be considered in the interpretation of PC2.

Figure 14. Contributions plot PC2. Figure from Mora-Jensen et al. (2024)(in review)[3], Appendix Study III, Section 15.



Note: PC, principal component, P, parent, C, child; f, frequency; i, intensity

We then looked at the Eigenvector table to see the specific loadings of these six variables on PC2 (see Table 11). In addition, we looked at the coding manual, to understand what a high or low score would indicate for these specific variables.

Table 11. Part of eigenvector table showing the loadings of the six most important variables for PC2. Part from table from Mora-Jensen et al. (2024)(in review)[3], Appendix Study III, Section 15.

Variable	Eigenvector
P.intrusiveness.f	-0.55
P.intrusiveness.i	-0.48
C.avoidance.f	0.19
C.narration.f	0.25
C.narration.i	0.20
C.reassurance.seeking.f	0.27

Note: PC, principal component, P, parent, C, child; f, frequency; i, intensity

How the loadings of the variables can be interpreted in relation to PC2 are in the two examples below:

Example of a negative loading: The variable “Parent intrusiveness - intensity” has a strong negative loading on PC2, which means that a parent-child pair with a low score on Parent Intrusiveness Intensity will have a high value on PC2 (however, also depending on the scores on other variables). The TEC-M coding manual describes Parent Intrusiveness and the scoring as seen in Figure 15.

Figure 15. Description of the code "Parent Intrusiveness – intensity " from the TEC-M manual[119]

> ***Parent intrusiveness – intensity (short description):*** *The behavior of a parent is considered intrusive when help is initiated too soon and/or in a manner that overrules the child's autonomy.*
>
> ***Scores:***
>
> *0: The behavior never occurs*
>
> *1: Providing verbal aid and/or interfering verbally when the child is already focused and working on the task, i.e. is not in need of help.*
>
> *2: Touching and/or rearranging the jigsaw pieces or pictures of the puzzles unless the child is in serious need of help.*
>
> *3: Obstructing the work of the child by moving his or her hands and taking over the puzzle.*

A parent-child interaction where parent intrusive behavior never occurred (scoring 0) or only verbal intrusiveness was observed (scoring 1) would therefore reach a higher score on PC2 (again, depending on scores on other variables).

Example of a positive loading: The variable “child reassurance seeking - frequency” has a strong positive loading on PC2, which means that a parent-child interaction with a score for this variable seeking will have a higher score on PC2. The description of this variable in the TEC-M manual is described in Figure 16.

Figure 16. Description of the code "Child reassurance seeking" from the TEC-M manual[119]

> ***Child reassurance seeking – frequency****: Comprises the child's need for support from the parent, either by looking up at the parent, or specifically or indirectly asking for guidance or reassurance (shortened description).*
>
> ***Scores****:*
>
> *0= The behavior never occurs*
>
> *1= One or two instances of reassurance-seeking behavior*
>
> *2 = Three or four instances of reassurance-seeking behavior*
>
> *3= Five or more instances of reassurance-seeking behavior*

Thus, a parent-child interaction, in which the child shows frequent reassurance seeking behavior (e.g., five or more times and receiving a score of 3) would get a higher value on PC2 (like in example above, depending on scores on other variables). Taken together, the score on PC2 was high when the child showed reassurance seeking and avoidance frequently during the task, and the child used narration frequently with high intensity, and when the mother did not show frequent or intense intrusive behavior. This could be a mother-child interaction, in which the child was verbal about the solving of the task **(narration)**, often elicited help or guidance from the parent (**reassurance seeking)** and often avoided the task by leaning away, playing with the puzzle instead of solving it or refusing to participate (**avoidance)**. The mother in this interaction did not overrule the child’s autonomy and did not help too soon or too much (**intrusiveness)**. Then, we discussed how this principal component should be interpreted and decided to label PC2 "*varied-coping interaction*",

thus, interpreting this as a child who shows different strategies for solving the task. This labeling process was done for all PCs. The variables that loaded significantly to the first six components are described in Table 12 for the mother-child interaction and Table 13 for the father-child interaction.

Table 12. Variables loadings for the first six principal components of the mother-child PCA. Table from Mora-Jensen et al. (2024)(in review)[3]

	Mother codes	**Child codes**	**Dyad codes**
Supportive interaction (PC1)	Positive expressions(f) Verbal reappraisal(f)	Narration(f,i) Positive expressions(f,i) Reassurance seeking(f)	Emotional warmth(i)
Varied-coping interaction (PC2)	Intrusive(f,i)	Reassurance seeking(f) Narration(f,i) Avoidance(f)	
Conflictual interaction (PC3)	Intrusive(f,i) Negative expressions(f,i) Tension(f,i) Support sensitivity(f)	Avoidance(f,i) Aggression(i) Control(f)	Emotional warmth(f)
Demanding interaction (PC4)	Negative expressions(f,i)	Avoidance(f,i)	
Overinvolved interaction (PC5)	Intrusive(f,i) Verbal reappraisal(f,i)	Narration(f,i) Avoidance(i)	
Supportive tense interaction (PC6)	Tension(f,i) Control(f) Support sensitivity(i) Avoidance(f,i)	Reassurance seeking(f,i) Avoidance(i) Negative expressions(f,i) Narration (i) Control (i)	

Note: Loading refers to the correlation between a variable and a PC. Green indicates positive loadings and red indicates negative loadings. f, frequency; i, intensity; PCA, Principal Component Analysis; PC, Principal Components.

Table 13. Variables loadings for the first six principal components of the father-child PCA. Table from Mora-Jensen et al. (2024)(in review)[3]

	Father codes	**Child codes**	**Dyad codes**
Supportive interaction (PC1)	Positive expressions(f) Intrusiveness(f,i) Negative expressions(i)	Positive expressions(f) Negative expressions(i) Avoidance(f,i)	Emotional warmth(f,i)
Disconnected interaction (PC2)	Intrusiveness(i) Avoidance(f,i)	Control(i) Avoidance(f,i) Narration(i) Reassurance seeking(f,i) Positive expressions(f,i) Negative expressions(i)	Emotional warmth(i)
Unengaged interaction (PC3)	P.intrusiveness(f,i) P.negative expressions(f,i) P.tension(f,i)	Avoidance.f Narration (f,i)	
Overinvolved interaction (PC4)	Intrusiveness(f,i) Positive expressions(f) Negative expressions(f,i)	Avoidance (f,i) Negative expressions (f,i)	
Supportive tense interaction (PC5)	Verbal reappraisal(f,i) Negative expressions(i) Tension(f,i) Intrusiveness(f,i)	Reassurance.seeking(f) Positive.expressions(f) Narration(f,i) Avoidance(f,i)	Emotional warmth(f)
Parent seeking interaction (PC6)	Control(f) Verbal reappraisal(f,i) Positive expressions(f,i) Negative expressions(f,i)	Reassurance.seeking(f,i)	

Note: Loading refers to the correlation between a variable and a PC. Green indicates positive loadings and red indicates negative loadings. f, frequency; i, intensity; PCA, Principal Component Analysis; PC, Principal Components.

7.3.3. Association between oxytocin and principal components from mother-child interaction and the father-child interaction

Next, we investigated if there was an association between children and parents' oxytocin levels and the scores on the principal components. For the mother-child pairs, child oxytocin was positively associated with the *overinvolved interaction* (PC5) (p=0.086, Table 14). Mother oxytocin was positively associated with *supportive interaction* (PC1), *varied-coping interaction* (PC2), and *overinvolved interaction* (PC5), and negatively associated with *conflictual interaction* (PC3) (Table 14). We found no association between child and father oxytocin and the six principal components from the father-child interaction (See Table 15).

Table 14. Regression analysis predicting mother and child oxytocin levels from principal components. Table from Mora-Jensen et al. (2024)(in review)[3]

Principal components	Child log oxytocin[A] (*n*= 99) Estimate (*SE*)	Adjusted *p*-value	Mother log oxytocin (*n*=99) Estimate (*SE*)	Adjusted p-value
Supportive interaction (PC1)	0.02 (0.02)	0.558	0.07 (0.02)	0.009*
Varied-coping interaction (PC2)	0.03 (0.03)	0.446	0.05 (0.03)	0.173*
Conflictual interaction (PC3)	-0.02 (0.03)	0.558	-0.05 (0.03)	0.193*
Demanding interaction (PC4)	-0.003 (0.03)	0.931	0.05 (0.03)	0.296
Overinvolved interaction (PC5)	0.09 (0.04)	0.086*	0.12 (0.04)	0.009*
Supportive tense interaction (PC6)	0.05 (0.05)	0.446	-0.02 (0.04)	0.724

*Note: SE, standard error; PC, Principal Component, [A]Log oxytocin adjusted for child pubertal stage (See Appendix study III, Section 15), *Significant with an accepted false discovery rate of 20% (p-value > 0.20).*

Table 15. Regression analysis predicting father and child oxytocin levels from principal component. Table from Mora-Jensen et al. (2024)(in review)[3]

Principal components	Child log oxytocin[A] (*n*=56) Estimate (*SE*)	Adjusted *p*-value	Father log oxytocin (*n*=60) Estimate (*SE*)	Adjusted *p*-value
Supportive interaction (PC1)	0.01 (0.03)	0.829	-0.01 (0.02)	0.829
Disconnected interaction (PC2)	0.04 (0.04)	0.829	0.01 (0.03)	0.829
Unengaged interaction (PC3)	-0.02 (0.04)	0.829	0.01 (0.03)	0.829
Overinvolved interaction (PC4)	-0.04 (0.05)	0.829	-0.02 (0.04)	0.829
Supportive tense interaction (PC5)	-0.05 (0.06)	0.829	0.04 (0.04)	0.829
Parent seeking interaction (PC6)	0.03 (0.06)	0.829	-0.03 (0.04)	0.829

*Note: SE, standard error; PC, Principal Component, [A]Log oxytocin adjusted for child pubertal stage (See Appendix study III, Section 15), *Significant with an accepted false discovery rate of 20% (p-value > 0.20).*

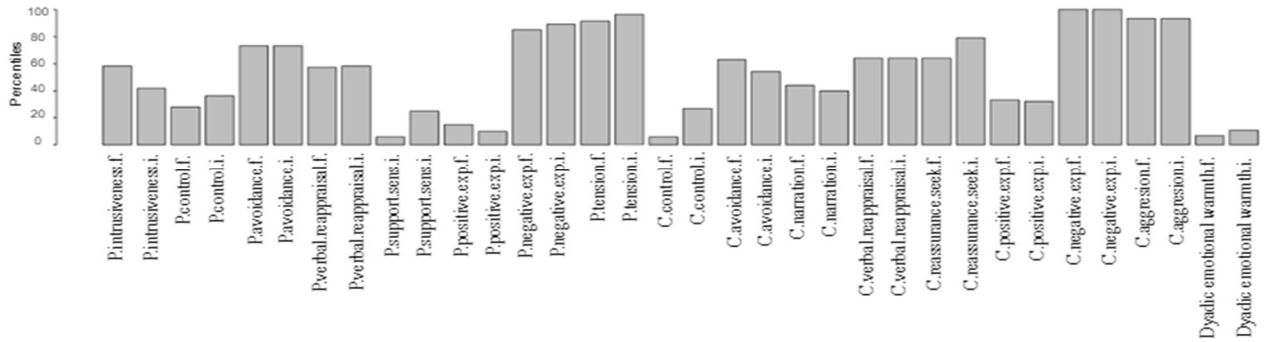
7.3.4. *Post hoc* analyses of the effect of child OCD on the relationships between oxytocin and principal components

We explored if the associations between the principal components and oxytocin differed between children with OCD and controls. We did not find a difference in the association between the principal components and both child and mother oxytocin, for children with versus without OCD (See Appendix study III, Section 15). For the father-child pairs, we found a different association between father oxytocin levels and *supportive interaction* (PC1), when comparing the children with OCD with the controls (adjusted p-value= 0.180, see Appendix study III, Section 15). Looking at the estimates and visual presentations of the model, we found that the association between father oxytocin levels and the *supportive interaction* (PC1) was positive in the children with OCD, but negative in controls (See Appendix study III, Section 15).

7.3.5. Computationally derived parent-child interaction patterns from archetype analysis

We used scree plots to determine the number of archetypes for further analysis. For the mother-child data, three archetypes best described the data, and for comparability, we chose to present three archetypes for the father-child data as well. We used percentile profiles of the archetypes to interpret the content of the archetypes and label the archetypes. To describe our use of percentile profiles, the labelling of Archetype I in the mother-child interaction will be described. Percentile profiles describe the value of the variables in an archetype, compared to the value of these variables in the data[132]. The percentile profile of Archetype I from the mother-child interaction is shown in Figure 17.

Figure 17. Archetype 1 from the mother-child archetype analysis. Table from Mora-Jensen et al. (2024)(in review)[3], Appendix, Section 15



Note: P, parent (in this plot "mothers); C, child; f, frequency; i, intensity

The x-axis shows the different variables. The height of the bars shows the percentile of this variable, thus, showing which percentile in the overall dataset for a given variable, that best describe this archetype.
For Archetype 1, we see that some variables are above the 75th percentile (parent negative expressions, parent tension, child reassurance seeking, child negative expressions and child aggression) and some below the 25th percentiles (parent support sensitivity, parent positive expressions, child control, and dyadic emotional warmth). This could indicate that a parent-child dyad scoring high on Archetype 1, would be a child not in control of the task, seeking help from the parent, displaying negative expressions and aggression. The parent in this type of interaction tended not to display support sensitivity nor positive expressions, but negative expressions and tension. The dyad was low in emotional warmth. We labelled Archetype I "*negative-low support interaction*". The percentile profiles of the archetypes from the mother-child and father-child interaction are illustrated in the Appendix study III, Section 15 and the variables with a percentile value below 25 or above 75 are shown in Table 16 for the three archetypes from the mother-child interaction, and in Table 17 for the father-child interaction

.

Table 16. Variables with percentile values below 25th(red) or above 75th(green) from the mother-child archetypes.

	Mother codes	Child codes	Dyad codes
Negative-low support interaction	Avoidance (f,i) Support sensitivity (f) Positive expressions (f,i) Negative expressions (f,i) Tension (f,i)	Control (f) Reassurance seeking (i) Negative expressions (f,i) Aggresions (f,i)	Emotional warmth (f,i)
Distant interaction	Control (f) Avoidance (f,i) Verbal reappraisal (f,i) Support sensitivity (i) Positive expressions (f,i) Tension (f,i)	Narration (f,i) Reassurance seeking (i) Positive expressions (f,i) Aggresion (f,i)	Emotional warmth (i)
Emotional interaction (Archetype 3)	Avoidance (f,i) Verbal reappraisal (f) Support sensitivity (f,i) Positive expressions (i) Tension (f,i)	Reassurance seeking (i) Positive expressions (i) Aggresion (f,i)	Emotional warmth (f,i)

Note: Red indicates around or below the 25th percentiles, green indicates around or above the 75th percentile.

Table 17. Variables with percentile values below 25th(red) or above 75th(green) from the father-child archetypes.

	Father codes	Child codes	Dyad codes
Negative-low support interaction	Intrusiveness (f,i) Avoidance (f,i) Support sensitivity (f) Positive expressions (i) Negative expressions (f,i) Tension (f,i)	Control (f,i) Avoidance (i) Narration (f,i) Reassurance seeking (i) Aggresion (f,i)	Emotional warmth (f,i)
Engaged interaction	Avoidance (f,i) Positive expressions (i) Negative expressions (i) Tension (f,i)	Control (f) Reassurance seeking (i) Aggresion (f,i)	
Emotional interaction (Archetype 3	Avoidance (f,i) Verbal reappraisal (f,i) Support sensitivity (f,i) Positive expressions (i)	Verbal reappraisal (f) Reassurance seeking (i) Aggresion (f,i)	Emotional warmth (f,i)

Note: Red indicates around or below the 25th percentiles, green indicates around or above the 75th percentile.

7.3.6. Association between oxytocin and archetypes of parent-child interactions

We used the scores of each archetype for the parent-child pairs to analyze the association between archetype scores and child and parent oxytocin. For the mother-child pairs, we found a negative association between child oxytocin and "*negative-low support interaction*" (Archetype 1) and positively associated with "*emotional interaction*" (Archetype 3) (see Table 18). We found a negative association between mother oxytocin and both "*negative-low support interaction*" (Archetype 1) and "*distant interaction*" (Archetype 2), and a positive association between mother oxytocin and "*emotional interaction*" (Archetype 3, Table 18).

For the father-child pairs, we found no association between child and father oxytocin and the archetypes (Table 19).

Table 18. Regression analysis predicting mother and child oxytocin levels from Archetypes. Table from Mora-Jensen et al. (2024)(in review)[3]

Archetypes	Child log oxytocin[A] (n=99) Estimate (*SE*)	Adjusted *p*-value	Mother log oxytocin (*n*=99) Estimate (*SE*)	Adjusted *p*-value
Negative-low support interaction	-0.36 (0.22)	0.184*	-0.33 (0.23)	0.184*
Distant interaction	-0.09 (0.18)	0.641	-0.48 (0.17)	0.023*
Emotional interaction	0.26 (0.17)	0.184*	0.57 (0.16)	0.002*

*Note: SE, standard error. [A]Log oxytocin adjusted for child pubertal stage (See Appendix study III, Section 15), *significant with an accepted false discovery rate of 20% (p-value > 0.20).*

Table 19. Regression analysis predicting father and child oxytocin levels from Archetypes. Table from Mora-Jensen et al. (2024)(in review)[3]

Archetypes	Child log oxytocin[A] (*n*=56) Estimate (*SE*)	Adjusted *p*-value	Father log oxytocin (*n*=60) Estimate (*SE*)	Adjusted *p*-value
Negative-low support interaction	-0.09 (0.21)	0.915	0.06 (0.15)	0.915
Engaged interaction	0.02 (0.22)	0.915	-0.04 (0.16)	0.915
Emotional and warm father-child interaction.	0.08 (0.22)	0.915	-0.02 (0.15)	0.915

*Note: SE, standard error. [A]Log oxytocin adjusted for child pubertal stage (See Appendix study III, Section 15), *Significant with an accepted false discovery rate of 20% (p-value > 0.20).*

7.3.7. *Post hoc* analyses of the effect of child OCD on the relationships between oxytocin and archetypes

We found no difference in the relationships between mother and child oxytocin and the mother-child archetypes, or father and child oxytocin and the father-child archetypes in children with OCD and controls (see Appendix study III, Section 15).

7.3.8. Summary of findings in study III

In summary, children's and mother's salivary oxytocin was related to various mother-child interaction patterns during a frustrating puzzle-task, and this was true for both children with OCD and controls. Children's and father's salivary oxytocin did not seem related to father-child interaction patters in children with OCD and control, when child OCD status was not included in the model. However, the relationship between father oxytocin and one of the father-child interactions, the "supportive interaction", seemed different depending on whether the child had OCD or did not have OCD.

8. Discussion

This section consists of a presentation of the main findings, a discussion of the results of each of the three studies in relation to previous studies, and considerations of the methodological limitations and strengths. Finally, it provides a discussion of how the three studies can contribute to answering the main question - to examine the relevance of salivary oxytocin in pediatric OCD.

8.1.Summary of main findings

8.1.1. Study I – main findings

Study I: The association between salivary oxytocin, age, and puberty in children with and without OCD

We found no evidence of a difference in salivary oxytocin levels between children with OCD and children without a psychiatric disorder using hypothesis significance testing. Including important covariates did not change this result and the findings were further supported by Bayesian statistical analyses; however, only with anecdotal to moderate support. Regarding covariates, we found a relationship between child oxytocin and child age and pubertal stage that seemed non-linear.

8.1.2. Study II – main findings

Study II: The role of oxytocin in family processes in the presence and absence of pediatric OCD

For children with OCD, we found no association between child oxytocin and mother-reported or father-reported family accommodation in children with OCD. When we examined the combined sample of children with OCD and controls, we found an association between child oxytocin and both family conflict and parental stress. Interestingly, we only found this associations in analyses with data from the fathers and the directions of the associations differed between children with OCD and controls. We found a positive relationship between child oxytocin levels and both mother's and father's oxytocin levels.

8.1.3. Study III – main findings

Study III: Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

We identified behavioral patterns describing the mother-child and father-child interactions. Child oxytocin was positively associated with two mother-child interaction patterns: "*overinvolved interaction*" and "*emotional interaction*". Child oxytocin was negatively associated with the interaction pattern "*distant interaction*". Mother oxytocin was positively associated with four of the mother-child interaction patterns: "*overinvolved interaction*", "*emotional interaction*" "*supportive interaction*" and "*varied-coping interaction*". Mother oxytocin was negatively associated with interaction patterns: "*conflictual interaction*", "*distant interaction*" and "*negative-low support interaction*". For the father-child interactions, we found no association between child or father oxytocin and the interaction patterns, when including all children, however, we found an association between father oxytocin and "*supportive interaction*" in the presence of child OCD.

8.2. Discussion of the three studies

8.2.1. Study I – discussion

Study I: The association between salivary oxytocin, age, and puberty in children with and without OCD

8.2.1.1. Are salivary oxytocin levels different in children with OCD compared to children without a psychiatric disorder?

Our study is the first to examine differences in salivary oxytocin levels in children with OCD compared with children without a psychiatric disorder. We found no overall mean group differences between children with OCD and controls, consistent with the findings from adult studies by Leckman et al.[62] and Altemus et al.[63], who also reported no significant differences, when comparing CSF oxytocin levels in adults with OCD compared to controls. Our findings were contrary to the study by Marazziti et al., who found higher plasma oxytocin in adults with OCD compared to controls[64]. The different findings between our study and the study by Marazziti et al, could be due to various factors, illustrating the complexity of comparing findings across studies in the oxytocin field.

First, the four studies, including ours, had different sample sizes, which may explain why some studies found a statistically significant difference, while the others reported null findings. The three studies in adults all described higher levels in adults with OCD, but the study by Marazziti et al. was the largest and was potentially the only one large enough to detect a significant group difference. Nevertheless, a meta-analysis combining the Leckman and Altemus study, thereby reaching a larger sample size, found no differences in CSF oxytocin between adult patients with OCD and controls[133]. A newer meta-analysis, combining data from all three studies in adults, found significantly higher levels of oxytocin (110 patients with OCD compared to 101 controls)[4]. In our study, the mean salivary oxytocin was higher in the children with OCD than in the controls, but the difference was small (26.61pg/ml compared to 25.61pg/ml) and the standard deviation large (14.28 pg/ml and 13.95 pg/ml) which fits with our non-significant findings. Interestingly, the variation in both groups in the Marazziti study were also considerable (mean(SD) OCD group: 4.27(1.36)pg/ml vs. mean(SD) control group: 2.55(2.18)pg/ml), but they still found a significant difference[64]. Thus, our null finding differ from the largest the study in adults[64] and the largest meta-analysis[4], but interestingly, we had a considerably larger sample than the three previous studies in adults and almost as large as the newest meta-analysis. However, our Bayesian statistics only showed anecdotal to moderate support for the null finding. This indicates that an even larger sample is necessary to have confidence in the results.

A second issue to consider, is that comparing findings from CSF, plasma and saliva may be problematic. The correlations between saliva and CSF oxytocin levels appears stronger, than the correlations between plasma and CSF oxytocin[90]. This could explain why our study is consistent with the two studies using CSF[62,63], but in contrast with the study using plasma[64]. In addition, this issue may limit our confidence in the meta-analysis combining results from studies using different measures of oxytocin levels[4].

Third, varying clinical characteristics in the patient samples of the four studies could cause different findings. The comorbid disorders differed in the four studies, which could impact the oxytocin levels of patients. In our study, we accepted a broad range of comorbidities, including Aspergers syndrome, eating disorders and separation anxiety, which have all been associated with lower oxytocin levels[4,57,133]. The three studies in adults did not include patients with these three comorbidities, but described patient samples with comorbid major depression, ADHD, generalized anxiety, and social phobia[62–64]. Thus, these differences among samples could affect the comparison of studies as the oxytocin levels in patients with OCD is also affected by the comorbidities.

A fourth aspect to consider, is the different age range in our study compared with the adult studies. Leckman et al. suggested that higher oxytocin levels could be related to OCD in "steroid-primed" individuals only, e.g., after puberty or pregnancy[5]. If Leckmans hypothesis of oxytocin and OCD was correct, higher levels of oxytocin would only be relevant, and expected, in patients with OCD after puberty[5]. Even though we corrected for pubertal stage in our study, we did perhaps not have enough power to show a relationship with oxytocin in the subgroups of patients in the later stages of puberty. That oxytocin could be relevant for disorders in only some age ranges, has been described previously in, for example, autism spectrum disorders (ASD), where oxytocin levels have been shown to be lower for children with ASD compared to controls, whereas no such difference was observed in adults[134].

In summary, we were unable to find evidence of a difference in salivary oxytocin concentrations in children with OCD compared to children without a psychiatric disorder, but without certain evidence based on Bayesian statistics. Levels differences in salivary oxytocin could be tested in a larger sample, but it might be more relevant to explore, whether oxytocin levels are related to more specific symptoms or subtypes of OCD in childhood and adolescence.

8.2.1.2. Are age, pubertal stage, and sex, associated with oxytocin levels?

Our examination of the association between age and pubertal stage showed interesting. but also complex, findings that we explored with *post hoc* tests. We found significant positive relationships between oxytocin and age and oxytocin and pubertal stage. However, when we visually inspected the data, the relationships seemed non-linear with an increase in oxytocin levels until around 16 years of age, or pubertal stage 4, followed by a decrease. We used *post hoc* analyses to explore and thereby increase our understanding of the results. A model predicting oxytocin from child age "squared" captured the increase followed by a decrease, but model comparison showed, that a model with pubertal stage was best to describe the changes in oxytocin levels.

When we broadened the age range and included the parents, we again found a positive linear association between oxytocin and age. However, with our knowledge from the models in the children, indicating a drop after age 16 years, it seemed unlikely that there is a linear relationship between oxytocin and age. When inspection the data in prediction plots, we noticed that the child models were not able to predict the oxytocin

levels for adults and vice versa. To explore a model that could fit the child data (with an increase and a decrease) and could fit the adult data, we performed a *post hoc* analysis of pubertal stage where adults were assigned "pubertal stage 6". This "puberty" model showed a better fit than the model for all participants with age. These results suggest that oxytocin change with age in a way that is complex and cannot simply be predicted with a linear relationship especially for children. As our sample did not include individuals of all ages, e.g., excluded the ages 18-33 years, we are unable to make inferences about that age range.

That the relationship between oxytocin and age is complex and not simply linear across ages means, that it is difficult to compare findings across studies, if they do not examine the same age range[93]. As described in the introduction, the previous literature the relationship between oxytocin levels and age in children is sparse, but we are aware of two studies describing the association between child oxytocin and age[57,96]. A study in children between 5 months and 7.5 years of age reported a *negative relationship* between salivary oxytocin and age[96]. However, this provided a scatterplot of their findings, showing high levels of oxytocin in the youngest children, then a steep decline until around 20 months of age, followed more stable oxytocin levels until 7.5 years of age[96]. Thus, the findings from this study also seem to describe a non-linear relationship in the age range 5 months to 7.5 years of age. A study examining salivary oxytocin in children between 7-16 years of age with clinical anxiety, described a *positive relationship* between salivary oxytocin and age in this age range[57]. As we found an increase in oxytocin levels until around 16 years of age, our findings could resemble the positive relationship, found in the study in children between 7-16 years of age[57]. Our findings differ from the relationship between salivary oxytocin and age described for the youngest children[96], but are perhaps describing the relationship in different age ranges.
In adults, the studies on age and oxytocin in adults are also scarce. A review from 2013 found, that the only study examining oxytocin and age in humans at that time, was a postmortem study investigating the number of oxytocin producing neurons between age 15 to 90 years, suggesting a decline with age[95,135]. Since then, a meta-analysis have described that studies with a higher mean age describe higher mean oxytocin levels (in studies with a mean age from 18 years to 72 years), suggesting an increase in oxytocin with age[89]. However, considering the complex changes across ages, indicated by our data, comparing the means of studies is perhaps not capturing the complexity of the change in oxytocin across ages.

That changes in oxytocin levels across ages is complex makes sense if we consider age as a proxy for the psychological and physiological changes across the lifespan[93]. Differences across the lifespan have also been suggested for oxytocin receptor expression and effects of intranasal oxytocin[93]. Changes in the oxytocin system with age could make sense if oxytocin is considered important for adapting to changes - the oxytocin system would then adapt to changes during development or across ages. If we consider the oxytocin levels in the two previous studies in children and our study in chronological order, they could describe complex changes, with high levels in the youngest children, a decline after 20 months, an increase until around 16

years, followed by a decrease in oxytocin levels after 16 years. In early life, the bonding and closeness with parents is important, which could correspond to the high oxytocin levels[96]. The decrease after two years of age and stable levels during early childhood could reflect a movement away from the parents and towards relationship with friends. The increase during adolescence could reflect an adjustment to the reproductive demands with romantic relationships and pubertal hormonal development. However, this is speculative, and as highlighted, by previous calls for oxytocin in relation to age, studies in both endogenous oxytocin levels, oxytocin receptor expression, genetic studies and intranasal studies could help provide understanding of the oxytocin system across the lifespan[93,94]

We did not find a sex or a sex-age interaction in our sample. However, the functions of oxytocin are perhaps important for males of females specifically in relation to, for example, reproduction that are of important in some stages of development, but not across all ages. It could therefore make sense to explore sex differences at different developmental stages, where the demands for reproduction or other aspects diverge for females and males.

8.2.2. Study II – discussion

Study II: The role of oxytocin in family processes in the presence and absence of pediatric OCD

8.2.2.1. Is child oxytocin associated with family accommodation in children with OCD?

Our study did not find support of a relationship between child oxytocin and aspects of family accommodation (participation and changing routines). The previous study by Lebowitz et al. found a relationship between family accommodation and child oxytocin in the context of anxiety disorders[57]. More specifically, they found a negative relationship between child oxytocin and a "total score" on family accommodation[57]. This relationship seemed driven by a negative relationship between child oxytocin and a factor describing "parent participation", but not "parent modification"[57]. In addition, the study in child anxiety disorders found a negative relationship between child oxytocin and parental distress related to accommodation and consequences when the parents do not accommodate[57].

It is difficult to compare the findings between our study, and the study in child anxiety disorders, as the samples and the scales used to measure family accommodation differ. Lebowitz et al. used the Family Accommodation Scale Anxiety (FASA)[34], which is developed for child anxiety disorders[57], whereas we used the FAS-SR[35], which is validated for OCD. The total accommodation scores in the FASA and FAS-SR vary in the number and phrasing of items, which reflects that family accommodation is not describing the same concept and describe parent behaviors across disorders[57]. This is important, as variations in the parent behaviors related to accommodation could be related differently to child oxytocin. Accommodation in separation anxiety could be centered around parent proximity - staying close to or sleeping next to the child, which could be related to oxytocin given previous findings from non-clinical samples between oxytocin and touch in parent child interaction[82]. However, in OCD, accommodation could take various forms that are perhaps less clearly linked to proximity or oxytocin e.g., a parent buying excessive amounts of soap or

cleaning the house in specific ways. Thus, future studies may benefit from examining child oxytocin in relation to more specific accommodating behaviors instead. Regarding the previous findings of a negative relationship between child oxytocin and distress and consequences in child anxiety disorder[57], this could not be addressed in our sample, as the FAS-SR does not include these items. However, given the significant findings from child anxiety disorders, this could be interesting aspects to address in future studies.

8.2.2.2. Is child oxytocin associated with family conflict, cohesion, and parental stress in children with OCD and controls?

When analyzing all children, we found an interesting pattern for the fathers: in children with OCD, child oxytocin was positively related to child-reported family conflict and negatively related to father-reported levels of parental stress. In contrast, for children without a psychiatric disorder, the directions were reversed and of larger magnitude. It is important to remember, that the study is cross-sectional, and we cannot determine whether child oxytocin is affected by levels of conflict or stress, whether child oxytocin "drives" levels of conflict or stress, whether these associations are bidirectional and whether the results are affected by confounding.

With this caveat in mind, we could speculate that differences could be explained by "family conflict" and "parental stress", being broad constructs, that may reflect different phenomena in families of children with OCD compared to families of controls. In OCD, family conflict could perhaps revolve around the child in distress and be more "attachment-related", than conflict in families of children without a psychiatric disorder. As described, children can react with coercive and disruptive behavior if parents do not provide accommodation, and children may try to force the parents to accommodate[27]. Thus, in families of children with OCD, conflict could reflect the families being involved in or near the child's OCD. In families of children with no psychiatric disorder, family conflict could describe another family environment, where conflict could be more related to non-attachment-related issues and everyday challenges like the household tasks, homework, and demands of caring for multiple children etc. However, it could also be, that family conflict and parental stress reflect the same, but that they are differently associated with child oxytocin, when the child has OCD.

In children with OCD, child oxytocin was positively associated with the child-reported level of conflict in their families and negatively associated to the level of father-reported parental stress. Thus, the lower the level of child oxytocin – the lower the level of conflict, but the higher the level of stress in the fathers. These findings are interesting in relation to the previous mentioned aspects on accommodation: (1) In child anxiety disorders, child oxytocin was negatively related to parental participation in accommodation[57], (2) If parents resist to accommodate, the child can react with disruptive behavior, which can cause conflict in the family[25], and (3) Accommodation can be stressful for the parents both when they participate and when they try to resist to accommodate[25]. Our findings could be interpreted in this context. *If low child oxytocin* is related to

more participation in accommodation (1), then it would make sense that the *level of conflict is low* (because the parents *do* accommodate) and the children do not have to react with disruptive behavior (2), but the *level of stress is high* because it is stressful for parents to accommodate (3). In the analysis of family accommodation using the FAS-SR, we did not measure components of family accommodation such as consequences or participation specifically, but the results on family conflict and parental stress could indicate that examination of more specific components of family accommodation could be relevant.

Interestingly, for the mothers, we did not find a relationship between child oxytocin and child-reported family conflict, cohesion, or mother-reported parental stress. Family conflict was reported in relation to the overall family and not related to either the mother or father. Therefore, it is surprising that we did not find the same association for family conflict in the models with the mother's information included as for the fathers information included. This could indicate that these findings are specific to fathers, and thus only when additional information from fathers were "present" in the model (so including father's oxytocin and fathers parental stress level), then child oxytocin is related to family conflict. The findings for fathers could also be discovered by chance (a Type I error). If the findings are true and they are specifically related to the fathers, it could be hypothesized that fathers' behaviors change when child OCD enters the family system, and the changed paternal behavior could then have different effect on child oxytocin levels. However, "the reverse" could also be hypothesized - that child oxytocin could be related to child behaviors that cause stress for fathers in the presence of pediatric OCD. Again, the cross-sectional nature of the study makes it impossible for us to conclude on the directions.

8.2.2.3. Are child and parent oxytocin levels associated?

We found a positive association between child oxytocin and mother and father oxytocin levels. Our findings align with the three previous studies, that found positive associations between child and mother oxytocin[84,85], and between child and parent oxytocin (including mothers and fathers oxytocin in the same statistical analysis)[83]. Our study is the first to show an association between child and father oxytocin levels in an analysis without mothers included. In addition, our study is the first to demonstrate an association between parent and child oxytocin levels in children older than 6 years. Previous studies included 4- to 6-month-old infants[83,84] and 6 year old children[85]. The positive relationship we found between child and parent oxytocin levels is interesting, as children's interactions with, and physical distance to their parents change throughout development. A study in children between 5 months and 7.5 years of age found decreasing levels of oxytocin in this age range, suggesting that the decrease in oxytocin reflects less direct interaction between children and mothers as the children gets older[96]. Our results indicate that the oxytocin systems of children and parents continue to interact with age. As mentioned in the introduction (Section 4.2.2.2), the interaction between child and parent oxytocin systems has been suggested to be driven by parent-child behaviors[83]. Thus, the results could reflect that despite changes in the children and parents' interactive behavior across development, they still affect each other's oxytocin systems. However, the associations could also be due to

shared genetics between children and parents, explained by a combined role of environment and genetics, or by epigenetics, which has been suggested as important in previous studies[136,137]. We did not observe any differences in the correlations between child and parent oxytocin levels in children with OCD versus controls, which could have been expected if pediatric OCD influences parent-child interactions and, in turn, oxytocin levels. However, given our sample size, this null finding should be interpreted with caution.

8.2.3. Study III - discussion

Study III: Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

When discussing the results from Study III, it is important to note that the naming of the interaction patterns in the results section is already subject to interpretation. Therefore, it is important to be aware, that this study is highly exploratory. In addition, we accepted a high risk of Type I errors in Study III, which increases the risk of false positive findings. In the discussion below, we will highlight some areas we found interesting in our interpretation of the results.

8.2.3.1. Are child oxytocin levels associated with computationally derived parent-child interaction patterns

Child oxytocin levels were positively associated with interaction patterns we named "*overinvolved interaction"* (PC5) and "*emotional interaction*" (Archetype 3), and negatively associated with "*negative-low support interaction"* (Archetype 1).

The positive association between child oxytocin and "*overinvolved interaction"* (PC5) could be explained by this interaction describing mother and children *engaged* in the interaction. The dyads with a high score on the "*overinvolved interaction*" were characterized by children, who participated in and verbalized the solving of the puzzle and did not show avoidance (positive load of child narration and negative load of avoidance), and mothers who were more involved than necessary and did not provide verbal reappraisal (positive load of mother intrusiveness and negative load of verbal reappraisal). This could be interpreted as involving an *engaged* child in a mother-child interaction. Two previous studies examined child oxytocin in relation to engagement in a mother-child interactions. Feldman et al. found a positive association between 4-6 months old infants salivary oxytocin levels and infant social engagement during an "play- and touch" interaction with their parents (infant social engagement described as composite score based on the time the infant looked at the parent and in the same time expressed positive affect or positive vocalizations)[83]. However, this study examined children interacting with either their mother or father, but combined the mothers and fathers in the analyses to describe "parents"[83]. Lebowitz et al. examined older children with anxiety disorders and found a positive association between child oxytocin response (i.e., change in oxytocin levels) and engagement during a mother-child interaction planning "their best day ever"[82]. The Lebowitz study used the same coding manual as the Feldman study in infants[83], but created an engagement score by averaging the behavioral codes: persistence, involvement, and cooperation[82]. Therefore, this study measured the dyadic mother-child engagement and not only the engagement of the child. Even though our findings could be interpreted in line

with the two studies by Feldman[83] and Lebowitz[82], it is important to note, that the definition of engagement and the type of interaction varies across studies, which makes comparison difficult.
The association between child oxytocin and the archetype "*emotional interaction*" (Archetype 3) could be explained by this describing a *warm and supportive interaction* (positive loadings of dyadic emotional warmth and support sensitivity). With reservation for different definitions, our finding of a positive association between child oxytocin and "*emotional interaction*" (Archetype 3) could resemble the previous research in younger children, showing a positive association between child oxytocin and mother-infant affect synchrony[83] (described in the Section 4.2.2).

There are two interesting things to highlight in relation to the negative association between child oxytocin and the *negative-low support interaction*" (Archetype 1). **First**, the archetypes "*negative-low support interaction*" (Archetype 1) and "*emotional interaction*" (Archetype 3) describes the same data and could be interpreted as describing the same spectrum but "reversed". When examining the original variables above the 75th percentile of the "*negative-low support interaction*" archetype, this archetype could be interpreted as an interaction with a child not in control, showing aggression and seeking reassurance from the mother, a mother showing tension and not support sensitivity, and a dyad low in dyadic warmth with low levels of positive expressions. The archetype could be considered as describing an interaction with low levels of parental sensitivity, which could explain a negative association with child oxytocin. **Second**, it is interesting to consider, that we did not find an association with the "*distant interaction"* (Archetype 2), which show many similarities with "*negative-low support interaction*" (Archetype 1). A dyad who scored high on the variable we named "*distant interaction",* could be a dyad displaying low on emotional warmth, with a child showing aggression but not seeking the mother, and a mother who is not supporting the child and who is not tense. An important difference could be the child reassurance-seeking behavior. In the study by Lebowitz et al. in children with anxiety disorders, child oxytocin was negatively associated with "child distress caused by parents not accommodating"[25]. Our negative correlation between child oxytocin and the "*negative-low support interaction"* could resemble this, as an interaction interpreted as a distressed, aggressive child seeking help from a tense parent who is not providing support to the child in the task.
Interestingly, we did not find a difference between mother-child pairs, where the child had OCD, compared to controls, which could indicate that our findings are relevant to both children with OCD and children without psychiatric illness. However, our null findings should be interpreted with the limited sample size in mind.
We did not find an association between child oxytocin and father-child interaction patterns. This is interesting in relation to the previous findings from Feldman et al., where they found a positive association between infant oxytocin levels and infant social engagement in parent-child interactions including both mothers and fathers[83]. Our findings of a relationship between child oxytocin and interactions describing child engagement in interaction with mothers, but not fathers, could indicate, that results from mothers and fathers should preferably be analyzed separately.

8.2.3.2. Are maternal oxytocin levels associated with mother-child interaction patterns?

We found a relationship between mother oxytocin and multiple mother-child interaction patterns - four of the six principal components and all three archetypes. There are four interesting aspects to highlight. **First**, of the four interactive patterns with a positive association with mother oxytocin, three had a positive load of "child narration", and the last one, Archetype 3, had high levels of child aggression and positive expressions. Such child narration and expressions could be interpreted as describing a child that is engaged in the interaction. With this interpretation, the findings of a positive association with mother oxytocin could resemble the findings from on parental oxytocin and engagement in a study by Feldman et al[83]. The study by Feldman et al. examining infant social engagement in a parent-child interaction, described in the previous section, also explored the relation to parental oxytocin level[83]. They found mother salivary oxytocin levels was positively related to infant social engagement[83]. However, as mentioned above, this study examined "parental" oxytocin combining the values from mothers and fathers. **Second**, we noticed that interactive patterns with *high* maternal intrusiveness in combination with *low* child avoidance was positively associated with mother oxytocin levels. In addition, *low* maternal intrusiveness and *high* child avoidance were also positively associated with mother oxytocin levels. Therefore, the relationship between mother oxytocin and these two behaviors, could be related to the combination of the two behaviors more than the behaviors separately. This could point to maternal oxytocin being related to "sensitive parenting" – intrusiveness could be appropriate when interacting with a child with high avoidance but less appropriate if the child is not avoiding the task. **Third**, the interactions that were positively associated with mother oxytocin could be interpreted as describing engaged maternal behavior without high levels of maternal negative expressions except the "*varied-coping interaction",* which is only described with the maternal code "low maternal intrusiveness". Parent positive engagement has been found to be associated with mothers oxytocin levels in a study in 112 mothers, where parent positive engagement defined as a composite of parent gaze to infant, positive affect, and 'motherese' vocalizations[138]. **Fourth**, the three interactive patterns that were negatively associated with mother oxytocin, all had positive loadings of child aggression, but without a positive loading of child positive emotions. In addition, there were high loadings of maternal negative expressions or negative loadings of maternal positive expressions. Finally, all three interactive patterns showed low emotional warmth.

8.2.3.3. Are paternal oxytocin levels associated with father-child interaction patterns

We did not find associations between father oxytocin and the interactive patterns. This could be due to various causes. It could indicate that the conditions of the task, elicited interactions that were more associated with maternal oxytocin than paternal oxytocin. Previous studies in younger children have shown that mothers and fathers' oxytocin are related to parenting behaviors, but that the oxytocin-related behaviors differ between mothers and fathers[79,80] (described in Section 4.2.2.2). The smaller sample size in the father-child analyses, compared to the mother-child analyses, could also explain why we have significant findings

in relation to mothers, but not fathers. Finally, the different findings for mothers and fathers could also be explained by differences among the children in the mother-child pairs and father-child pairs, which is possible since each child interacted with only one parent, either the mother or father.

8.2.3.4. Does child OCD have an interactive effect on the associations between oxytocin and parent-child interactions?

We found no difference in the association between child oxytocin and mother-child interactive patterns, nor in the relationship between mother oxytocin and mother-child interactive patterns, when comparing children with OCD to the controls. This may suggest that the associations between mother and child oxytocin and the interactive patterns could be consistent in clinical and non-clinical samples. However, it could also be a null finding based on too low power to detect a difference.

Regarding father oxytocin and father-child interactive patterns, the presence of child OCD seemed to affect the results. Specifically, in the presence of child OCD, father oxytocin was positively associated with "*supportive interaction*" (PC1), whereas it was negatively associated in the control group. The "*supportive interaction*" in father-child interactions shared similarities with the "*supportive interaction*" in mother-child interactions, although the variable loadings were not identical. This "*supportive interaction*" was positively associated with maternal oxytocin. If this interaction pattern is viewed as indicative of warm, affectionate, and sensitive maternal parenting, these findings could align with a study on non-clinical infants and mothers[79]. For example, one study showed high levels of maternal affectionate touch during a mother-infant interaction were associated with increased maternal oxytocin levels[79]. In the same study, this pattern was not observed for fathers; instead, father oxytocin levels were associated with higher levels of stimulatory touch[79]. A hypothesis could be that in our non-clinical sample, our experiment does not measure the "normal" oxytocin-activated father parenting, but when a child has OCD and often experience distress, other oxytocin-driven behaviors come into play - and that father's oxytocin, that is related to supportive parenting, is more appropriate and necessary in the situation with children with OCD. However, this is highly speculative and should be explored in well-designed studies.

8.3. Methodological considerations – strengths and limitations

When interpreting the results in this Ph.D. project, it is necessary to be aware of some limitations and strengths in the design. Overall, the sample size estimation of the larger TECTO RCT trial had implications for the sample sizes in the exploratory sub-studies. For example, the sample size was not always suitable for the specific outcomes in the sub-studies. Additionally, when selecting measurements for the sub-studies, we ensured they did not interfere with the RCT or were adapted to align with the RCT visits. This, at times, required compromises in the scope or choice of measurements for the sub-studies. These limitations are embedded in exploratory sub-studies of a larger trial, but they come with the great advantage of allowing us to explore interesting and novel outcomes in a large and well-characterized sample. The section below

provides a discussion of general and study-specific methodological limitations and strengths of this Ph.D. project.

8.3.1. Sample size

The sample size calculation in TECTO was driven by the primary research question of the TECTO RCT that required at least 128 children. The control study aimed to match the patient number, but only 90 controls were included before the end of the trial. The three studies in this PhD had different sample sizes due to varying degrees of missing data and the methodological problems associated with sample sizes were handled differently.

In Study I, the primary aim was to investigate the difference in oxytocin levels between patients and controls. It has been recommended to perform a power analysis based on the smallest effect size of interest and to use effect size distributions specific to a given field or sub-field[139]. With only three previous studies in adults[62–64], we were unsure about which differential estimate in oxytocin levels would be meaningful or sufficient to create field specific effect size distributions. This also means, that even under ideal circumstances, where we could design a study specifically for our research aim, we would be unsure about how to perform a qualified power analysis. With the sample size of patients and controls with available oxytocin levels for analysis, our power calculation showed that we could reliably detect a Cohens *d*=0.40. Null-findings in our study could thus either be true null-findings or due to the true effect size being smaller than Cohens *d*=0.40. A common limitation is the lack of information on effect sizes, which could have enhanced our interpretation of findings, especially, in relation to the power calculation.

In Study II, we conducted a power analysis based on our primary objective of investigating an association between child oxytocin and family accommodation. Again, with only one previous study conducted in a different population and using another measure of family accommodation, we were unsure of the minimal clinically relevant effect size and only provided the effect size (eta squared) that we could reliably detect. In Study III, we did not perform a power calculation as this study was highly exploratory. In both study II and III, we did not use pre-registration or Bayesian statistics, which could be considered as limitations. Knowledge from Bayesian statistics could provide information on the null-findings; however, the use of Bayesian statistics also showed us important limitations with Bayesian statistics (especially the problems with prior sensitivity). Providing effect sizes in the two studies could be a way to enhance the interpretation and usability of the findings and the lack of effect sizes is a limitation in the studies.

8.3.2. Risk of Type I errors – controlling the family-wise error rate

A Type I error rate describes the probability of rejecting the null hypothesis when it is true, and a Type II error rate describes the probability of failing to reject the null hypothesis when it is false[139,140]. In studies with multiple hypotheses tests, it is important to consider the family-wise error rate, which is the Type I error rate for all hypotheses being tested, i.e., as the number of tests increases, the Type I error rate increases[140]. In

Study I and II, we chose not to correct for multiple testing thereby accepting a risk for Type I errors. This was based on the studies being exploratory and since we considered the number of tests in these two studies relatively limited. However, that we do not complete mathematical correction for multiple testing could be considered a limitation as we at risk of providing false positive results to a field, that has already had problems with replicating findings (see Section 4.2). In Study III, we were unsure of the number of interactive patterns the PCA and archetype analyses would yield. Therefore, we were unsure of the number of tests, and we controlled for the family-wise error rate using the Benjamin Hochberg approach.

8.3.3. Salivary oxytocin as a proxy of central oxytocin levels

As mentioned in the introduction (Section 4.2.3.1), the use of peripheral oxytocin levels as an indicator of central levels is an area of debate. We chose salivary oxytocin, as a proxy for central oxytocin, since we considered it a feasible measure in a sub-study of a larger trial, where oxytocin was not the primary focus. A more accurate alternative could have been measuring CSF oxytocin. However, we found this infeasible in a study where a lumbar puncture was not clinically indicated. We did not find the use of plasma oxytocin relevant or feasible due to the smaller correlation between plasma and CSF oxytocin compared to saliva and CSF oxytocin. A limitation is our use of single-point and baseline measurements. One study found that levels of salivary oxytocin were inconsistent across different days[91]. A meta-analysis found that correlations between peripheral oxytocin and central oxytocin were better after stimulation (e.g., by an interaction) than baseline measures[87]. Therefore, to increase confidence in our saliva results as accurately reflecting the oxytocin levels for a given period, the study could be improved by measuring oxytocin levels at multiple time points. To strengthen our trust in salivary oxytocin, as reflecting central levels, we could have explored oxytocin reactivity or oxytocin sampled after an experimental manipulation (e.g., changes from baseline to post interaction).

8.3.4. Assessing pubertal stage

We assessed pubertal stage as a self-reported measure. The use of self-reported pubertal stage, without clinical examination, is widely used but the reliability of self-assessment has been questioned[141]. Studies have recommended that the choice of measure should be guided by the research question and the benefits and risk of each method[141,142]. The gold standard, when measuring pubertal stage, is clinical assessment[142]. One study investigated the acceptability of clinical assessment and found low acceptability of children participating in research, which can affect feasibility of recruitment[142]. Self-assessment is perhaps more feasible and less labor-intensive, but has been suggested to have a higher risk of misclassifications[141,142]. However, a meta-analysis investigating agreement between clinical assessment and self-reported pubertal stage found moderate to substantial overall agreement with higher agreement for some pubertal stages than others (most accurate for Tanner Stage 1 and 5)[143]. We chose self-report as we considered this method feasible, faster, and less invasive in a trial with children with OCD, where puberty was not the main focus. However, we acknowledge that self-assessment could perhaps provide more detailed information.

8.3.5. Estimating menstrual cycle phase

The estimation of menstrual phase based on self-reported first day of menstrual blood flow onset, has been subject to criticism[116]. Research has suggest that only the identification of "menses" and early follicular subphases can be validly estimated by self-report[116]. Furthermore, the high variability in the length of menstrual phase adds to the risk of mis-classification[116], which is particularly relevant when studying children, as the length of the menstrual cycle often varies more[144]. However, researchers have also suggested that calculating menstrual phase from self-reported first day of last menstruation can be used when budget or other constraints do not allow for more precise methods (e.g. sonography or measurement of hormones)[116]. We considered self-reported first day of last menses feasible in our study. However, it could have been improved by obtaining this information in an interview instead of a self-report questionnaire and could benefit from questions on the stability of length in menstrual cycle.

8.3.6. Cross-sectional design

We used a cross-sectional design in all three studies, which limits our ability to make inferences about causality and complicates our interpretations of variables across development and the directions of associations. As an example, in Study I, we investigated the associations between oxytocin and pubertal stage and age. The cross-sectional design of Study I, makes inferences across development difficult, and we can only compare participants with different ages or pubertal stages. Here, a study with multiple measurements of oxytocin within the same participant, across development, could clarify how oxytocin change in individuals across development or with age. In Study II and III, the cross-sectional design limits our ability to conclude on direction of associations. For example, we do not know whether high oxytocin in the mothers leads to more supportive interactions, whether more supportive interaction leads to higher mother oxytocin or whether both directions are relevant. In all our studies, bidirectional effects and influence of confounding is plausible, which complicates the analyses and interpretations. Using experimental design, with a controlled setting of oxytocin measurements before and after an interaction or using a design with intranasal oxytocin administration could provide information on the directions of associations.

8.3.7. Clinical assessment of the children and comorbid disorders

All children and adolescents in the TECTO trial were thoroughly assessed with K-SADS for their diagnostic status and the clinical picture was discussed with a specialist in child and adolescent psychiatry (for controls only in case of doubt). In addition, all assessors completed training in K-SADS and received supervision. This is a strength in especially Study I, where the comparison of oxytocin between children with OCD and controls was our primary aim. We considered the sample of children with OCD a clinical representative group with multiple comorbidities, which is common in children with OCD[8]. However, comorbid disorders can cause variation in oxytocin levels (e.g., found in child autism, separation anxiety and eating

disorders[4,57,133,134]) thus comorbid disorders could potentially cause heterogeneity in the group of children with OCD.

8.3.8. The sample of parents

In Study II and III, we included information on aspects of the family and parent-child interactions from mothers and fathers in separate models, which we consider a strength compared to combining data mothers and fathers. By including information from mothers and fathers separately, we can examine the relationship with child oxytocin specially for mothers and fathers. However, it is important to remember, that we do not examine the total amount of, for example, family accommodation for a child. A child could be involved in high levels of family accommodation by a father, in which case the analysis of child oxytocin in relation to the family-accommodation reported by the mother would not reflect the family accommodation for this specific child. This is not a limitation, but merely not the question we asked. However, whether the total amount of family accommodation is linked to child oxytocin could be interesting for future studies.

We lacked information on several factors that could improve our understanding of the association between parent oxytocin and aspects of the family and parent behaviors such as the parents' psychiatric and somatic status. Such factors could affect the family measures and parental oxytocin. Thus, the lack of information complicates our analyses and interpretation of the results.

In Study III, we analyzed each child's interaction with either their mother or father. We did therefore not have information on the same child interacting with both the mother and father, which limits our ability to draw conclusions on differences between mother-child and father-child interactions within the same family.

8.3.9. The measures of family aspects and parent-child interaction

In Study II, we used information on questionnaires reported by children and parents. The use of questionnaires can introduce self-report bias[145]. Especially our use of child-reported family conflict and cohesion could be an area of concern. The Family Environmental Scale (FES)[44] has not been validated in children below 11 years of age and the manual describes a pictorial version that could be used for children between 7 and 11 years. However, we used the same version for all children and adolescents (8 to 17 years both inclusive) and their parents. The FES consists of 90 items which could be difficult to answer for children with age-related limited reading abilities. If responses are reported by the child without support from the parents, there could be a risk of misunderstanding the items. If parents provide help, there could be a risk of bias as the children could respond in a manner, they think would be acceptable for the parents. For the parent reporting, there could be a concern of social desirability bias, where the participants' answers are influenced by the general social desirability of the investigated topic[145]. For example, the PSS includes the item:" If I had it to do over again, I might decide not to have child(ren)"[36], for which the answers could perhaps be affected by what the parents think is appropriate.

For the subscales on the FES used in Study II, we found an internal reliability (Cronbach-α), i.e., reflecting how consistently different items within the FES measure the same construct, of 0.703 for conflict and 0.63

for cohesion. The Cronbach-α reported in the FES manual is higher (0.78 and 0.78, respectively)[44]. However, to our understanding, these were based on both adolescent and parent reports. The FES and its internal reliability has been subject to criticism[146]. One study found low to moderate internal reliabilities for most subscales but better internal reliability for conflict (Cronbach-α=0.72), cohesion (Cronbach-α=0.67) and moral-religious emphasis (Cronbach-α=0.71)[146]. In summary, the use of questionnaires holds a risk of bias and for us perhaps especially for the reporting by children. One way to improve this, could be to include multiple perspectives, e.g., by including both responses from children and parents.

Regarding the FAS-SR used in Study II, the score "total family accommodation" could suggest that this score measures how much family accommodation takes place in the family. However, this scale measures how many different accommodation behaviors the parents engage in and how many days for each behavior in the preceding week. A parent engaging in 10 different accommodating behaviors, but only one day per week (and perhaps on the same day), will reach a score of 10. A parent engaging in only one accommodating behavior, but every day (and perhaps all through the day), will reach a score of 4.

In Study III, we used behavioral observations with the TEC-M coding manual[119] in an attempt to reduce the risk of the sources of bias from self-reporting and to explore interactions between children and parents[145]. However, the TEC-M has been developed to assess children's emotion regulation skills within a parent participating setting[120] and not specifically for examining parent-child interactions. Our use of behavioral observation holds some interpretation for example in the naming of the interaction patterns. To qualify the interpretation of parent-child interaction patterns, three researchers (two psychologists and one medical doctor within psychiatry) interpreted the results from the parent-child interactions and named the interactions in collaboration after thorough discussion of the interactions patterns. A limitation regarding the TEC-M coding is, that we did not assess interrater reliability; however, consensus coding was performed.

8.3.10. Statistical considerations

It is important to consider our studies as exploratory, that were inspired by, but not replications of, previous studies. Although previous studies have tested similar hypotheses (e.g., oxytocin level differences in patients with OCD compared to controls and child oxytocin in relation to family accommodation), there are large differences between study populations and methods used to measure, e.g., oxytocin and family accommodation. Regarding reproducibility, we have tried to thoroughly describe our methods on the analysis of oxytocin concentrations and the statistical procedures. We plan to make our code and data available on the Zenodo database, which will enable other researchers to reproduce our results. However, this is not completed, yet. We addressed the robustness in our results by including NHST and Bayesian statistic in Study I, which increases our certainty in our results as both techniques yielded findings in the same directions.

We chose to remove outliers in all three studies. The outliers could be explained by errors in the procedures of sampling or measuring oxytocin; however, it could also be due to natural variation. We tried to explored explanations for the outliers in the studies, but we did not find explanations with the data we had available. The analyses could be improved by conducting sensitivity analysis both with and without outliers included.

We addressed the uncertainty of the power calculation with Bayesian statistics to provide information on the null findings and consider the use of Bayesian statistics a strength in study I. However, Bayesian statistics are complicated and have important limitations[104], for instance that the results can depend on the choice of prior[104]. To address this limitation, we pre-registered our choice of prior, and tested the robustness in the Bayesian statistics by including different priors in sensitivity analyses. These analyses showed that our results were dependent on the priors we used and were therefore not robust.

In Study III, we used machine learning to explore patterns of parent-child interactions. It is important to consider this highly exploratory, as we cannot know whether similar patterns would appear from other datasets and the naming and interpretation of the interaction patters holds much subjectively. However, we consider this data-driven approach a valuable method in the process of exploring associations to generate future hypotheses.

8.3.11. Summary of strength and limitations

In summary, this PhD holds strengths and limitations. The limitations include some measures we used (e.g., salivary oxytocin instead of central oxytocin levels, the uncertainty in self-reports), factors embedded in the study design (the cross-sectional nature of the study and the sample size), and the lack of information that could qualify our findings such as parental psychopathology. This study also has imports strengths, which include a large, well-described sample that allows exploration of novel outcomes. Additionally, the inclusion of mothers and fathers, children with OCD and children without psychiatric disorders, information on self-reported and observational data regarding the families and thorough examination and inclusion of the covariates - age and pubertal stage.

8.4.Overall discussion of thesis and possible directions of future research

The overall aim of this PhD project was to explore the relevance of oxytocin in pediatric OCD. Examining differences in oxytocin levels between children with OCD and controls seemed like an important but perhaps a too simple first step. Finding differences in oxytocin levels, could have implications for our understanding of the possible relationship between oxytocin and OCD and direct future studies and potentially clinical practices. However, in Study I, we found no significant group differences in oxytocin levels between children with OCD and children without a psychiatric disorder. The lack of overall group differences is perhaps not surprising given the heterogeneity in OCD phenomenology and oxytocin's various functions. We

included all children with OCD in the same "OCD group", but studies have pointed to symptom dimensions of OCD[8]. A recent study of 1,366 children, adolescents, and adults with OCD found 13 symptom dimensions[7]. The issue of OCD heterogeneity was already discussed by Leckman et al., suggesting that oxytocin could perhaps be linked to some, but not all subtypes of OCD[5]. Leckman's observation of behaviors in conditions with high oxytocin[5], could support a link between oxytocin and some subdimensions of OCD but not all. As an example, the observation in animal studies of auto-grooming behavior after central administration of oxytocin could support a link to the "contamination" symptom dimension of OCD. Future research could explore whether oxytocin is related to specific symptom dimension of OCD instead of overall group differences. This could be interesting as different biological underpinnings of subtypes of OCD could improve our understanding of symptoms dimensions and perhaps point to differences in how these should be treated. However, this is speculative and require much exploring before hypotheses can be generated and we can understand possible clinical implications. If future studies examine oxytocin in relation to OCD symptoms dimensions, the sample size should be considered carefully. In our sample of 130 children with OCD, we may very likely have too few to explore the association with subtypes especially given the high variation in oxytocin.

We also considered whether oxytocin was related to processes that are common within OCD, specifically family accommodation, instead of overall group differences. Here, we looked towards the child anxiety literature[57], with a hope to extend the findings of a link between oxytocin and family accommodation. The findings on child oxytocin and family accommodation in the context of child anxiety, has contributed to improved interventions with a higher focus on reducing family accommodation and improving other ways parents can help regulate child distress[32]. We did not find a relationship between child oxytocin and family accommodation in our sample of children with OCD. This could very like be due to difference in scales used in the Lebowitz study[57] and our study. Another thing to consider is that, even though the process of family accommodation has been found relevant across multiple disorders, it may likely describe different accommodating behaviors between disorders[57]. Therefore, it is perhaps unlikely that oxytocin would be similarly related to family accommodation across disorders. Even within OCD, family accommodation is perhaps describing multiple different interactions between children and parents, that might be differently or not all related to oxytocin. Future studies could address this by exploring oxytocin in relation to more specific aspects of family accommodation instead of a total score combining information on different behaviors.

We worked with the family accommodation total score as a measure of how much family accommodation takes place in the family with the hypothesis that low levels of child oxytocin would be related to more family accommodation. However, our work with the FAS-SR[35] made us realize that the total score provides information on *how many* types of behaviors *and how much* each behavior takes place. An interesting area of future research could be the creation of a scale of family accommodation that uses a structure like the CY-

BOCS[110] thereby measuring severity based on: time used, functional impairment and distress related to the accommodation.

When trying to increase our understanding by also examining controls, we found that the results for fathers were particularly interesting. In Study II, our results indicated that child oxytocin was differently associated with information from fathers depending on whether the child had OCD or not. Our findings in Study III also indicated that father's oxytocin was differently associated with one father-child interaction pattern depending on whether the child had OCD. In both studies, we highlighted that these results are exploratory and should be explored further and replicated.

We did not aim to test oxytocin level differences between fathers of children with OCD to controls; however the descriptive statistics in study II showed higher oxytocin levels in fathers with OCD (but only borderline significance, see Section 7.2.1). We did not find difference in oxytocin levels between mothers of children with OCD and mothers of children without a psychiatric disorder. Interestingly, the oxytocin levels of fathers of children with OCD seemed to resemble the oxytocin levels of the mothers, but the oxytocin levels of the fathers of the control group was lower. In study III, we found indications of a different relationship between father oxytocin and father-child interactive behaviors, when the child did or did not have OCD. Differently, mother's oxytocin seemed similarly related to mother-child interactive behaviors when the children had or did not have OCD. In total, the high oxytocin levels in fathers of children with OCD could be related to the interaction effects of OCD on the relationship between father oxytocin and interactive patterns.
The findings regarding the fathers, made us consider what happens to the fathers when OCD enters the family system. Future studies could explore changes in fathers' behavior and OCD levels with a longitudinal design both before and after the child is diagnosed with OCD, thereby informing on the directions of a possible relationship. However, such studies would be time consuming and expensive. We suggest that our results show differences between mothers and fathers, but we did not include the same children interacting with both their mothers and fathers. Future studies could include a child and both parents thereby facilitating the comparison.

Previous studies have shown that paternal behaviors are important in OCD and speculated that we perhaps, in general, tend to believe, that the inclusion of one parent in therapy sessions will change the overall parent behavior[147]. However, differences in what drives behaviors of mothers and fathers or what affects their behaviors could have clinical implications. Studies that inform how parents should be provided with information on disorders and how parents are involved in treatments are often guided by studies including only mothers or combining information on mothers and father to a "parent" score[148]. Our study could indicate that fathers and mothers should perhaps be understood differently in how they are affected by their child's OCD and/or how they affect the child with OCD. Studies examining differences between mother and fathers' behaviors and what drives these behaviors could provide a more nuanced understanding of parents in pediatric OCD that could help improve treatments.

9. Conclusion

The aim of this thesis was to examine the relevance of oxytocin in pediatric OCD. We explored this in three studies examining both children with OCD and children without a psychiatric disorder.

In study I, we found that oxytocin change with age and pubertal development in both children with OCD and controls and the relationship seemed non-linear. We did not find a group difference in salivary oxytocin levels. We investigated whether oxytocin might play a more specific role in pediatric OCD in study II but found no evidence for an association between child oxytocin and family accommodation. We also investigated oxytocin's role in more general non-OCD related parent-child interactions. We found that children's oxytocin levels were associated with mother and father oxytocin levels, and that this did not differ between children with OCD and children without a psychiatric disorder. We found that oxytocin was related to family conflict and parental stress in relation to information from fathers in Study II and that the relationship was different for children with OCD and controls. In study III, we found that oxytocin was related to specific types of parent-child interactions of both mothers and fathers, but for fathers the relationship was dependent on whether the child had OCD. The associations between child and mother oxytocin and mother-child interaction patterns were not different in children with OCD compared to controls.

In summary, the three studies of this thesis do not provide a clear understanding of oxytocin's role in OCD but highlight interesting and more general aspects related to developmental changes in oxytocin during childhood and adolescence, and a relevance of oxytocin in parent-child interactions with differences in the associations with mothers and fathers. These findings are relevant for the understanding of oxytocin's functions when children grow older, and for the understanding of oxytocins involvements in interactions in the father-child and mother-child dyad. In future studies, exploration of associations between child oxytocin and OCD symptom dimensions, examination of child oxytocin's relation with more specific components of family accommodation, and a focus on differences between mothers and father's behaviors and their associations with oxytocin appears relevant to investigate in pediatric OCD.

10. References

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11. Abstract English

Background: Pediatric obsessive-compulsive disorder (OCD) is a common disorder that often causes high levels of distress for children and their families. One challenge is family accommodation in which parents change their parenting behavior when attempting to relieve the child's OCD related negative emotions. However, family accommodation often maintains and reinforces OCD. Oxytocin, a neuropeptide involved in parenting behavior and anxiety, has been suggested to play a role in psychiatric disorders including OCD.

Aims and objectives: The aim of this thesis was to examine the relevance of oxytocin in pediatric OCD. The objectives of the three papers were to: I) Examine differences in salivary oxytocin between children with OCD and children without a psychiatric disorder, and to explore covariates with a focus on age, pubertal stage and sex; II) Examine the association between child oxytocin and family accommodation, family conflict, family cohesion, parental stress and parent oxytocin levels; III) Explore the association between child, mother and father oxytocin and parent-child interaction patterns.

Methods: Baseline data from 130 children with OCD and 90 age- and sex matched children without a psychiatric disorder (controls) from a larger OCD trial (the TECTO trial). Saliva samples for measuring oxytocin were collected from children and parents and analyzed using enzyme-linked immunosorbent assay (ELISA). In Study I, we used null hypothesis significance testing (NHST) and Bayesian statistics to explore oxytocin level differences between children with OCD and controls and to explore relevant covariates. In addition, we used a machine learning technique (random forest analysis) to explore nonlinear relationships. In Study II, we examined the relationship between child oxytocin and mother- and father reported family accommodation in children with OCD. We used data from children with OCD and controls to explore associations between child oxytocin and child-reported family conflict and cohesion, the mother- and father-reported parental stress, and mother and father oxytocin levels. Additionally, we explored interactive effects of child OCD on these relationships. In Study III, we used behavioral observations from videos of parent-child interactions and examined parent-child interactive patterns using two machine learning approaches – principal component analysis and archetype analyses. We explored the associations between parent-child interactive patterns and child, mother and father oxytocin, and interactive effects of child OCD status.

Results: We did not find a difference in oxytocin levels between children with OCD and controls. In both children with OCD and controls, oxytocin was associated with age and pubertal stage and the relationship seemed to be non-linear. We found no association between child oxytocin and family accommodation in pediatric OCD. When we examined all children, we found a relationship between child oxytocin and family conflict and parental stress in models including fathers, and child OCD status had an interactive effect on this relationship. Child oxytocin was related to mother and father oxytocin regardless of child OCD status. Child oxytocin and mother oxytocin was associated with multiple mother-child interactive patterns. Child and father oxytocin was not associated with father-child interactive patterns in models of both children with OCD and controls. However, when including OCD status, one interaction pattern (father-child supportive interaction) was related to father oxytocin, and this relationship differed for children with OCD and controls.

Conclusions: The studies of this thesis are the first to examine differences in salivary oxytocin levels and parent-child interactions in children with OCD compared to children without a psychiatric disorder. This thesis does not provide a clear understanding of oxytocin's relevance in OCD. However, the results indicate developmental changes in oxytocin levels during childhood and adolescence, and support oxytocin's association with parent-child interactions. Future studies could explore oxytocin's relation to more specific symptom dimensions of OCD, and specific components of family accommodation. In addition, a focus on differences between father's and mother's interaction with their child, what causes parental stress and how this is related to child OCD and oxytocin could be relevant to investigate.

12. Abstract Dansk

Baggrund: Pædiatrisk obsessiv-kompulsiv tilstand (OCD) er en almindelig tilstand, der ofte påvirker børn og deres familier meget. En udfordring i familier med OCD er familietilpasning, hvor forældre ændrer deres adfærd i forsøget på at mindske deres barns ubehag. Familietilpasning kan dog vedligeholde og forstærke barnets OCD. Oxytocin er et neuropeptid, der både har betydning for relationen mellem forældre og børn, og betydning for angst. Det er også blevet foreslået af betydning for flere psykiatriske tilstande inklusiv OCD.
Formål: Formålet med dette Ph.d. projekt var at undersøge betydningen af oxytocin hos børn med OCD. De specifikke formål med de tre studier var: I) At undersøge om børn med OCD og børn uden psykiatriske tilstande har forskellige niveauer af oxytocin målt i spytprøver, samt at eksplorere betydningen af kovariater med et fokus på alder, pubertet og køn; II) At undersøge om børns oxytocinniveau er associeret med familietilpasning, konflikt og samhørighed i familien, niveauet af forældrestress samt forældrenes oxytocinniveauer; III) At eksplorere relationen mellem mønstre af forældre-barn interaktioner og oxytocinniveauer hos børn, mødre og fædre.
Metode: Vi anvendte baseline data fra 130 børn med OCD og 90 alder- og køns-matchede børn uden en psykiatrisk tilstand (kontroller) fra et større OCD projekt (TECTO). Spytprøver til måling af oxytocin blev indsamlet fra forældre og børn. Koncentrationen af oxytocin blev analyseret enzymbundet immunosorbent test (ELISA). I studie I anvendte vi test af nulhypotesens signifikans og Bayesiansk statistik til at undersøge om oxytocinniveauer er forskellige hos børn med OCD sammenlignet med børn uden OCD, samt til at undersøge betydningen af kovariater. Vi anvendte maskinlæring (random forest) til at undersøge ikke-lineære sammenhænge. I studie II undersøgte vi sammenhængen mellem børns oxytocinniveauer og familietilpasning rapporteret af mødre og fædre til børn med OCD. Derudover anvendte vi data fra børn med OCD og kontroller til at undersøge sammenhængen mellem børns oxytocin og familiekonklift og -samhørighed rapporteret af børnene, forældrestress rapporteret af mor og far, samt mor og fars oxytocinniveauer. Vi undersøgte også om sammenhængene var forskellige for børnene med OCD og kontrollerne. I studie III anvendte vi adfærdskodning fra videoer af interaktioner mellem forædler og børn. Vi brugte to maskinlæringsteknikker (principal komponentanalyse og archetype-analyse) til at undersøge forældre-børne interaktionsmønstre. Vi undersøgte derefter sammenhængen mellem interaktionsmønstre og børn, mødre og fædres oxytocin, samt om sammenhængene var forskellige for børn med og uden OCD.
Resultater: Vi fandt ikke en forskel i oxytocinniveauer, når vi sammenlignede børn med OCD og børn uden en psykiatrisk tilstand. Hos både børn med OCD og kontroller fandt vi en ikke-lineær sammenhæng mellem oxytocin og alder og pubertetsstadie. Vi fandt ikke en association mellem børns oxytocinniveauer og familietilpasning hos børn med OCD. Når vi undersøgte alle børn, fandt vi en sammenhæng mellem børns oxytocinniveau og familiekonflikt og forældrestress, men kun i modeller, der inkluderede fædre, og disse sammenhænge var forskellige hos børn med og uden OCD. Børns oxytocinniveauer var associeret med mødre og fædres oxytocinniveauer hos både børn med OCD og kontroller. Børn og mødres oxytocin var relateret til mange mødre-barn interaktionsmønstre. Børn og fædres oxytocin var ikke associeret med far-

barn interaktionsmønstre, men når barnets OCD status blev inkluderet var én far-barn interaktion (”støttende far-barn interaktion”) relateret til fars oxytocinniveau. Denne sammenhæng var forskellig hos børn med OCD og børn uden en psykiatrisk tilstand.

Konklusion: Studierne i dette Ph.d. projekt er de første til at undersøge forskelle i oxytocinniveauer i spytprøver samt forskelle i forældre-barn interaktioner hos børn med OCD sammenlignet med børn uden en psykiatrisk tilstand. Denne Ph.d. giver ikke en endegyldig beskrivelse af, hvordan oxytocin kan være relevant i pædiatrisk OCD. Resultaterne indikerer dog ændringer i oxytocin gennem pubertetenog understøtter at oxytocin er af betydning for interaktionen mellem forældre og børn. Fremtidige studier kan undersøge oxytocins mulige betydning for mere specifikke symptomdimensioner af OCD og specifikke komponenter af familietilpasning. Derudover kunne et fokus på forskelle mellem fars og mors interaktion med deres barn, hvad der forårsager forældres stress og hvordan dette hænger sammen med børns OCD og oxytocin være relevant at undersøge.

13. Appendix I of thesis: Study I and Appendix for study I

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The association between salivary oxytocin, age, and puberty in children with and without OCD

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The oxytocin system has been thought to contribute to obsessive-compulsive disorder (OCD). Few studies, only involving adults, have investigated this hypothesis and have found inconsistent results regarding oxytocin system activity and OCD. We investigated whether salivary oxytocin concentrations differed between children and adolescents with and without OCD and qualified our comparative analysis by investigating the possible covariates age, pubertal stage, and sex. Participants included 113 children and adolescents (8–17 years) with OCD and 88 children and adolescents without any previous or current psychiatric disorder and their parents (254 parents included). Salivary oxytocin concentrations were measured in children and parents with enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed using frequentist and Bayesian approaches. We found no evidence of a difference in mean salivary oxytocin concentrations between children and adolescents with and without OCD. Bayesian analysis indicated anecdotal to moderate support for the null hypothesis. We found an association between oxytocin and age and between oxytocin and pubertal stage, which by visual inspection of plots and post-hoc tests indicated nonlinear relationships. We found no association between oxytocin concentration and sex. Our findings do not suggest elevated oxytocin concentrations in pediatric OCD. Nonlinear changes in oxytocin across development show the importance of accounting for hormonal and behavioral changes during puberty.

Keywords Oxytocin, Obsessive-compulsive disorder, Children, Adolescents, Pubertal stage, Age

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Obsessive-compulsive disorder (OCD) is a common and impairing psychiatric disorder[1] marked by persistent obsessions compulsions or both, that are time consuming, cause distress and/or impairment[2,3]. Obsessions are unwanted and repetitive thoughts, images or urges that often cause distress. Compulsions are repetitive behaviors or mental acts that the individual feels driven to perform to suppress obsessions, to follow rigid rules, to feel "complete" and/or reduce distress[2,3]. The biological mechanisms underlying OCD are not fully understood.

Oxytocin, a neuropeptide mainly synthesized in the hypothalamus, has been proposed to play a key role in OCD[4]. OCD-like behaviors in animals and humans have been observed under conditions of elevated oxytocin[4]. For example, injecting oxytocin into the central nervous system of rats and squirrel monkeys induced licking and grooming behavior – possibly analogous to cleaning compulsions[4]. Pregnant and postpartum women, groups marked by elevated oxytocin concentrations, have reported obsessions about and compulsive checking of their child's well-being[4]. Combined with the knowledge that oxytocin receptors are present in brain areas that are important in OCD this led to the hypothesis that high levels of oxytocin may intensify and prolong obsessions, discomfort, and compulsions and that oxytocin levels therefore may be elevated in patients with OCD[4]. This hypothesis has been investigated in three studies so far. The first study of 29 adults with OCD, 23 with Tourette's syndrome, and 31 controls found no differences in oxytocin concentrations in cerebrospinal fluid (CSF) among the three groups[5]. Another study of 14 adults with OCD and 26 controls found no significant difference in CSF oxytocin between the two groups[6]. A more recent study—the largest to date (44 adult patients with OCD and 44 controls) – found significantly higher concentrations of plasma oxytocin in patients with OCD[7]. Studies that investigate oxytocin concentrations in children and adolescents with OCD are needed as OCD often onsets in childhood and oxytocin system functioning changes across the lifespan[8]. Given the invasive and stressful nature of lumbar puncture for extracting CSF and the risk of selection bias in collecting plasma samples (only children without needle phobia would consent), salivary oxytocin is the most viable alternative. Salivary oxytocin has been extensively used in studies on parenting behavior and infant development[9].

Peripheral oxytocin concentrations often show high variability, which can complicate the detection of group differences[10,11]. Thus, studies that identify the sources of this variation and attempt to account for it before performing group comparisons could contribute with important knowledge. In children and adolescents, age, sex, and pubertal stage may account for variation in oxytocin concentrations as major physiological and behavioral changes occur during this stage in life and sex differences in oxytocin have been observed[8,12,13].

Thus, we investigated whether salivary oxytocin concentrations differed between children and adolescents with OCD and children and adolescents without OCD, while controlling for age, pubertal stage, and sex. We also explored whether and how these variables contributed to variation in salivary oxytocin.

Materials and methods

Study design

This is a sub-study of the TECTO trial (Treatment Effects of Family Based Cognitive Therapy in Children and Adolescents with Obsessive-Compulsive Disorder)[14]. The TECTO trial is a randomized clinical trial (RCT) comparing family-based cognitive behavioral therapy with family-based psychoeducation and relaxation training in 130 children and adolescents with OCD[14] combined with a case-control study including 90 age- and sex-matched controls without current or previous psychiatric diagnoses (described in this paper as "children and adolescents without OCD"). In the present study, we used baseline data only. Data collection started July 2018 and finished September 2022.

The TECTO trial complies to the Helsinki Declaration[15] and was approved by the ethics committee (Scientific Ethics Committees for the Capital Region of Denmark) (H-18010607). The trial was approved by the Knowledge Centre on Data Protection Compliance in The Capital Region of Denmark and was registered with clinical trials.gov (NCT03595098).

Participants and procedures

We included children and adolescents (8–17 years) and their parents as participants. Presence or absence of psychiatric diagnoses were based on the Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL)[16] and the criteria for International Classification of Diseases-10 (ICD-10)[17]. All children were assessed for eligibility with full-scale Wechsler Intelligence Scales (either WISC-V[18] for children aged 16.9 years or below or WAIS-IV[19] for children above 16.9 years). The children with OCD were also assessed with the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)[20]. The assessors performing the K-SADS-PL were medical doctors or psychologists (and for the children and adolescents without OCD also psychology students) with experience in child- and adolescent psychiatry. All assessors performing the K-SADS-PL completed a course in the method and were provided continuous supervision. Parents or legal guardians provided written informed consent concerning their child's and their own participation.

Inclusion criteria for children and adolescents with OCD were a primary diagnosis of OCD according to ICD-10[21], CY-BOCS total score ≥ 16, age 8 to 17 years both inclusive, and signed informed consent from parents/legal caregivers. Exclusion criteria for children with OCD were comorbid illness with pervasive developmental disorder (not including Asperger's syndrome), schizophrenia/psychosis, mania or bipolar disorder, depressive psychotic disorders, substance dependence syndrome, IQ < 70, treatment with cognitive behavioral therapy, psychoeducation with relaxation training, antidepressant, or antipsychotic medications within the last six months prior to trial entry. Inclusion criteria for children and adolescents without OCD were age 8 to 17 years both inclusive, sex and age matched with an included child with OCD (+/- 3 months of birthday) and signed informed consent from parents/legal caretakers. Exclusion criteria for children without OCD were any current or previous psychiatric disorder according to ICD-10 criteria, non-Danish-speaking, and IQ < 70. We included parents with a child participating in the study and with informed consent to participation.

Oxytocin was assessed in saliva samples from children and parents. Saliva samples were collected with Salivettes (Sarstedt, Rommelsdorf, Germany). Participants were asked to refrain from eating two hours and drinking liquids 30 min prior to sampling. A detailed description of the handling of the saliva samples are available in Appendix A. The samples were analyzed with immunoassay using Enzo® (NY, USA) oxytocin kits according to the manufacturer's instructions, and concentrations were calculated using WorkOut 2.5 (Dazdaq Solutions Ltd, Brighton, UK). The intra-assay coefficient of variation was 13% and the inter-assay coefficient of variation was 20%.

Children and adolescents self-reported their pubertal status after instruction from a trained investigator using Tanner staging[22,23]. The children and adolescents chose the most fitting pubertal stage among pictorial presentations of secondary sex characteristics on a 5 point scale ranging from 1 (pre-pubertal development) to 5 (full adult development)[22,23]. We did not use classification of the specific sex characteristics but asked the children and adolescents to report the best fitting total pubertal stage. In one post hoc analysis, we assigned parents "pubertal stage 6" indicating "adults". However, this is not a part of the original Tanner staging. Throughout the paper, we use the term "pubertal stage" to describe Tanner stage.

Statistical analysis

We registered our statistical analysis plan prior to any data analysis (date of registration June 7, 2023, https://osf.io/hmc43). Changes from the pre-registered plan are described in Appendix B. We used frequentist statistics and Bayesian statistics. Bayesian statistics has been recommended as a valuable supplement to null hypothesis significance testing (NHST), as it can quantify the degree to which the data favor the alternative hypothesis (H_1) or the null hypothesis (H_0)[24]. Traditional NHST cannot evaluate the evidence for the null hypothesis[24]. Bayesian statistics can be used to describe the probability of observing data by updating prior information about the data with the observed data. One approach to compare models in Bayesian statistics is by using Bayes Factors[24]. The Bayes factor (BF_{10}) is the ratio between the support of the alternative hypothesis (H_1) and the null hypothesis (H_0)[24,25].

We interpreted the Bayes Factors using a classification scheme by Lee and Wagenmakers[25] with a BF_{10} of 1 indicating no difference in support of the H_1- compared to the H_0. Values above 1 indicate support of the H_1, and values below 1 indicate support of the H_0. The degree of support is divided by cutoffs describing anecdotal, moderate, strong, very strong, or extreme evidence (see description of cutoffs in Appendix C). We used Bayes factor, when we tested a discrete parameter (such as OCD-status of the children). When the variables were not discrete, we used the expected log-pointwise predictive density estimated by leave-one-out cross validation (ELPD-LOO). For a more detailed description of the Bayesian methods and analyses of sensitivity to the prior distributions, see Appendix C. Analyses were performed using R (version 4.2.0 and 4.3.0 for random forest analyses).

We excluded outliers in oxytocin concentrations (values ± 3 SDs from the mean) in accordance with previous studies in salivary oxytocin[26,27] and our pre-registration, and applied a logarithmic transformation with the natural logarithm. We have added a description of the outliers in Appendix B. In our pre-registration, assuming $n = 220$ (children and adolescents with OCD: 130, healthy controls: 90), $\alpha = 0.05$ (2-sided), and $\beta = 0.80$, we noted that we were able to reliably detect a Cohen's d of at least 0.4 (calculated using r package "pwr" as a Cohens d of 0.39). Our sample size was slightly reduced by missing information and outliers, however, without major change in the effect size we could detect ($n = 201$ (children and adolescents with OCD: 113, children and adolescents without OCD: 88), $\alpha = 0.05$ (2-sided), and $\beta = 0.80$, Cohens d of 0.40). We did not adjust for multiple comparisons as the study was exploratory, but we reported all comparisons and considered the risk of false discoveries.

The following primary statistical models were run (see also Appendix E):

Model 1: Using a Student's t-test we investigated the difference in oxytocin concentrations (dependent variable) between children and adolescents with and without OCD (OCD-status; independent variable). A Bayes factor was calculated to compare H_1 (an effect of OCD-status) to H_0 (no effect of OCD-status).

Model 2: A backward stepwise regression explored the variance in children and adolescent's oxytocin concentrations (dependent variable) explained by OCD-status, age, sex, and age-sex interaction (independent variables). The reduced model from the stepwise regression was used to analyze the effect of OCD-status on oxytocin concentrations. We used Bayesian analysis to compare H_1 (the reduced model plus an effect of OCD-status) to H_0 (the reduced model). Additionally, we used Bayesian analysis to compare the results of the stepwise regression and thereby the significant effects of relevant variables by comparing H_1 (the reduced model) to H_0 (intercept model).

Model 3: We investigated the variance explained by child and adolescent pubertal stage by repeating Model 2 with pubertal stage instead of age.

Model 4: We explored the effect of age and sex by analyzing all participants (children and adolescents, and parents). A backward stepwise regression of a mixed effect model was performed with participant oxytocin concentration (dependent variable), age, sex, and the interaction between age and sex (fixed effects) and a "family-variable" (random effect). The family-variable (a nominal variable denoting family membership) was included because previous research has shown associations between the oxytocin concentrations of parent couples (mothers and fathers)[27] as well as parents and their children[9].

To further explore the data, we ran the following *post hoc* models and tests that were not pre-registered:

Post Hoc Model A: We explored the observed nonlinearity in the child and adolescent data by repeating Model 2 with a quadratic age variable (age^2).

Post Hoc test B: To investigate if either age or pubertal stage best accounts for variation in oxytocin levels, we compared Model 3 with OCD and Post Hoc Model A with OCD using Bayesian analysis.

Post Hoc Model C: We repeated Model 4 with pubertal stage instead of age for all participants assigning parents an adult "pubertal stage 6". Bayesian analyses were used to compare the reduced Post Hoc Model C to the reduced Model 4.

Post Hoc Model D: To further explore the adult data we repeated Model 4 in adults alone.

Post Hoc test with random forest: A random forest approach (described in Appendix D) explored non-linear associations between salivary oxytocin and relevant covariates. We explored sex, age, pubertal stage, and information on menarche status, menopause status and use of hormonal contraception.

Complete case analyses were performed. Sample selection is presented in Fig. 1. Due to missing information on pubertal stage for some children and adolescents, we refitted models without child and adolescent pubertal stage to data which excluded children and adolescents with missing pubertal stage, when comparing models with pubertal stage to models without pubertal stage. We excluded cases with less than two family members from the models that included the 'family-variable'. We report supplementary analyses (described in Appendix F) of variables that could cause variation in oxytocin but were not main objectives of this study (time of sample, psychosocial functioning, OCD severity score, social responsiveness, menarche, and menstrual cycle phase).

Results

Participant characteristics are presented in Table 1 (children and adolescents) and Table 2 (parents). The children and adolescents with OCD did not differ on age, sex or pubertal stage from the children and adolescents without OCD. We did not have information on medication use or somatic disorders on the children without OCD.

In Model 1, salivary oxytocin concentrations did not significantly differ between children and adolescents with and without OCD ($p = 0.2682$, Table 3 and in Appendix E for further details) and the Bayes factor was below 1 indicating support for the null hypothesis. However, the size of the Bayes factor showed only anecdotal support, i.e., the lowest degree of support ($BF_{10} = 0.37$, Table 3).

When including age and sex and an age-sex interaction in a model with OCD-status (Model 2) only age remained significantly associated with child oxytocin in the reduced model showing a significant positive linear relationship between age and child and adolescent oxytocin ($p = 0.0031$, Table 4, and see Appendix E) [Figs. 2 and 4 (pink line)].

The Bayesian analysis showed that the model including child and adolescent age had a better predictive performance but a large standard error (*SE*) (ELPD-LOO = -3.4, *SE* = 2.7, Table 4). When we reexamined the relationship between child and adolescent oxytocin and OCD-status while controlling for age, OCD-status did not significantly account for variation in oxytocin ($p = 0.3605$, Table 4, and see Appendix E). The corresponding Bayes factor again showed support for the null hypothesis with a value below 1. The size of the Bayes factor indicated moderate support for no difference in oxytocin concentrations between children and adolescents with and without OCD controlling for age ($BF_{10} = 0.31$, Table 3) but with considerable prior sensitivity (see Appendix C).

When we included pubertal stage and age and their interaction (Model 3) all variables except pubertal stage were dropped in the model reduction (see Appendix E). The reduced model showed a significant relationship between child and adolescent oxytocin and pubertal stage ($p = 0.0004$, Table 4 and see Appendix E) (Fig. 3).

Bayesian analysis showed a better predictive performance of a model including pubertal stage, but with a large standard error (ELPD-LOO=-6.5, *SE* 4.1, Table 4). Controlling for pubertal stage in a model with OCD-status did not change the results for OCD-status, which was still not significantly associated with variation in oxytocin ($p = 0.1477$, Table 4 and Appendix E). The corresponding Bayes factor was below 1, thus again showing support for no difference in oxytocin concentrations between children and adolescents with and without OCD controlling for pubertal stage, and the size of the Bayes factor indicated anecdotal support ($BF_{10} = 0.52$, Table 4) with considerable prior sensitivity (see Appendix C).

In Model 4, we included children and adolescents, and parents. Again, we found no association between oxytocin and the age-sex interaction and sex, which were removed from the model in the first two steps (see Appendix E). The reduced model showed a significant positive, linear relationship between participant age and oxytocin ($p < 0.0003$, see Appendix E) [Fig. 4 (blue line)].

Including quadratic age in the exploratory Post Hoc Model A showed a significant relationship between oxytocin and quadratic age [Figs. 2 and 4 (purple line); $p = 0.0496$, see Appendix E], but again sex and the age-sex interaction was dropped in the model reduction (Appendix E).

Post Hoc test B showed that the predictive performance of the model including pubertal stage and OCD (reduced Model 3 with OCD) was slightly better than the model with quadratic age and OCD (reduced Post Hoc Model A and OCD) (ELPD-LOO = 2.9, *SE* 3.0, see Appendix C). However, the standard error of the difference in predictive performance was large.

Visual inspection of Fig. 4, illustrating the reduced Model 2 (child and adolescent age, pink line), the reduced Post Hoc Model A (child and adolescent quadratic age, purple line), and the reduced Model 4 (child, adolescent, and parent age, blue line), indicated that the models in children and adolescents cannot be extrapolated to adults and the model in all participants seemed problematic in estimating in children and adolescents.

In Post Hoc Model C, we used "pubertal stage 6" for the adults and found a significant relationship (Figure E.1 in Appendix E) between salivary oxytocin and the modified pubertal stage in the sample including children, adolescents, and adults ($p = 0.005$, see Appendix E). The Bayesian analysis showed a better predictive performance of the model with the modified pubertal stage compared to the model with age (ELPD-LOO = 5.9, *SE* 5.1, see Appendix C).

In Post Hoc Model D, including only adults, the age-sex interaction, sex, and age did not contribute to the variation in oxytocin ($p = 0.10$, see Appendix E).

Sensitivity analyses of the Bayesian analyses of the models showed a general sensitivity to priors and little difference in predictive performance between the compared models with high standard errors (Appendix C).

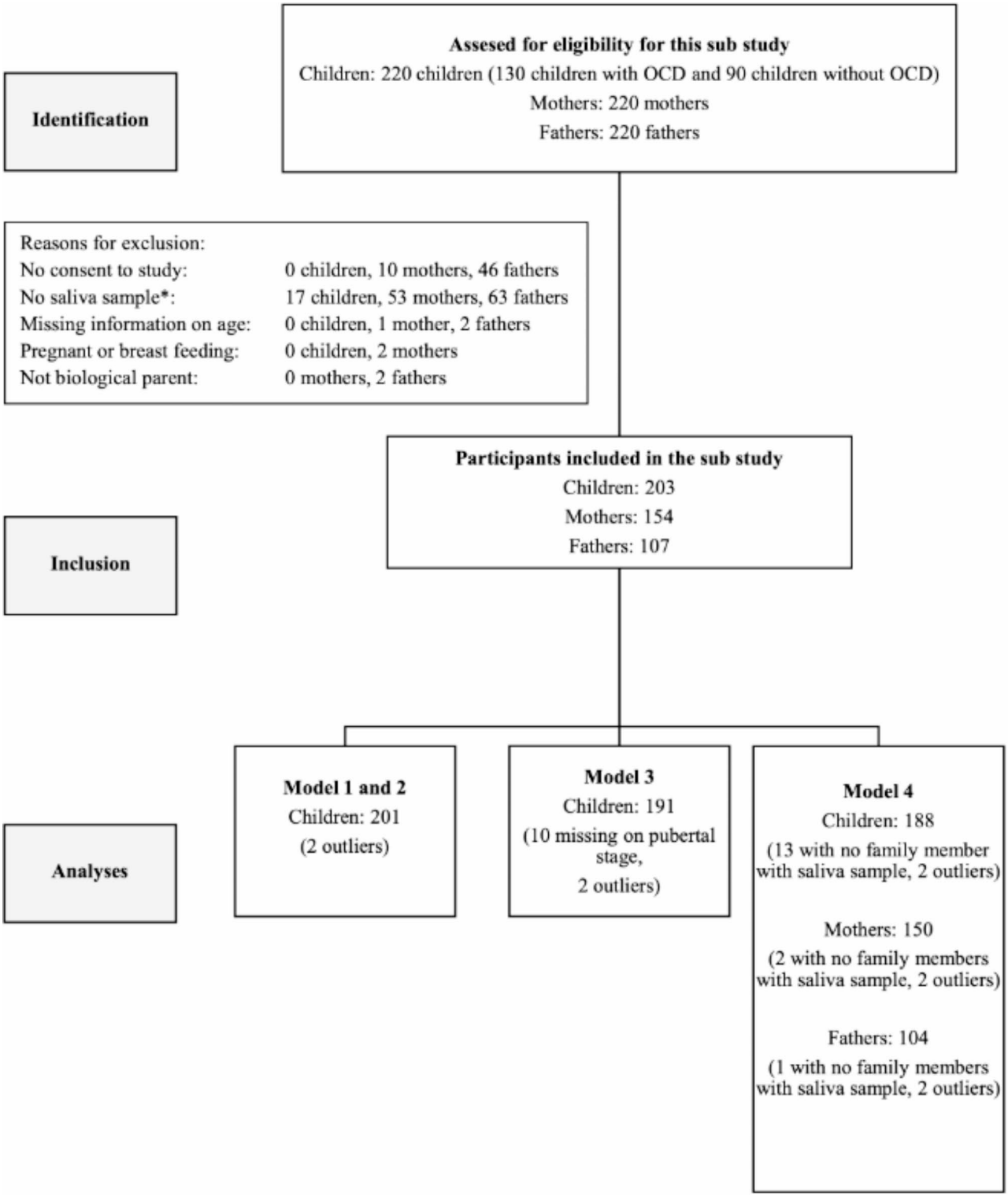

Fig. 1. Participant flow chart.*Reasons for missing saliva sample on children: 5 with too high anxiety on the day, 6 cancelled due to covid, 4 procedure mistakes, 2 with no reason registered. Reasons for missing sample on parents not registered. OCD, obsessive-compulsive disorder.

The random forest analysis in children and adolescents alone and in children, adolescents, and adults indicated that pubertal stage/modified pubertal stage and age were the most important variables. Furthermore, these findings both in visual presentation of the predictions and the mean squared error (*MSE*) resembled the findings from the linear models (see Appendix D).

	Children and adolescents with OCD	Children and adolescents without OCD	Test (*p*-value)
Child salivary oxytocin (pg/ml)			
Mean (*SD*)	26.61 (14.28)	25.61 (13.95)	
Missing	0	0	
n	113	88	
Age (years)			0.26_A
Mean (*SD*)	13.37 (2.83)	12.92 (2.78)	
Median (min, max)	13.81 (8.08, 17.99)	12.37 (8.09,17.83)	
Missing (*n*)	0	0	
Sex (*n* (%))			0.98_B
Female	58 (51.3%)	45 (51.1%)	
Male	55 (48.7%)	43 (48.9%)	
Missing	0	0	
Pubertal stage (*n* (%))			0.39_B
Pubertal stage 1	32 (28.3%)	23 (26.1%)	
Pubertal stage 2	13 (11.5%)	20 (22.7%)	
Pubertal stage 3	20 (17.7%)	14 (15.9%)	
Pubertal stage 4	25 (22.1%)	23 (26.1%)	
Pubertal stage 5	13 (11.5%)	8 (9.1%)	
Missing	10 (8.8%)	0 (0%)	
Nationality (*n* (%))			–
Danish	95 (84,1%)	63 (71.6%)	
Other	3 (2.7%)	2 (2.3%)	
Missing	15 (13.3%)	23 (26.1%)	
Comorbid psychiatric disorders (*n* (%))		*na*	–
Anxiety disorders ICD-10 F40-41, F93	12 (10.6%)		
Stress and adjustment ICD-10 F43	12 (10.6%)		
Asperger's Syndrome ICD-10 F84.5	14 (12.4%)		
Hyperkinetic disorders ICD-10 F90.0	13 (11.5%)		
Tic disorders ICD-10 F95	13 (11.5%)		
Other*	20 (17.7%)		
Medication (*n* (%))		*na*	–
Children receiving medication	24 (21.2%)		
Somatic disorders (*n* (%))		*na*	–
Children with a somatic disorder	32 (28.3%)		

Table 1. Descriptive characteristics of the children and adolescents included in Model 1 and model 2. Children in model 3 and model 4 are a subsample of the children. *n*, number; *SD*, standard deviation; OCD, obsessive-compulsive disorder; ICD-10, International Classification of Diseases-10, *na* refers to the information not being available. For some variables, statistical testing was not relevant or possible marked by "–". * "Other" describes all other diagnoses. For detailed information on patient medication and comorbid and somatic disorders see Appendix A. $_A$t-test, $_B$Chi-squared test.

In the additional analyses in our appendix, we found that adult females had significantly higher oxytocin concentrations during ovulation than during menstruation (estimate = 0.53, $p = 0.0008$, for more details see Appendix F). The additional analyses did not show a significant association between child oxytocin and OCD symptoms severity measured with CY-BOCS ($r = 0.003$, $p = 0.73$, for more details see Appendix F). The remaining analyses were not statistically significant.

Discussion

We evaluated the hypothesis that salivary oxytocin concentrations are elevated in children and adolescents with OCD. We included a relatively large sample of children and adolescents aged 8–17 years with and without OCD while controlling for potential variance in oxytocin concentrations related to age, pubertal stage, and sex. Overall, we found no support for the hypothesis of elevated oxytocin concentrations in OCD. Our results contrast with those of Marazziti et al. who found higher oxytocin concentrations in adults with OCD[7]. The contrasting findings could be explained by the use of different physiological fluids (i.e., saliva versus plasma). The correlation between salivary and plasma oxytocin has been found small-to-moderate (rho = 0.361)[26] raising the question of whether plasma and saliva measure the same thing[28]. A modest-to-strong positive correlation between salivary and CSF oxytocin has been found (rho = 0.711)[26] whereas a small-to-modest positive correlation has been found between plasma and CSF (rho = 0.417)[26]. Our findings are consistent with findings on oxytocin in CSF in adults with OCD

	Children and adolescents with OCD	Children and adolescents without OCD
n		
Mothers	92	58
Fathers	72	32
Mother age (years)		
Mean (*SD*)	45.72 (5.03)	45.97 (5.10)
Median (min, max)	45.61 (32.76, 56.24)	46.45 (34.97, 57.19)
Missing (*n*)	0	0
Father age (years)		
Mean (*SD*)	48.17 (5.67)	47.47 (6.78)
Median (min, max)	48.91 (33.1, 73.51)	47.08 (34.45, 62.73)
Missing (n)	0	0
Nationality Mother (*n* (%))		
Danish	77 (83.7%)	46 (79.3%)
Other	5 (5.4%)	3 (5.2%)
Missing	10 (10.9%)	9 (15.5%)
Nationality Father (*n* (%))		
Danish	57 (79.2%)	24 (75%)
Other	5 (6.9%)	1 (3.1%)
Missing (*n*)	10 (13.9%)	7 (21.9%)
Parental education mothers (*n* (%))		
Upper secondary education or below	8 (8.70%)	1 (1.7%)
Short-cycle tertiary education	14 (15.2%)	3 (3.2%)
Bachelor's degree or equivalent	38 (41.3%)	22 (37.9%)
Master's degree or above	22 (23.9%)	23 (39.7%)
Missing (*n*)	10 (10.9%)	9 (15.5%)
Parental education fathers (*n* (%))		
Upper secondary education or below	12 (16.7%)	3 (9.4%)
Short-cycle tertiary education	8 (11.1%)	3 (9.4%)
Bachelor's degree or equivalent	16 (22.2%)	6 (18.8%)
Master's degree or above	25 (34.7%)	13 (40.6%)
Missing (*n*)	11 (15.3%)	7 (21.9%)

Table 2. Descriptive characteristics of parents included in model 4. *n*, number; *SD*, standard deviation; OCD, obsessive-compulsive disorder.

			t-statistics	*p*-value	Bayesian analysis
Model 1					
1. Test	H_1	OCD-status	1.111	0.2682	$BF_{10} = 0.37$
	H_0	Intercept			

Table 3. Result of student's *t*-test of salivary oxytocin levels in children and adolescents with and without OCD (OCD-status) and bayesian statistics (model 1). BF, Bayes factor; H_1, alternative hypothesis; H_0, null hypothesis.

by Leckman and colleagues[5] and Altemus and colleagues[6]. A meta-analysis pooling the two aforementioned studies in adults also found no significant difference[29], however, a more recent meta-analysis also including the study by Marazziti et al., found significantly higher levels in patients with OCD compared to healthy controls[30]. Our complementary Bayesian analyses showed only anecdotal to moderate support for the null hypothesis, even with a larger sample size than the previous studies and almost as large as the sample in the recent meta-analysis. While it may be that oxytocin concentrations do not differ between children and adolescents with and without OCD, the null findings could be due to sample sizes too small to detect a discrete group difference. Another consideration of the null findings is the heterogeneous nature of OCD with multiple different phenomenological manifestations[31] which perhaps makes assuming overall group differences in biological markers too simplistic. The heterogeneous nature of OCD could also explain the contrasting findings between our study and the study by Marazziti et al. As proposed by Leckman et al., OCD is a highly heterogenous disorder, and oxytocin could be of importance in some subtypes of OCD[4] which is also indicated by their findings of higher levels in a subgroup of OCD patients[5]. A previous study has shown different symptom dimensions in OCD and suggested

			F-statistics	*p*-value	Bayesian analysis
Model 2					
1. Test	H_1	Reduced model (age)	8.913	0.0031	H_1- H_0 ELPD-LOO (*SE*) = 3.5(2.7)
	H_0	Intercept			
2. Test	H_1	Reduced model (age) and OCD-status	0.840	0.3605	$BF_{10=}$0.31
	H_0	Reduced model (age)			
Model 3					
1. Test	H_1	Reduced model (pubertal stage)	5.384	0.0004	H_1- H_0 ELPD-LOO (*SE*) = 6.6(4.1)
	H_0	Intercept			
2. Test	H_1	Reduced model (pubertal stage) and OCD-status	2.113	0.1477	$BF_{10=}$0.52
	H_0	Reduced model (pubertal stage)			

Table 4. Results of reduced models, reduced models with OCD, and bayesian statistics of model 2 and model 3. BF, Bayes factor; H_1, alternative hypothesis; H_0, null hypothesis; ELPD-LOO, expected log-pointwise predictive density estimated by leave-one-out cross validation; *SE*, standard error.

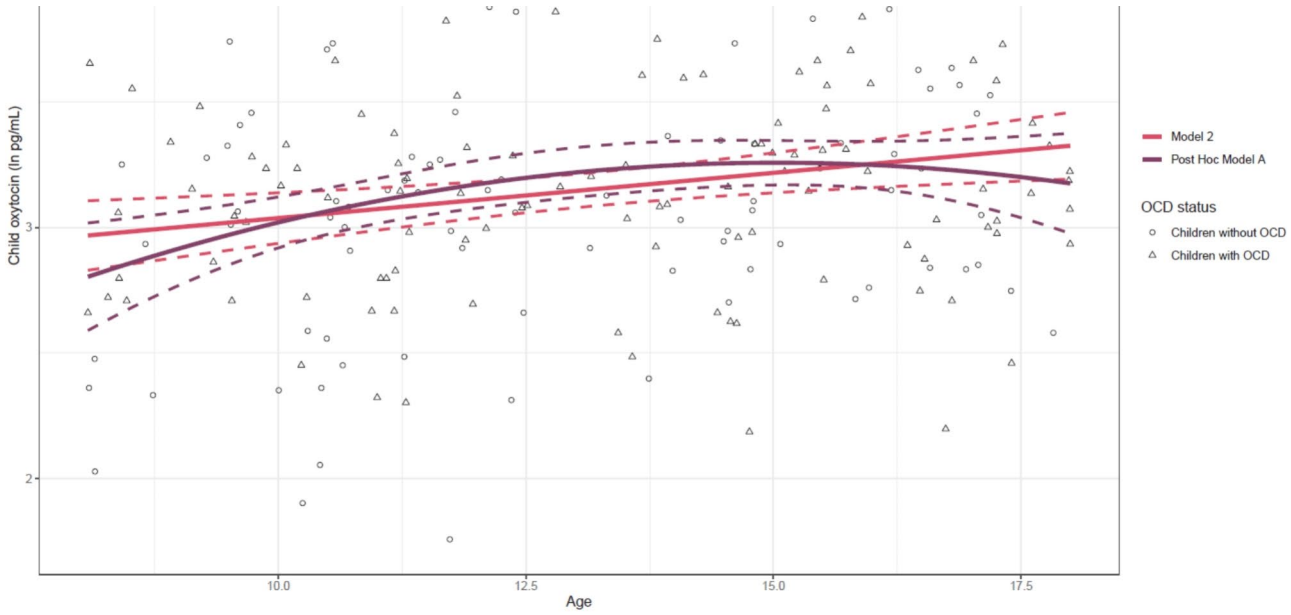

Fig. 2. The relationship between oxytocin and age predicted by the reduced model 2 with age (pink line) and the reduced post hoc model A with quadratic age (purple line). Note: The dashed lines represent the 95 confidence interval of the mean. Ln, natural logarithm.

that this could help our understanding of OCD including the underlying neurobiology[32]. Oxytocin could be linked to specific OCD dimensions e.g., the observed aggressive and sexual behavior in animals after oxytocin administration which could support a link between higher oxytocin and "disturbing thoughts" in OCD. A focus on trans-diagnostic mechanisms have also been suggested as important in OCD research[31] which could also be relevant in the research of oxytocin and OCD. Oxytocin has been implicated in regulation of anxiety and interpersonal behaviors[33]. One research group investigated a theory of oxytocin as a biochemical signal of safety with anxiolytic effects and associated with behaviors, such as family accommodation, which is common in OCD and anxiety disorders[34]. They investigated 50 children with clinical anxiety and found negative associations between salivary oxytocin, anxiety levels, and subscales of family accommodation[34]. Family accommodation is frequent in children with OCD[35] and associated with a poor treatment outcome[36]. A focus on oxytocin's relation to family behaviors, like family accommodation, rather than the overall OCD diagnosis could help understand poor treatment outcomes.

Our findings indicate that peripheral oxytocin concentrations increase with age from 8 to 17 years. This finding is consistent with findings from a study of children and adolescents (7–16 years) with clinical anxiety[34]. Another study in children (5–90 months of age) concluded that oxytocin decreases from 5 months to 90 months[37]. However, a scatter plot visualizing this data suggests an elbow shaped curve may fit this data better than a straight line[37]. Several of our analyses (scatter plot, quadratic age, and pubertal stage) supported a nonlinear relationship with oxytocin concentrations slightly increasing from 8 to 15 years, and from pubertal stage 1 to 4, followed by a slight decrease. The exploration of the relationship between age and oxytocin in our parent sample

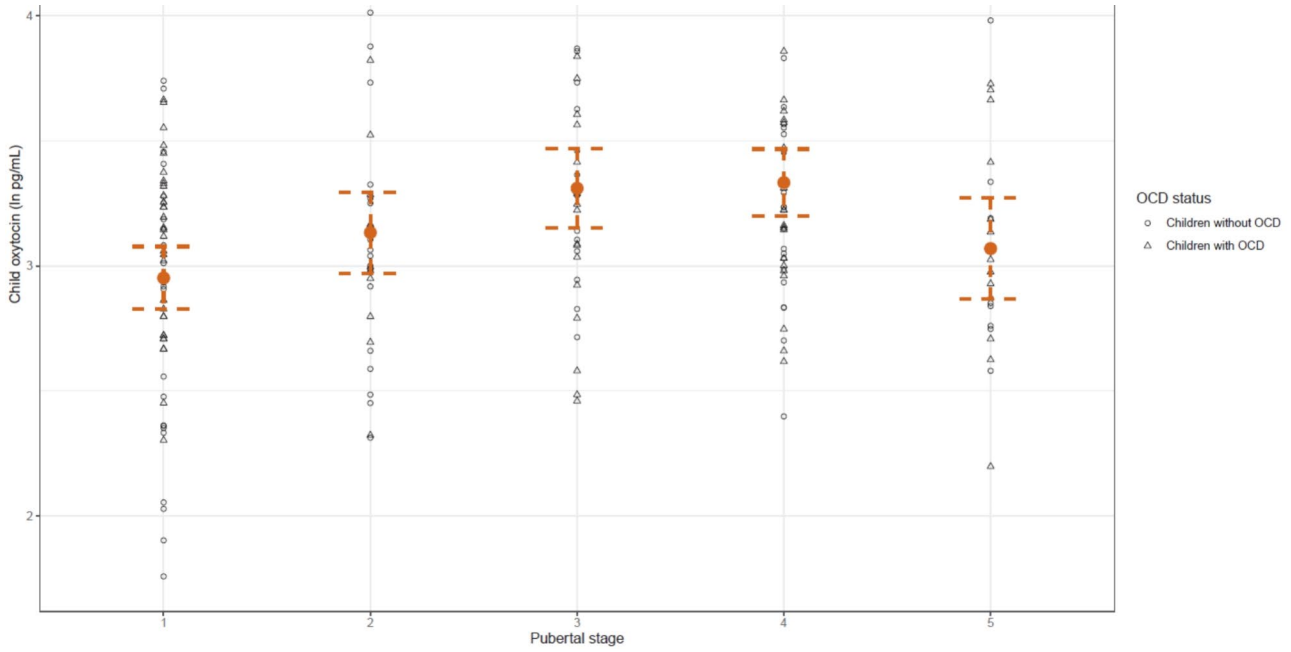

Fig. 3. The relationship between oxytocin and age predicted by the reduced model 4 with age of all participants (blue line), the reduced model 2 with age in children (pink line) and the reduced post hoc model A with age and quadratic age in children (purple line). *Note*: The dashed lines represent the 95 confidence interval. Ln, natural logarithm.

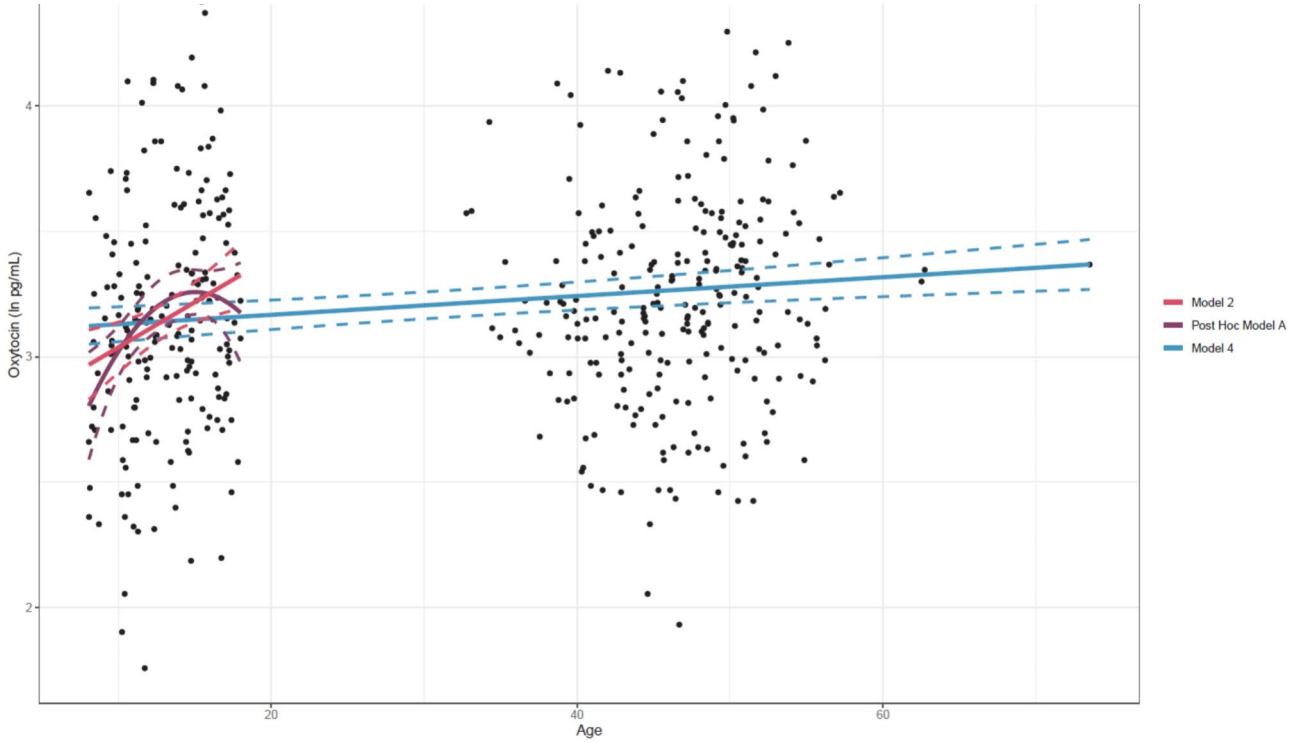

Fig. 4. The relationship between oxytocin and pubertal stage predicted by the reduced model 3 with pubertal stage. *Note*: The dashed lines represent the 95 confidence interval of the mean. Ln, natural logarithm.

(age range ≈ 33–73 years) indicated that oxytocin increases sometime after puberty, which is indicative of its coupling to sex hormones. The importance of pubertal stage could support that hormonal or behavioral changes during puberty cause important variation in oxytocin activity[13]. Our exploration with random forest analyses resembled the results from the models using quadratic age and pubertal stage suggesting that these models capture the main signal in this age range.

Our results did not indicate that sex accounts for variation in oxytocin levels in children and adolescents or adults. Previous findings on sex differences in oxytocin in adults are mixed[38–41]. The contrasting results could be due to studies investigating different ages and sex differences may only occur in some age ranges.

This study has both strengths and limitations, which should be considered when interpreting our results. Our sample is relatively large compared to prior research in the field and used frequentist and Bayesian statistics providing additional information regarding relative evidence for null hypotheses. Other studies has found an association between salivary and CSF oxytocin[26], however, whether salivary and other peripheral measures of oxytocin reflect central oxytocin concentrations under basal conditions is unclear[10,28,42]. Thus, inferences about central oxytocin activity based on peripheral measures might not be reliable. However, saliva sampling is a noninvasive and feasible method in youth. We included a clinically representative group of children and adolescents with OCD with multiple comorbidities, however comorbidity can cause variability in oxytocin[29]. We used a cross-sectional design which is a limitation when making inferences about oxytocin changes across development where a longitudinal approach would be more precise. We used no correction for multiple testing, thus statistical significance should be interpreted carefully. With the goal of transparency, we pre-registered our analysis plan. Pubertal stage was assessed without clinical examination, which decreases the accuracy[43]. Yet, pubertal stage has not previously been examined in relation to oxytocin.

Conclusion

Salivary oxytocin does not appear to be related to OCD in children and adolescents. Rather than focusing on group differences, future studies on oxytocin and OCD may focus on more specific components of OCD like family factors or family behaviors and specific features or symptom dimension of OCD that are plausible influenced by oxytocin. Oxytocin concentration fluctuations across development suggest that hormonal or behavioral changes during puberty may influence oxytocin system activity. Future studies across a wider age range and using nonlinear models may increase the understanding of oxytocin concentrations across the lifespan.

Data availability

After publication of all results from the TECTO trial, we plan to make a depersonalized data set publicly available on the EU ZENODO database.

Code availability

Analysis scripts from R are available on request to the authors.

Biological material availability

The saliva samples have been dispersed after the final analysis to follow our protocol and the biological material is therefore no longer available.

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Author contributions

Conceptualization: ACM, LC, EL, LP, KJP, AKP, NNLMethodology: ACM, LC, MG, EL, DQ, NRJ, CSL, LB, GC, NNLValidation: ELFormal analysis: ACM, MGInvestigation: ACM, CSL, LP, VU, SC, CU, NK, CT, JH, MR, KJP, AKP, NNLResources: LC, NRJ, AKP, NNLWriting - Original Draft: ACM, MGWriting - Review & Editing: LC, MG, EL, DQ, NRJ, CSL, LB, GC, LP, VU, SC, CU, NK, CT, JH, MR, KJP, AKP, NNLVisualization: ACM, MG-Supervision: LC, AKP, NNLProject administration: SC, NNLFunding acquisition: ACM, LP, KJP, AKP, NNL.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Appendix

Content of Appendix

A. Additional information on methods

Details on eligibility screening

All children and adolescents were screened for eligibility with the Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL)[1], full-scale Wechsler Intelligence Scales (either WISC-V for children aged 16.9 years or below or WAIS-IV for adolescents above age 16.9)[2,3]. Children and adolescents with OCD also completed The Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)[4]. Diagnoses were based on the criteria for International Classification of Diseases-10 (ICD-10)[5] and diagnostic status was confirmed by a psychiatrist or psychologist specialized in child and adolescent psychiatry.

Details on informed consent procedures

Children and adolescents, and parents received verbal information and children above 15 years and parents/legal guardians also received written information about the child's participation in the trial. Parents or legal guardians signed informed consent concerning participation of their child. Parents were given verbal and written information on their own participation and gave informed consent to their own participation.

Saliva handling and preparation for analysis

After sampling, the saliva was stored in a -20 °C freezer. In the preparation for analysis, the samples were 1) thawed, 2) centrifuged at 3,000 rpm for five minutes at 4 °C, 3) frozen to -80 °C for at least one day, 4) thawed at 5 °C, 5) centrifuged at 4 °C at 1,500 × g for 20 minutes, 6) stored at -80 °C, 7) lyophilized (freeze dried) for 17 hours, and 8) stored at -20 °C. On the assay day, the samples were 1) thawed, 2) reconstituted in assay buffer, 3) mixed twice for five minutes at 2700 rpm with a 5-minute interval, and 4) centrifuged for five minutes at 10,000 rpm. We did not use extraction of the saliva samples.

B. Deviation from pre-registered analysis plan and consideration of outliers

We chose the following deviation from the pre-registered statistical analysis plan:

- We tested variance in OCD status groups prior to model 1. Because the difference in variance was not significant, we performed the analysis as a student's t-test and not as a Welsch t-test as described in the pre-registration.
- The family variable in model 4 was included as a random intercept effect which was not specified in the analysis plan.
- The Bayesian statistics was only specified regarding the use of a Cauchy Prior in analysis plan. Full description of method and priors used are described in the method section.
- In the supplemental analysis of menstrual cycle, we chose to analyze girls and mothers separately as we would have many missing variables in the family variable should be included.
- Analyses without logarithmic transformation and with outliers included would break the assumptions of normality and we therefore did not include these sensitivity analyses.
- Analyses was performed in R studio only and not JASP as mentioned in the pre-registration.

Consideration of outliers

We pre-registered removal of outliers as this have been used in other studies. The variation in oxytocin is often considerable and a outliers can complicate the statistical analyses. However, outliers could be due to natural variation in oxytocin levels in which case removal would reduce our understanding. The evaluation of whether outliers are due to sampling or measurement errors or due to natural variation requires information on variables such as other conditions or diseases, medication, pregnancy, and breast feeding. We had six outliers – two in children where one child did have OCD and the other did not (values 414.3 and 198.1pg/ml), two in mothers (340.3 and 166.8 pg/ml) and two in fathers (338.4 and 121.7pg/ml). We did not find any explanation for the outliers in the information we had available (pregnancy and breast feeding in female participants, or medication and somatic disorders in the children with OCD). We lack information on e.g. medication or somatic disorders in both parents and children without OCD, but chose to exclude the outliers even though we cannot be sure that the values are not caused by natural variation. However, we acknowledge that exclusion of outliers holds uncertainty.

C. Description of Bayesian methods and results including sensitivity analyses of priors

Description of Bayesian methods

Bayesian statistics was performed using R-studio with R (version 4.2.0) and the packages *brm* (version 2.20.4) and *stan (version 2.32.3)*.

Bayesian statistics is provided as a supplement to the frequentist model reduction and hypothesis testing. We will mainly use Bayes Factor to compare models, as suggested by Quintana et al. who consider hypothesis testing in psychiatry[6].

However, Bayes Factor has been criticized for being highly sensitive to the choice of prior and the problem structure. Gelman et al. therefore generally advise against the use of this measure to compare models[7]. They particularly advise against the use of Bayes Factor for hypotheses which are not truly discrete[7].

In the light of these recommendations, we will supplement the selected frequentist analyses by a measure of Bayes factor, when we test a discrete parameter. We supplement all selected frequentist analyses by a measure of the predictive performance based on the expected log-pointwise predictive density estimated by leave-one-out cross validation (ELPD-LOO).

We use Cauchy priors with scale r on the fixed effects and Jeffrey's prior on the variance (JZS priors) to compare models by Bayes Factor as suggested by these references[6,8]. Bayes factor is very sensitive to the choice of scale of the prior and provides increased evidence for the null-hypothesis with increasing values of r[8]. We therefore provide results for both r=0.5 and r=1.0. The ELPD-LOO estimates are based on the default priors provided in the brms package.

Interpretation was made with the classification for the Bayes factor described by Lee and Wagenmaker[9]. The Bayes factor, BF_{10}, describes the evidence of the alternative hypothesis (H_1) compared to the null hypothesis (H_0). Values above 1 describes evidence for H_1 with increasing values describing increased degree of evidence (1-3 "anecdotal", 3-10 "moderate", 10-30 "strong", 30-100 "very strong", >100 "extreme). Values below 1 describes evidence of H_0 with decreasing values describing increased level of evidence (1- 1/3 "anecdotal", 1/3-1/10 "moderate", 1-10-1/30 "strong", 1/30-1/100 "very strong", <1/100 "extreme)[9].

We estimate all Bayesian models using MCMC with 4 chains which each have 8000 iterations. We use the first 1000 iterations for warmup.

Results of Bayesian analyses

Table C..1. Results from the Bayesian analyses including sensitivity-analyses with different prior and reporting of predictive performance in terms of expected log pointwise predictive density (ELPD-LOO)

Models	**Hypotheses**		**BF_{10} (r=0.5)**	**BF_{10} (r=1.0)**	**ELPD-LOO (*SE*) (H_1- H_0)**
Model 1					
1. Bayes factor	H_1	OCD-status	0.37	0.21	-0.3 (1.2)
	H_0	Intercept			
Model 2					
1. Bayes factor	H_1	Red. model (age)	-	-	3.5 (2.7)
	H_0	Intercept			
2. Bayes factor	H_1	Red. model (age) and OCD-status	0.31	0.17	-0.6 (0.9)
	H_0	Red. model (age)			
Model 3					
1. Bayes factor	H_1	Red. model (pubertal stage)	-	-	6.6 (4.1)
	H_0	Intercept			
2. Bayes factor	H_1	Red. model (pubertal stage) and OCD-status	0.52	0.31	0.0 (1.5)
	H_0	Red. model (pubertal stage)			
Post Hoc test B					
1. Bayes factor	H_1	Red. Model 3 (pubertal stage) with OCD-status	-	-	2.9 (3.0)
	H_0	Red. Post Hoc Model A (age and age^2) and OCD-status			
Post Hoc Model C					
1. Bayes factor	H_1	Red. Post Hoc Model C (pubertal stage)	-	-	5.9 (5.1)
	H_0	Red. Model 4 (age)			

Note: ELPD-LOO, expected log-pointwise predictive density estimated by leave-one-out cross validation; *SE*, standard error. Red, reduced; OCD, Obsessive Compulsive Disorder, (H_1) the alternative hypothesis; H_0, the null hypothesis

D. Exploration of non-linearities by random forest

Random forests are ensemble models based on a collection of decision trees. We used random forests to explore non-linear associations between the response (oxytocin) and the relevant covariates/predictors.
We explored random forest models on the data set only including children and adolescents (RF child and adolescent) and the data set including children and parents (RF all). We used R (version 4.3.0) to perform the analysis.

RF child and adolescent:
This random forest regression used the ln-transformed oxytocin as the dependent variable and took the five predictors - OCD-status, sex, age, pubertal stage, and hormonal stage (hormonal stage here denotes menarche status) (levels: pre-menarche, menarche, male, missing) - as input. Note, that here sex was a subset of hormonal stage.

The models were fitted and optimized using the R-package *randomForest* (version 4.7-1.1).

We split each data set in a training set (~70%) and a test set (~30%) to evaluate the performance of the random forests and compare them to the linear models. The dataset has imbalanced groups. We therefore used stratified sampling to split the data in a training set and a test set and when building the random forest models. We used the R -package *splitTools* (version 1.0.1) to generate the strata. We normalized both the training set and the test set before fitting the random forests. Random forests include several hyperparameters which we optimized by a crude grid-search. The hyper-parameters and the range over which they were optimized are found in Table D.1. We used out-of-bag (OOB) error estimates to evaluate the performance of the hyperparameter settings. We grew 5000 trees.
We handled missing values in the categorical predictors in random forest by adding an extra category named “missing”.

Table D.1. Description of hyper-parameters and range of the RF child and adolescent analyses

Hyperparameter	**Name in *RandomForest* package**	**Range**
Number of variables randomly sampled as candidates in each split	mtry	1, 2, 3, 4
Sample size	sampsize	50%, 60%,70%,80%, 90%
Minimum size of the terminal nodes	nodesize	5, 10, 15, …, 95, 100

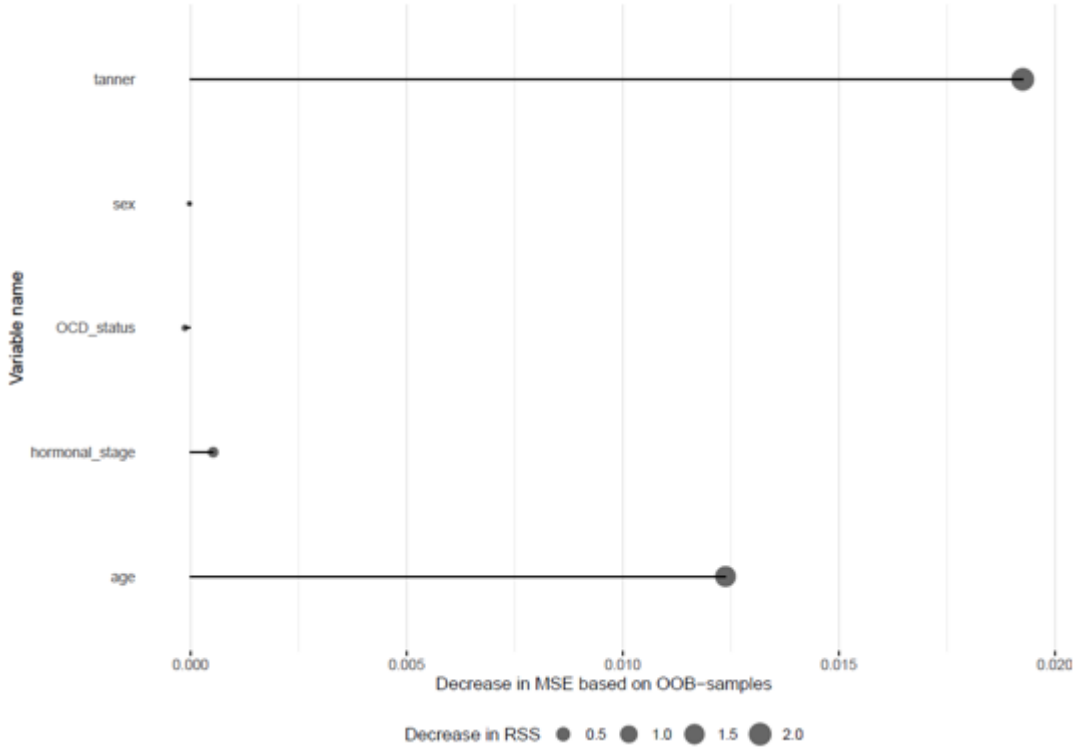
Optimization of the hyperparameters based on OOB error estimates found the combination

(mtry, sampsize, nodesize) = (4, 90%, 100)

of hyperparameters.

Variable importance is plotted in Figure D.1.

Figure D.1. Variable importance for the Random forest children model



We found that tanner and age are the most important variables. This is similar to the findings using traditional linear regression. Looking at the predictions from the random forest model on the test set (Figure D.2. and D.3.) it is evident that the random forest captures the same tendencies in the data as the linear models.

Figure D.2. The relationship between oxytocin and age predicted by the reduced Model 2 (pink line), the reduced Post Hoc Model A (purple line) and Random Forest approach (green line). Predictors are based on the test set. The black dots are data in the test set.

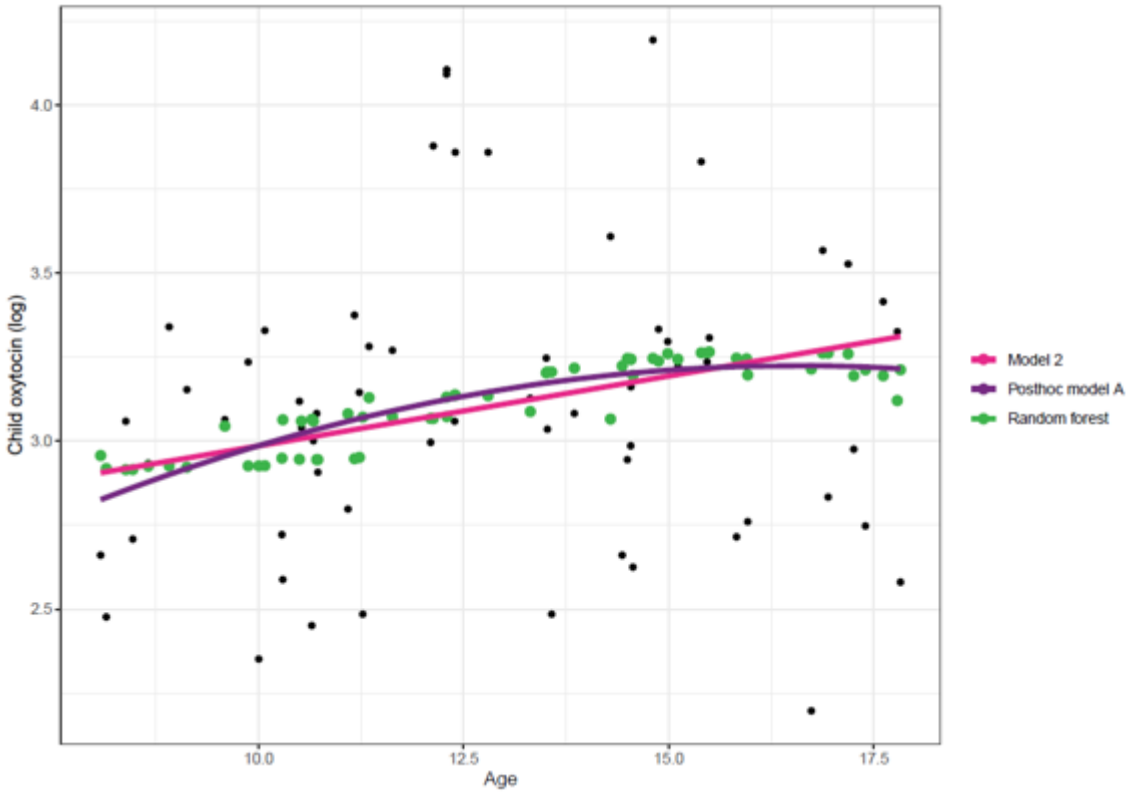
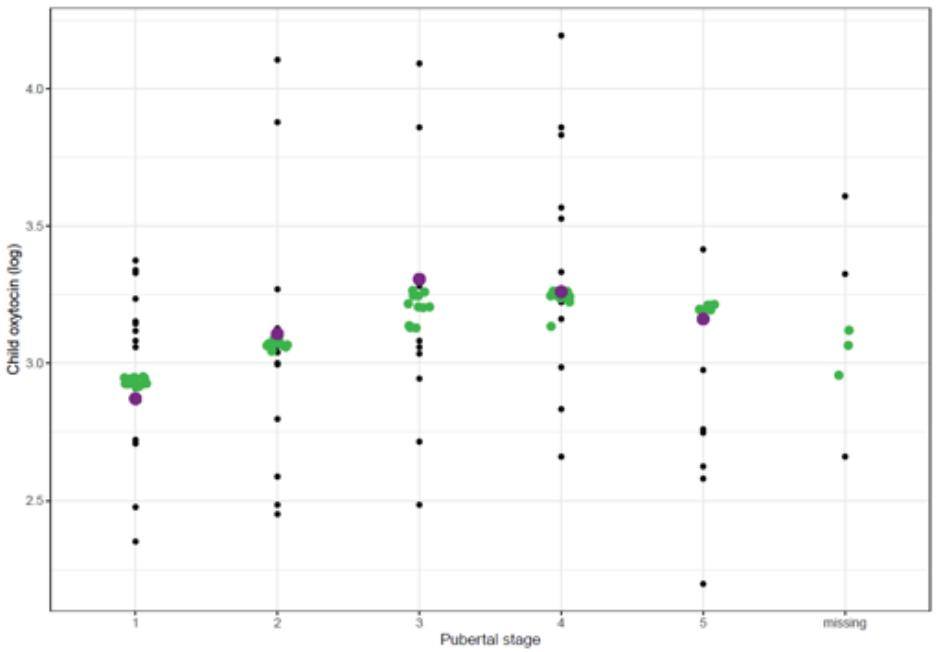


Figure D.3. The relationship between oxytocin and pubertal stage predicted by the reduced model 3 (purple dots) and random forest (green dots). Predictors are based on the test set. The black dots are data in the test set.



The MSE of random forest on the test set is similar to the MSE of the linear models (see Table D.2.) All in all, this indicates that the random forest has not captured signals in the data, which were not captured by the linear models.

Table D.2. Mean Squared Error of the RF child and adolescent, Model 1, Post Hoc Model A and Model 3

	RF child and adolescent	Model 1	Post Hoc model A	Model 3
MSE (test set)	0.190	0.190	0.181	0.187

Note: MSE, Mean Squared Error; RF, Random Forest

RF all:

This random forest regression used the ln-transformed oxytocin as response and takes as input the five predictors, sex, age, tanner, hormonal stage (levels: pre-menarche, menarche, menopause, male, missing) and hormonal protection (levels: Yes (oral), Yes (not oral), Yes (no specification), No, Not mother, missing). Note that sex is a subset of hormonal stage.

Observations from the same family are correlated, which makes this data, which contain information on both children, adolescents, and parents, correlated. We did not take this into account when modelling the data. Tools such as Mixed Effect Random Forests (MERF) can account for correlation in data, but tools for these models are sparse in R. We will therefore neglect the correlation when modelling the data.

This data set also has imbalanced groups. We split the data into a training set (~70%) and test set (~30%) based on family and strata hereof. This means that observations from the same family will not be split between training and test set. This ensures no leak of information between the two data sets. We used the R -package *splitTools* (version 1.0.1) to generate the strata. We normalized both the training set and the test set before fitting the random forests.
We used a random forest to model. We optimized the hyper-parameters listed in Table D.1. We used out-of-bag (OOB) error estimates to evaluate the performance of the hyperparameter settings. Note that OOB error estimates might be biased because of the correlated data and imbalanced groups. We grew 5000 trees. We handled missing values in the categorical predictors in random forest by adding an extra category named “missing”.

Optimization of the hyperparameter based on OOB error estimates finds

(mtry, sampsize, nodesize) = (3, 100, 50%).

Variable importance is plotted in Figure D.4. We found that pubertal stage and age were the most important variables. This is similar to the findings using traditional linear regression. Looking at the predictions from the random forest model on the test set (Figure D.5. and D.6.) it is evident that the random forest captures the quadratic-like effect of age for the children and adolescents and the linear effect of age for the parents.

Figure D.4. Variable importance for the Random Forest all model

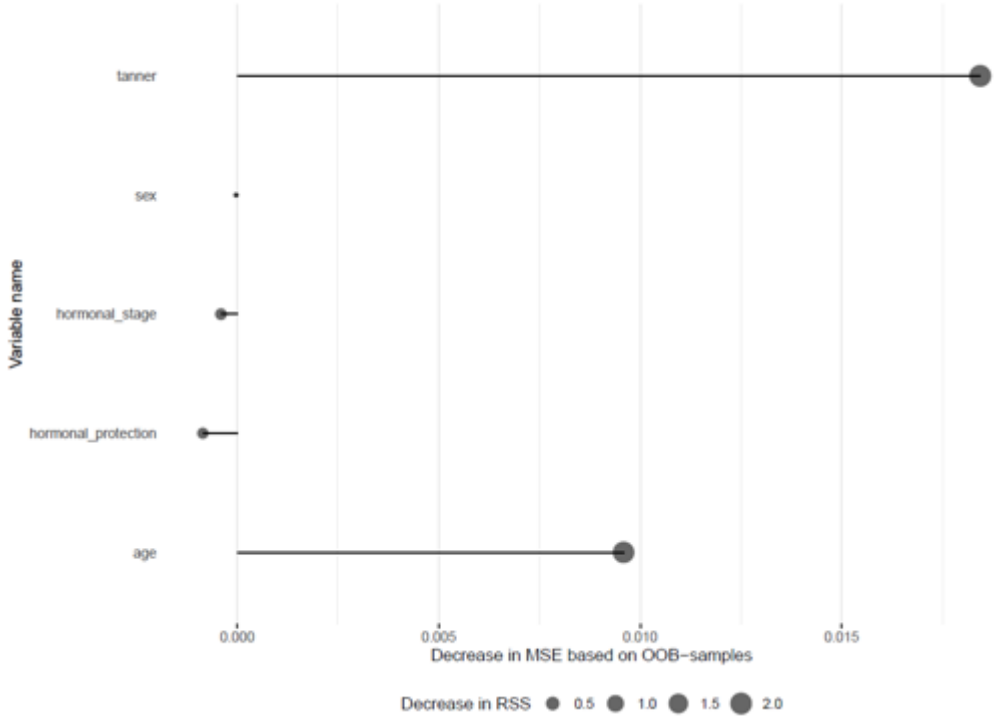


Figure D.5. The relationship between oxytocin and age predicted by the reduced model 4 (blue line) and random forest (green dots). Predictors are based on the test set. The black dots are data in the test set.

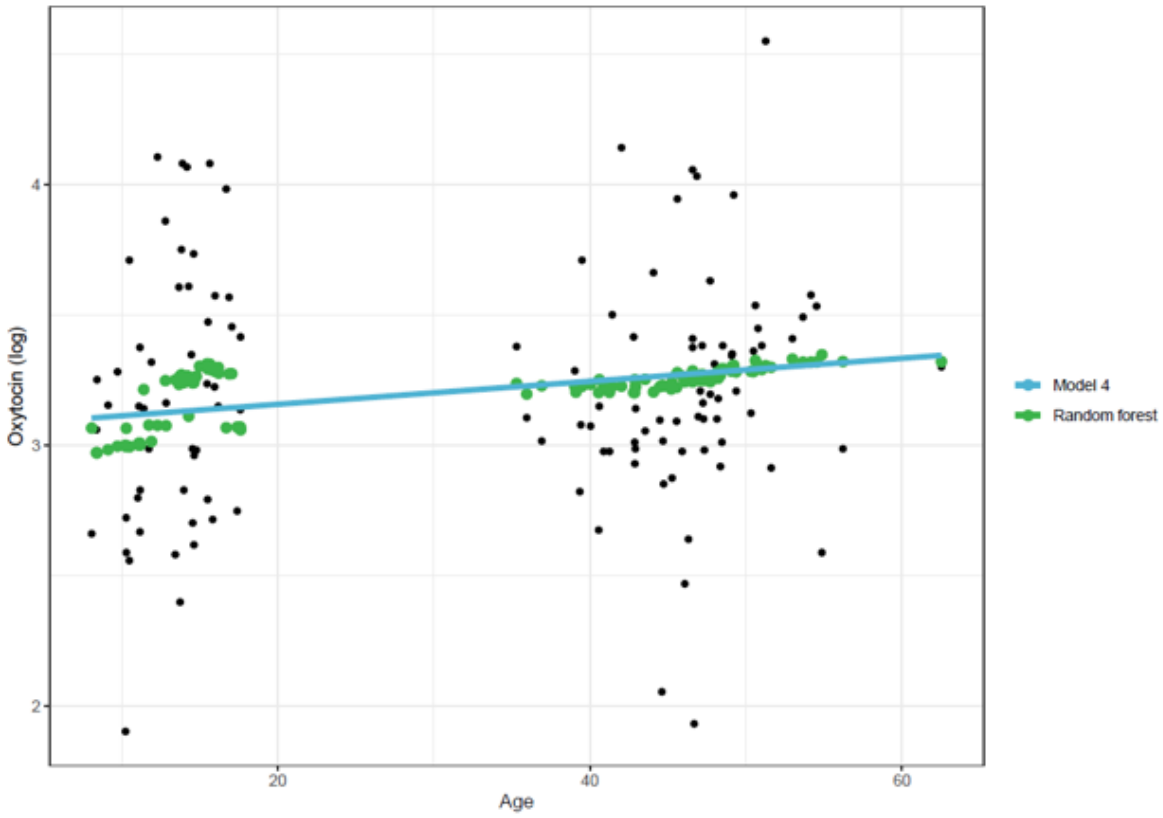
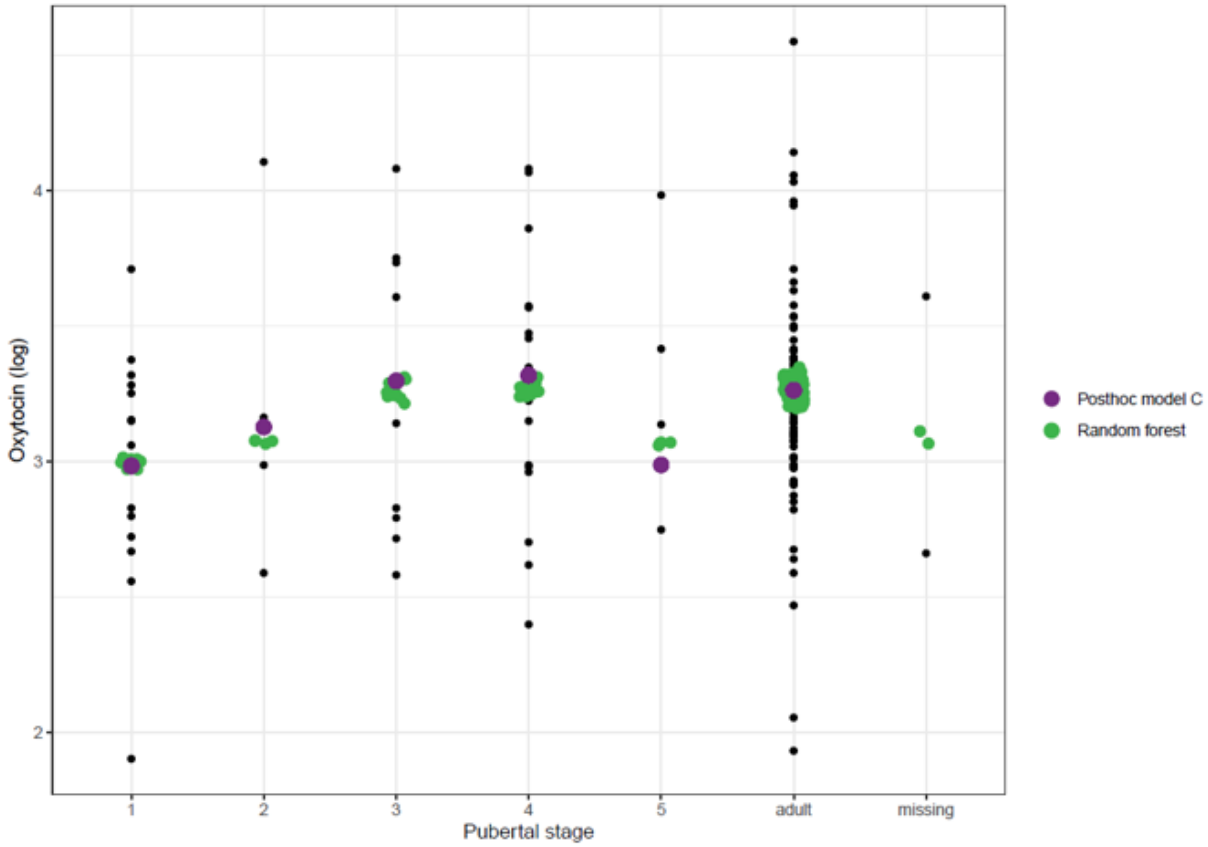


Figure D.6. The relationship between oxytocin and tanner predicted by the reduced post hoc model C (purple dots) and random forest (green dots). Predictions are based on the test set. The black dots are data in the test set.



The MSE of random forest on the test set is similar to the MSE of the mixed effect model (see Table D.3). All in all, this indicates that the random forest has not captured signals in the data, which were not captured by the linear models.

Table D.3. Mean Squared Error of the RF all, Model 4, and Post Hoc Model C

	RF all	Model 4	Post Hoc Model C
MSE (test set)	0.192	0.200	0.198

Note: MSE, Mean Squared Error; RF, Random Forest

E. Detailed description of full and reduced models and results from frequentist analyses

Model 1

Eq. E.1. Model 1:

$$\ln(oxytocin\_i) = \mu + \beta(OCD\ status_i) + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

Ln denotes the natural logarithm and $i = 1, \dots, 201$ describes the individual participants (in this model children and adolescents). The intercept is denoted by μ, β represents the regression coefficient, and ϵ_i represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation σ.

Table E.1. Descriptive statistics of child and adolescent salivary oxytocin concentrations

Child salivary oxytocin concentrations	Children and adolescents with OCD	Children and adolescents without OCD
Mean (*SD*)	26.61 (14.28)	25.61 (13.95)
Missing (*n*)	0	0

Note. *n*, number of observations; *SD*, standard deviation; OCD, obsessive-compulsive disorder

Table E.2. Results on parameters from reduced model 1

Parameter	Estimate	Standard Error
μ	3.107	0.052
β (OCD status)	0.079	0.069
σ	0.488	

Model 2

Eq. E.2. Full Model 2:

$$\ln(oxytocin_i) = \mu + \beta_1(OCD\ status_i) + \beta_2 \cdot age_i + \beta_3(sex_i) + \beta_4(sex_i) \cdot age_i + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma^2)$$

Ln denotes the natural logarithm and $i = 1, \dots, 201$ describes the individual participants (in this model children and adolescents). The intercept is denoted by μ, β represent the regression coefficient, and ϵ_i represent the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation σ.

Eq. E.3. Reduced Model 2

$$\ln(oxytocin_i) = \mu + \beta_2 \cdot age_i + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma^2)$$

Table E.3. Stepwise reduction of model 2

Drop	Variable	*F*-statistics	*p*-value
1	Interaction between age and sex	0.112	0.7387
2	Sex	0.068	0.7953
3	OCD-status	0.840	0.3605
	Age	8.913	0.0031

Note: OCD, Obsessive Compulsive Disorder

TableE.4. Parameter estimates and standard errors from reduced Model 2

Parameter	Estimate	Standard Error
μ	2.677	0.163
β_2 (age)	0.036	0.012
σ	0.479	

Model 3

Eq. E.4. Full Model 3

$$\ln(oxytocin\_i) = \mu + \beta_1(OCD\ status_i) + \beta_2(pubertal\ stage_i) + \beta_3(sex_i) + \beta_4(sex_i \times pubertal\ stage_i) \quad + \epsilon_i$$

$$\epsilon_i \sim N(0, \sigma^2)$$

Ln denotes the natural logarithm and $i = 1, \dots, 191$ describes the individual participant (in this model children and adolescents). The intercept is denoted by μ , β_x represents the regression coefficients, and ϵ_i represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation σ.

Eq. E.5.Reduced Model 3

$$\ln(oxytocin_i) = \mu + \beta_2(pubertal\ stage_i) + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma^2)$$

Table E.5. Stepwise reduction of model 3.

Drop	Variable	*F*-statistics	*p*-value
1	Interaction between pubertal stage and sex	0.571	0.6838
2	Sex	0.010	0.9192
3	OCD-status	2.113	0.1477
	Pubertal stage	5.384	0.0004

Note: OCD, Obsessive Compulsive Disorder

Table E.6. Parameter estimates and standard errors from reduced model 3

Parameter	Estimate	Standard Error
μ	2.951	0.064
β_2 (pubertal stage 2)	0.181	0.104
β_2 (pubertal stage 3)	0.359	0.103
β_2 (pubertal stage 4)	0.381	0.093
β_2 (pubertal stage 5)	0.118	0.121
σ	0.471	

Model 4

Eq. E.6.. Full Model 4

$$\ln(oxytocin\_i) = a(family_i) + \beta_1 \cdot age_i + \beta_2(sex_i) + \beta_3(sex_i) \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2) \quad a_i \sim N(0, \sigma_a^2)$$

Ln denotes the natural logarithm and $i = 1, \dots, 442$ describes the individual participant (in this model children, adolescents, and parents). The intercept is denoted by μ, β_x represents the coefficients of the fixed effects, a denotes the random effects and ε represents the residuals.

Eq. E.7. Reduced Model 4

$$\ln(oxytocin_i) = \mu + a(family_i) + \beta_1 \cdot age_i + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma^2), \quad a(family_i) \sim N(0, \sigma_a^2)$$

Table E.7. Stepwise reduction of the from the mixed effect model 4 (model reduction table). Family-variable included as random effect in all steps

Drop	Variable	*F*-statistics	*p*-value
1	Interaction between age and sex	1.499	0.2228
2	Sex	0.074	0.7857
	Age	13.281	0.0003

Table E.8. Parameter estimates and standard errors from reduced model 4

Parameter	Estimate	Standard Error
μ	3.092	0.042
β_1 (age)	0.004	0.001
σ_a	0.082	
σ	0.130	

Post Hoc model A

Eq. E.8. Full Post Hoc model Aodel

$$\ln(oxytocin\_i) = \beta_1(OCD\ status_i) + \beta_2 \cdot age_i + \beta_3 \cdot age_i^2 + \beta_4(sex_i) + \beta_5(sex_i) \cdot age_i + \beta_6(sex_i) \cdot age_i^2 + \epsilon_i$$
$$\epsilon_i \sim N(0, \sigma^2)$$

Log describes the natural logarithm", $i = 1, \dots, 201$, (describes the individual participant - in this model children and adolescents), μ represent the intercept-mean, β_x the coefficients, ε the residual, σ the standard deviation and N a normal distribution

Eq. E.9. Reduced Post Hoc model A

$$\ln(oxytocin_i) = \mu + \beta_2 \cdot age_i + \beta_3 \cdot age_i^2 + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma^2)$$

Table E.9. Stepwise reduction of Post Hoc Model A (model reduction table).

Drop	Variable	*F*-statistics	*p*-value
1	Interaction between age^2 and sex	3.028	0.0834
2	Interaction between age and sex	0.005	0.942
3	Sex	0.286	0.593
4	OCD-status	0.946	0.332
	Age^2 (with age)	3.904	0.0496

Note: OCD, Obsessive Compulsive Disorder

Table E.10. Parameter estimates and standard errors from reduced Post hoc model A

Parameter	Estimate	Standard Error
μ	1.137	0.796
β_2 (age)	0.282	0.125
β_3 (age^2)	-0.009	0.005
σ	0.476	

Post Hoc Model C

Eq. E.10. Full Post Hoc model C

$$\ln(oxytocin\_i) = a(family_i) + \beta_1(modified\ \ pubetal\ stage_i) + \beta_2(sex_i) + \beta_3(sex_i \times modified\ pubertal\ stage_i) + \epsilon_i$$

$$\epsilon_i \sim N(0, \sigma^2) \quad a_i \sim N(0, \sigma_a^2)$$

Ln denotes the natural logarithm and $i = 1, \dots, 188$ describes the individual participant (in this model children, adolescents, and parents). The intercept is denoted by μ, β_x represents coefficients of the fixed effects, *a* denotes random effects and ε represents residuals.

Eq. E.11. Reduced Post Hoc model C

$$\ln(oxytocin\_i) = \mu + a(family_i) + \beta_1(modified\ pubetal\ stage_i) + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2) \quad a_i \sim N(0, \sigma_a^2)$$

Table E.11. Stepwise reduction of Post Hoc Model C (model reduction table). Family-variable included as random effect in all steps

Drop	Variable	*F*-statistics	*p*-value
1	Interaction between pubertal stage and sex	0.576	0.7187
2	Sex	0.022	0.8836
	Modified pubertal stage	4.562	0.0005

Table E.12. Parameter estimates and standard errors from reduced Post hoc model C

Parameter	Estimate	Standard Error
μ	3.002	0.060
β_1 (pubertal stage 2)	0.133	0.097
β_1 (pubertal stage 3)	0.265	0.092
β_1 (pubertal stage 4)	0.284	0.085
β_1 (pubertal stage 5)	0.045	0.109
β_1 (pubertal stage adult)	0.247	0.061
σ_α	0.081	
σ	0.128	

Post Hoc model D

Eq. E.12. Full Post Hoc model D

$$\ln(oxytocin\_i) = \mu + a(family_i) + \beta_1 \cdot age_i + \beta_2(sex_i) + \beta_3(sex_i) \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2) \quad a_i \sim N(0, \sigma_a^2)$$

Ln denotes the natural logarithm and $i = 1, \ldots, 254$ describes the individual participant (in this model youths and parents). The intercept is denoted by μ, β_x represent the coefficients of the fixed effects, a denotes the random effects and ε represents the residuals

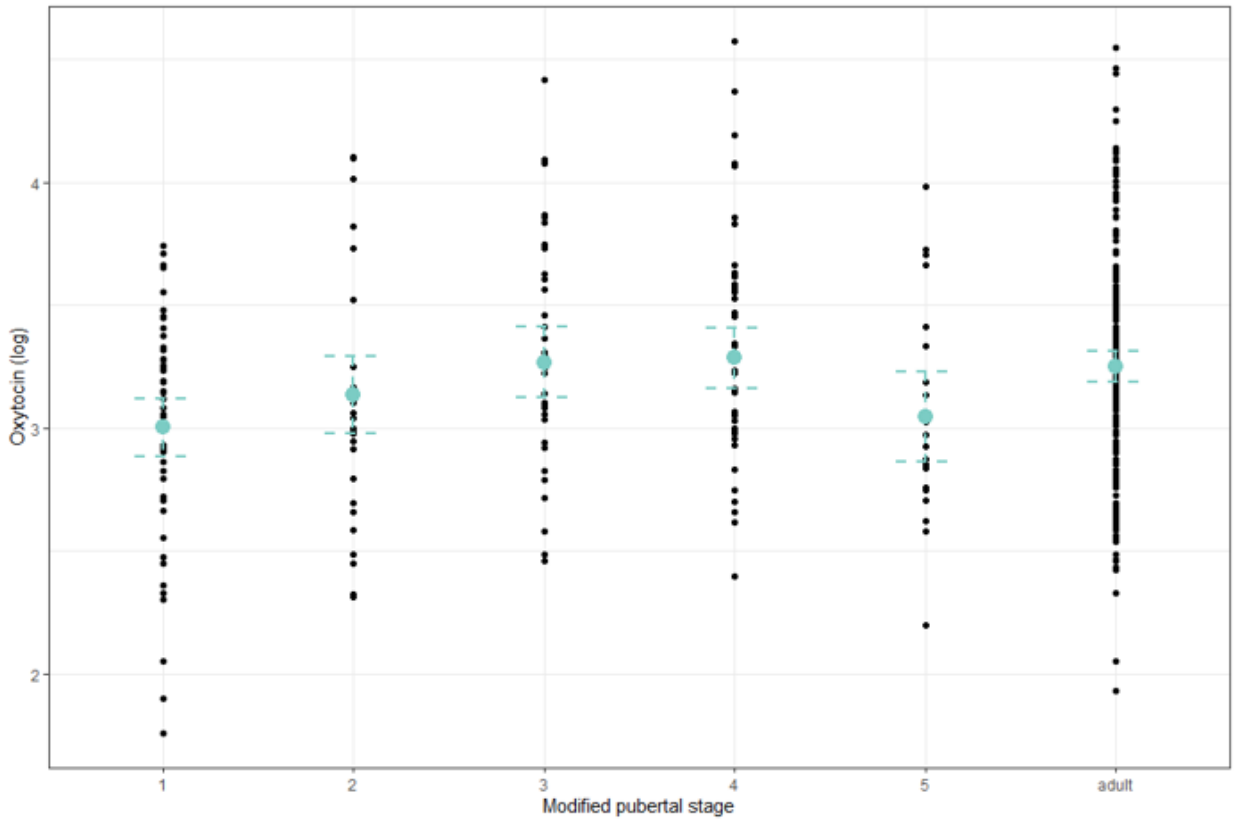
Eq. E.13. Reduced Post Hoc model D

$$\ln(oxytocin_i) = \mu + a(family_i) + \beta_1 \cdot age_i + \epsilon_i, \qquad \epsilon_i \sim N(0, \sigma^2), \quad a(family_i) \sim N(0, \sigma_a^2)$$

Table E.14. Stepwise reduction of Post Hoc Model D (adults alone) (model reduction table). Family-variable included as random effect in all steps

Drop	Variable	*F*-statistics	*p*-value
1	Interaction between age and sex	0.035	0.8516
2	Sex	0.146	0.7033
3	Age	2.696	0.1019

Figure E.1. The relationship between oxytocin and modified pubertal stage predicted by the reduced post hoc model C. The dashed lines represent the 95-confidence interval of the mean.



F. Exploratory analyses

We chose to analyze additional variables that could cause variation in oxytocin, but since these analyses were not the primary focus of the present study, we provide them as supplementary analyses.

Methods exploratory tests in supplement

For all models we used transformation with natural logarithm and exclusion of outliers as for the primary analyses. We provide unadjusted results and results adjusted for age when relevant.

Time of sample

Time of the saliva sample was registered by investigators at the time of sampling. We analyzed time of sample in all participants (both children, adolescents, and parents). There were no missing values on time of sample. Time of sample was included as a categorical variable with four categories (8am-09:59am, 10am-11:59am, 12am-01:59pm, 2pm-03:59pm). Age was included as covariate. We included the family-variable as a random effect variable for the same reason as described for Model 4 (described in main text). We used a mixed effect model.

Eq. F.1.

$$\ln(oxytocin_i) = \mu + a(family_i) + \beta_1(time\ of\ sample_i) + \beta_2 \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2) \qquad a(family_i) \sim N(0, \sigma_a^2)$$

CGAS

Children and adolescents functional level was measured with the Children's Global Assessment Scale (CGAS)[10]. The scoring was scored by trained investigators after the initial clinical assessment of the children and adolescents. CGAS has a range from 0 to 100 with higher values indicating better function. It was included as a continuous variable. We used a partial correlation analysis with age as covariate.

Eq. F.2.

$$\ln(oxytocin\_i) = \mu + \beta_1 \cdot CGAS_i + \beta_2 \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

HIK

We planned to investigate IQ with age as a covariate. When investigating assumptions, we found age and IQ correlated in our sample. We therefore chose not to analyze oxytocin and IQ.

CYBOCS

We used the CYBOCS total score as a measure of children and adolescents OCD severity[4]. Trained investigators performed and scored the CYBOCS semi-structured interview. The CYBOCS total score has a range from 0-40 with higher levels indicating more severe OCD. We included CYBOCS total score as a continuous variable and used a partial correlation analysis age as covariate.

Eq. F.3.

$$\ln(oxytocin_i) = \mu + \beta_1 \cdot CYBOCS_i + \beta_2 \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

Social responsiveness scale

Social responsiveness was assessed in patients by parent answering the Social Responsiveness Scale questionnaire (SRS-2)[11]. The consist of 65 questions with answers on a 4-point scale. We used a total score that can be calculated from the questionnaire with higher values indicating problem with social responsiveness. The total score was included as a continuous variable. We analyzed the total scores for mothers and fathers separately. We used a partial correlation with age as a covariate.

Eq. F.4.

$$\ln(oxytocin\_i) = \mu + \beta_1 \cdot SRS_i + \beta_2 \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

Menarche status

The menarche status was reported in a questionnaire by the youths. We included menarche status as a binary variable and tested for a difference in child and adolescent oxytocin concentrations in the binary variable "Menarche" with a Welsch t-test. Age was not included as a covariate as it is closely associated with menarche.

Eq. F.5.

$$\ln(oxytocin\_i) = \mu + \beta_1(menarche\ status_i) + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

We estimated the menstrual phase of the female participants from the first day of last menstruation which was reported by the participants in a questionnaire. The participants were estimated as in menstrual phase if the day of saliva sampling was two days before or two days after first day of last menstruation. The participants were estimated in ovulatory phase if day of saliva sampling was 10 till 14 days after first day of last menstruation. We chose to analyze girls and mothers separately as we only had information on two pairs of mothers and daughters and could therefore not include the family variable.

For the mothers, we used a Welsch t-test to investigate oxytocin differences between the two menstrual phases.

Eq. F.6.

$$\ln(oxytocin\_i) = \mu + \beta_1(menstrual\ phase_i) + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

For the children and adolescents, we used an ANCOVA with age as a covariate.

Eq. F.7.

$$\ln(oxytocin\_i) = \mu + \beta_1(menstrual\ phase_i) + \beta_2 \cdot age_i + \epsilon_i \qquad \epsilon_i \sim N(0, \sigma^2)$$

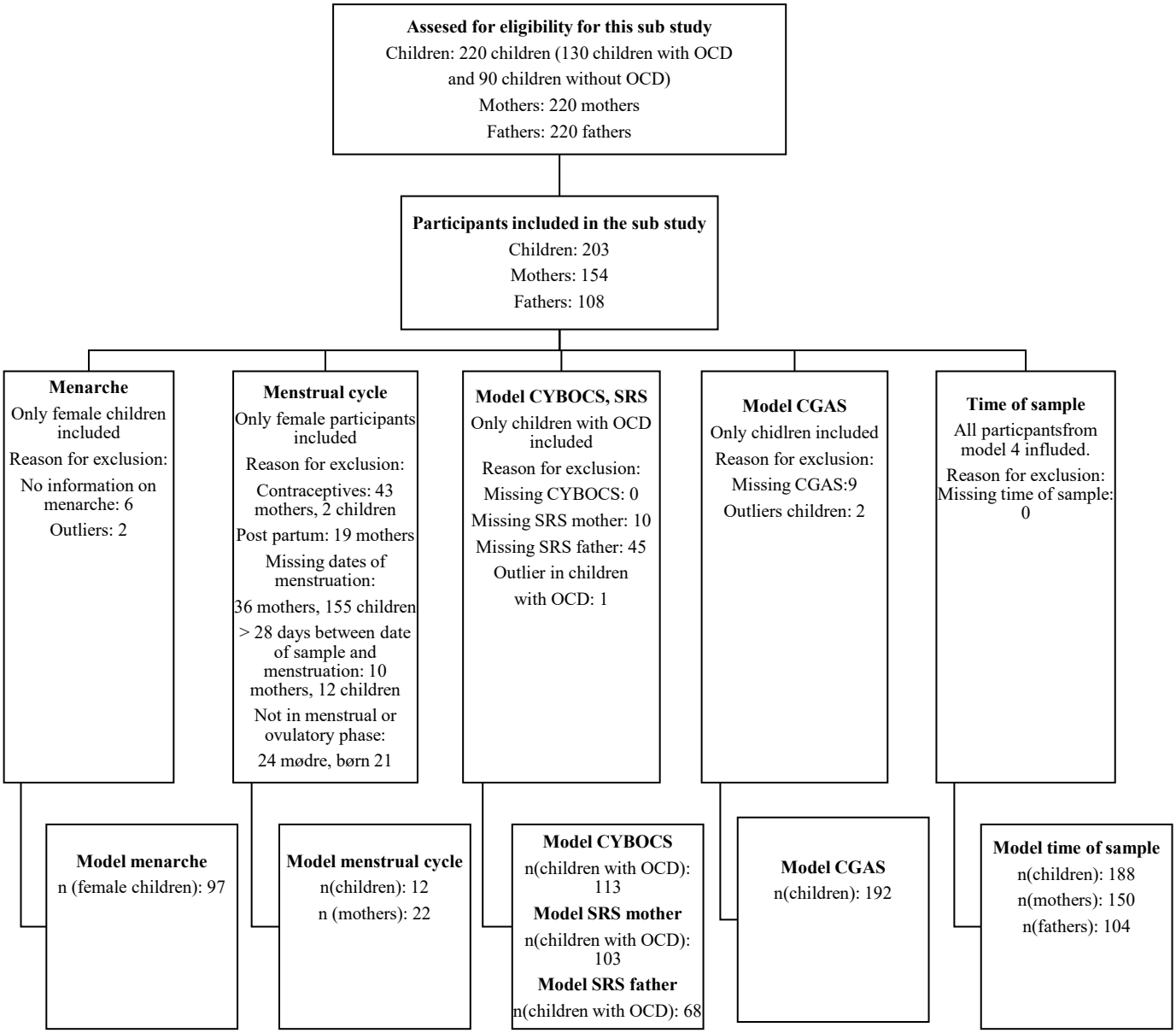
Results

The flowchart of participants in each exploratory analysis can be seen in Figure F.1. Descriptive statistics and results of all analyses can be seen in Table F.1-12.

We only found a significant difference between oxytocin concentrations of adult females in menstrual phase compared to adult females in menstrual phase. This should be interpreted with caution since it is part of exploratory analyses with no adjustment of multiple testing. The results of the analyses should be replicated in studies designed to investigate the specific variables with adequate sample sizes.

In addition, the measure of menstrual cycle is uncertain as it is self-reported as last day of menstruation which is a very insecure measure of menstrual cycle phase. The results can however be interesting in planning future studies.

Figure F.1. Flow chart of participants in supplementary analyses



Note: OCD, Obsessive Compulsive Disorder; n, number; SRS, Social Responsiveness Scale, CGAS, Children's Global Assessment Scale

All participants

Time of day

Table F.1. Descriptive on time of day.

Time of sample (n (%))	
8am – 09:59am	165 (37.33%)
10am-11:59am	166 (37.56%)
12am-13:59am	71 (16.06%)
14am-15:59am	40 (9.05%)

Table F.2. Time of sample results from mixed effect model **Family-variable included as random effect in all steps.

	Sum Sq	F- statistics	*p*-value
Time of sample (corrected for age)	0.86	2.22	0.09
Time of sample (not correction)	0.95	2.37	0.07

All child and adolescent participants

CGAS

Table F.3. Descriptive on CGAS

CGAS	
Mean (SD)	69.85 (18.44)
Median (min, max)	65 (35,100)

Table F.4. CGAS results from partial correlation

	Estimate	t-statistics	*p*-value
CGAS (corrected for age)	-0.0002	-0.11	0.90
CGAS (no correction)	-0.0006	-0.033	0.74

Children and adolescents with OCD only

CYBOCS

Table F.5. Descriptive on CYBOCS

CYBOCS Total score	
Mean (SD)	25.32 (4.91)
Median (min, max)	25 (16,36)

Table F.6. CYBOCS results from partial correlation

	Estimate	t-statistics	*p*-value
CYBOCS (corrected for age)	0.003	0.34	0.73
CYBOCS (no correction)	0.004	0.44	0.66

SRS

Table F.7. Descriptive on SRS

SRS	
SRS mor	
Mean (SD)	52.58 (8.05)
Median (min, max)	50 (39,76)
SRS far	
Mean (SD)	52.89 (8.83)
Median (min, max)	52 (41,83)

Table F.8. SRS results from partial correlation

	Estimate	t-statistics	*p*-value
SRS total mor (corrected for age)	0.003	0.48	0.63
SRS total mor (no correction)	0.002	0.40	0.69
SRS total far (corrected for age)	-0.005	-0.80	0.43
SRS total far (no correction)	-0.006	-0.79	0.43

Female children and adolescents only

Menarche

Table F.9. Descriptive on menarche

Menarche (n (%))	
Yes	62 (64%)
No	35 (36%)

Table F.10. Menarche results from t-test

	Estimate	t-statistics	*p*-value
Menarche (no correction)	0.17	1.53	0.13

Female participants only

Menstrual cycle

Table F.11. Descriptive on menstrual cycle

Menstrual cycle (*n* (%))	Female children and adolescents	Female adults
Menstrual phase	7 (58%)	9 (41%)
Ovulatory phase	5 (42%)	13 (59%)

Table F.12. Menstrual cycle results from t-test

	Estimate	t- statistics	*p*-value
Menstrual phase children (corrected for age)	0.33	0.79	0.45
Menstrual phase children (no correction)	0.20	0.57	0.58
Menstrual phase mothers (no correction)	0.53	2.94	0.008

G. Patient information on comorbidity, somatic disorders, and medication

Table G.1. Information on comorbid psychiatric disorders, medicine and somatic disorders on children and adolescents with OCD included in model 1 (patients with saliva samples available and outliers excluded)

	Children and adolescents with OCD
Comorbid psychiatric disorders (n (%))	
Depressive disorders, ICD-10 F32	1 (0.88%)
Anxiety disorders, ICD-10 F40-41, F93	12 (10.62%)
Stress and adjustment disorders, ICD-10 F43	12 (10.62)
Eating disorders, ICD-10 F50	4 (3.54%)
Sleeping disorders, ICD-10 F51	1 (0.88%)
Personality disorders, ICD-10 F60-69	1 (0.88%)
Asperger's Syndrome, ICD-10 F84·5	14 (12.39%)
Other developmental disorders, ICD-10 F88	4 (3.54%)
Hyperkinetic disorders ICD-10 F90.0	13 (11.50%)
Hyperkinetic conduct disorders ICD-10 F90.1	2 (1.77%)
Conduct disorders ICD-10 F91.3	1 (0.88%)
Attachment disorders, ICD-10 F94	1 (0.88%)
Tic disorders, ICD-10 F95	13 (11.50%)
Enuresis, ICD-10 F98.0	3 (2.65%)
Attention deficit disorder, ICD-10 F98.8	2 (1.77%)
Medication (n (%))	
Methylphenidate	2 (1.77%)
Melatonin	9 (7.96%)
Phenergan	1 (0.88%)
Antiepileptic medication (Valproat)	1 (0.88%)
Medication for asthma or allergy	13 (11.50%)
Antihormonal contraception	2 (1.77%)
Thyroid hormone (Levothyroxine)	1 (0.88%)
Somatic disorders (n (%))	
Epilepsy	1 (0.88%)
Migraine	3 (2.65%)
Hypothyroidism	1 (0.88%)
Allergy	14 (12.39%)
Asthma	6 (5.31%)
Skin conditions (eczema, psoriasis, vitiligo)	14 (12.39%)
Kidney disease (hydronephrosis, Henoch-Schonlein purpura)	2 (1.77%)
Sticklers' syndrome	1(0.88%)

Note: ICD-10, International Classification of Diseases-10; n, number; OCD, Obsessive Compulsive Disorder

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14. Appendix II of thesis: Study II and Appendix for study II

The Role of Oxytocin in Family Processes in the Presence and Absence of Pediatric OCD

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Abstract

Purpose: Shared neuroendocrine mechanisms between the attachment and anxiety systems have led to an interest in the role of neuroendocrine mechanisms in parent involvement in children's mental disorders. Research on child anxiety disorders has suggested a role of the neuropeptide oxytocin in family accommodation. In this study, we aimed to determine whether these findings could be extended to pediatric OCD. Specifically, we explored whether child oxytocin levels are associated with family accommodation in children with OCD. In addition, we examined whether child oxytocin is associated with family conflict, cohesion, parental stress, and parental oxytocin levels in families of children with and without OCD.

Methods: We conducted a cross-sectional study. including 120 children (71 with OCD, 49 controls), 97 mothers, and 58 fathers. Salivary oxytocin from children and parents was analyzed with ELISA. Parents completed the Family Accommodation Scale- Self-Report (FAS-SR) and the Parental Stress Scale (PSS). Children completed the Family Environmental Scale (FES). Regressions analyses were used to estimate associations between child oxytocin as the outcome and four models containing different variables.

Results: We found no association between child oxytocin and mother- or father-reported family accommodation. Variance in child oxytocin levels was associated with child-rated family conflict and with parental stress but only in the models with information from fathers. The direction of associations differed for children with OCD and controls. Child oxytocin was associated with mother and father oxytocin levels.

Conclusions: Our results shed light on a potential link between child oxytocin and family conflict and parental stress. The cross-sectional design complicates interpretations, but we discussed different possible causes for the different directions for children with OCD and controls, and different directions for in relation to data from the mothers and fathers. Our findings suggest that child and parent oxytocinergic systems continues to be associated after infancy. Our study is exploratory and cross-sectional, but the results point to possible future studies.

Keywords: Oxytocin, Obsessive Compulsive Disorder, Parental stress, Children, Adolescents, Family Accommodation

1. Introduction

From birth, children depend on caregivers for protection and rely on help from their parents when they experience distress or fear[1]. The attachment to and the interaction with parents therefore serve as important safety signals during childhood[2,3]. The connection between the systems of attachment and anxiety is evident from research showing shared neuroendocrine regulating mechanism[1,4,5]. This has led to an interest in neuroendocrine mechanisms underlying parent involvement in children's mental disorders, such as anxiety and OCD [5].

Obsessive-compulsive disorder (OCD) is a serious and debilitating psychiatric disorder that often debuts in childhood[6,7]. In pediatric OCD, children experience frequent and high levels of negative emotions such as fear and anxiety, and parents are often involved in trying to manage this distress[8]. Impairment and burden of the disease are not limited to the child; the disorder affects the entire family, especially the parents[6,9,10]. The clinical presentation of families with children who have OCD has been characterized by high levels of parental stress (the feelings and levels of stress associated with parenting), high levels of family conflict (i.e. expressed anger and disagreement or argument between family members) and low levels of family cohesion (i.e. the support, warmth, and commitment between family members)[11,12]. These family aspects are important, as they can influence the course of pediatric OCD and are often addressed in therapy sessions[13].

Families of children with OCD have also been characterized by high levels of involvement in managing the disorder, particularly through family accommodation. Family accommodation is a frequent change in family interactions in pediatric OCD and a key target for treatment, as reducing accommodation can lead to improvements in the child's symptoms[6,14,15]. Family accommodation refers to a process in which family members, often parents, adjust their behavior to relieve or prevent the child's OCD-related negative emotions[10]. Changing behavior to help one's distressed child is a natural part of parenting; however, family accommodation can maintain and reinforce OCD by increasing the child's avoidance and safety behaviors[5]. Accommodation can be time consuming and stressful for the parents[5]. However, when parents do not accommodate, children often react with coercive and disruptive behaviors leading to heightened conflict between the child and parent[5]. The disruptive child behavior and high level conflict can in turn lead to high levels of parent stress[10].

Neuroendocrine mechanisms are involved in the regulation of attachment and anxiety; however, it is not clear if neuroendocrine mechanisms are involved in maladaptive parent-child interactions in the context of pediatric OCD. Oxytocin is a neuropeptide and a neurohormone that may play a role in family accommodation due to its role in regulation of anxiety and interpersonal behavior[5]. The association between child salivary oxytocin and family accommodation has been investigated in a study of 50 children with anxiety disorders and their parents[16]. The study showed a negative relationship between child oxytocin and anxiety ratings, and a negative relationship between oxytocin and components of family accommodation

(child consequences when parents do not accommodate and maternal participation in accommodation)[16]. The findings on oxytocin's possible role in pediatric anxiety inspired treatment interventions with increased focus on the interpersonal behavior between children and parents[14,17,18] and research aiming to identify ways to guide treatment choices[19]. To our knowledge, no studies have investigated the role of oxytocin in family accommodation in pediatric OCD. Addressing this knowledge gap could provide valuable insights that may guide future studies and, in the long term, inform the development of improved or new treatments in pediatric OCD. The previous study in pediatric anxiety pointed to important areas that could be addressed in future studies on oxytocin and family accommodation[16].

First, inclusion of a non-clinical control group could be included to explore whether similar patterns of associations exist in a non-clinical population[16]. The construct of family accommodation and its measurement has been developed in the context of psychopathology[20]. However, other family factors emerge and can be measured in clinical and non-clinical samples. Family conflict, cohesion and parental stress are important in pediatric OCD, but manifest to varying extents across all families[9] and can be measured in non-clinical samples. Second, based on evidence concerning multi-generational and epigenetic influences on the oxytocin system, the previous study called for research on the interplay between the maternal oxytocinergic system, maternal care, child oxytocin and anxiety[16]. Lastly, the authors highlighted the importance of also including paternal oxytocin in the study of to improve the overall understanding of parental involvement[16]. To explore whether the findings on the role of oxytocin in family accommodation in pediatric anxiety can be extended to pediatric OCD, we included a sample of children with OCD and a age- and sex-matched control group to investigate: 1) whether child oxytocin is negatively associated with the degree of family accommodation in pediatric OCD (primary aim), and 2) whether child oxytocin is associated with family conflict and cohesion, parental stress and parental oxytocin, in children with OCD and children without a psychiatric disorder and whether associations differ between children with OCD and controls (secondary aim).

2. Methods

2.1. Study design

This is an exploratory sub-study using baseline data from the TECTO-trial (Treatment Effects of Cognitive Therapy in Adolescents with OCD) – a randomized clinical trial (RCT) investigating the efficacy of cognitive behavioral therapy (CBT) in pediatric OCD[21]. The TECTO trial included children in the age range 8-17 years, recruited from the Child and Adolescent Mental Health Center, Copenhagen University Hospital in Denmark. We initiated the data collection in July 2018 and ended the data collection in September 2022. The TECTO trial complies to the Helsinki Declaration[22]. The trial was approved by the ethics committee (H-18010607) and the Knowledge Centre on Data Protection Compliance in The Capital Region of Denmark. The TECTO trial was registered with clinical trials.gov (NCT03595098).

2.2. Participants

The TECTO trial included 130 children and adolescents with OCD, 90 age- and sex-matched children and adolescents without any psychiatric disorder (from now on referred to as "controls"), and their parents. Here, we will use the term "children" to describe children and adolescents included in the study. All children were thoroughly screened for eligibility with the Kiddie-Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version (K-SADS-PL)[23]. In addition, all children were assessed withfull-scale Wechsler Intelligence Scales (WISC-V[24] for children aged 16.9 years or below or WAIS-IV[25] for children above 16.9 years). We assessed the children with OCD with the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)[26]. All children received verbal information about the study. Adolescents above 15 years also received written information about participation in the trial. Parents were given verbal and written information on their child's and their own participation and gave signed informed consent. Inclusion and exclusion criteria have also been described in previous TECTO papers[21,27]. The inclusion criteria for children with OCD were a primary diagnosis of OCD according to International Classification of Diseases-10 (ICD-10)[28], a CY-BOCS total score ≥16, age 8 to 17 years both inclusive, and signed informed consent from parents/legal guardians. Exclusion criteria for children with OCD were comorbid illness with pervasive developmental disorder (not including Asperger's syndrome), schizophrenia/psychosis, mania or bipolar disorder, depressive psychotic disorders, substance dependence syndrome, IQ<70, treatment with CBT, psychoeducation with relaxation training, antidepressant, or antipsychotic medications within the last six months prior to trial entry. Inclusion criteria for the controls were age 8 to 17 years both inclusive, sex and age matched with an included child with OCD (+/- 3 months of birthday) and signed informed consent from parents/legal caregivers. Exclusion criteria for the controls were any current or previous psychiatric disorders according to ICD-10 criteria, non-Danish-speaking, and IQ<70.Parents were included if they had a participating child in the study and if the parents gave informed consent for parent participation.

2.3. Measurements

2.3.1. Oxytocin – collection, sample preparation and immunoassay

Saliva was collected from children and parents using Salivettes® (Sarstedt, Rommelsdorf, Germany). Participants were instructed not to eat two hours or drink 30 min prior to sampling. The samples were collected between 8 AM and 4 PM and were stored at -20 °C until the preparation for analysis.
The analysis was prepared with the following steps: 1) thawing at room temperature, 2) centrifugation at 2,000 × g × 5 min × 4°C, 3) freezing to -80°C for at least one day, 4) thawing at 4°C, 5) centrifugation at 1,500 × g × 5 min × 4°C, 6) storing at -80°C, 7) lyophilization overnight, and 8) storing at -20°C until the analysis was performed. On the day of analysis, the samples were 1) thawed at room temperature, 2) reconstituted in assay buffer, 3) mixed twice at 2,700 rpm (MixMate, Eppendorf, Hamburg, Germany) for five min with a 5-min interval. We did not employ any procedure for extraction in the preparation of the

samples. The samples were analyzed with a commercial immunoassay (cat. no. ADI-900-153A; Enzo® oxytocin kit, NY, USA) following the manufacturer's instructions. We used WorkOut 2.5 (Dazdaq Solutions Ltd, Brighton, UK) for calculating the oxytocin concentrations as pg oxytocin per mL of saliva. The analyses showed an intra-assay coefficient of variation of 13% and an inter-assay coefficient of variation of 20%. Oxytocin levels were included as a continuous variable.

2.3.2. Family accommodation

Mothers and fathers completed the Family Accommodation Scale – Self-Report (FAS-SR) to assess the degree of family accommodation in OCD[29]. The FAS-SR is specifically designed for use with families of individuals with OCD, focusing on the ways in which family members may inadvertently support or accommodate OCD-related symptoms[29]. In the FAS-SR, the respondents estimate how often they engaged in 19 accommodating behaviors in the preceding week. The 19 items describe different accommodating behaviors, e.g. giving reassurance, facilitating avoidance, participating in compulsions or facilitating or modifying behaviors[29]. The items are answered on a 5-point scale ("never", "1 day", "2-3 days", "4-5 days", "every day"). The 19 items are summed to obtain a total score with a range from 0-76. The FAS-SR has shown acceptable internal reliability with a Cronbach-$\alpha = 0.9$[29] and acceptable convergent validity with FAS-SR correlating with OCD severity, and patient and family functioning[29]. The internal reliability in our study was similar (Cronbach-$\alpha = 0.9$) (calculated with R-studio package 'psych'). We included the FAS-SR total score in our analyses as a continuous variable.

2.3.3. Family cohesion and family conflict

Family cohesion and conflict was measured with The Family Environment Scale (FES)[30]. The FES includes 90 statements to each of which the participant should answer "true" or "false"[30]. The FES consists of ten subscales assessing different aspects of the family environment[30]. We used the child-rated responses on two subscales– family conflict and family cohesion. The FES defines family conflict as, "the amount of openly expressed anger and conflict among family members" and family cohesion as, "the degree of commitment, help, and support family members provide for one another"[30]. Each of the two subscales consist of nine true/false questions resulting in a score, ranging from 0 to 9 with higher scores indicating higher cohesion or higher conflict. Both subscales have demonstrated acceptable internal reliability (family conflict Cronbach-$\alpha = 0.75$ and family cohesion Cronbach $\alpha = 0.78$)[30]. The internal reliabilities in our study were acceptable for family conflict Cronbach $\alpha = 0.70$ and family cohesion Cronbach-$\alpha = 0.63$ (calculated with R-studio package 'psych'). The subscale scores were included in our analyses as continuous variables.

2.3.4. Parental stress level

Mothers and fathers completed the Parental Stress Scale (PSS) to assess their individual perceived levels of stress associated with parenting their child/children[11]. A study investigating the psychometric properties of

the PSS has suggested a two factor structure with to unidimensional subscales – parental stress and lack of parental satisfaction[31,32]. We used the Parental Stress subscale which contains nine items that is answered on a 5-point scale and the answers can be used to create a total score ranging from 9 to 45[32]. The Parental Stress Subscale has showed acceptable reliability for answers from parents of children from a community sample and parents of children with behavioral problems[32]. Our sample showed a good internal reliability with a Cronbach $\alpha = 0.82$ (calculated with R-studio package 'psych'). The subscale scores were included in our analyses as a continuous variable.

2.3.5. Child OCD status

We used the variable "child OCD status" describing whether the child had OCD or belonged to the control group. The diagnostic information was available from eligibility screening and was based on ICD-10 criteria[28]. Child OCD status was included as a binary variable in our analyses.

2.4. Statistical procedure

Due to the cross-sectional design, we were not able to make causal inferences and regression analyses were used to explore relationships between variables. The statistical analyses were conducted in two parts. First, we tested the statistical hypothesis that child oxytocin is negatively related to parent reported family accommodation in children with OCD (primary aim). Second, we explored whether child oxytocin is related to family conflict, family cohesion, parental stress, and parent oxytocin levels in children with OCD and controls and whether the relationships differed between children with OCD and controls in terms of magnitude or direction (secondary aim). Data from mothers and fathers were included in separate models.

The analyses were performed with regression models. Child oxytocin level was included as the dependent variable in all models. Child pubertal stage was included and retained in all models as a covariate based on a previous study from the TECTO trial showing a positive relationship between child salivary oxytocin and pubertal stage[27] (see Appendix A.1. for details on the assessment of pubertal stage).
The primary aim was investigated with two analyses of covariance regressions with child oxytocin as outcome, mother/father oxytocin as independent variables (in separate models) and child pubertal stage as a covariate. The secondary aim was investigated with backwards multiple regression analyses with child oxytocin as outcome, pubertal stage as a covariate. Family conflict, family cohesion, mother/father reported parental stress, mother/father oxytocin, OCD status, interactions between OCD and all independent variables, and the interaction between mother/father parental stress and mother/father oxytocin were included as independent variables (for a detailed description of the models see Appendix B-E). In the backwards stepwise regression, the initial model included all variables and interactions of interest. We excluded the variable with the highest p-value based on F-statistics in steps, until only variables with a significance level below $p = 0.05$ remained, i.e., the final model.

Analyses were performed in R studio (version 4.2.0) with the 'drop1' function for the backwards stepwise regression. We excluded outliers in oxytocin levels (defined as oxytocin levels three standard deviations from the mean). We applied a natural logarithmic transformation (ln) of oxytocin levels. Scores on family accommodation, family cohesion and conflict, and parental stress were only calculated for participants with complete item responses on the respective scales. We excluded data from participants with missing values on any given variable in a model and participants who were breastfeeding or pregnant. Due to the potential influence of genetics on oxytocin levels[33], only biological child-caregiver pairs were included.
We performed post hoc power calculations for our primary aim. In the models including mother family accommodation, assuming n=83, $\alpha = 0.05$ (2-sided), and $\beta = 0.80$, and pubertal stage included as a covariate, we were able to detect a large effect size (eta squared =0.135). In the models including fathers accommodation, assuming n=72, $\alpha = 0.05$ (2-sided), and $\beta = 0.80$, and pubertal stage included as a covariate, we were able to detect a large effect size (eta squared =0.152) (calculated using r package "pwrss"[34]).
Our sample size was based on the larger TECTO trial, and we considered this sub-study exploratory even though we tested a statistical hypothesis for both our primary and secondary aim. We did not pre-register the analysis plan or adjust for multiple comparisons. However, we reported all comparisons and considered the risk of false discoveries. We used a significance level threshold of $p<0.05$. We investigated assumptions for all models and the Variance Inflation Factor (VIF) of the final models (for details see Appendix B-E).

3. Results

In all models, we included only children with information on child oxytocin and child pubertal stage. In the two primary models investigating family accommodation, we included 83 children with OCD who had information on mother reported family accommodation and 72 children with information on father reported family accommodation (See flowchart in Appendix F). In the two exploratory models, we included 97 mother-child pairs and 58 father-child pairs with information on all relevant questionnaires and mother/father oxytocin. Some children were included in both the analyses with information for mothers and fathers and the number of children in the models do therefore sum up to the number of children included in the study. Table 1 displays the characteristics of the 120 children, 97 mothers and 58 fathers that were included in the models comparing children with OCD and controls. We found no difference in age, sex, or pubertal stage between the children with OCD and controls (Table 1). Child salivary oxytocin levels ranged from 6.7 pg/mL to 79.1 pg/mL after outliers were removed (for information on and consideration of the outliers see Appendix G). The children with OCD had moderate to severe illness severity measured with the CY-BOCS[26] total score with a range from 16 to 36. Family cohesion was lower and parental stress was significantly higher in the OCD group than the control group (Table 1). Fathers to children with OCD had higher oxytocin levels than fathers of controls (Table 2).

Table 1. Participant characteristics of children with OCD and controls

	Children with OCD	Controls	OCD vs Controls
Number of children *n* (% of sample)	71 (59%)	49 (41%)	
Child age, years (Mean (SD))	13.24 (2.73)	13.55 (2.84)	*p*=0.57[a]
Missing (*n*)	0	0	
Child sex (*n* (%))			*p*=0.59[b]
Female	37 (52%)	21 (57%)	
Male	34 (48%)	28 (43%)	
Missing	0	0	
Child pubertal Tanner stage (*n* (%))			*p*=0.92[b]
Pubertal stage 1	22 (31%)	13 (27%)	
Pubertal stage 2	9 (13%)	8 (16%)	
Pubertal stage 3	130 (18%)	7 (14%)	
Pubertal stage 4	18 (25%)	14 (29%)	
Pubertal stage 5	9 (13%)	7 (14%)	
OCD severity			-
CY-BOCS total score (Mean (SD)	25.78 (4.94)	-	
Child psychiatric comorbidity (*n* (%))			-
Asperger's syndrome F84·5	10 (14%)	-	
Hyperkinetic disorders F90.0	9 (13%)	-	
Anxiety disorders F40-41, F93	8 (11%)	-	
Stress and adjustment disorders F43	8 (11%)	-	
Tic disorders F95	8 (11%)	-	
Other disorders (*n*)	9	-	
Child Nationality (*n* (%))			-
Danish	69 (97%)	46 (94%)	
Other	1 (1.5%)	2 (4%)	
Missing	1 (1.5%)	1 (2%)	
Parental education mothers (*n* (%))			
Upper secondary education or below	7 (10%)	0	
Short-cycle tertiary education	11 (15%)	2 (4%)	
Bachelor's degree or equivalent	24 (34%)	20 (41%)	
Master's degree or above	26 (37%)	24 (49%)	
Missing	3 (4%)	3 (6%)	
Parental education fathers (*n* (%))			
Upper secondary education or below	13 (16%)	2 (4%)	
Short-cycle tertiary education	8 (10%)	3 (6%)	
Bachelor's degree or equivalent	16 (23%)	12 (24%)	
Master's degree or above	22 (31%)	16 (33%)	
Missing	14 (20%)	16 (33%)	
Salivary oxytocin in pg/ml* (Mean (SD))			
Child	26.21 (14.45)	27.01 (12.92)	*p*= 0.94[a]
Mother	28.66 (13.5)	28.71 (16.2)	*p*= 0.76[a]
Father	27.72 (9.3)	22.48 (6.78)	*p*= 0.05[a]
Family accommodation[1] (Mean (SD))			
Mother reported	21.99 (16.5)		
Father reported	15.46 (14.4)		
Family cohesion[2] (Mean (SD))			
Child reported	6.81 (1.8)	7.47 (1.7)	*p*=0.05[a]
Family conflict[2] (Mean (SD))			
Child reported	2.32 (2.2)	1.65 (1.7)	*p*= 0.07[a]
Parental stress[3] (Mean (SD))			
Mother reported	23.03 (6.54)	19.68 (5.43)	<0.01[a]
Father reported	23.36 (5.50)	18.76 (4.70)	<0.01[a]

Note: OCD = Obsessive Compulsive Disorder, SD, standard deviation.The t-tests on oxytocin levels were performed on the natural logarithm of oxytocin levels to meet the assumptions of the t-test; however, the summary statistics shows the values without natural logarithm. [1]Family Accommodation Scale-SR (FAS-SR), [2]Family Environmental Scale (FES), [3]Parental Stress Scale (PSS).

[a]= t-test

[b]= chi-square test

3.1. The between child oxytocin and family accommodation in children with OCD

We did not find an association between child oxytocin and mother-reported family accommodation (Model 1) or between child oxytocin and father-reported family accommodation (Model 2).

Table 2. Associations between the natural logarithm of child oxytocin and mother and father reported family accommodation

Final model	Intercept (μ (*SE*))	Parameter (β (*SE*))	F-statistics	*p*-value	Adj. R^2
Model 1*	2.970 (0.11)			0.239	0.02
Mother family accommodation	-	0.003 (0.003)	0.866	<0.355	-
Model 2*	3.037 (0.12)			0.421	0.000
Father family accommodation	-	0.005 (0.005)	0.009	0.926	-

*Note: *Adjusted for pubertal stage (detailed results on pubertal stage for these models in Appendix D and E). SE, standard error.*

3.2 The association between child oxytocin and family conflict, family cohesion, parental stress and parent oxytocin in children with OCD and controls

In the analysis of Model 3, when mother oxytocin, mother parental stress, family conflict and family cohesion, and the interactions were included in a backwards stepwise regression, all variables except mother oxytocin were eliminated (pubertal stage retained as a covariate) (Table 4, details in Appendix D). A correlation matrix of the included variables showed significant correlations between child-reported family cohesion and conflict (r= -0.44), between mother perceived parental stress and child-rated family conflict (r= 0.32) and between mother perceived parental stress and child OCD status (r= 0.26) (Appendix D). All correlation coefficients were below 0.517 and the VIFs were acceptable for the final model (Described in Appendix). Sensitivity analyses found no difference using a forward stepwise approach (Appendix D).

In the model with data from all children and fathers, father oxytocin, father parental stress, family conflict and family cohesion were entered into a backwards stepwise regression. Here, father oxytocin level, the interactions between child OCD status and child -reported family conflict, as well as the interaction between child OCD status and father reported parental stress were retained as these were all significant (and pubertal stage retained as a covariate) (See Table 4, details in Appendix E). A sensitivity analysis using a forward stepwise procedure only included father oxytocin (pubertal stage again retained as a covariate), which highlights that the analysis is sensitive to the model selection strategy. The adjusted R^2 for the backwards

regression was 0.20 and for the forward regression 0.15. Therefore, we chose to conclude on the backwards approach. A correlation matrix showed significant correlations between child-reported family cohesion and conflict (r= -0.26), between father reported parental stress and child pubertal stage (r= -0.38) and child OCD status (r= 0.37), and between father oxytocin level and child OCD status (r= 0.26). All correlation coefficients were below 0.440 Sensitivity analyses with a forward stepwise approach did not change the results (Appendix E).

Table 4. Results from final step in backwards multiple regression Model 3 and 4 predicting the natural logarithm of child oxytocin from family conflict, cohesion, parental stress and oxytocin levels in children with OCD and controls

Final model	Intercept (μ (*SE*))	Parameter (β (*SE*))	F-statistics	*p*-value	Adj. R^2
Model 3*	1.38 (0.32)			<0.001	0.25
Ln mother oxytocin	-	0.53 (0.10)	29.32	<0.001	-
Model 4*	0.44 (0.67)			<0.05	0.20
Ln father oxytocin	-	0.54 (0.17)	10.57	<0.01	-
Father reported parental stress		0.06 (0.03)	5.43	0.02	
Child reported family conflict		-0.18 (0.08)	4.75	0.03	
Child OCD status		0.99 (0.58)	2.94	0.09	
Father reported parental stress x child OCD status		-0.07 (0.03)	5.76	0.02	
Child reported family conflict x child OCD status		0.20 (0.09)	5.50	0.02	

Note: Ln, natural logarithm, SE, standard error. OCD; Obsessive Compulsive Disorder

**Adjusted for pubertal stage (detailed results on pubertal stage for these models in Appendix D and E).*

4. Discussion

In this study, we explored the role of oxytocin in family accommodation in the context of pediatric OCD. Additionally, we expanded our analysis by comparing children with OCD to matched controls, examining child oxytocin levels in relation to family conflict, family cohesion, parental stress, and the association between child and parent oxytocin levels. We did not find an association between child oxytocin and mother-reported or father-reported family accommodation in the children with OCD. When we looked at both the children with OCD and controls, we found an association between child oxytocin and both family conflict and parental stress in analyses that included data from fathers. Interestingly, the directions of these associations differed between children with OCD and controls. We found a positive association between child oxytocin and both mother's and father's oxytocin.

4.1. Child oxytocin and family accommodation in children with OCD

We found no association between child oxytocin levels and family accommodation in children with OCD. A study in children with anxiety disorders used the Family Accommodation Anxiety Scale (FASA) which

provides a total family accommodation score based on nine items, and also includes measures of parental distress related to accommodation and children's responses to not being accommodated[16]. This study found an association between child oxytocin and the family accommodation total score, but more than describing an overall relationship between child oxytocin and family accommodation, this was perhaps driven by a relationship between child oxytocin and maternal participation[16]. The total score from the FAS-SR and the FASA are based on a different number of items and direct comparison is therefore difficult. The study in pediatric anxiety disorders found a negative relationship between child oxytocin and with the FASA response on "child consequences of not accommodating". In addition, they found an negative relationship between child oxytocin and a factor consisting of all items regarding "parent participation"[16]. We chose to use the FAS-SR as this is validated and specific to family accommodation in OCD[29]. The FAS-SR does not measure "child consequences of not accommodating" and the factor structure of FAS-SR has not been investigated in children. We therefore only used the total score and cannot conclude on the same subscales as the FASA.

4.2. Child oxytocin and family conflict, cohesion, and parental stress in children with OCD and controls

We found that the variance in children's oxytocin levels was associated with variables from a model that included data from fathers: father oxytocin, father-reported parental stress, and child-reported conflict. For both parental stress and conflict, the relationships were different for the children with OCD compared to controls. When discussing interpretations of the associations, it is important to remember, that our study is cross-sectional, and we cannot infer causality. It is therefore important to consider multiple possible causes and potential reciprocal relationships when interpreting the associations.

In the children without a psychiatric disorder (controls), child oxytocin was negatively related to the child-reported family conflict and positively related to the father-reported level of parental stress. In the children with OCD, these associations were in opposite direction and of lesser magnitude. The different findings for family conflict and father-reported parental stress in children with OCD and controls, may indicate that these broad constructs are not describing the same phenomena in children with OCD and controls, and are thereby differently related to the child oxytocin system. In families of children with OCD, the family conflict and parental stress in the fathers could be related to parenting and supporting a child experiencing OCD-related distress and therefore related to the attachment between the children and parents. In children without OCD or other psychiatric disorders, conflict in the family could revolve around non-attachment-related issues (e.g., household tasks or schoolwork). Additionally, the stress experienced by parents of children without psychiatric disorders may be more influenced by everyday challenges, rather than concerns about supporting a distressed child. It could also be that family conflict and father-reported parental stress describe the same phenomena in pediatric OCD but that have different effects on the oxytocin system in the context of OCD.

However, these speculations must be grounded in empirical evidence. In children with OCD, child oxytocin was slightly positively related to children's perceptions of the level of conflict in their families and negatively related to the level of parental stress experienced by fathers. To attempt to understand these findings, it may be helpful to consider them in light of previous findings. Lebowitz et al. found that low child oxytocin was associated with high parental participation in child symptoms and child consequences for not accommodating[16]. Our findings may indicate that when children have OCD and low oxytocin, children elicit more accommodation from parents, possibly through means of coercion, which does not result in more conflict because fathers provide accommodation, but it does come at a cost to fathers in the form of high parental stress. This is speculative at this stage and warrants further investigation.

In contrast to the results from fathers, the variation in child oxytocin was not associated with child-rated family conflict nor parental stress in mothers. It is important to consider, that family conflict reflects children's perceptions of family conflict in general and not conflict specifically between children and mothers or fathers. It is therefore surprising that this relationship was not significant in the models with the mothers, but only when father-reported parental stress and oxytocin were kept in the model. This could be due to a Type I error, i.e., the significant findings in fathers were simply discovered by chance. However, it could also be that the associations with child oxytocin are related to fathers in the presence of child OCD but not the mothers. It could be hypothesized that fathers change behavior when child OCD enters the family system, and the changed paternal behavior affects child oxytocin levels. An alternative hypothesis is that child oxytocin could be related to child behaviors in a way that cause more stress for fathers than mothers in the presence of pediatric OCD. However, again, this warrants further investigation.

4.3. Associations between child oxytocin and parental oxytocin

We found a positive association between child salivary oxytocin (both children with OCD and controls) and both mother and father oxytocin. Our findings of an association between parent- and child oxytocin levels are consistent with three previous studies. Two studies showed positive correlations between parent and child salivary oxytocin – one study in 4- to 6-month-old children (n=55) and their mothers and fathers[35], and the other in 4- month-old children (n=53) and their mothers[36]. A third study investigated 6 year old children (n=97) and their mothers and found a positive correlation in urinary oxytocin levels[37]. Our study is the first to show an association between parent- and child oxytocin levels in children above 6 years of age. This novel finding may indicate that parents' and children's oxytocin levels continue to be associated across later childhood and adolescence, a period of rapid development associated with more independence and more physical space between children and parents than during early childhood. The correlation between parent and child oxytocin levels has been suggested to be driven by parent-child behaviors based on studies showing that the strength of correlations in parent-child oxytocin levels are affected by behaviors observed during parent-child interactions[35].

We did not observe any difference in the correlations in child and parent oxytocin between children with OCD and controls, which could be hypothesized if the presence of pediatric OCD affects how the parent-child interact and thereby affect oxytocin levels. However, with our sample size, this null finding should be interpreted with caution. The associations in oxytocin levels between children and parents could be explained by shared genetics, which have been indicated by studies finding alleles related to plasma oxytocin levels[38]. A combined role of both environment and genetics as well as epigenetics has been suggested[39].

4.4. Strengths and limitations

This study has limitations that must be considered in the interpretation of the findings. First, we used peripheral oxytocin measures as a proxy for central oxytocin levels, however, this is a topic of discussion[40,41]. We chose to use peripherally oxytocin the measurement of central levels too invasive for the children in our sample. Furthermore, a previous study found that oxytocin measured in CSF had higher correlations with salivary oxytocin than oxytocin measured in plasma[42]. However, whether salivary oxytocin can be used as a proxy for central levels under basal conditions is still unclear [40,41,43]. Second, we did not have information on the parents' somatic or diagnostic status, which could affect parental oxytocin levels and the relational aspects we investigate. Third, we had a considerable amount of missing data especially on the questionnaires (see flow chart in Appendix F) and our power calculation showed that we could only detect larger effect sizes. Even though our sample size is larger than the previous study investigating child oxytocin and family accommodation in pediatric anxiety, we did not quantify our null findings and the results should therefore be interpreted carefully[44]. A fourth consideration is the two subscales from FES that we used to investigate the family conflict and cohesion. Family conflict and cohesion have internal reliability measured by Cronbach-α of 0.703 and 0.63 respectively, which raises concerns of the usability of these scales. Fifth, we only used self-reported family conflict and cohesion. Self-reported measures can be biased by e.g., misunderstanding the questions, but the inclusion of information from multiple sources could be a strength in the study – here children's perspective on family environment, and mothers' and fathers' perspective on their own stress and family accommodation.

The study also has several important strengths. First, we included information from fathers as well as mothers, as recommended by a previous study since it can increase the understanding of family aspects compared to studies focusing on mothers only[16]. Second, we included thoroughly assessed children with OCD and controls without any psychiatric disorder, which strengthens our ability to investigate differences in the two groups as they are well characterized. Third, we included pubertal stage which can cause variation in oxytocin in the age range we investigate.

5. Conclusion

We did not find an association between child oxytocin levels and family accommodation in pediatric OCD. However, our results suggest a potential link between child oxytocin and family conflict and parental stress,

which varied between children with OCD and controls, and differed in relation to data from the mothers and fathers. In addition, we found that child oxytocin levels were associated with both mother and father oxytocin levels. Our study is exploratory but can be used to generate new research questions. Future studies could improve our knowledge on the underlying directions with appropriate designs, for example, by investigating oxytocin response during an interaction with mothers and fathers, using experimental designs with intranasal oxytocin or using longitudinal studies for example exploring the associations before and after child OCD enters the family system. Additionally, studies could explore more detailed components of family accommodation using scales developed for pediatric OCD, as well as examine whether parenting-related stress differs between children with pediatric OCD and controls, and whether this varies in mothers and fathers. Finally, knowledge on the interplay between child and parent oxytocin levels could be improved by investigating whether the relationship between parent and child oxytocin is mediated by specific parent and child behaviors.

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7. Data availability statement

After publication of all results from the TECTO trial, we plan to make a depersonalized data set publicly available on the EU ZENODO database.

8. Declarations of interest:

Valdemar Funch Uhre is currently employed by Novo Nordisk A/S. The authors declare no other conflicts of interests.

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Appendix

Content

A. Supplementary information in methods

Pubertal stage

We used child-reported pubertal stage using the Marshall-Tanner stages[1,2]. The children received instruction from one of the investigators (that was trained in the procedure) to report the most fitting pubertal stage from 1 (describing pre-pubertal development) to 5 (describing full adult development) after visual presentation of secondary sex characteristics based on the Marshall-Tanner criteria[1,2]. We did not use classification of the specific sex characteristics but the best fitting overall pubertal stage. The pubertal stage had a range from 1-5 and was included as a categorical variable.
Pubertal stage was retained as a possible covariate in the stepwise procedure regardless of the p-value (as insignificance would be due to a smaller sample size in the models in this study).

Method for checking assumptions of statistical models.

Assumptions were investigated with:

- Correlation matrix of all variables included to check co-linearity of the independent variables (R package GGally)
- Residual vs. fitted plot to check linearity of the model and autocorrelation (Autoplot in R package 'GGfortify'[3])
- qqplot to check normality of residuals (Autoplot in R package 'GGfortify'[3])
- Scale-location plot to check for homoscedasticity (Autoplot in R package 'GGfortify'[3])
- Residuals vs Leverage to check for influential cases (Autoplot in R package 'GGfortify'[3])

B. Model 1

Model 1

Ln(Child oxytocin) =$\mu + \beta_1 \cdot$ mother $FAS_i + \beta_2 \cdot$ child pubertal $stage_i + \varepsilon_I$ ($\epsilon_i \sim N(0, \sigma^2)$)

Ln denotes the natural logarithm and $i = 1, \ldots, 83$ *describes the individual participants. The intercept is denoted by* μ, β_j *represents the jth regression coefficient, and* ϵ_i *represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation* σ. *FAS, Family Accommodation Scale*

Table 1. Table on assumptions check of Model 1

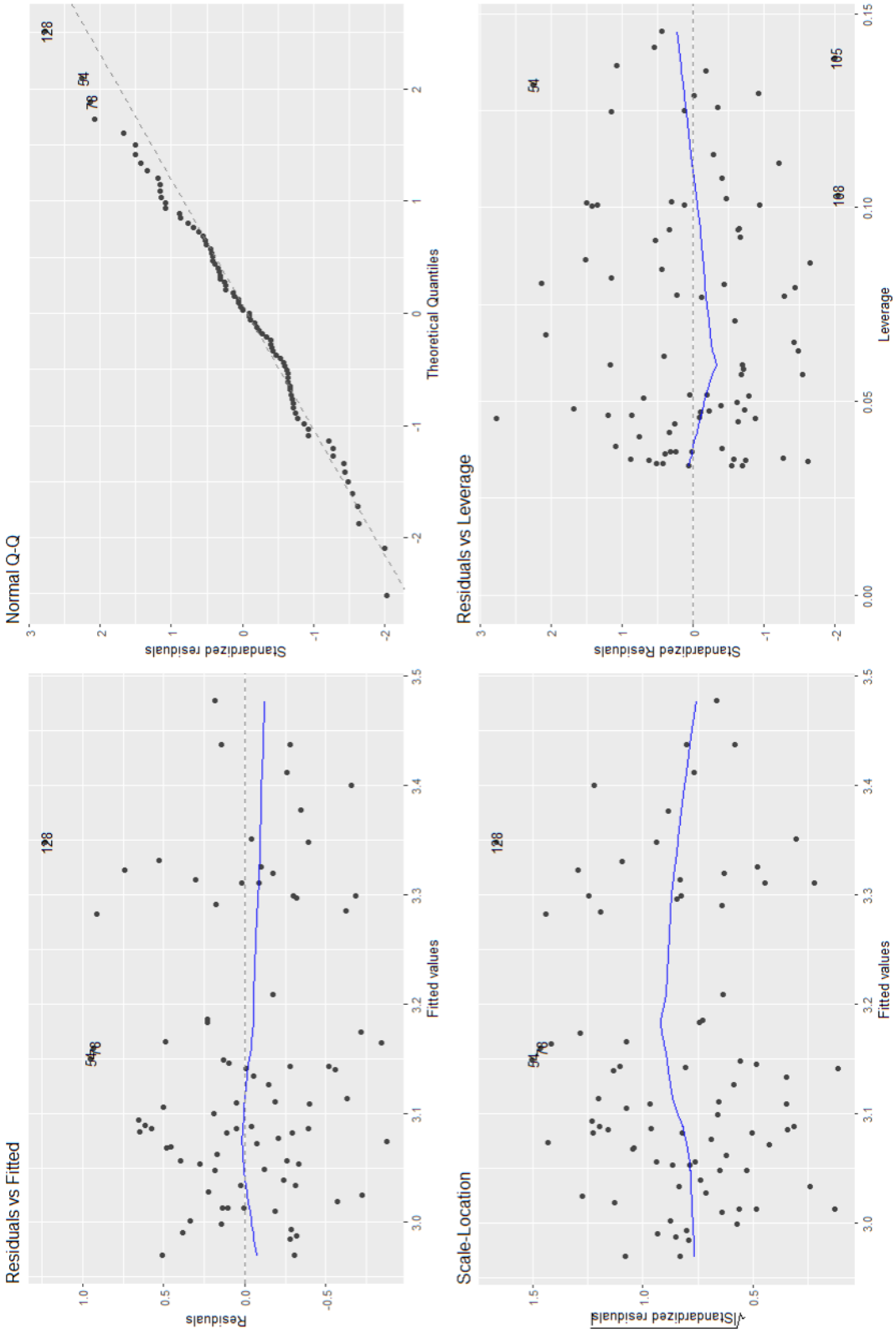
Check	Plot/Test	Assumptions met
Linearity between independent variables	Figure E.1. Correlation matrix	Yes
Linearity of model	Figure E.2. Residual vs. fitted plot	Yes
Homoscedasticity	Figure E.2. Scale-location plot	Yes
Influential cases	Figure E.2. Residuals vs Leverage	Yes
Autocorrelation between residuals	Figure E.2. Residual vs. fitted plot	Yes

Table 2. Detailed information of results Model 1

Parameter	Estimate	*SE*	*GVIF*
μ	2.97	011	
β_1 (mother FAS)	0.003	0.003	1.02
β_2 (child pubertal stage)			1.00
2	0.10	0.18	
3	0.10	0.15	
4	0.31	0.13	
5	0.08	0.17	
σ		0.45	

Note: Ln, natural logarithm; SE, Standard Error; GVIF, Generalized variance inflation factor

Figure 1. Plots to investigate assumptions for Model 1



C. Model 2

Model 2

Ln(Child oxytocin) $=\mu + \beta_1 \cdot$ father $FAS_i + \beta_2 \cdot$ child pubertal $stage_i + \varepsilon_I$ ($\epsilon_i \sim N(0, \sigma^2)$)

Ln denotes the natural logarithm and $i = 1, \ldots, 72$ describes the individual participants. The intercept is denoted by μ, β_j represents the jth regression coefficient, and ϵ_i represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation σ; FAS, Family Accommodation Scale

Table 3. Table on assumptions check of Model 2

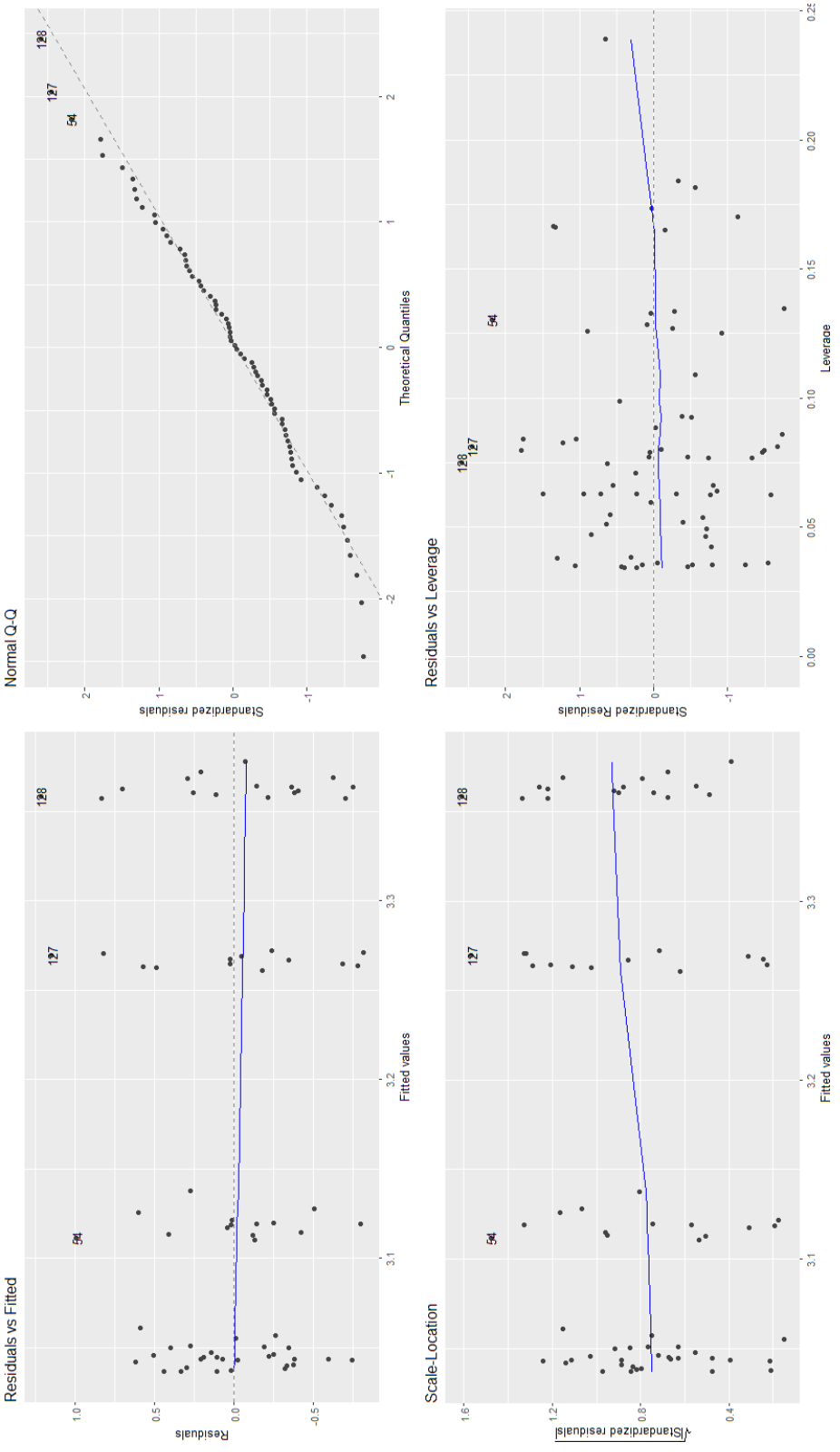
Check	**Plot/Test**	**Assumptions met**
Linearity between independent variables	Figure F.1. Correlation matrix	Yes
Linearity of model	Figure F.2. Residual vs. fitted plot	Yes
Homoscedasticity	Figure F.2. Scale-location plot	Yes
Influential cases	Figure F.2. Residuals vs Leverage	Yes
Autocorrelation between residuals	Figure F.2. Scale-location plot	Yes

Table 4. Detailed information of results Model 2

Parameter	**Estimate**	***SE***	***GVIF***
μ	3.04	0.12	
β_1 (father FAS)	0.0005	0.09	1.01
β_2 (child pubertal stage)			1.00
2	0.07	0.20	
3	0.22	0.16	
4	0.32	0.15	
5	0.08	0.22	
σ		0.49	

Note: Ln, natural logarithm; SE, Standard Error; GVIF, Generalized variance inflation factor

Figure 2. Plots to investigate assumptions for Model 2



D. Model 3

Initial/full Model 3

$\text{Ln(Child oxytocin)} = \mu + \beta_1 \cdot \ln(\text{mother oxytocin})_i + \beta_2 \cdot \text{mother parental stress}_i + \beta_3 \cdot \text{child family cohesion}_i + \beta_4 \cdot \text{child family conflict}_i + \beta_5 \cdot \text{child pubertal stage}_i + \beta_6 \cdot \text{child OCD status}_i + \beta_7 \cdot \ln(\text{mother oxytocin})_i \text{ x mother parental stress}_i + \beta_8 \cdot \text{child OCD status}_i \text{ x ln(mother oxytocin)}_i + \beta_9 \cdot \text{child OCD status}_i \text{ x mother parental stress}_i + \beta_{10} \cdot \text{child OCD status}_i \text{ x child family cohesion}_i + \beta_{11} \cdot \text{child OCD status}_i \text{ x child family conflict}_i + \varepsilon_I \quad \varepsilon_I\ (\epsilon_i \sim N(0, \sigma^2))$

Ln denotes the natural logarithm and $i = 1, \ldots, 97$ describes the individual participants. The intercept is denoted by μ, β_j represents the jth regression coefficient, and ϵ_i represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation σ. OCD, Obsessive Compulsive Disorder; PS, Parental Stress.

Table 5. Table on assumptions check of the full/initial Model 3

Check	Plot/Test	Assumptions met
Linearity between independent variables	Figure B.2. Correlation matrix	Yes
Linearity of model	Figure B.3. Residual vs. fitted plot	Yes
Homoscedasticity	Figure B.3. Scale-location plot	Yes
Influential cases	Figure B.3. Residuals vs Leverage	Yes
Autocorrelation between residuals	Figure B.3. Residual vs. fitted plot	Yes

Table 6. Reduction table Model 3

Drop	Variable	F-statistics	*p*-value	Estimate	*SE*
1	Child reported family conflict x child OCD status	0.001	0.98	0.001	0.061
2	Mother oxytocin (ln) x Child OCD status	0.032	0.86	0.038	0.212
3	Mother reported parental stress x Child OCD status	0.698	0.41	-0.013	0.016
4	Child reported family conflict	0.799	0.37	0.024	0.027
5	Mother oxytocin (ln) x mother reported parental stress	2.175	0.14	0.023	0.016
6	Mother reported parental stress	0.402	0.53	0.005	0.007
7	Child reported family cohesion x child OCD status	2.685	0.11	0.085	0.052
8	Child reported family cohesion	0.477	0.49	-0.018	0.026
9	Child OCD status	0.410	0.52	-0.057	0.090

Note: Ln, natural logarithm; SE, Standard Error; OCD, Obsessive Compulsive Disorder; PS, Parental Stress

Final Model 3

$\text{Ln(Child oxytocin)} = \mu + \beta_1 \cdot \ln(\text{mother oxytocin})_i + \beta_5 \cdot \text{child pubertal stage}_i + \varepsilon_I \quad \varepsilon_I\ (\epsilon_i \sim N(0, \sigma^2))$

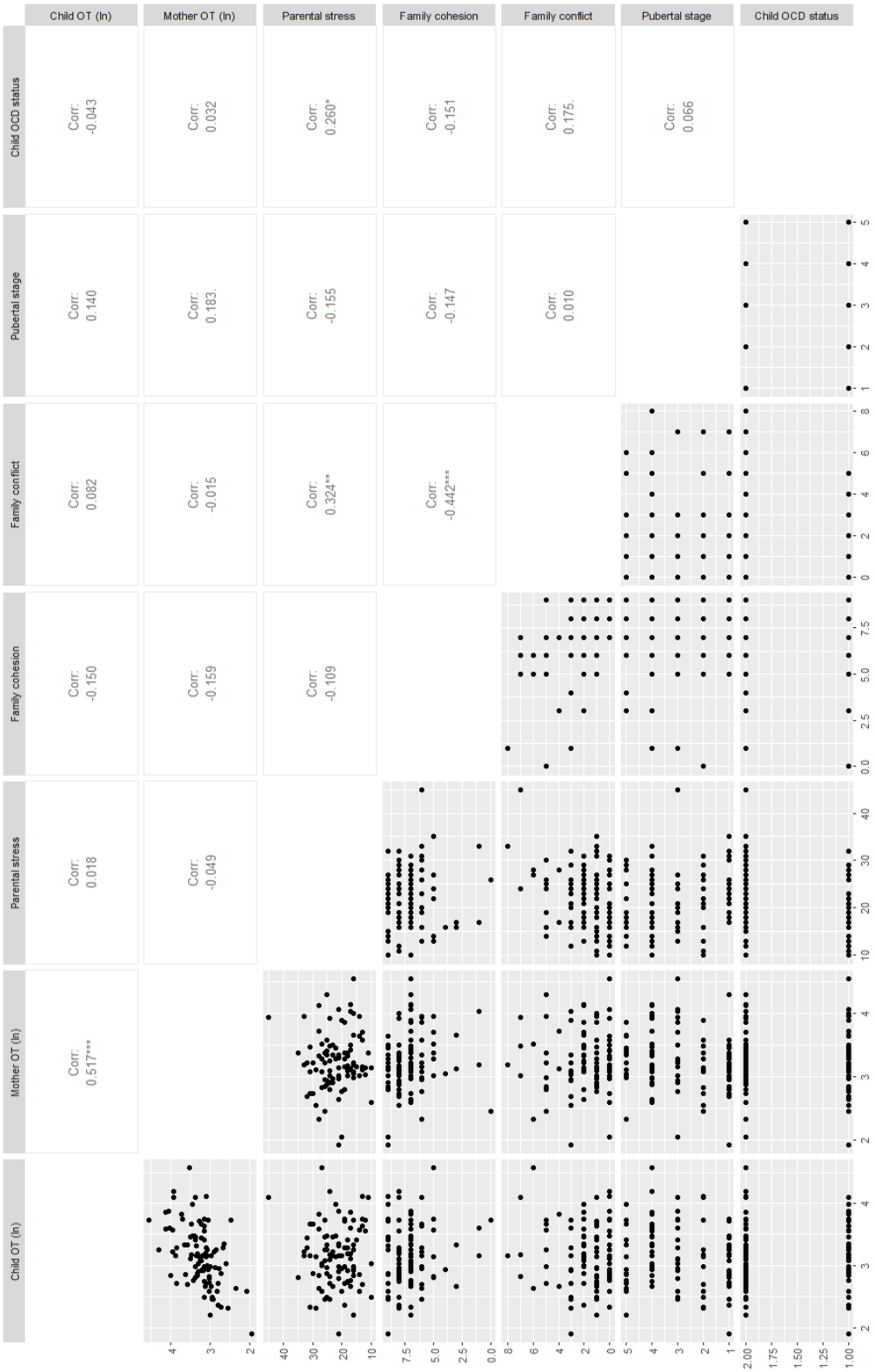
Ln denotes the natural logarithm and $i = 1, \ldots, 97$ describes the individual participants. The intercept is denoted by μ, β_j represents the jth regression coefficient, and ϵ_i represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation σ.

Table 7. Detailed information of results Model 3

Parameter	Estimate	*SE*	*GVIF*
μ	1.38	0.32	
$\beta 1$ (Mother oxytocin (ln)	0.53	0.10	1.07
β_5 (child pubertal stage)			1.07
2	0.05	0.14	
3	0.03	0.14	
4	0.16	0.12	
5	-0.04	0.14	
σ		0.42	

Note: Ln, natural logarithm; SE, Standard Error; GVIF, Generalized variance inflation factor

Figure 3. Correlation Matrix of all variables in Model 3



Note: Ln, natural logarithm; OT, oxytocin; OCD, Obsessive Compulsive Disorder; Corr, Correlation

Figure 4. Plots to investigate assumptions for the initial/full Model 3

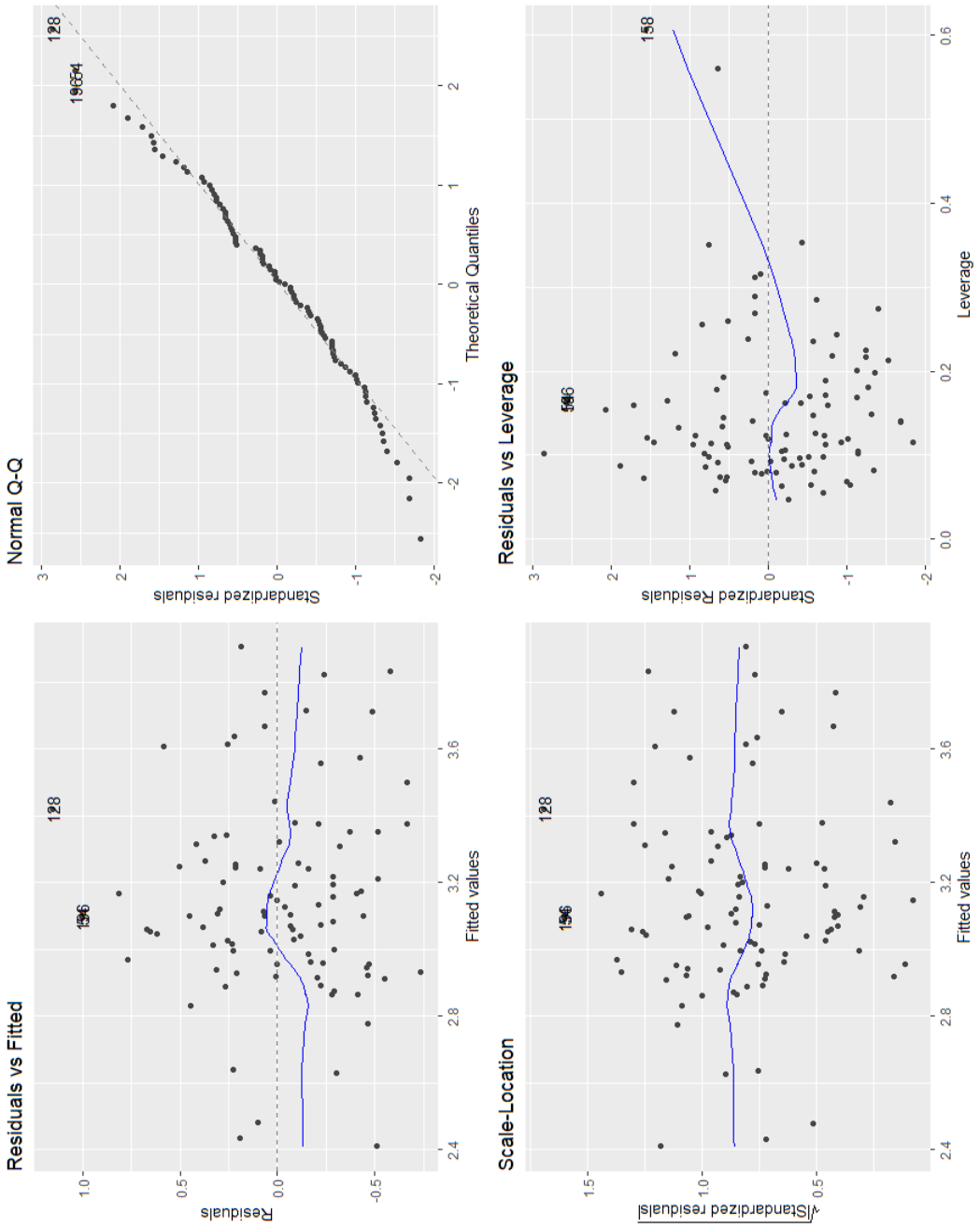


Table 8. Table on assumptions check of the final Model 3

Check	Plot/Test	Assumptions met
Linearity between independent variables	Figure B.2. Correlation matrix	Yes
Linearity of model	Figure B.3. Residual vs. fitted plot	Yes
Homoscedasticity	Figure B.3. Scale-location plot	Yes
Influential cases	Figure B.3. Residuals vs Leverage	Yes
Autocorrelation between residuals	Figure B.3. Scale-location plot Durbin-Watson Statistics: Auto correlation: 0.133 D-W Statistics: 1.713 p-value: 0.166	Yes

Figure 5. Plots to investigate assumptions for the reduced Model 3

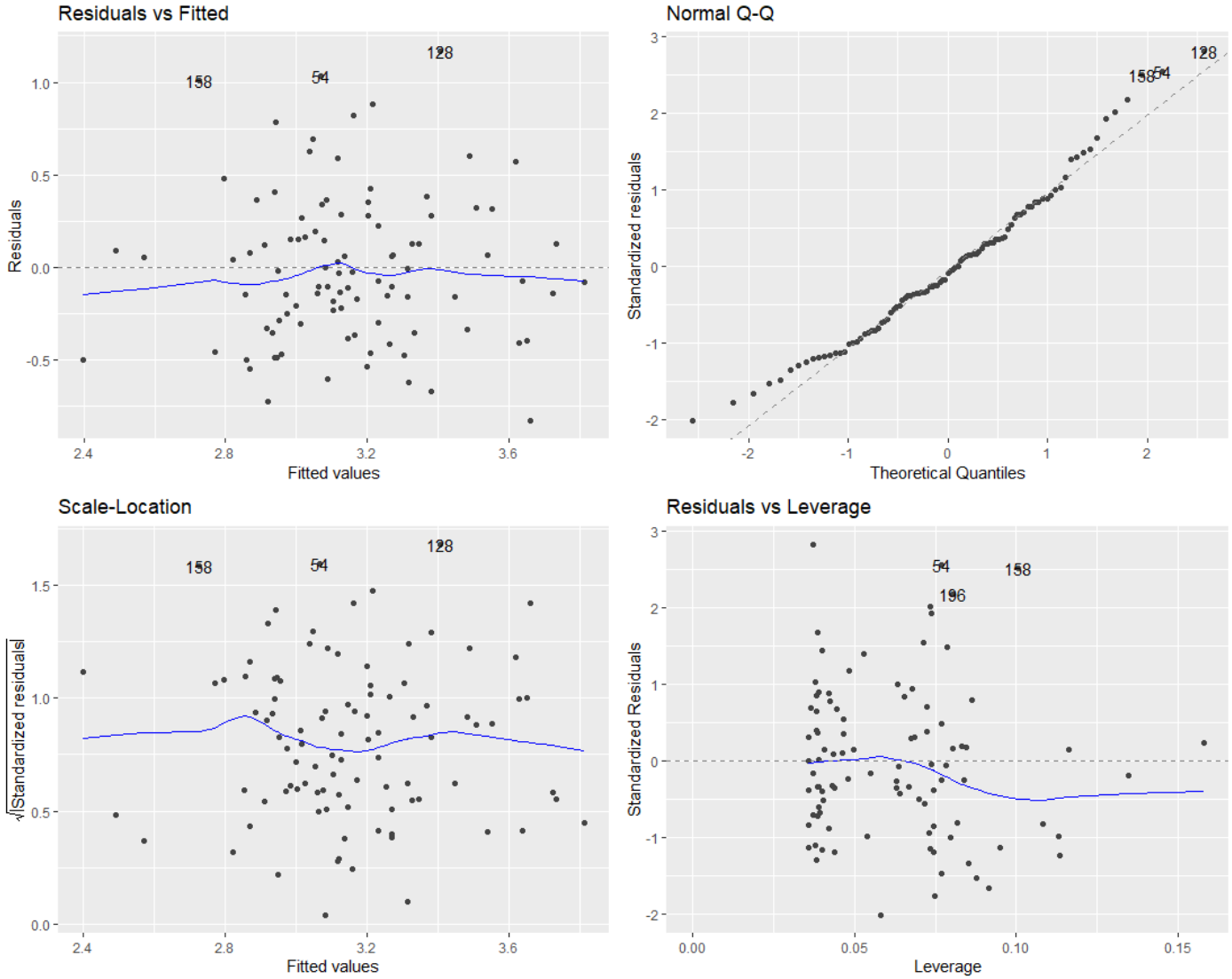


Table 9. Sensitivity analyses – Reduction table Model 3 using forward stepwise regression

Step	Variable	Variable kept in from previous step, covariate, or main effect when testing interaction	*p*-value	Estimate	*SE*
1	Mother oxytocin (ln)	Child pubertal stage	<0.0001	0.530	0.098
2	Child reported family cohesion	Child pubertal stage Mother oxytocin (log)	0.559	-0.015	0.026
3	Mother oxytocin (ln) x mother reported parental stress	Child pubertal stage Mother oxytocin (ln) Mother reported parental stress	0.244	0.018	0.015
4	Child reported family cohesion x child OCD status	Child pubertal stage Mother oxytocin (ln) Child reported family cohesion Child OCD status	0.105	0.085	0.052
5	Mother oxytocin (ln) x child OCD status	Child pubertal stage Mother oxytocin (ln) Child OCD status	0.469	0.142	0.195
6	Child reported family conflict	Child pubertal stage Mother oxytocin (ln)	0.320	0.023	0.023
7	Child OCD status	Child pubertal stage Mother oxytocin (ln)	0.524	-0.057	0.090
8	Mother reported parental stress x child OCD status	Child pubertal stage Mother oxytocin (ln) Mother reported parental stress Child OCD status	0.520	-0.010	0.016
9	Child reported family conflict x child OCD status	Child pubertal stage Mother oxytocin (ln) Child reported family conflict Child OCD status	0.443	-0.041	0.053
10	Mother reported parental stress	Child pubertal stage Mother oxytocin (ln)	0.644	0.003	0.007

Note: Ln, natural logarithm; SE, Standard Error; OCD, Obsessive Compulsive Disorder

E. Model 4

Initial/full Model 4

$\text{Ln(Child oxytocin)} = \mu + \beta_1 \cdot \ln(\text{father oxytocin})_i + \beta_2 \cdot \text{father parental stress}_i + \beta_3 \cdot \text{child family cohesion}_i + \beta_4 \cdot \text{child family conflict}_i + \beta_5 \cdot \text{child pubertal stage}_i + \beta_6 \cdot \text{child OCD status}_i + \beta_7 \cdot \ln(\text{father oxytocin})_i \text{ x father parental stress}_i + \beta_8 \cdot \text{child OCD status}_i \text{ x } \ln(\text{father oxytocin})_i + \beta_9 \cdot \text{child OCD status}_i \text{ x father parental stress}_i + \beta_{10} \cdot \text{child OCD status}_i \text{ x child family cohesion}_i + \beta_{11} \cdot \text{child OCD status}_i \text{ x child family conflict}_i + \varepsilon_I \qquad \varepsilon_I\ (\epsilon_i \sim N(0, \sigma^2))$

Ln denotes the natural logarithm and $i = 1, \ldots, 58$ *describes the individual participants. The intercept is denoted by* μ*,* β_j *represents the jth regression coefficient, and* ϵ_i *represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation* σ*. OCD denotes Obsessive Compulsive Disorder, PS denotes Parental Stress.*

Table 10. Table on assumptions check of the full/initial Model 4

Check	Plot/Test	Assumptions met
Linearity between independent variables	Figure C.1. Correlation matrix	Yes
Linearity of model	Figure C.2. Residual vs. fitted plot	Yes
Homoscedasticity	Figure C.2. Scale-location plot	Yes
Influential cases	Figure C.2. Residuals vs Leverage	Yes
Autocorrelation between residuals	Figure C.2. Residual vs. fitted plot	Yes

Table 11. Reduction table Model 4

Drop	Variable	F-statistics	*p*-value	Estimate	*SE*
1	Father oxytocin (ln) x father parental stress	0.141	0.71	0.015	0.039
2	Child reported family cohesion x child OCD status	0.9745	0.33	-0.083	0.084
3	Father oxytocin (ln) x child OCD status	1.413	0.24	0.489	0.412
4	FES cohesion	1.018	0.32	0.034	0.034

Note: SE, Standard Error; OCD, Obsessive Compulsive Disorder; ln, natural logarithm; PS, Parental Stress

Final Model 4

$\text{Ln(Child oxytocin)} = \mu + \beta_1 \cdot \text{ln(father oxytocin)}_i + \beta_2 \cdot \text{father parental stress}_i + \beta_3 \cdot \text{child family conflict}_i + \beta_4 \cdot \text{child pubertal stage}_i + \beta_5 \cdot \text{child OCD status}_i + \beta_6 \cdot \text{child OCD status}_i \text{ x father parental stress}_i + \beta_7 \cdot \text{child OCD status}_i \text{ x child family conflict}_i + \varepsilon_I$

$\varepsilon_I \ (\epsilon_i \sim N(0, \sigma^2))$

Ln denotes the natural logarithm and $i = 1, \dots, 58$ *describes the individual participants. The intercept is denoted by* μ*,* β_j *represents the jth regression coefficient, and* ϵ_i *represents the residuals. The residuals are assumed to be independent and identically normal distributed with mean zero and standard deviation* σ*. OCD denotes Obsessive Compulsive Disorder*

Table 12. Detailed information of results Model 4

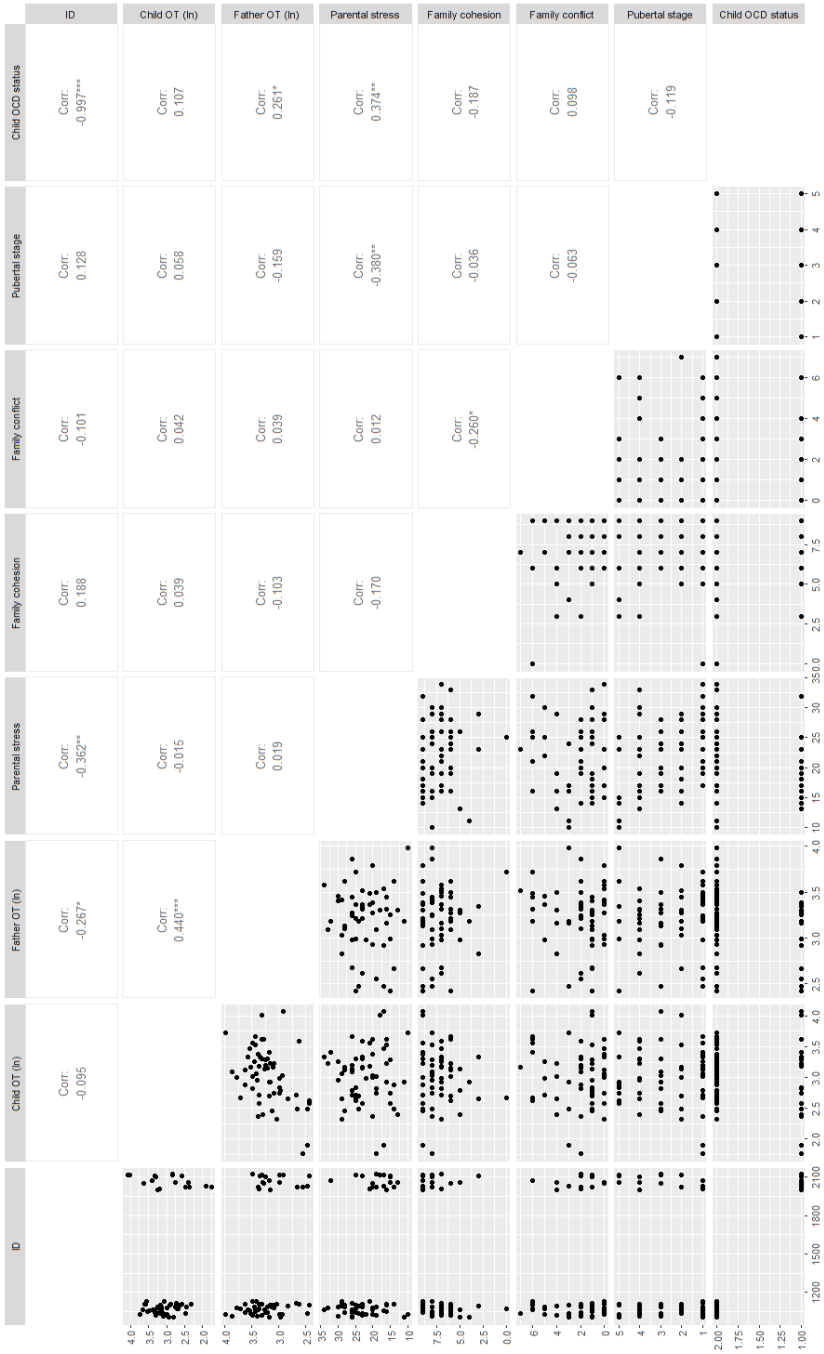
Parameter	Estimate	*SE*
μ	0.44	0.67
$\beta 1$ (Father oxytocin (ln))	0.54	0.17
β_2 (father parental stress)	0.06	0.03
β_3 (child family conflict)	-0.18	0.08
β_4 (child pubertal stage)		
2	0.10	0.18
3	0.07	0.17
4	0.33	0.16
5	-0.12	0.21
β_5 (child OCD status)	0.99	0.58
β_6 (child OCD status x father parental stress)	-0.07	0.03
β_7 (child OCD status x child family conflict)	0.20	0.09
σ		0.43

Note: Ln, logarithmic; SE, Standard Error; GVIF, Generalized variance inflation factor

Table 13. Table on assumptions check of the final Model 4

Check	Plot/Test	Assumptions met
Linearity between independent variables	Figure C.1. Correlation matrix	Yes
Linearity of model	Figure C.3. Residual vs. fitted plot	Yes
Homoscedasticity	Figure C.3 Scale-location plot	Yes
Influential cases	Figure C.3 Residuals vs Leverage	Yes
Autocorrelation between residuals	Figure C.3 Residual vs. fitted plot	Yes

Figure 6. Correlation Matrix of all variables in Model 4



Note: OT, oxytocin; ln, logarithm; OCD, Obsessive Compulsive Disorder; Corr, Correlation

Figure 7. Plots to investigate assumptions for the initial/full Model 4

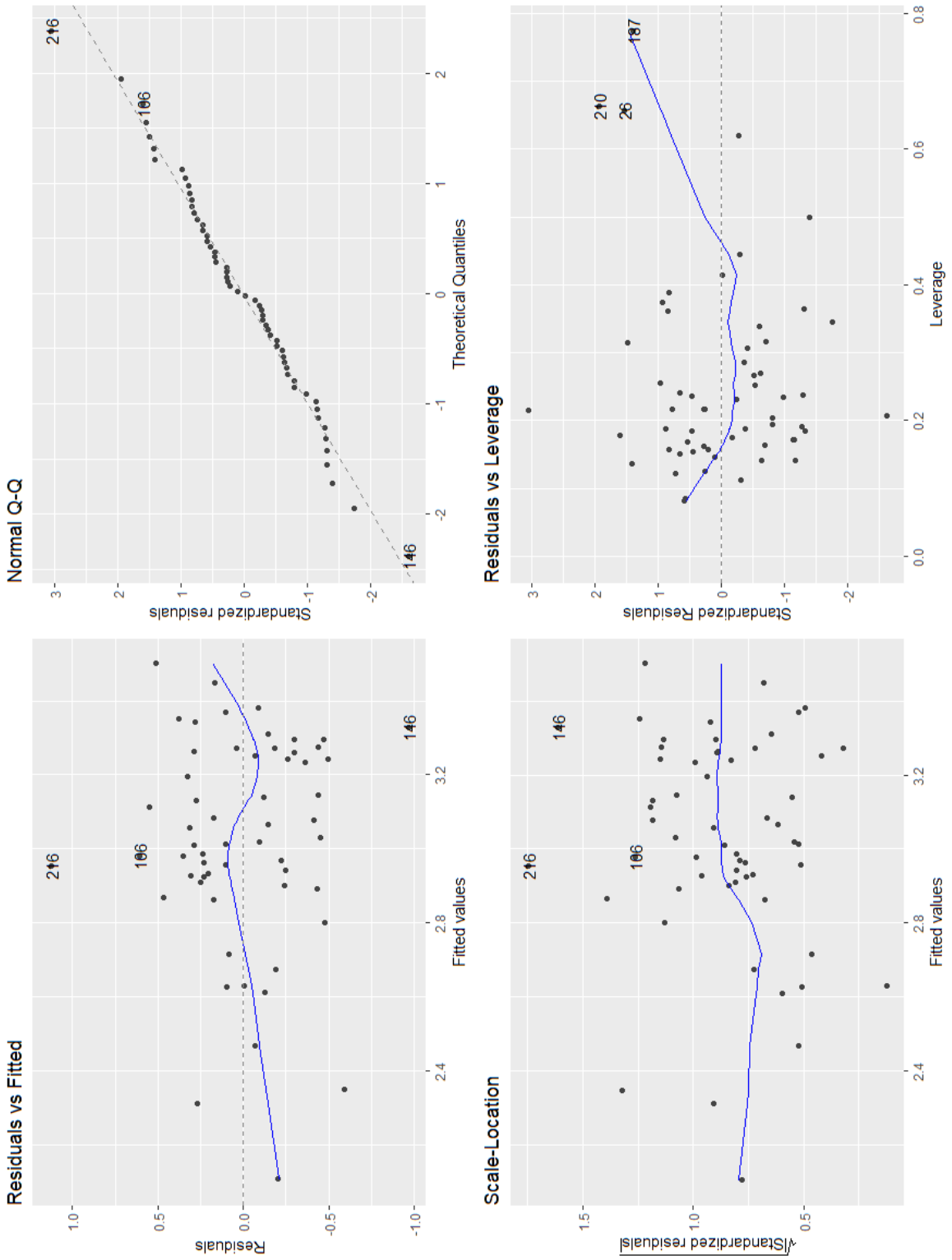


Figure 8. Plots to investigate assumptions for the reduced Model 4

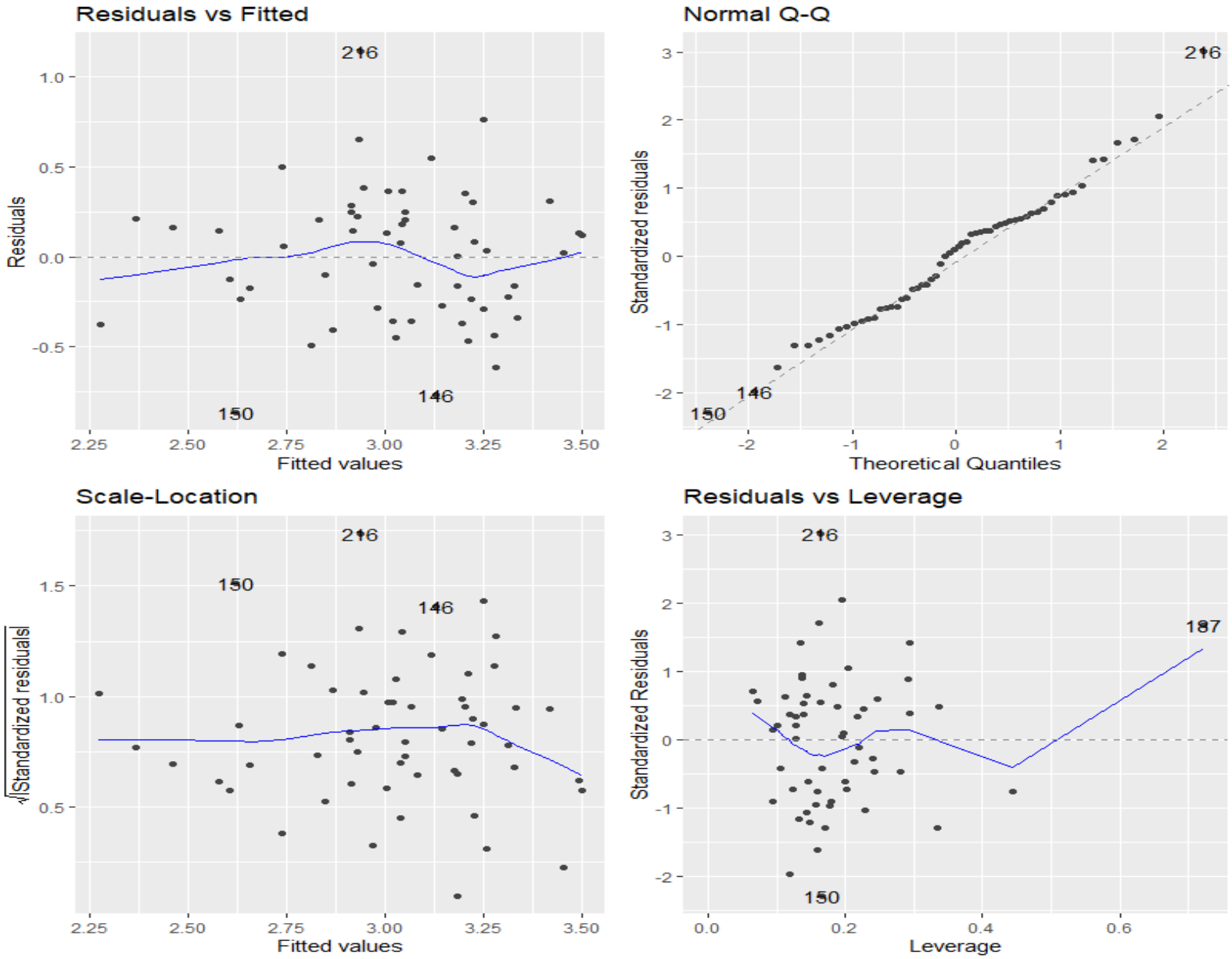

Residuals vs Fitted
216
150
146
Residuals
Fitted values
Normal Q-Q
216
146
150
Standardized residuals
Theoretical Quantiles
Scale-Location
216
150
146
√|Standardized residuals|
Fitted values
Residuals vs Leverage
216
187
150
Standardized Residuals
Leverage

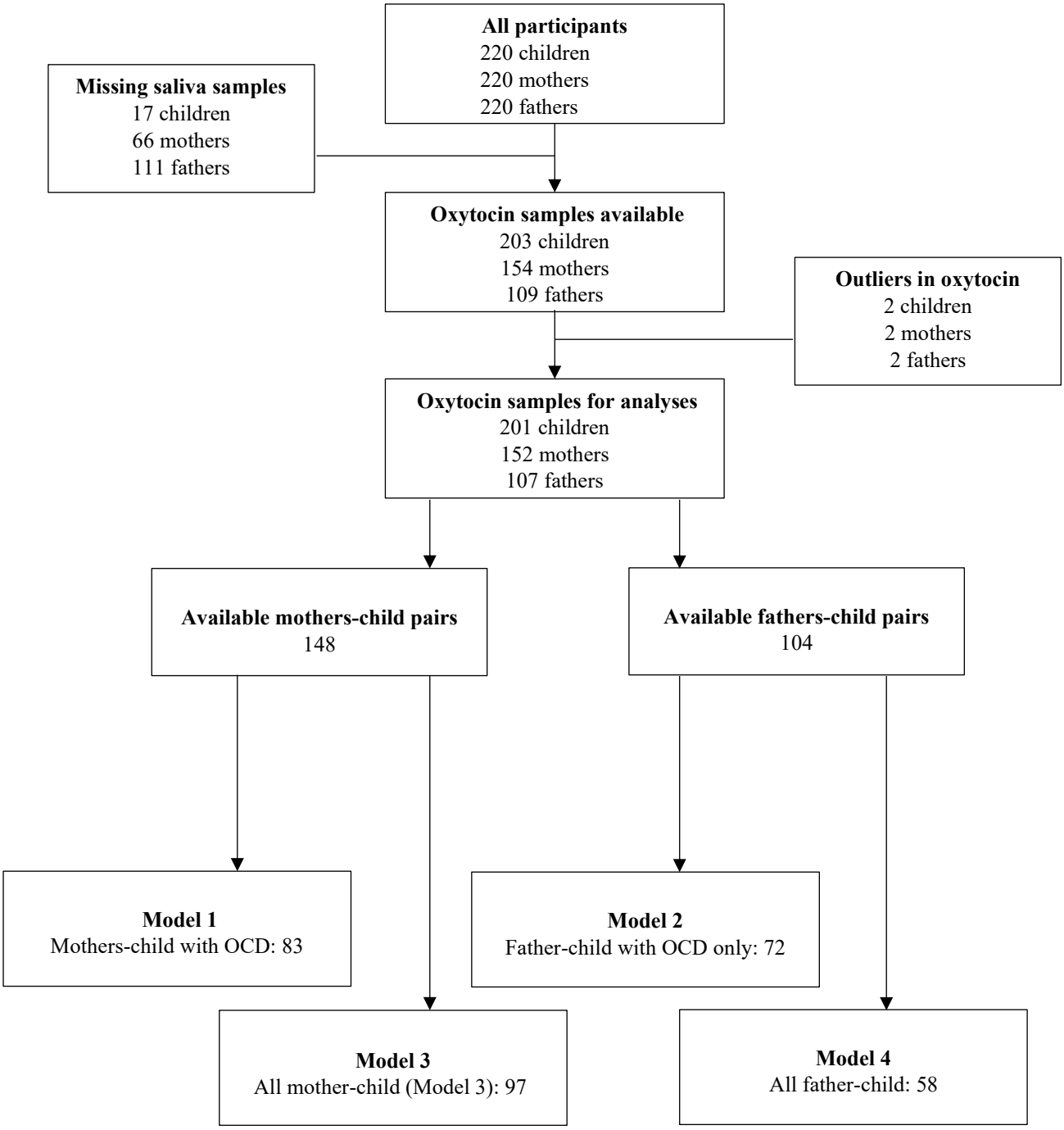
Table 14. Sensitivity analyses – Reduction table Model 4 using forward stepwise regression

Step	Variable	Variable kept in from previous step, covariate, or main effect when testing interaction	*p*-value	Estimate	*SE*
1	Father oxytocin (ln)	Child pubertal stage	0.0005	0.598	0.160
2	Father parental stress x child OCD status	Child pubertal stage Father oxytocin (ln) Father parental stress Child OCD status	0.127	-0.043	0.028
3	Father oxytocin (ln) x child OCD status	Child pubertal stage Father oxytocin (ln) Child OCD status	0.217	-0.481	0.385
4	Child reported family conflict x child OCD status	Child pubertal stage Father oxytocin (ln) Child reported family conflict Child OCD status	0.173	0.111	0.081
5	Father oxytocin (ln) x father parental stress	Child pubertal stage Father oxytocin (ln) Father parental stress	0.127	-0.043	0.028
6	Child OCD status	Child pubertal stage Father oxytocin (ln)	0.482	0.099	0.140
7	Child reported family cohesion x child OCD status	Child pubertal stage Father oxytocin (ln) Child reported family cohesion Child OCD status	0.535	-0.051	0.081
8	Child family conflict	Child pubertal stage Father oxytocin (ln)	0.751	0.010	0.030
9	Child family cohesion	Child pubertal stage Father oxytocin (ln)	0.475	0.023	0.033
10	Father parental stress	Child pubertal stage Father oxytocin (ln)	0.921	0.001	0.011

Note: ln, logarithm; SE, standard error; OCD, Obsessive Compulsive Disorder

F. Flowchart

Figure 9. Flowchart of participants in analyses



*Note: *Reasons for missing: 17 children missing saliva sample (5 with to high anxiety on the day, 6 cancelled due to covid, 4 procedure mistakes, 2 with no reason registered); 10 mothers without consent to study, 5 without consent to saliva sampling, 1 pregnant, 2 breast feeding, 48 missing saliva sample (reasons not registered); 45 fathers without consent to study, 5 without consent to saliva sampling, 5 not biological parent, 56 with missing saliva sample (reasons not registered). OCD, Obsessive Compulsive-Disorder*

G. Information on and consideration of outliers

We removed outliers defined as oxytocin levels 3SD from the mean. The removal of outliers have also been used in previous studies, as oxytocin levels often show large variations and outliers can complicate the statistical analyses. In this study we had six outliers (two in children (values 414.3 and 198.1pg/ml), two in mothers (340.3 and 166.8 pg/ml) and two in fathers (338.4 and 121.7pg/ml)). We explored whether the six outliers were due to sampling or measurement errors or due to natural variation and did not find any explanation for the outliers in the information we had available (pregnancy and breast feeding in female participants, or medication and somatic disorders in the children with OCD). However, we lack information on parameters that could be important e.g. medication or somatic disorders in parents and children without OCD, and our choice to exclude the outliers holds some uncertainty.

15. Appendix III of thesis: Study III and Appendix for study III

Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

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Abstract

Background: Parent-child interactive processes are important factors in pediatric OCD. Understanding biological mechanisms of parent-child interactive behavior could help improve treatment of pediatric OCD. Oxytocin has been suggested as a biological mechanism in parent-child interactions. However, no studies in pediatric OCD exist. We used machine learning to discover latent patterns in parent-child interactive behaviors and explored associations with oxytocin in children with and without OCD.

Methods: We used parent and child salivary oxytocin levels measured with enzyme-linked immunosorbent assay (ELISA) and investigator-rated parent-child behaviors during a frustration task. Children with or without OCD and their parents – 107 mother-child and 62 father-child pairs were included. We used machine learning to generate data-driven, theory agnostic behavioral variables; and regression to estimate their associations with oxytocin.

Results: Principal component and archetype analyses identified behavioral patterns describing the mother-child and father-child interactions. We found an association between child and mother oxytocin and the interaction patterns "overinvolved interaction" and "emotional interaction" and a negative relation to "distant interaction". Additionally, mother oxytocin was associated with "supportive interaction" and "varied-coping interaction", and negatively related to "conflictual interaction" and "negative-low support interaction". Father oxytocin was associated with “supportive interactions” only in the presence of child OCD.

Conclusion: Child and mother oxytocin seem related with mother-child interactive patterns. Fathers’ oxytocin was related with interaction patterns only in children with OCD. Implications for future clinical research are discussed.

Highlights

- Machine learning techniques revealed latent variables for parent-child interaction patterns.
- Child and mother oxytocin levels were associated with mother-child interaction patterns.
- Child and father oxytocin levels were not associated with father-child interaction patterns.
- In fathers of children with OCD, oxytocin showed an association with interaction patterns.

1. Introduction

Obsessive-compulsive disorder (OCD) is a highly distressing mental illness affecting up to 3% of children worldwide[1]. OCD presents with two key symptom clusters: 1) obsessive thoughts and images, which cause distress in their repetitive and intrusive nature, and 2) compulsive behaviors intended to neutralize the obsessive beliefs and distress[2]. Cognitive behavioral therapy (CBT) with exposure and response prevention is the first-line treatment for OCD[3]. For children, clinical practice guidelines emphasize the importance of involving families in treatment to optimize treatment outcome (National Institute for Health and Care Excellence (NICE, 2005)[4]). Although family-enhanced CBT appears to be effective in reducing OCD symptoms, up to 40% of children do not or only partially respond to treatment[5]. These findings highlight the need for continued work on factors affecting the trajectory of the illness.

Family interactive behaviors play a critical role in pediatric OCD[6–9]. Several studies have demonstrated that lower levels of conflict, blame, and family accommodation predict greater response to CBT treatment[10,11]. Notably, evidence for these associations comes predominantly from questionnaire data[12] with less work on observed behaviors and biological underpinnings of the behaviors[13].

Non-clinical studies suggest the neuropeptide oxytocin as an underlying biological mechanism in parent-child interactions[14]. Findings indicate that observed sensitive parental behaviors, including affect synchrony, adaptive engagement, and stimulatory play, are positively associated with oxytocin levels in parents[15–17] and infants[14]. Clinical studies within childhood anxiety disorders indicate that lower child oxytocin levels are related to more observed anxious behavior in children during a mother-child interaction and higher levels of mother-rated family accommodation[18]. This is of particular interest to pediatric OCD, where family members often modify their behavior to accommodate the child's OCD[19]. Contrary to the aim of relieving the child's distress, family accommodation reinforces the child's OCD beliefs, thereby maintaining symptoms and distress[13]. In sum, clinical and non-clinical research suggest that oxytocin play a role in how parents and children interact. Given that the family play a critical role in pediatric OCD examining the extent to which oxytocin is implicated in parent-child interactions within the context of pediatric OCD could lead to greater understanding of the disorder and its treatment. However, studies on oxytocin and family interactive behaviors in pediatric OCD are lacking[13].

Thus, the current study aims to enhance knowledge on family factors in pediatric OCD by exploring the associations between oxytocin and observed parent-child interactive behavior patterns in dyads with pediatric OCD and dyads without pediatric psychiatric disorder. Exploratory studies are essential for generating hypotheses in areas with limited evidence. We extended our exploratory approach to the generation of latent parent-child interactive patterns. Observations of parent-child behaviors often create numerous variables and generate highly dimensional data. Instead of allowing theory to decide how to create latent variables, we have taken a data-driven approach using two machine learning techniques. First, we used

principal component analysis (PCA) to reduce the number of variables while ensuring they are uncorrelated[20]. Then, we created archetypes of parent-child interaction patterns. Subsequently, we explored if these computationally derived parent-child interaction patterns were associated with mother, father, and child oxytocin levels. Previous studies in non-clinical families highlight that oxytocin-related behaviors may differ between mothers and fathers. We therefore investigated associations between oxytocin and parent-child behaviors in mothers and fathers separately. To increase our understanding of OCD, we investigated if the associations differed depending on the presence of child OCD.

2. Material and methods

2.1. Study design and participants

We included baseline data from 1) the TECTO trial (Treatment Effects of Family Based Cognitive Therapy in Children with Obsessive-Compulsive Disorder)[21] including 130 children with OCD and their parents and 2) a parallel case-control study with 90 non-psychiatric control children (from here on denoted control children) and their parents (for information on the case-control study see protocol[21]).

The inclusion and exclusion criteria have also been described in the TECTO protocol paper and previous TECTO papers[21,22]. The inclusion criteria for the children with OCD were 1) a primary diagnosis of OCD according to the International Classification of Diseases-10 (ICD-10)[23], 2) The Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS)[24] total score ≥16, 3) age 8 to 17 years both inclusive, 4) signed informed consent from parents/legal caretakers. Exclusion criteria for children with OCD were 1) comorbid illness with pervasive developmental disorder not including Asperger's syndrome, schizophrenia/psychosis, mania or bipolar disorder, depressive psychotic disorders, substance dependence syndrome, 2) IQ<70, 3) treatment with cognitive behavioral therapy, psychoeducation with relaxation training, antidepressant, or antipsychotic medications within the last six months prior to trial entry.

The control children were matched 1:1 on sex and age with an included child with OCD (+/- 3 months of birthday). Signed informed consent from parents/legal caretakers was mandatory for inclusion in the study. Exclusion criteria were 1) any current or previous psychiatric disorder according to ICD-10 criteria[23], and 3) IQ<70.

The inclusion criteria for parents were 1) a child participating in the study, 2) signed informed consent. The TECTO trial complies to the Helsinki Declaration and was approved by the ethics committee (Scientific Ethics Committees for the Capital Region of Denmark) (H-18010607) and the Knowledge Centre on Data Protection Compliance in The Capital Region of Denmark[21]. We started the data collection in July 2018, and we finished the collection in September 2022.

2.2. Measures

2.2.1. Salivary oxytocin

Saliva was collected from children and parents with Salivettes® (Sarsted, Rommelsdorf, Germany). Participants were instructed not to eat two hours and drink 30 minutes before the sampling. The samples were prepared for analysis with centrifugations, lyophilization (freeze-drying), and concentration (for details on the procedure, see Appendix 1.1). Oxytocin was analyzed with Enzyme Linked Immunosorbent Assay (ELISA) from Enzo® (NY, USA) and the concentrations calculated with WorkOut 2.5 (Dazdaq Solutions Ltd, Brighton, UK).

2.2.2. Parent-child interaction during a frustration task

Parent-child interaction was assessed during a five-minute complex Tangram puzzle between one parent and one child. The child was instructed to solve as many of the six available puzzles as possible, while the parent was given a solution booklet, instructed not to reveal the solutions and to assist only if truly necessary. After instructions, the assessor left the room, and the entire five-minute interaction was recorded on video for subsequent behavioral coding. Parent-child behaviors were coded using The Tangram Emotion Coding Manual (TEC-M)[25,26]. Originally developed to assess the child's emotion regulation abilities, the manual consists of eight items for parental behavior (intrusiveness, control, avoidance, verbal reappraisal, support/sensitivity, positive expressions, negative expressions, and tension), eight items for child behavior (control, avoidance/resignation, narration, verbal reappraisal, reassurance-seeking behavior, aggression, positive expressions, and negative expressions), one item for the dyadic relationship (emotional warmth), and a global score for the child's emotion regulation abilities (EmReg)[26]. Items are scored on frequency (never, rarely, sometimes, often) and intensity (mild, moderate, marked)[26]. The TEC-M has been validated and employed in studies assessing clinical as well as non-clinical groups[27,28]. In this study, all codes were used, except the global EmReg score. Videos were coded by trained investigators, medical doctors, and psychologists (graduates and non-graduates), and consensus coding was frequently performed to ensure consistency in coding.

2.3. Statistical Analyses

Analyses followed a four-step process for mothers and fathers separately. First, we employed two data driven methods to create latent constructs or interaction patterns. Principal component analysis (PCA) was used to reduce dimensions of the parent-child behaviors while ensuring the new variables are uncorrelated. Archetypal analysis, "archetypes" or "pure types", were used to reduce dimension to create constructs from extreme values in the parent-child behavior data set. Second, regression analyses were run with child, mother, and father oxytocin as the outcome and the retained principal components in three separate models. Third, a series of regression models were run with child, mother, and father oxytocin as the outcome and archetypes as the predictors in six separate models. Finally, all models were run again testing for interaction effects, depending on child OCD status.

To investigate maternal oxytocin in relation to only maternal behavior and paternal only in relation to paternal behavior, all dimension reductions were performed on dataset including only mothers or only fathers. We excluded outliers of oxytocin defined as mean values +/- 3 standard deviations. In the models with child oxytocin as the outcome, we adjusted for child pubertal stage as we have previously shown an association between child pubertal stage and child oxytocin[22] (for details on method for adjusting see Appendix 1.2). We included observations with complete data on the variables of interest. The final mother-child data set included 107 mother-child interactions of which we had available oxytocin samples on mothers of 99 children (49 with OCD, 50 without OCD) and available oxytocin and pubertal stage on 99 children (47 with OCD, 52 without OCD) (see flow chart in Appendix Figure A.2.1). The final father-child dataset included 62 father-child interactions of which we had oxytocin on fathers of 60 children (31 with OCD; 29 without OCD) and oxytocin and pubertal stage on 56 children (27 with OCD, 29 without OCD). All analyses were performed with R (version 4.2.0).

2.3.1. Identifying parent-child interaction patterns using Principal Components Analyses

In PCA, new variables, or principal components (PC), are created as orthogonal linear functions of the original variables. The original variables will each have a load on the principal components that can either be positive (high values on original variable will increase the score of the PC) or negative (low values of the original variable will increase the score of the PC). The principal components are created to successively explain as much of the variance in the data set as possible (with the first PC accounting for most variance, and the second PC accounting for second-most variance and so forth)[20].

The PCA was made for mothers and fathers separately. We conducted the PCA with both frequency and intensity measures of the 17 behavioral codes from the parent-child interaction task: mother and father behaviors (intrusiveness, control, avoidance, verbal reappraisal, support sensitivity, positive expressions, negative expressions, and tension), child behaviors (control, avoidance/resignation, narration, verbal reappraisal, reassurance-seeking behavior, aggression, positive expressions, and negative expressions), and dyadic emotional warmth[26] thus a total of 34 variables entered in the PCA. The PCA was based on the covariance matrix as all behaviors are measured on the same scale[20]. We used the R package 'FactoMineR'[29] to perform the PCA. To determine the number of PCs to be retained, we visually inspected a graphical representation, the scree plot, for each individual PC.

2.3.2. Identifying parent-child interaction patterns using Archetypal analysis

Archetype analyses were conducted on the same 34 variables as the PCA on mothers and fathers separately. We used the R package 'archetypes'[30,31]. To determine the number of archetypes to retain, we used visual inspected scree plots of the residual sum of squares (RSS).

2.3.3. Exploring relationships between derived parent-child interaction patterns and oxytocin

In separate models, we used linear regression to analyze the association between the level of oxytocin and the principal components from the PCA and the alpha coefficients from the archetype analysis describing the score of each archetype assigned to an individual. We included child, mother, or father oxytocin level (child oxytocin adjusted for child pubertal stage, for method see Appendix 1.2) as the outcome in separate models. The first six principal components from the PCA were entered in the same model as these components are orthogonal. The archetypes were analyzed in separate models as these may correlate. We used the Benjamin-Hochberg method to adjust for multiple testing with a false discovery rate of 20% as the tests are exploratory. In post hoc analyses, we used regression analyses to investigate if the relationships were moderated by the presence of child OCD.

3. Results

The characteristics of children, mothers, and fathers are described in Table 1.

Table 1. Descriptive characteristics of children, mothers, and fathers

	Children with OCD	Children without OCD
Children		
Number of children (*n*)	74	81
Age (years) Mean (*SD*)	12.96 (2.82)	12.95 (2.74)
Sex (*n* (%))		
Female	37 (50%)	42 (52%)
Male	37 (50%)	39 (48%)
Pubertal stage (*n* (%))		
Pubertal stage 1	26 (35%)	21 (26%)
Pubertal stage 2	8 (11%)	18 (22%)
Pubertal stage 3	16 (22%)	14 (17%)
Pubertal stage 4	18 (24%)	20 (25%)
Pubertal stage 5	6 (8%)	8 (10%)
Nationality (*n* (%))		
Danish	64 (86%)	59 (73%)
Other	2 (11%)	1 (26%)
Missing	8 (3%)	21 (1%)
Comorbid psychiatric disorders (*n* (%))		
Anxiety disorders, ICD-10 F40-41, F93	8	-
Stress and adjustment disorders, ICD-10 F43	7	-
Asperger's Syndrome, ICD-10 F84.5	13	-
Hyperkinetic disorders ICD-10 F90.0	10	-
Tic disorders, ICD-10 F95	9	-
Other*	11	-
Oxytocin concentration (log) Mean (*SD*)	27.17 (15.26)	25.96 (14.31)
Mothers		

Number of mothers (n)	55	52
Age (years) Mean (*SD*)	44.81 (5.09)	45.55 (4.87)
Nationality (*n* (%))		
Danish	41 (75%)	41 (79%
Other	2 (3%)	2 (4%)
Missing	12 22%)	9 (17%)
Oxytocin (log) Mean (*SD*)	28.91 (15.01)	31.13 (17.90)
Fathers		
Number of fathers	33	29
Age (years) Mean (*SD*)	47.30 (4.23)	47.24 (7.01)
Nationality (*n* (%))		
Danish	29 (88%)	21 (72%)
Other	1 (3%)	1 (4%)
Missing	3 (9%)	7 (24%)
Oxytocin (log) Mean (*SD*)	28.79 (8.90)	24.99 (11.81)

*Other describes all other diagnoses (for information on these see Appendix Table A.2.3). Note: *n*, number; *SD*, standard deviation; OCD, obsessive-compulsive disorder; ICD-10, International Classification of Diseases-10.

3.1. Computationally derived parent-child interaction patterns

3.1.1. Principal components in mother-child interaction

The PCA, comprising the 34 behavioral codes, showed that the first six principal components explained around 60% of the variance, and the eigenvalue spectrum flattened out subsequently (See appendix Table A.3.1 with Eigenvalues and Figure A.3.2 for scree plot). The first six principal components are shown in Table 2.

Mother-child dyads scored high on PC1 when the scores were high on child narration, child reassurance seeking, child and mother positive expressions, and mother-child emotional warmth (See Appendix Figure A.3.3). We label PC1 "*supportive interaction"* (see Table 2).

PC2 was high when child reassurance seeking, task avoidance, and narration were high and mother intrusiveness was low (Appendix Table A.3.3). We label PC2 "*varied-coping interaction*" (see Table 2).

PC3 was high when child task avoidance, aggression, mother negative expression, intrusiveness, and tension were high and mother support sensitivity and mother-child emotional warmth were low (See appendix Figure A.3.4). We label PC3 "*conflictual interaction*" (see Table 2).

PC4 was high when mother negativity was high and child avoidance was low (See appendix Figure A.3.4). We label PC4 "*demanding interaction*" (see Table 2).

PC5 was high when child narration and mother intrusiveness were high and child avoidance and mother verbal reappraisal were low (Appendix Figure A.3.5). We label PC5 "*overinvolved interaction*" (see Table 2).

PC6 was high when child reassurance seeking and mother tension, support sensitivity, and control were high and child control, negative expression, narration, and mother avoidance were low (See appendix Figure A.3.5). We label PC6 "*supportive tense interaction*" (see Table 2).

Table 2. Variables loadings for the first six principal components of the mother-child PCA

	Mother codes	Child codes	Dyad codes
Supportive interaction (PC1)	Positive expressions (f) Verbal reappraisal (f)	Narration (f,i) Positive expressions (f,i) Reassurance seeking (f)	Emotional warmth (i)
Varied-coping interaction (PC2)	Intrusive (f,i)	Reassurance seeking (f) Narration (f,i) Avoidance (f)	
Conflictual interaction (PC3)	Intrusive (f,i) Negative expressions (f,i) Tension (f,i) Support sensitivity (f)	Avoidance (f,i) Aggression (i) Control (f)	Emotional warmth (f)
Demanding interaction (PC4)	Negative expressions (f,i)	Avoidance (f,i)	
Overinvolved interaction (PC5)	Intrusive (f,i) Verbal reappraisal (f,i)	Narration (f,i) Avoidance (i)	
Supportive tense Interaction (PC6)	Tension (f,i) Control (f) Support sensitivity (i) Avoidance (f,i)	Reassurance seeking (f,i) Avoidance (i) Negative expressions. (f,i) Narration (i) Control (i)	

Note: Loading refers to the correlation between a variable and a PC. Green indicates positive loadings and red indicates negative loadings. f, frequency; i, intensity; PCA, Principal Component Analysis; PC, Principal Components

3.1.2. Principal components in father-child interaction

The PCA showed that the first six principal components explained 63% of the variance (See table 3 and appendix Figure A.3.7 for details).

Father-child dyads scored high on PC1 when scores on child narration, child and father positive expressions, and father-child emotional warmth where high and scores on child avoidance, father intrusiveness, and father negative expressions were low (Appendix Figure A.3.8). We label PC1 "*supportive interaction*" (see Table 3).

PC2 was high when child control was high, and child avoidance, positive and negative expressions, narration, and reassurance seeking and father avoidance, intrusiveness, and father-child emotional warmth were low (Appendix Figure A.3.8). We label PC2 "*disconnected interaction*" (see Table 3).

PC3 was high when child avoidance was high and child narration, father negative expressions, intrusiveness, and tension were low (Appendix Figure A.3.9). We label PC3 "*unengaged interaction*" (see Table 3).

PC4 was high when father intrusiveness and positive expressions were high and child avoidance and both child and father negative expressions were low (Appendix Figure A.3.9). We label PC4 "*overinvolved interaction*" (see Table 3).

PC5 was high when child reassurance seeking, and positive expression and father tension, negative expressions and verbal reappraisal were high and child narration, and avoidance, and father intrusiveness and father-child emotional warmth were low (Appendix Figure A.3.10). We label PC5 "*supportive tense interaction*" (see Table 3).

PC6 was high when child reassurance-seeking and father control were high and father verbal reappraisal, control, and positive and negative expressions were low (Appendix Figure A.3.10). We label PC6 "*parent seeking interaction*" (see Table 3).

Table 3. Variables loadings for the first six principal components of the father-child PCA

	Father codes	Child codes	Dyad codes
Supportive interaction (PC1)	Positive expressions (f)	Positive expressions (f)	Emotional warmth (f,i)
	Intrusiveness (f,i)	Negative expressions (i)	
	Negative expressions (i)	Avoidance (f,i)	
Disconnected interaction (PC2)	Intrusiveness (i)	Control (i)	Emotional warmth (i)
	Avoidance (f,i)	Avoidance (f,i)	
		Narration (i)	
		Reassurance seeking (f,i)	
		Positive expressions (f,i)	
		Negative expressions (i)	
Unengaged interaction (PC3)	P.intrusiveness (f,i)	Avoidance.f	
	P.negative expressions (f,i)	Narration (f,i)	
	P.tension (f,i)		
Overinvolved interaction (PC4)	Intrusiveness (f,i)	Avoidance (f,i)	
	Positive expressions (f)	Negative expressions (f,i)	
	Negative expressions (f,i)		
Supportive tense interaction (PC5)	Verbal reappraisal (f,i)	Reassurance.seeking (f)	Emotional warmth (f)
	Negative expressions (i)	Positive.expressions (f)	
	Tension (f,i)	Narration. (f,i)	
	Intrusiveness (f,i)	Avoidance. (f,i)	
Parent seeking interaction (PC6)	Control (f)	Reassurance.seeking (f,i)	
	Verbal reappraisal (f,i)		
	Positive expressions (f,i)		
	Negative expressions (f,i)		

Note: Loading refers to the correlation between a variable and a PC. Green indicates positive loadings and red indicates negative loadings. f, frequency; i, intensity; PCA, Principal Component Analysis; PC, Principal Components

3.1.3. Association between oxytocin and principal components from mother-child interaction

We found an association between child oxytocin levels and *overinvolved interaction* (PC5) (Table 4). Further, we found an association between mother oxytocin levels and *supportive interaction* (PC1), *varied-coping interaction* (PC2), *conflictual interaction* (PC3), and *overinvolved interaction* (PC5) (Table 4).

Table 4. Regression analysis predicting mother and child oxytocin levels from principal components

	Child log oxytocin[A] (n= 99)		Mother log oxytocin (n=99)	
Principal components	Estimate (SE)	Adjusted p-value	Estimate (SE)	Adjusted p-value
Supportive interaction (PC1)	0.02 (0.02)	0.558	0.07 (0.02)	0.009*
Varied-coping interaction (PC2)	0.03 (0.03)	0.446	0.05 (0.03)	0.173*
Conflictual interaction (PC3)	-0.02 (0.03)	0.558	-0.05 (0.03)	0.193*
Demanding interaction (PC4)	-0.003 (0.03)	0.931	0.05 (0.03)	0.296
Overinvolved interaction (PC5)	0.09 (0.04)	0.086*	0.12 (0.04)	0.009*
Supportive tense interaction (PC6)	0.05 (0.05)	0.446	-0.02 (0.04)	0.724

Note: *SE*, standard error; PC, Principal Component, [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2), *Significant with an accepted false discovery rate of 20% (*p*-value > 0.20).

3.1.4. Association between oxytocin and principal components from father-child interaction

We found no association between child and father oxytocin levels and the first six principal components (Table 5).

Table 5. Regression analysis predicting father and child oxytocin levels from principal components

	Child log oxytocin[A] (*n*=56)		Father log oxytocin (*n*=60)	
Principal components	Estimate (*SE*)	Adjusted *p*-value	Estimate (*SE*)	Adjusted *p*-value
Supportive interaction (PC1)	0.01 (0.03)	0.829	-0.01 (0.02)	0.829
Disconnected interaction (PC2)	0.04 (0.04)	0.829	0.01 (0.03)	0.829
Unengaged interaction (PC3)	-0.02 (0.04)	0.829	0.01 (0.03)	0.829
Overinvolved interaction (PC4)	-0.04 (0.05)	0.829	-0.02 (0.04)	0.829
Supportive tense interaction (PC5)	-0.05 (0.06)	0.829	0.04 (0.04)	0.829
Parent seeking interaction (PC6)	0.03 (0.06)	0.829	-0.03 (0.04)	0.829

Note: *SE*, standard error; PC, Principal Component, [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2), *Significant with an accepted false discovery rate of 20% (*p*-value > 0.20).

3.1.5. Post hoc analyses of the effect of child OCD on the relationships between oxytocin and principal components

We found no difference in the associations between oxytocin levels and latent constructs in mother-child interaction patterns comparing children with and without OCD (Appendix Table A.3.3).

For father-child interaction patterns the analyses showed that the association between father oxytocin levels and *supportive interaction* (PC1) differed for children with and without OCD (See Appendix Table A.3.4). A plot of the data showed that the association was positive for children with OCD and negative for non-clinical control children (See Appendix Figure A.311).

3.2. Archetypes in parent-child interactions

3.2.1. Archetypes in the mother-child interaction

Data showed that three archetypes were best to describe data from the mother-child interaction (see scree plot appendix Figure A.4.1). The three identified archetypes are seen in Appendix Figure A.4.2.

Archetype 1 represents an interaction pattern with high child aggression, high reassurance seeking, high child and mother negative expressions, and high mother tension. Further, low child control, low child and mother positive expressions, low mother support sensitivity, and low mother-child emotional warmth. We label this archetype "*negative-low support interaction*".

Archetype 2 represents an interaction pattern with high child aggression, low child and mother positive expressions, low child verbal reappraisal, low maternal tension, and low mother-child warmth. We label this archetype "*distant interaction*".

Archetype 3 represents an interaction pattern with high child aggression, high positive expressions, high mother support sensitivity, high tension, high mother verbal reappraisal, and high mother-child emotional warmth. We label this archetype "*emotional interaction*".

3.2.2. Archetypes in the father-child interaction

For comparability to the three archetypes found for mother-child interaction patterns, we chose to present the three archetypes best describing the observations for fathers. The identified archetypes are seen in Appendix Figure A.4.4.

Archetype 1 represents an interaction pattern with high child aggression, high father tension, high father negative expression and intrusiveness, low child control, low father verbal reappraisal and low father positive expressions. We label this archetype "*negative-low support interaction*".

Archetype 2 represent an interaction pattern with high child control, high father positive expressions, high tension, low child aggression, and low father avoidance. We label this archetype "*engaged interaction*".

Archetype 3 represents interaction patterns with low child aggression, high child reassurance seeking, high child and father verbal reappraisal, high support sensitivity, high father avoidance, high child and father positive expressions, and high father-child emotional warmth. We label this archetype "*emotional interaction*".

3.2.3. Association between oxytocin and archetypes in mother-child interaction

Child and mother oxytocin were both negatively related to "*negative-low support interaction*" (Archetype 1) and positively associated with "*emotional interaction*" (Archetype 3) (Table 6). Mother oxytocin was also negatively related to "*distant interaction*" (Archetype 2) (Table 6).

Table 6. Regression analysis predicting mother and child oxytocin levels from Archetypes.

	Child log oxytocin[A] (n=99)		Mother log oxytocin (*n*=99)	
Archetypes	Estimate (*SE*)	Adjusted *p*-value	Estimate (*SE*)	Adjusted *p*-value
Negative-low support interaction	-0.36 (0.22)	0.184*	-0.33 (0.23)	0.184*
Distant interaction	-0.09 (0.18)	0.641	-0.48 (0.17)	0.023*
Emotional interaction	0.26 (0.17)	0.184*	0.57 (0.16)	0.002*

Note: *SE*, standard error. [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2), *significant with an accepted false discovery rate of 20% (*p*-value > 0.20).

3.2.4. Association between oxytocin and archetypes in father-child interaction

We found no association between child and father oxytocin and Archetype 1-3 (Table7).

Table 7. Regression analysis predicting father and child oxytocin levels from Archetypes.

	Child log oxytocin[A] (*n*=56)		Father log oxytocin (*n*=60)	
Archetypes	Estimate (*SE)*	Adjusted *p*-value	Estimate (*SE*)	Adjusted *p*-value
Negative-low support interaction	-0.09 (0.21)	0.915	0.06 (0.15)	0.915
Engaged interaction	0.02 (0.22)	0.915	-0.04 (0.16)	0.915
Emotional and warm father-child interaction.	0.08 (0.22)	0.915	-0.02 (0.15)	0.915

Note: *SE*, standard error. [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2), *Significant with an accepted false discovery rate of 20% (*p*-value > 0.20).

3.2.5. Post hoc analyses of the effect of child OCD on the relationships between oxytocin and archetypes

We found no difference in the relationships between mother and child oxytocin and the mother-child archetypes, or father and child oxytocin and the father-child archetypes in children with and without OCD (see Appendix Table A.4.1 and A.5.4).

4. Discussion

Our study is the first to investigate the association between oxytocin and parent-child interactive behaviors in pediatric OCD. With machine learning techniques, we created new latent constructs for mother-child and father-child interaction patterns. Behavioral patterns in the mother-child interaction were associated with

child and mother oxytocin levels in that most of the mother-child interactive patterns, indicating engagement, were positively associated with mother and child oxytocin levels. No behavioral patterns in the father-child interaction were associated with father and child oxytocin. However, post hoc analysis indicated a possible effect of presence of childhood OCD on the father-child associations between interactive patters and oxytocin. First, we discuss potential implications from results on child oxytocin, followed by two paragraphs concerning mother and father oxytocin respectively. Finally, we discuss the results from the post hoc analyses.

4.1. Child oxytocin levels and computationally derived parent-child interaction patterns

Child oxytocin was associated with certain mother-child interaction patterns but not with any father-child interaction patterns. From mother-child interactions, child oxytocin was positively related to the principal component labelled "*overinvolved interaction*" (PC5). Dyads scoring high on this component involve children who did not avoid the task, but actively verbalized how they solved the puzzle. Mothers in these dyads appeared overinvolved (high score on intrusiveness) considering the child's need and did not verbally reappraise the situation for the child. Thus, the child and the mother were both engaged in the situation albeit using different strategies. The role of engagement may account for the positive relationship with child oxytocin. Previous studies have found engagement during a mother-child interaction to be positively associated with baseline infant oxytocin levels[14] and oxytocin response in youths[32]. Child oxytocin was negatively related to the "*negative-low support interaction*" (Archetype 1). Dyads scoring high here were rated high on child aggression, reassurance seeking, child and mother negative expressions, and mother tension. Further, they were low on child control, child and mother positive expressions, mother support sensitivity, and dyadic emotional warmth. No association was found between child oxytocin and the "*distant interaction"* (Archetype 2) consisting of high child aggression, low child and mother positive expressions, low child verbal reappraisal, low maternal tension, and low dyadic warmth. While this may be surprising given the behavioral similarity to "*negative-low support interaction*" (Archetype 1), the differences in levels of child reassurance-seeking behavior, as well as maternal negativity and tension observed in "*negative-low support interaction*" (Archetype 1), may be crucial in understanding why this "*distant interaction*" archetype (Archetype 2) was not associated with oxytocin. Our findings may align with research showing strong negative correlations between child oxytocin and mother-rated family accommodation-related distress and aggression in children with anxiety disorders[18]. In family accommodation, distress refers to parental distress caused by parents aiding children to avoid anxiety and child distress caused by parents not helping[13]. Family accommodation-related child aggression refers to angry or abusive expressions, when parents do not accommodate anxiety[13]. With these previous findings in mind, the distinctions between the "*negative-low support interaction*" (Archetype 1) and the "*distant interaction*" archetype (Archetype 2) may explain why we find a relationship between child oxytocin and "*negative-low support interaction*" (Archetype 1) only.

Like family accommodation, *negative-low support interaction*" (Archetype 1) is marked by a distressed, aggressive child seeking help from a tense parent who may not support the child in the task but provides verbal reappraisals and intrusive actions in the same interaction.
Child oxytocin was positively related to the archetype "*emotional interaction*" (Archetype 3). This archetype includes high parental sensitivity and dyadic emotional warmth. This finding is consistent with previous research indicating a positive association between infant oxytocin and mother-infant affect synchrony[14].

4.2. Mother oxytocin levels and computationally derived parent-child interaction patterns

For mother oxytocin, we found an association with multiple principal components and all archetypes highlighting the significance of maternal oxytocin in mother-child interaction behaviors. Specifically, mother oxytocin was positively associated with "*supportive interaction*" (PC1), "*varied-coping interaction*" (PC2), and "*overinvolved interaction*" (PC5) and "*emotional interaction*" (Archetype 3). Interestingly, child narration loads positively on all these latent constructs. Child narration may be a proxy for child engagement, which has previously been found to associate with child and mother oxytocin[14,33]. Importantly, our study is cross-sectional, and thus we cannot determine whether higher oxytocin levels drive maternal behavior leading to child engagement, or if mother oxytocin is responding to an already engaged parent-child interaction. Another potential explanation for the positive association with mother oxytocin could be that these latent constructs entail engaged maternal behavior without direct negativity or hostility.
Relevant for the interpretation of potentially more strained interactions, we found a negative association between mother oxytocin and the "*conflictual interaction*" (PC3), the "*negative-low support interaction*" (Archetype 1) and the "*distant interaction*" (Archetype 2). All these constructs described interactions with low dyadic emotional warmth, a child with more aggression, and higher levels of negative expressions in the dyad.

4.3. Father oxytocin and computationally derived parent-child interaction patterns

We found no associations between father oxytocin and any of the latent constructs. These results could suggest that father and mother oxytocin levels relate differently to the situation they are in, and design of the task potentially required behaviors more aligned with mother oxytocin. Further, findings from both the PCA and the archetype analyses could suggest that children interact differently with mothers and fathers. Emotional warmth and expressed emotions loaded more on father-child interaction patterns and could thus have more importance in explaining the variance here compared to the mother-child interaction. Importantly, future research should address this hypothesis by assessing children's interaction with both parents separately.

4.4. The effect of pediatric OCD on associations between oxytocin and computationally derived parent-child interaction patterns

To increase our understanding of the role of pediatric OCD, we performed post hoc analyses differentiating between parent-child dyads from the OCD and the non-clinical group.

The presence of child OCD did not moderate associations between child or mother oxytocin and parent-child interactive behaviors. This suggests that the associations with oxytocin may apply to mother-child interactions generally. The post hoc analyses suggest that the presence of child OCD affects the associations between father oxytocin and father-child behaviors. For children with OCD father oxytocin was positively associated with "*supportive interaction*" (PC1). For "*negative-low support interaction*" (Archetype 1), post hoc analyses suggested child OCD and father oxytocin to be associated. These findings could indicate that fathers' behavior differ or perhaps change when a child has OCD. Such hypothesis could be investigated in future prospective child cohorts.

4.5 Strengths and limitations

The findings in this study should be interpreted with consideration of some limitations. First, peripheral oxytocin as a measure of central levels is a topic of discussion[34,35]. Nonetheless, salivary oxytocin assessment is the least invasive, which is an important consideration when working with children. Second, parental psychiatric diagnostic status could have been informative for parental behavior and oxytocin levels. Third, each child interacted with either their mother or father, which limits our ability to draw comparable conclusions between mother and father behavior. Future research could benefit from studying each child interacting separately with their mother and father. Fourth, the TEC-M behavioral codes were originally developed to assess the child's emotion regulation, potentially overlooking aspects of the parent-child interaction. Finally, while extensive consensus coding was performed to improve reliability, the inter-rater reliability for the TEC-M was not assessed in this study.

Despite the shortcomings, the study also has several strengths. First, we included both mothers and fathers, which provides a comprehensive examination of the complex behavioral and biological dynamics in parent-child interactions – a practice that is often recommended but rarely implemented in the literature[18,36]. Second, in using an observational design, we aimed at reducing the risk of the major sources of bias from self-reporting[37]. Finally, we used machine learning which can help generate knowledge in new areas where exploratory data driven research is important before planning more clinical studies.

4.6. Future directions

With this study, we aim to guide future research questions and hypotheses. We highlight the following perspectives for future investigations. Child oxytocin was negatively related to incongruous engagement during distress in mother-child interaction. Thus, future studies in pediatric OCD could explore if low child

oxytocin levels are related to maladaptive family engagement, i.e., family accommodation, and child emotional regulation abilities. Father oxytocin may be differently associated with interactive behaviors when the child has OCD. Studies investigating father-child interaction longitudinally before and after OCD enters the father-child dyad and after treatment could be important in understanding this further.

5. Conclusion

In conclusion, the present study is the first to explore oxytocin and parent-child behaviors in pediatric OCD. Machine learning techniques offered new insights to highly dimensional latent constructs in the parent-child behaviors and provided an opportunity to investigate the behavioral constructs in relation to both child, mother, and father oxytocin. With the findings, we highlight important considerations for future studies in oxytocin and parent-child behaviors in pediatric OCD.

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8. Data availability statement

When all results from the TECTO trial have been published,, we plan to make a depersonalized data set publicly available on the EU ZENODO database.

9. Conflict of interest

Professor Line Katrine Harder Clemmensen is Co-founder and CSO of Interhuman AI. Valdemar Funch Uhre is currently employed by Novo Nordisk A/S. The authors declare no other conflicts of interests.

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Appendix - Computationally derived parent-child interaction patterns and oxytocin in children with and without OCD

Content of Appendix

1. Details on methods

1.1. Details on oxytocin analyses

The preparation for analysis was done with the following steps: 1) thawing, 2) centrifugation 5-minutes at 3,000 rpm at 4°C, 3) freezing at least one day to -80°C for, 4) thawing at 5°C, 5) centrifugation 20 minutes at 1,500 × g at 4°C, 6) storing at -80°C, 7) lyophilizing for 17 hours, 8) storing at -20°C.

On the assay day, the samples were handled with the following steps: 1) thawing, 2) reconstitution in assay buffer, 3) mixing twice at 2700 rpm for five minutes with a five-minute interval, 4) centrifugation five minutes at 10,000 rpm. We did not use any procedure for extraction in the preparation of the samples.

The samples were analyzed with immunoassay using Enzo® oxytocin kits (NY, USA). WorkOut 2.5 (Dazdaq Solutions Ltd, Brighton, UK) was used to calculate concentrations of oxytocin. The analyses ox oxytocin showed an intra-assay coefficient of variation of 13% and an inter-assay coefficient of variation of 20%.

1.2. Details on assessment of pubertal stage and adjustment in analyses

Trained investigators instructed children to report the best fitting pubertal stage from line drawings corresponding to Tanner stages[1,2]. The pubertal stages ranged from 1 (pre-pubertal development) to 5 (full adult development). Pubertal stage was included in analyses as a categorial variable. The statistical adjustment was carried out by using the residuals from a model with child oxytocin as the outcome and child pubertal stage as a predictor.

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2. Additional descriptive statistics

Table A.2.1. Counts of behaviors in the mother-child interaction

	Scores on the TEC-M			
	0	**1**	**2**	**3**
Maternal behaviors				
Intrusiveness f	32	30	16	29
Intrusiveness i	32	13	46	16
Control f	0	5	25	77
Control i	0	6	33	68
Avoidance f	78	23	5	1
Avoidance i	78	24	3	2
Verbal reappraisal f	61	26	11	9
Verbal reappraisal i	62	26	17	2
Support sensitivity f	0	6	24	77
Support sensitivity i	0	6	21	80
Positive expressions f	0	16	29	62
Positive expressions i	0	11	68	28
Negative expressions f	32	46	13	16
Negative expressions i	32	56	7	12
Tension f	88	9	7	3
Tension i	88	15	2	2
Child behaviors				
Control f	0	6	27	74
Control i	0	29	20	58
Avoidance f	19	23	25	40
Avoidance i	19	39	43	6
Narration f	22	25	13	47
Narration i	22	21	33	31
Verbal reappraisal f	69	27	10	1
Verbal reappraisal i	69	35	3	0
Reassurance seeking f	5	33	31	38
Reassurance seeking i	5	7	73	22
Positive expressions f	6	29	23	49
Positive expressions i	6	28	59	14
Negative expressions f	1	9	31	66
Negative expressions i	1	24	38	44
Aggression f	86	13	8	0
Aggression i	86	13	3	5
Dyadic codes				
Dyadic emotional warmth f	0	8	25	74
Dyadic emotional warmth i	0	12	40	55

Note: Based on the 107 mother-child interaction (See flow chart Figure A.2.1). f, frequency; i, Intensity

Table A.2.2. Counts of behaviors in the father-child interaction

	Scores on the TEC-M			
	0	**1**	**2**	**3**
Paternal behaviors				
Intrusiveness f	21	14	10	17
Intrusiveness i	21	7	24	10
Control f	0	5	18	39
Control i	0	3	22	37
Avoidance f	45	11	4	2
Avoidance i	45	11	4	2
Verbal reappraisal f	33	20	7	2
Verbal reappraisal i	22	17	10	2
Support sensitivity f	0	4	14	44
Support sensitivity i	0	5	11	46
Positive expressions f	0	19	11	32
Positive expressions i	1	6	40	15
Negative expressions f	18	27	11	6
Negative expressions i	17	30	6	9
Tension f	42	13	6	1
Tension i	42	13	6	1
Child behaviors				
Control f	0	4	16	42
Control i	0	13	7	42
Avoidance f	16	8	15	23
Avoidance i	16	16	25	5
Narration f	13	10	11	28
Narration i	14	8	21	19
Verbal reappraisal f	43	10	8	1
Verbal reappraisal i	43	18	1	0
Reassurance seeking f	6	20	21	15
Reassurance seeking i	6	6	46	4
Positive expressions f	6	14	14	28
Positive expressions i	6	10	33	13
Negative expressions f	1	10	16	35
Negative expressions i	1	16	26	19
Aggression f	56	4	1	1
Aggression i	56	3	1	2
Dyadic codes				
Dyadic emotional warmth f	0	7	10	45
Dyadic emotional warmth i	0	11	22	29

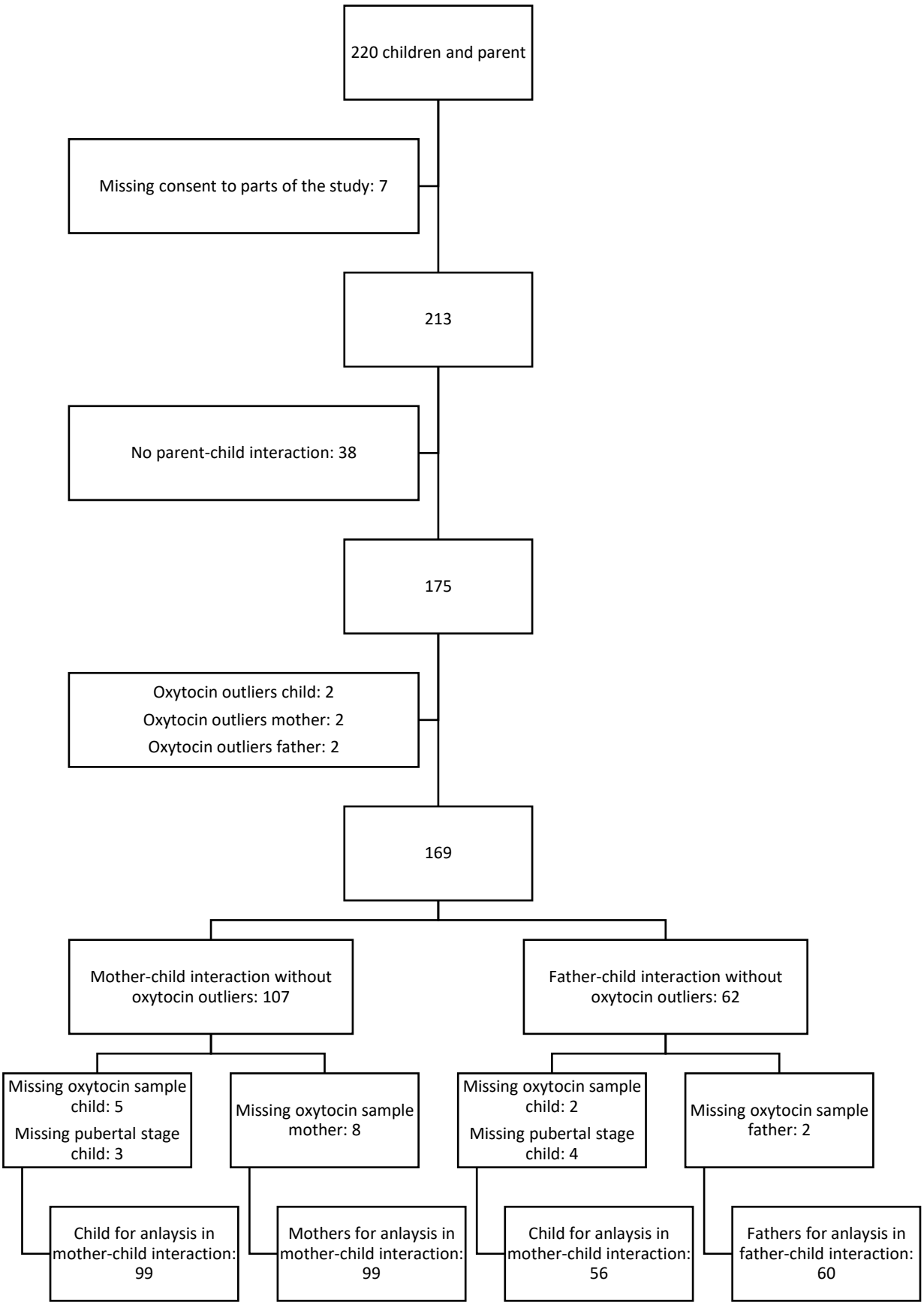
Note: Based on the 62 father-child interaction (See flow chart Figure A.2.1). f, frequency; i, Intensity

Table A.2.3. Count of comorbid psychiatric disorders in the children with OCD

Comorbid psychiatric disorders (*n* (%))	Children and adolescents with OCD
Depressive disorders, ICD-10 F32	0
Anxiety disorders, ICD-10 F40-41, F93	8
Stress and adjustment disorders, ICD-10 F43	7
Eating disorders, ICD-10 F50	2
Sleeping disorders, ICD-10 F51	1
Personality disorders, ICD-10 F60-69	1
Asperger's Syndrome, ICD-10 F84·5	13
Other developmental disorders, ICD-10 F88	2
Hyperkinetic disorders ICD-10 F90.0	10
Hyperkinetic conduct disorders ICD-10 F90.1	0
Conduct disorders ICD-10 F91.3	1
Attachment disorders, ICD-10 F94	0
Tic disorders, ICD-10 F95	9
Enuresis, ICD-10 F98.0	2
Attention deficit disorder, ICD-10 F98.8	2

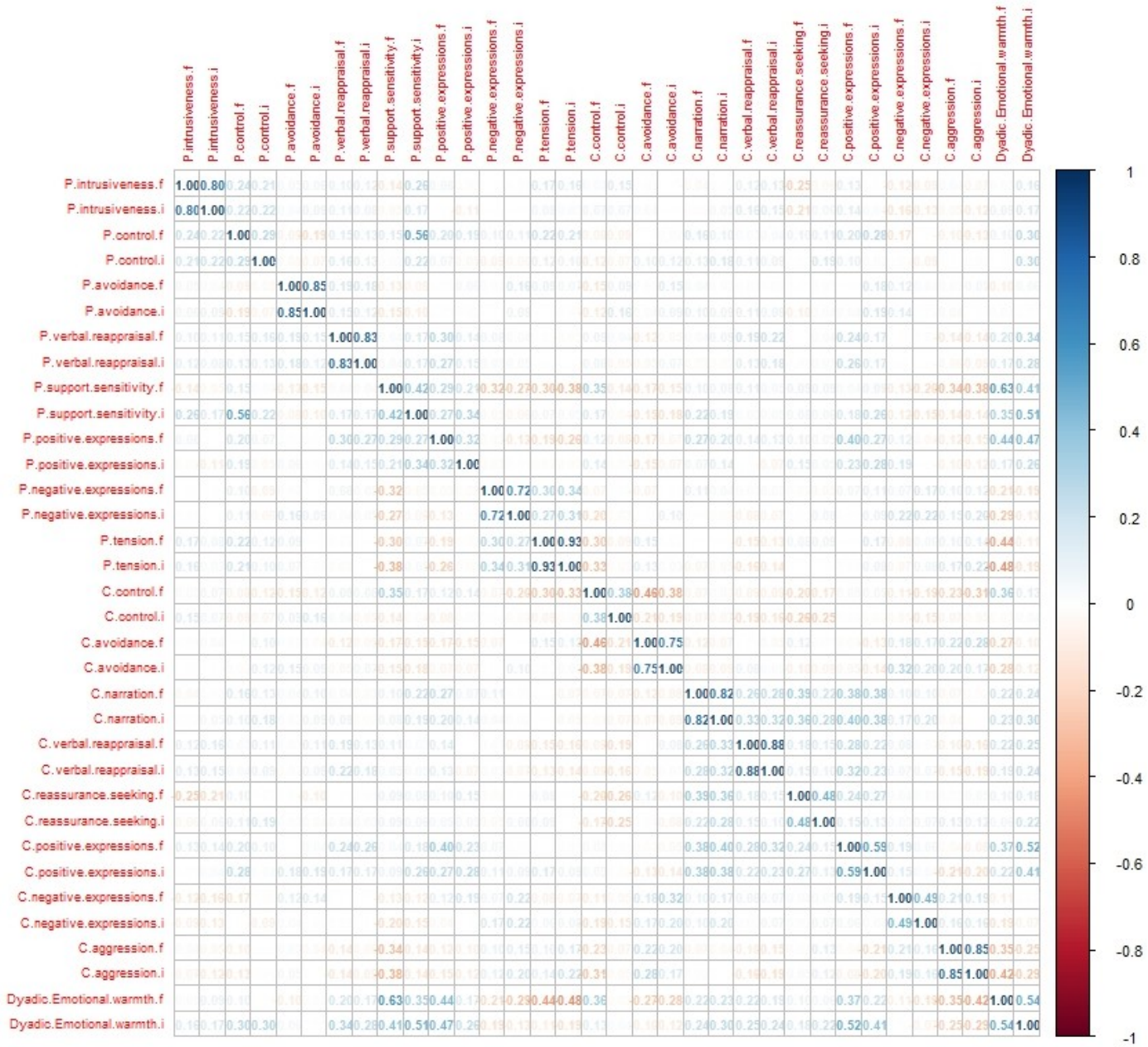
Note: n, number; ICD-10, International Classification of Diseases-10; OCD, Obsessive Compulsive Disorder

Figure A.2.1. Flow chart of participants



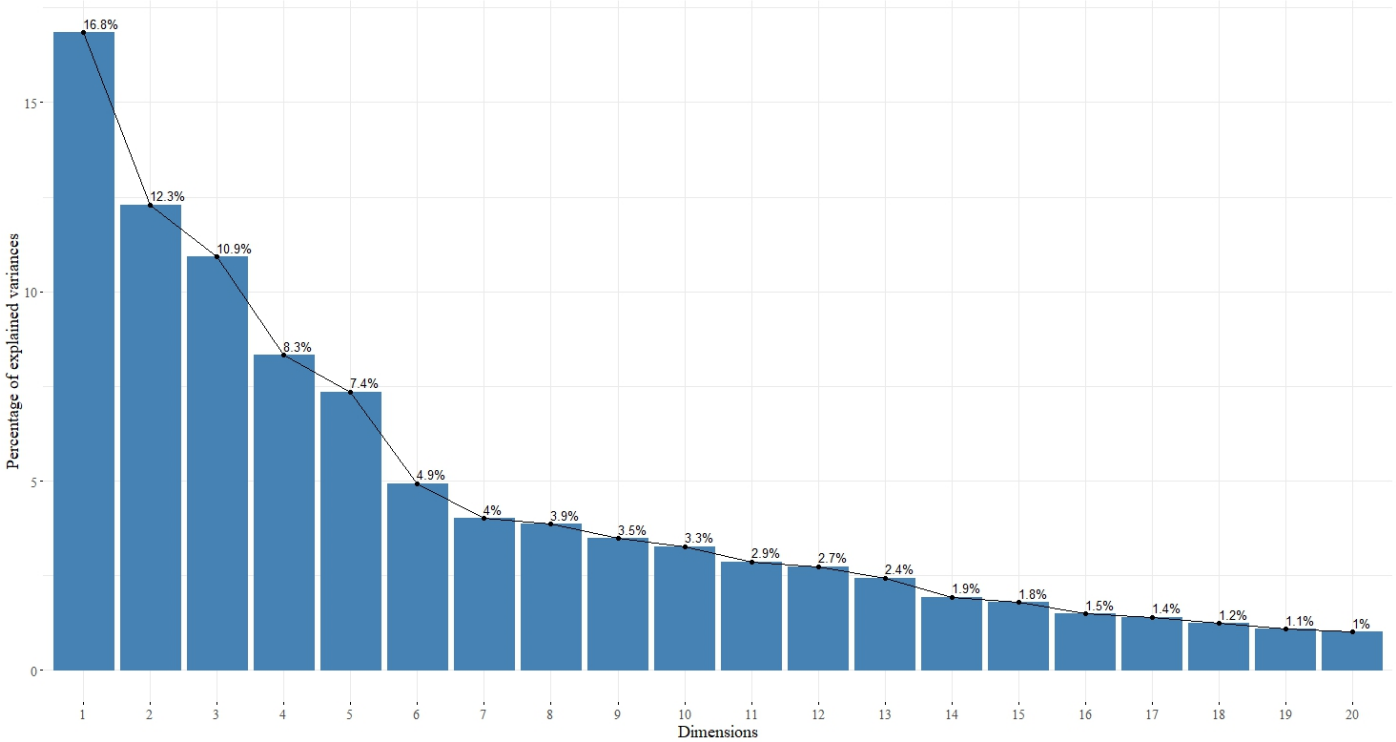
3. Principal Component Analysis

Figure A.3.1. Correlation matrix of mother-child behaviors



Note: p, parent (in this plot "mother); C, child; f, frequency; i, intensity

Figure A.3.2. Scree plot of mother-child PCA



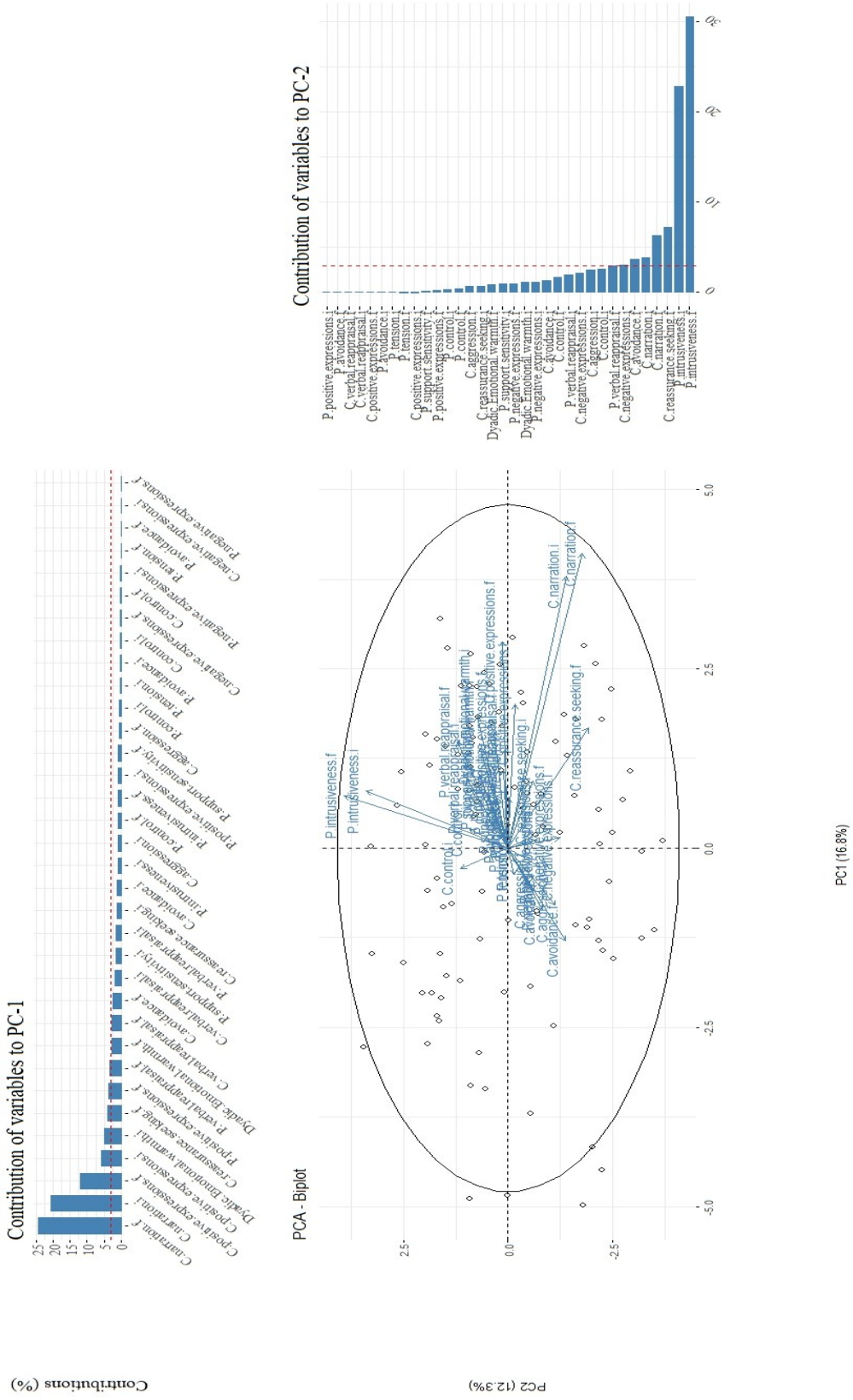
Note: Dimensions describes “principal components” in this plot

Table A.3.1. Eigenvectors from mother-child PCA

	PC1	PC2	PC3	PC4	PC5	PC6
P.intrusiveness.f	0.09	-0.55	0.36	-0.07	0.22	-0.01
P.intrusiveness.i	0.10	-0.48	0.31	-0.11	0.21	0.01
P.control.f	0.09	-0.06	0.07	0.01	-0.02	0.23
P.control.i	0.07	-0.06	0.08	-0.09	-0.01	0.10
P.avoidance.f	0.02	0.00	0.08	0.05	-0.11	-0.19
P.avoidance.i	0.03	-0.02	0.06	0.05	-0.06	-0.29
P.verbal.reappraisal.f	0.18	-0.17	0.03	0.12	-0.54	-0.06
P.verbal.reappraisal.i	0.12	-0.14	0.04	0.07	-0.49	0.00
P.support.sensitivity.f	0.08	-0.04	-0.19	-0.11	-0.04	0.11
P.support.sensitivity.i	0.12	-0.10	-0.02	-0.01	0.00	0.18
P.positive.expressions.f	0.19	-0.05	-0.08	-0.03	-0.17	-0.01
P.positive.expressions.i	0.08	0.00	-0.05	0.03	-0.09	0.06
P.negative.expressions.f	0.01	0.10	0.29	0.54	-0.02	0.05
P.negative.expressions.i	-0.03	0.11	0.29	0.43	-0.07	0.05
P.tension.f	-0.02	0.02	0.23	0.14	0.05	0.31
P.tension.i	-0.04	0.02	0.19	0.14	0.04	0.21
C.control.f	0.03	-0.13	-0.19	0.09	0.02	-0.09
C.control.i	-0.03	-0.16	-0.08	0.17	0.10	-0.37
C.avoidance.f	-0.15	0.19	0.41	-0.50	-0.17	0.03
C.avoidance.i	-0.10	0.11	0.28	-0.29	-0.20	-0.18
C.narration.f	0.49	0.25	0.06	0.00	0.30	-0.17
C.narration.i	0.45	0.20	0.09	-0.05	0.25	-0.19
C.verbal.reappraisal.f	0.16	-0.01	0.05	-0.10	-0.06	-0.06
C.verbal.reappraisal.i	0.13	-0.02	0.04	-0.05	-0.05	-0.05
C.reassurance.seeking.f	0.20	0.27	0.00	-0.10	0.00	0.40
C.reassurance.seeking.i	0.11	0.08	0.04	-0.05	0.02	0.23
C.positive.expressions.f	0.35	-0.02	0.09	-0.01	-0.16	0.01
C.positive.expressions.i	0.24	0.02	0.03	0.09	-0.08	0.07
C.negative.expressions.f	0.03	0.14	0.11	-0.01	-0.11	-0.28
C.negative.expressions.i	0.02	0.17	0.16	0.04	-0.07	-0.23
C.aggression.f	-0.07	0.08	0.14	-0.01	0.03	-0.05
C.aggression.i	-0.10	0.16	0.19	0.01	0.04	-0.06
Dyadic.Emotional.warmth.f	0.17	-0.09	-0.18	-0.08	-0.06	0.01
Dyadic.Emotional.warmth.i	0.22	-0.11	-0.05	-0.09	-0.14	0.09

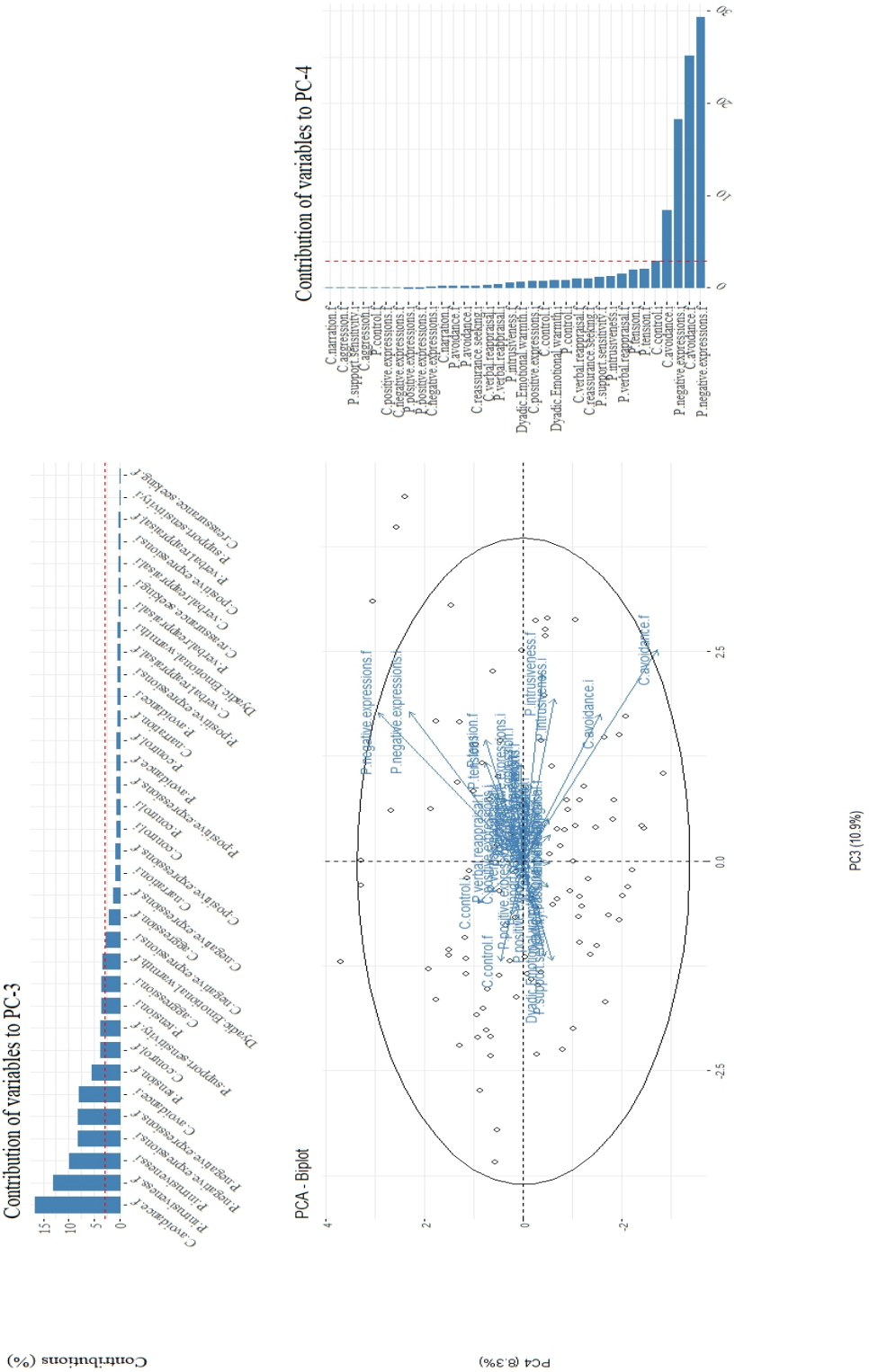
Note: P, parent (here describing “mother”); C, child; f, frequency; i, intensity; PC, Principal Component

Figure A.3.3. Biplot and contribution plots of PC1 and PC2 from the mother-child PCA



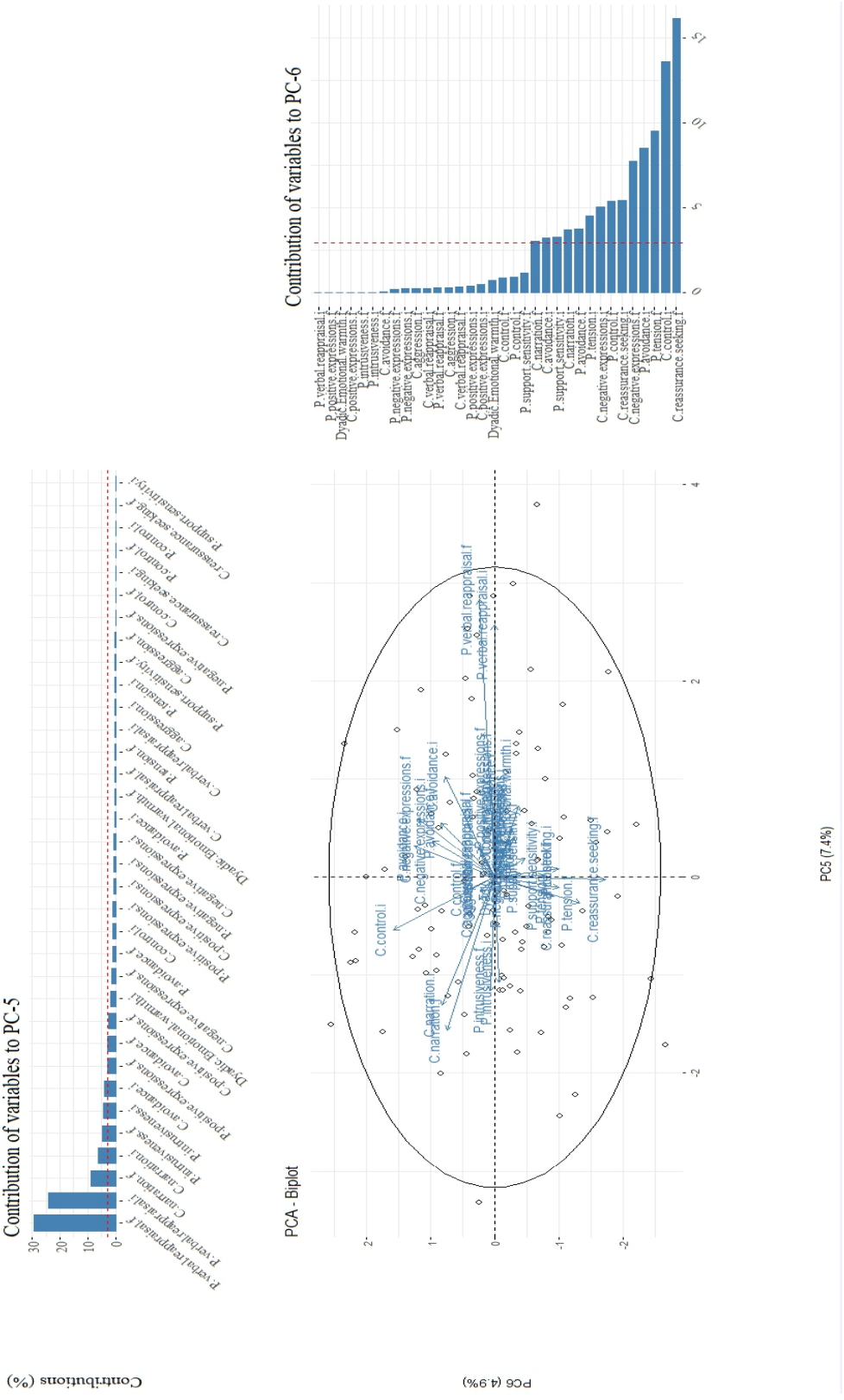
Note: P, parent (in this plot “mother); C, child; f, frequency; i, intensity; PC, Principal Component;

Figure A.3.4. Biplot and contribution plots of PC3 and PC4 from the mother-child PCA



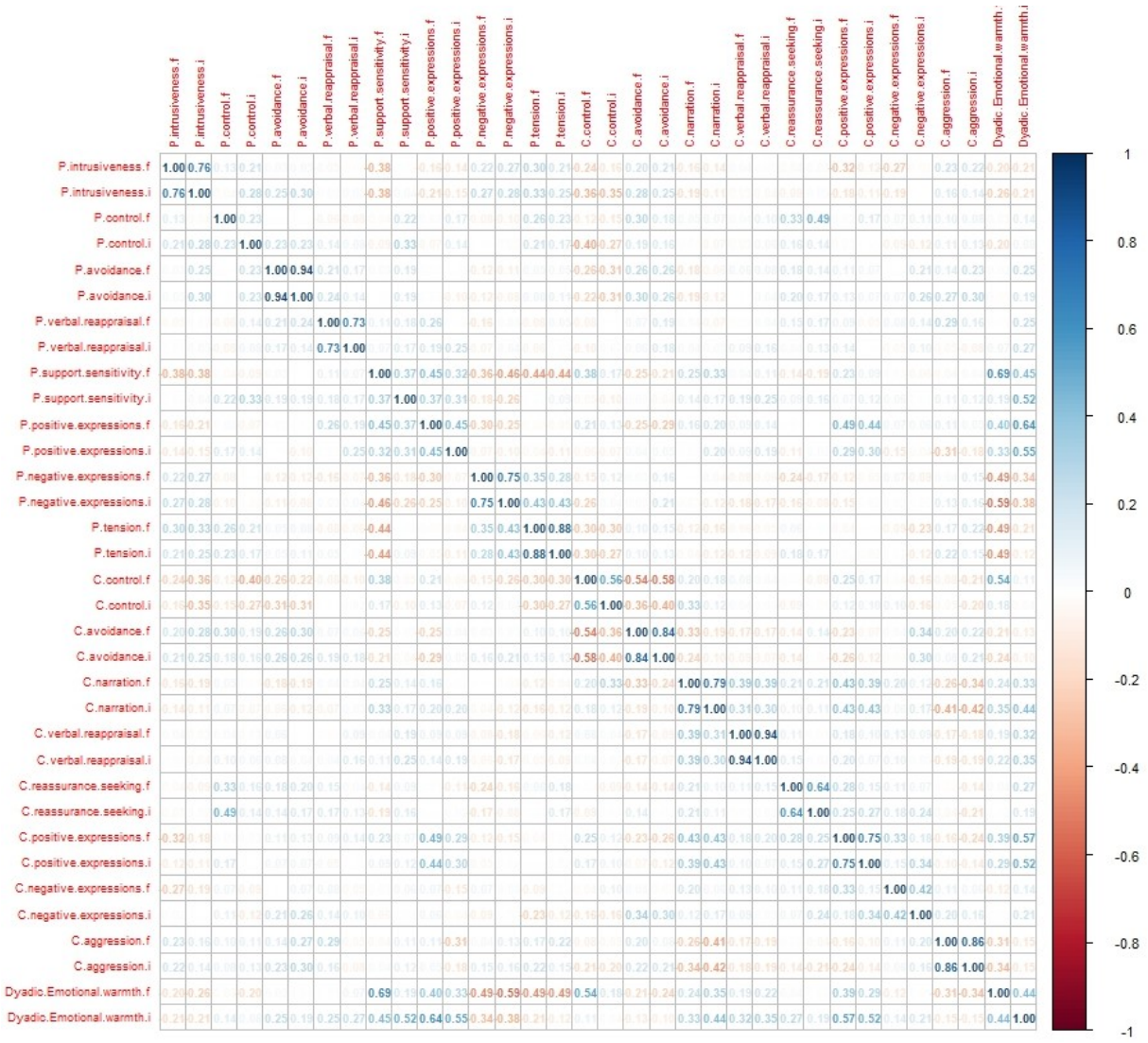
Note: P, parent (in this plot "mother); C, child; f, frequency; i, intensity; PC, Principal Component;

Figure A.3.5. Biplot and contribution plots of PC5 and PC6 from the mother-child PCA



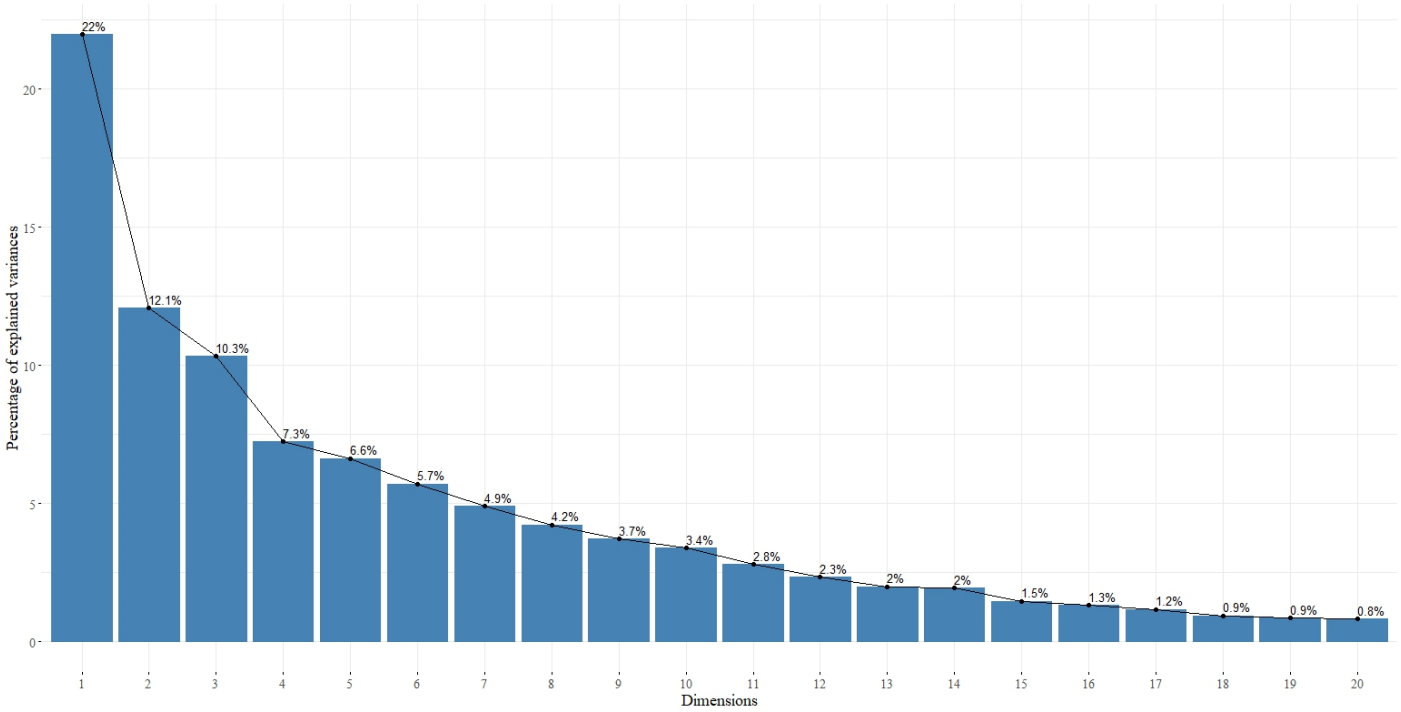
Note: P, parent (in this plot "mother); C, child; f, frequency; i, intensity; PC, Principal Component

Figure A.3.6. Correlation matrix of father-child behaviors



Note: p, parent (in this plot “father); C, child; f, frequency; i, intensity

Figure A.3.7. Scree plot of father -child PCA



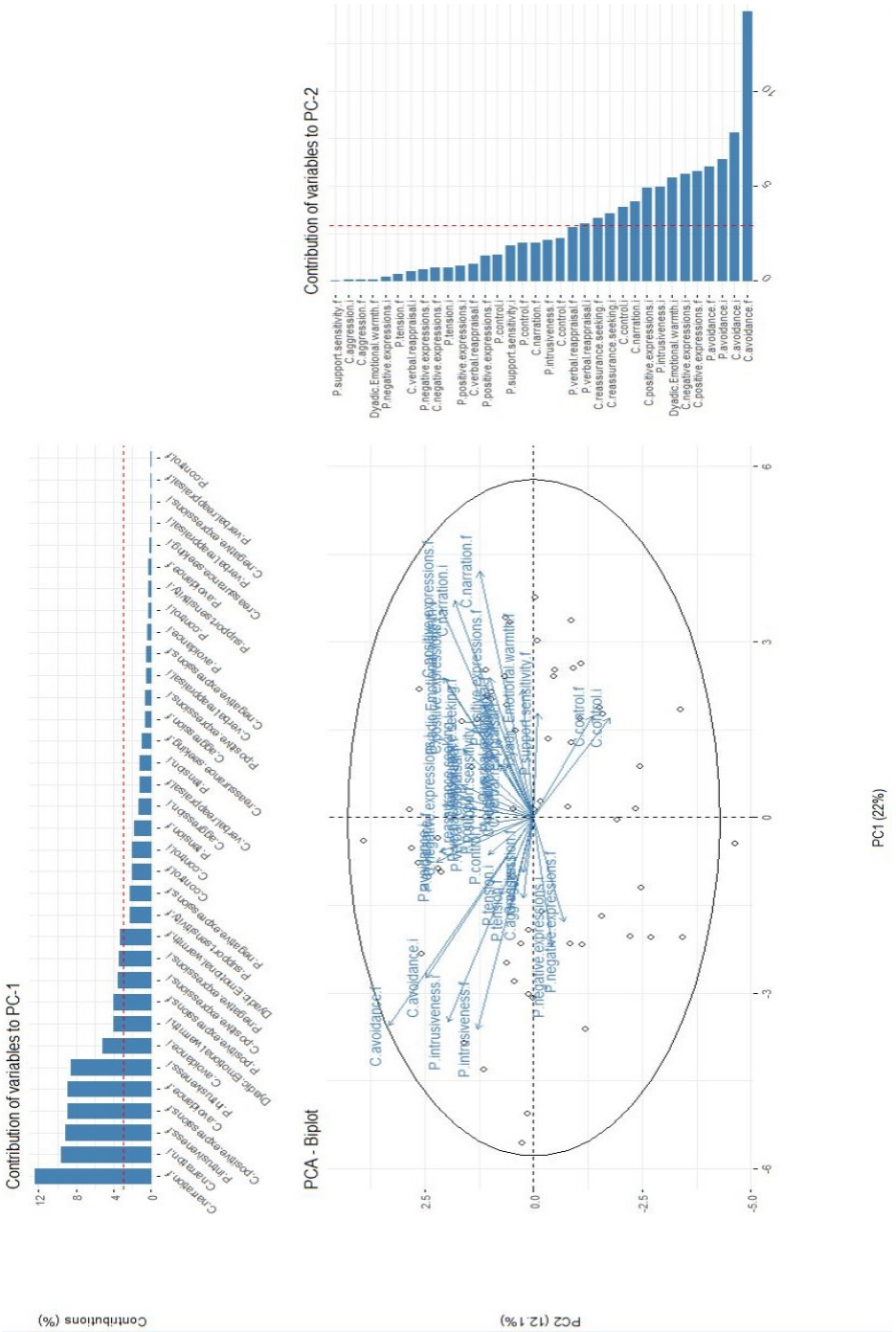
Note: Dimensions describes “principal components” in this plot

Table A.3.2. Eigenvectors from father-child PCA

	PC1	PC2	PC3	PC4	PC5	PC6
P.intrusiveness.f	-0.30	-0.15	-0.34	0.45	-0.20	-0.01
P.intrusiveness.i	-0.29	-0.22	-0.28	0.36	-0.11	-0.06
P.control.f	-0.01	-0.14	0.00	-0.03	0.03	0.21
P.control.i	-0.05	-0.12	-0.02	0.09	0.04	0.05
P.avoidance.f	-0.05	-0.25	0.13	0.10	0.10	0.01
P.avoidance.i	-0.06	-0.25	0.13	0.09	0.14	0.02
P.verbal.reappraisal.f	0.01	-0.17	0.11	0.05	0.19	-0.28
P.verbal.reappraisal.i	0.03	-0.17	0.06	0.05	0.09	-0.36
P.support.sensitivity.f	0.15	0.01	0.13	0.07	-0.12	-0.14
P.support.sensitivity.i	0.05	-0.14	0.04	0.14	0.00	-0.05
P.positive.expressions.f	0.20	-0.12	0.08	0.20	0.17	-0.34
P.positive.expressions.i	0.08	-0.09	0.05	0.03	-0.04	-0.21
P.negative.expressions.f	-0.15	0.08	-0.35	-0.25	0.09	-0.22
P.negative.expressions.i	-0.18	0.05	-0.37	-0.26	0.22	-0.25
P.tension.f	-0.13	-0.06	-0.20	0.02	0.25	0.03
P.tension.i	-0.11	-0.09	-0.18	-0.03	0.30	0.07
C.control.f	0.14	0.15	0.00	0.09	0.00	-0.03
C.control.i	0.14	0.20	-0.09	-0.06	0.00	-0.13
C.avoidance.f	-0.30	-0.38	0.23	-0.33	-0.28	0.03
C.avoidance.i	-0.23	-0.28	0.10	-0.29	-0.22	-0.09
C.narration.f	0.35	-0.14	-0.41	-0.15	-0.18	0.10
C.narration.i	0.31	-0.20	-0.30	-0.10	-0.36	0.01
C.verbal.reappraisal.f	0.11	-0.10	-0.11	0.09	-0.14	0.05
C.verbal.reappraisal.i	0.08	-0.07	-0.06	0.06	-0.07	0.02
C.reassurance.seeking.f	0.10	-0.18	-0.03	0.10	0.31	0.51
C.reassurance.seeking.i	0.05	-0.19	-0.02	-0.03	0.16	0.30
C.positive.expressions.f	0.30	-0.24	-0.08	-0.04	0.25	-0.11
C.positive.expressions.i	0.19	-0.22	-0.11	-0.07	0.10	-0.10
C.negative.expressions.f	0.08	-0.08	0.00	-0.28	0.17	0.05
C.negative.expressions.i	0.02	-0.24	0.06	-0.22	-0.05	-0.01
C.aggression.f	-0.08	-0.03	0.04	0.03	0.14	-0.06
C.aggression.i	-0.12	-0.03	0.07	0.02	0.12	-0.11
Dyadic.Emotional.warmth.f	0.18	-0.03	0.12	0.17	-0.18	-0.05
Dyadic.Emotional.warmth.i	0.20	-0.23	0.07	0.11	0.03	-0.12

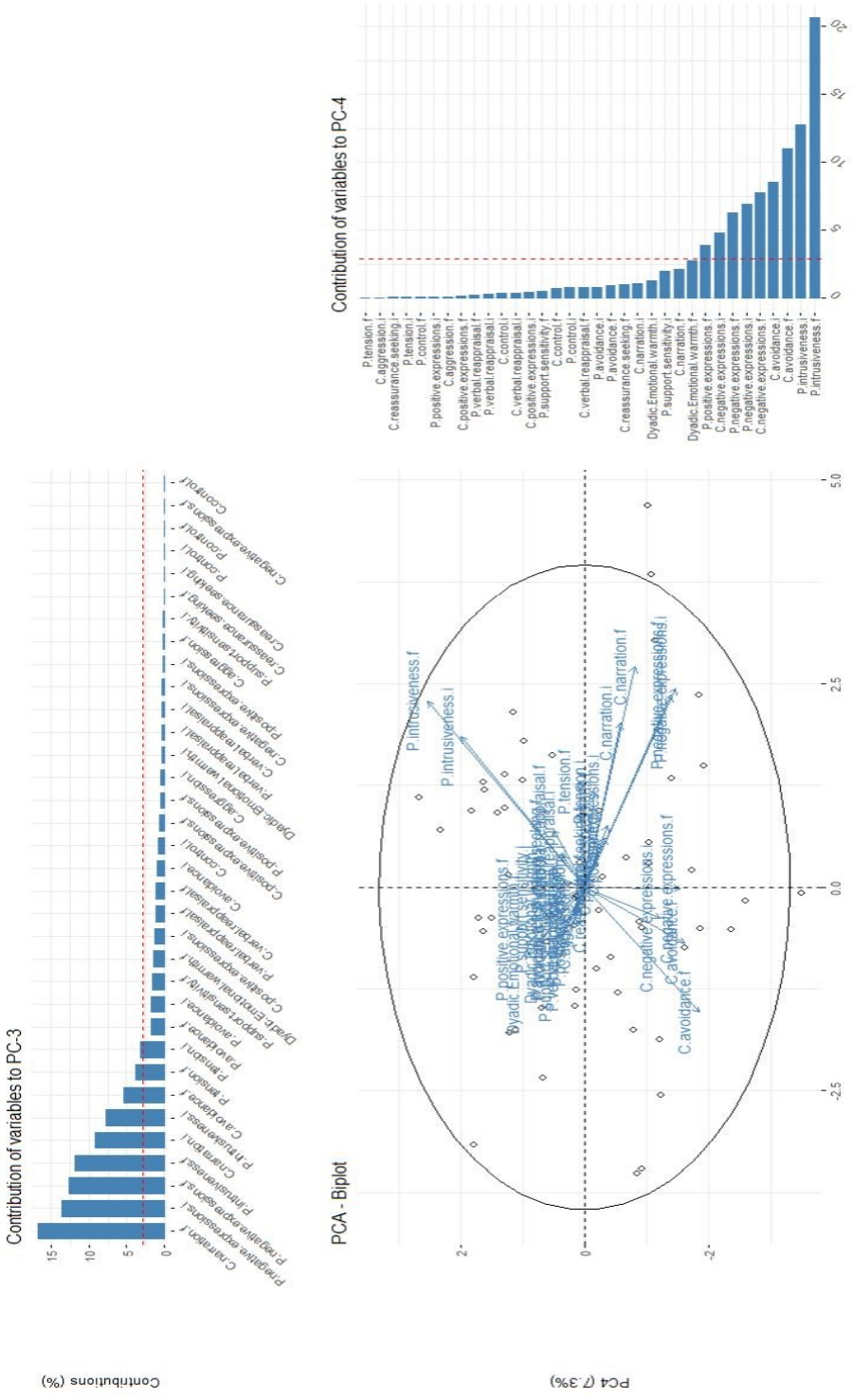
Note: P, parent (here describing “father”); C, child; f, frequency; i, intensity; PC, Principal Component

Figure A.3.8. Biplot and contribution plots of PC1 and PC2 from the father-child PCA



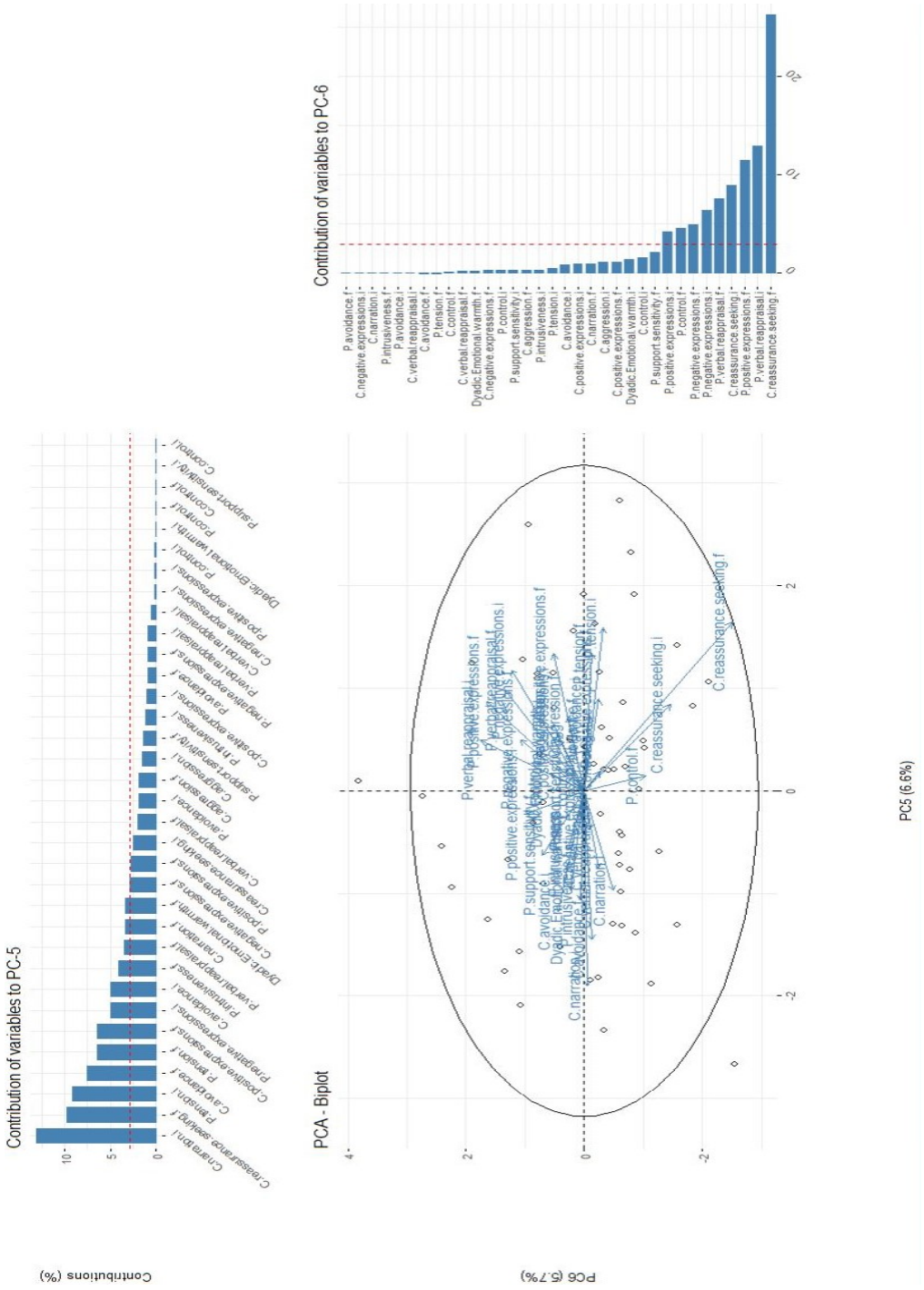
Note: P, parent (in this plot "father); C, child; f, frequency; i, intensity; PC, Principal Component

Figure A.3.9. Biplot and contribution plots of PC3 and PC4 from the father-child PCA



Note: P, parent (in this plot "father); C, child; f, frequency; i, intensity; PC, Principal Component;

Figure A.3.10. Biplot and contribution plots of PC5 and PC6 from the father-child PCA



Note: P, parent (in this plot "father); C, child; f, frequency; i, intensity; PC, Principal Component

Table A.3.3. Regression analyses predicting child and mother oxytocin from PC1-PC6 and the interaction between PC1-PC6 and child OCD status.

	Child log oxytocin[A] (*n*=99)		Mother log oxytocin (*n*=99)	
	Estimate (*SE*)	Adjusted *p*-value	Estimate (SE)	Adjusted *p*-value
PC1				
PC1	0.03 (0.04)	0.807	0.11 (0.04)	0.162*
OCD	0.05 (0.10)	0.807	-0.07 (0.09)	0.807
PC1:OCD	-0.02 (0.05)	0.807	-0.06 (0.05)	0.768
PC2				
PC2	0.02 (0.02)	0.807	0.06 (0.04)	0.768
OCD	0.05 (0.05)	0.807	-0.07 (0.10)	0.807
PC2:OCD	0.02 (0.02)	0.808	-0.003 (0.06)	0.961
PC3				
PC3	-0.04 (0.05)	0.807	-0.08 (0.05)	0.768
OCD	0.06 (0.10)	0.807	-0.03 (0.10)	0.808
PC4:OCD	0.03 (0.06)	0.807	0.08 (0.06)	0.768
PC4				
PC4	-0.03 (0.05)	0.807	0.03 (0.05)	0.807
OCD	0.05 (0.10)	0.807	-0.04 (0.10)	0.808
PC3:OCD	0.04 (0.07)	0.807	0.02 (0.07)	0.808
PC5				
PC5	0.04 (0.05)	0.807	0.08 (0.05)	0.768
OCD	0.03 (0.09)	0.808	-0.09 (0.09)	0.807
PC5:OCD	0.133 (0.08)	0.768	0.092 (0.08)	0.781
PC6				
PC6	-0.051 (0.07)	0.807	-0.09 (0.07)	0.768
OCD	0.056 (0.09)	0.807	-0.06 (0.10)	0.807
PC6:OCD	0.183 (0.09)	0.768	0.12 (0.09)	0.768

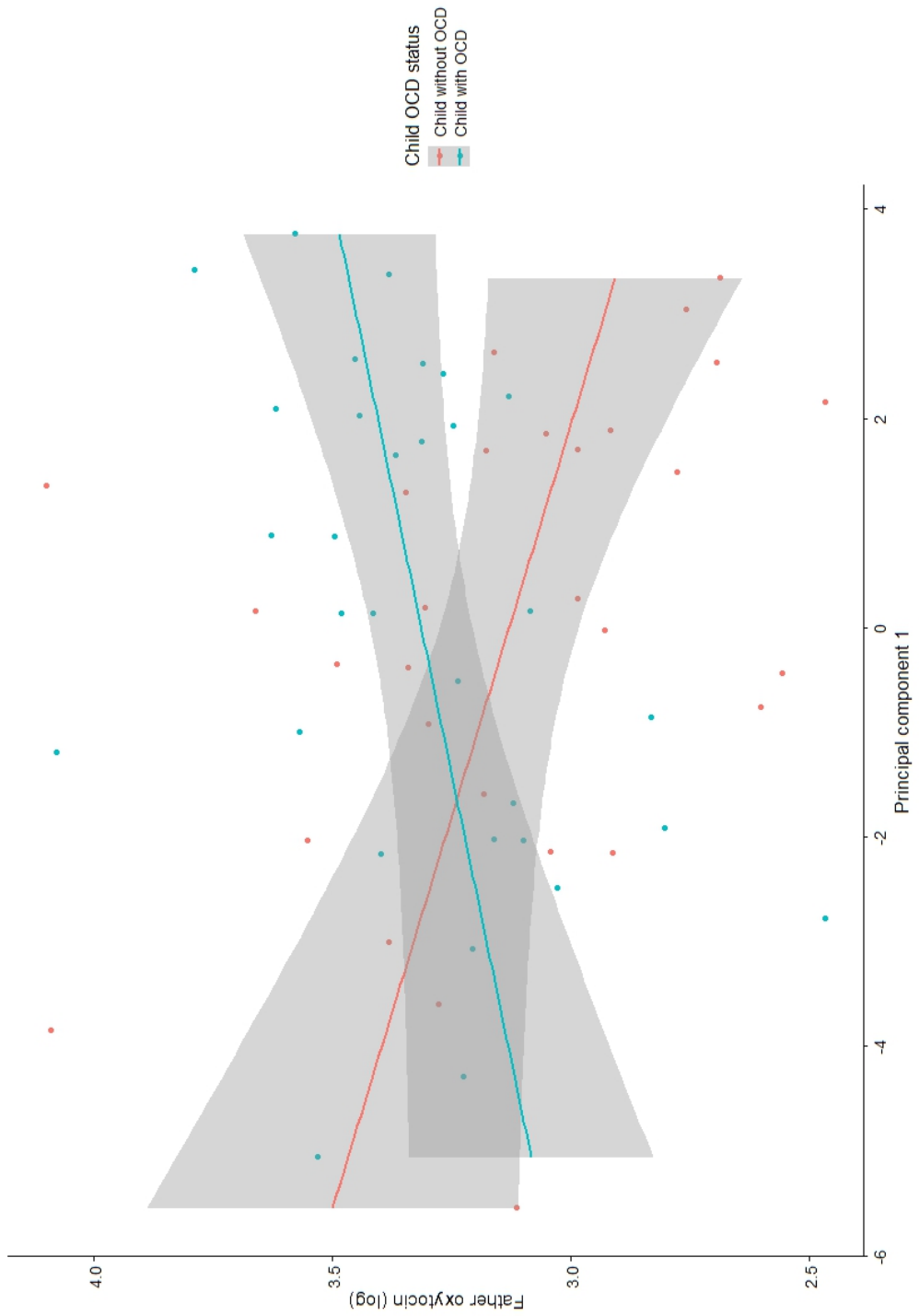
Note: log, logarithmic; OCD, Obsessive Compulsive Disorder; *SE*, standard error; PC, Principal Component; [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2). *Significant with an accepted false discovery rate of 20% (p-value > 0.20).

Table A.3.4. Regression analyses predicting child and father oxytocin from PC1-PC6 and the interaction between PC1-PC6 and child OCD status.

	Child log oxytocin[A] (*n*=56)		Father log oxytocin (*n*= 60)	
	Estimate (*SE*)	Adjusted *p*-value	Estimate (*SE*)	Adjusted *p*-value
PC1				
PC1	-0.05 (0.04)	0.628	-0.07 (0.03)	0.306
OCD	0.14 (0.13)	0.628	0.18 (0.09)	0.306
PC1:OCD	0.11 (0.06)	0.306	0.11 (0.04)	0.180*
PC2				
PC2	0.06 (0.05)	0.628	0.03 (0.04)	0.683
OCD	0.13 (0.14)	0.628	0.18 (0.09)	0.306
PC2:OCD	-0.06 (0.08)	0.688	-0.05 (0.06)	0.628
PC3				
PC3	-0.03 (0.05)	0.733	-0.01 (0.04)	0.927
OCD	0.15 (0.14)	0.628	0.18 (0.09)	0.306
PC3:OCD	0.01 (0.10)	0.927	0.04 (0.06)	0.733
PC4				
PC4	-0.06 (0.07)	0.674	-0.07 (0.05)	0.628
OCD	0.16 (0.14)	0.628	0.20 (0.09)	0.306
PC4:OCD	0.04 (0.11)	0.837	0.07 (0.07)	0.628
PC5				
PC5	-0.07 (0.07)	0.628	0.03 (0.05)	0.719
OCD	0.15 (0.14)	0.628	0.18 (0.09)	0.306
PC5:OCD	0.05 (0.12)	0.781	0.01 (0.09)	0.927
PC6				
PC6	0.03 (0.09)	0.837	-0.06 (0.06)	0.628
OCD	0.15 (0.14)	0.628	0.17 (0.10)	0.306
PC6:OCD	0.01 (0.12)	0.927	0.06 (0.08)	0.688

Note: log, logarithmic; OCD, Obsessive Compulsive Disorder; *SE*, standard error; PC, Principal Component; [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2). *Significant with an accepted false discovery rate of 20% (p-value > 0.20).

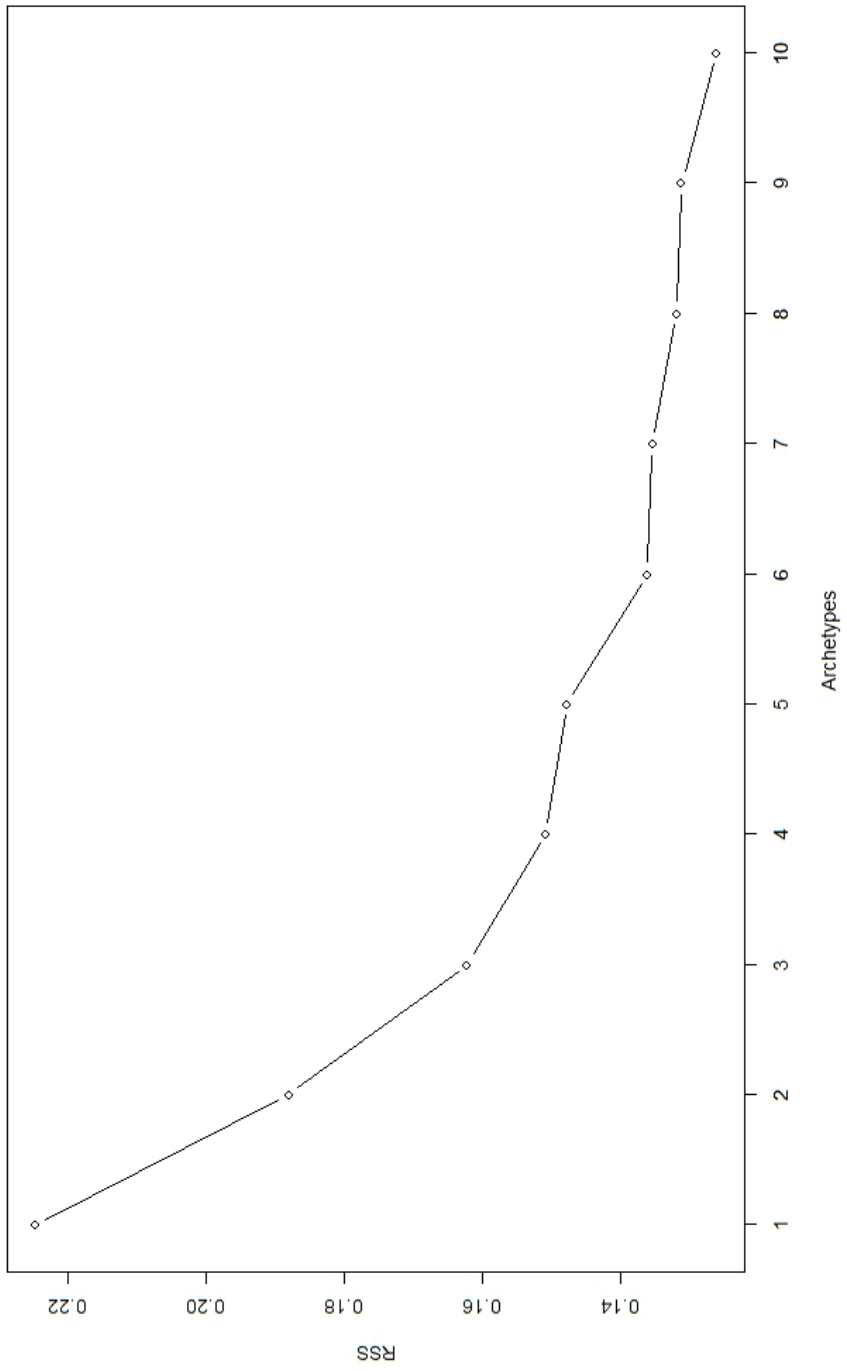
Figure A.3.11. Scatter plot of principal component 1 (PC1) and father oxytocin (log) by child OCD status



Note: log, logarithmic; OCD, Obsessive Compulsive Disorder

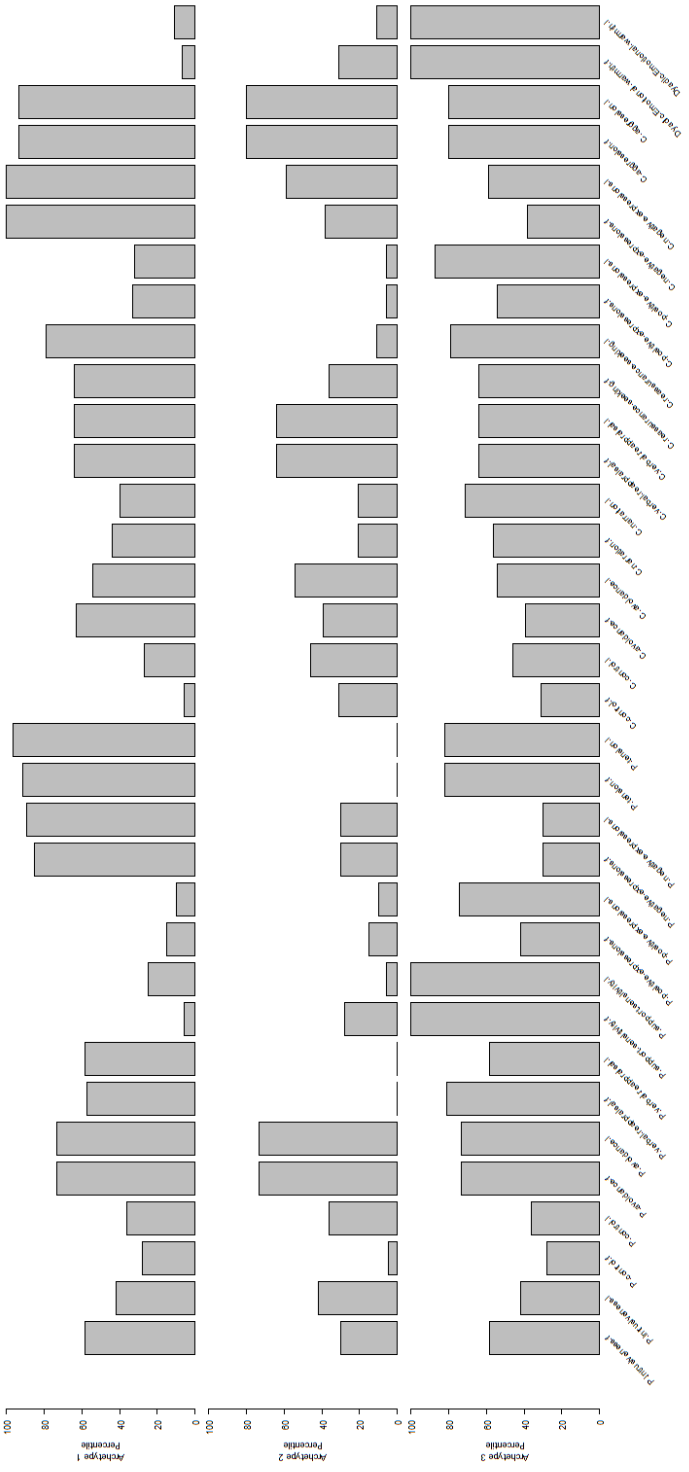
4. Archetype analyses

Figure A.4.1. Scree plot of archetypes from the mother-child archetype analyses



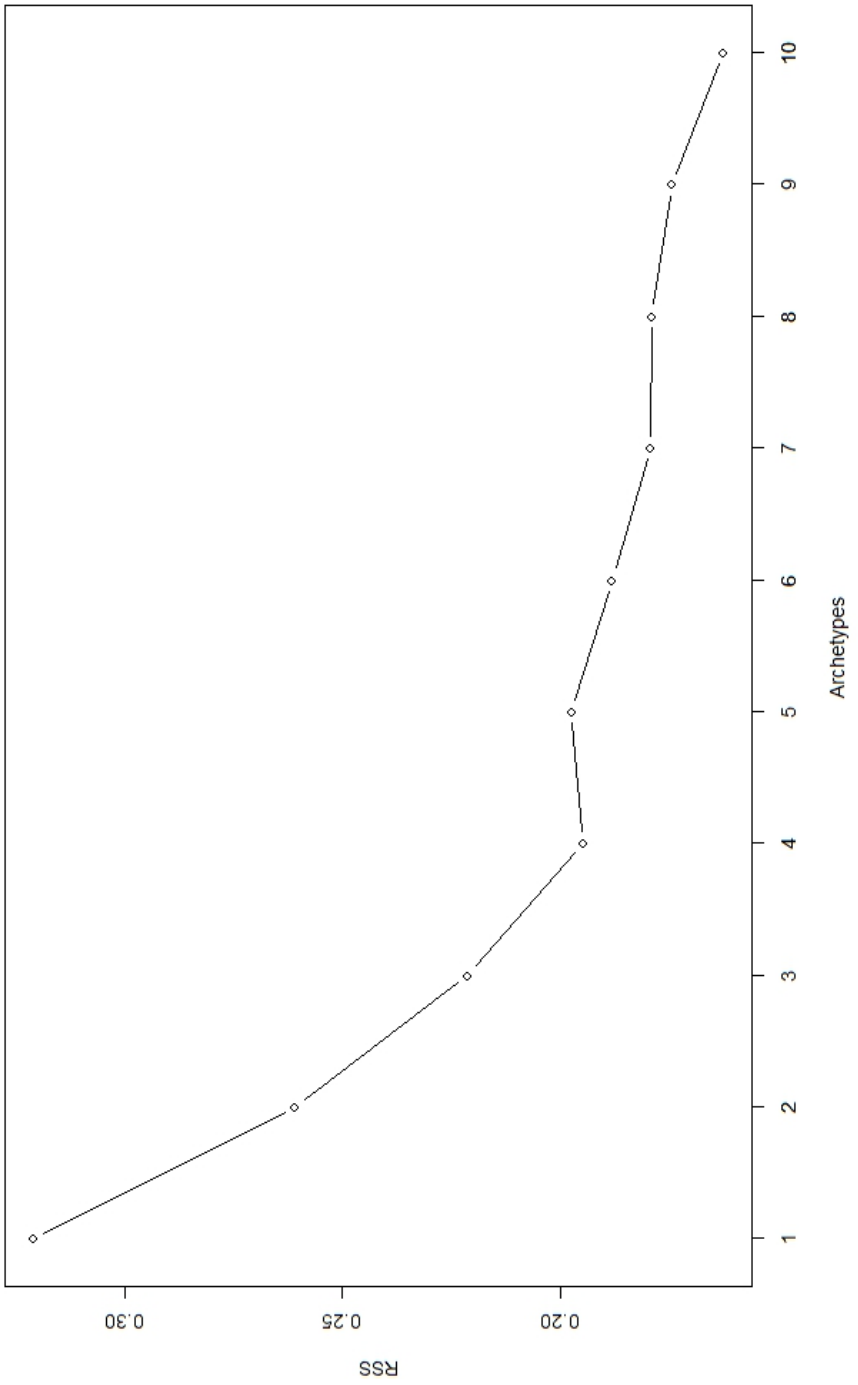
Note: RSS, Residual sum of squares

Figure A.4.2. Archetypes from the mother-child archetype analysis



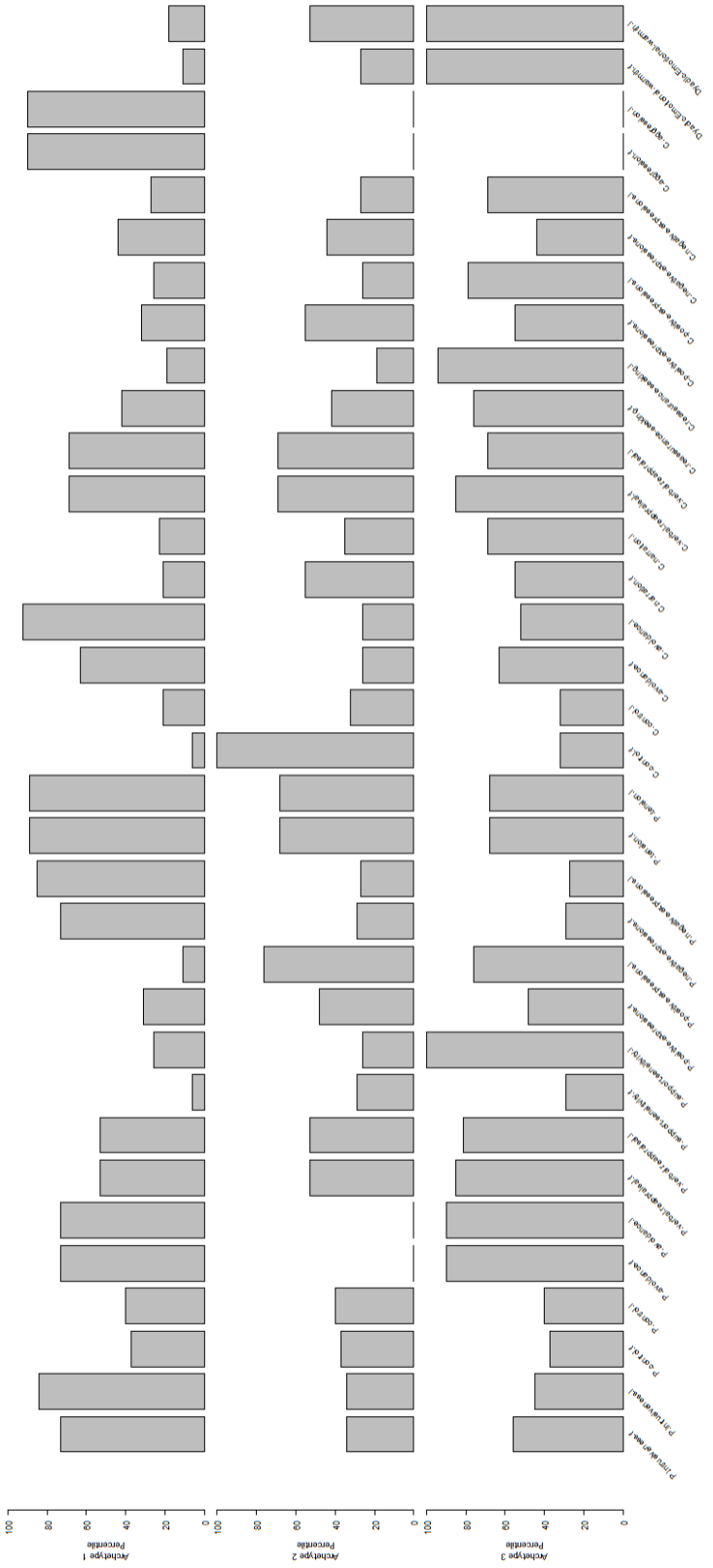
Note: P, parent (in this plot “father); C, child; f, frequency; i, intensity

Figure A.4.3. Scree plot of archetypes from the father-child archetype analyses



Note: RSS, Residual sum of squares

Figure A.4.4. Archetypes from the father-child archetype analysis



Note: P, parent (in this plot “father); C, child; f, frequency; i, intensity

Table A.4.1 Regression analyses predicting child and mother oxytocin from archetype 1-3 and the interaction between archetype 1-3and child OCD status.

	Child log oxytocin[A] (*n*=56)		Mother log oxytocin[A] (*n*=60)	
	Estimate (*SE*)	Adj. *p*-value	Estimate (SE)	Adj. *p*-value
Archetype 1				
Archetype1	-0.25 (0.35)	0.624	-0.55 (0.35)	0.624
OCD	0.09 (0.12)	0.624	-0.10 (0.12)	0.624
Archetype 1:OCD	-0.15 (0.45)	0.776	0.39 (0.46)	0.624
Archetype 2				
Archetype 2	-0.25 (0.28)	0.624	-0.66 (0.27)	0.170*
OCD	-0.03 (0.13)	0.833	-0.15 (0.13)	0.624
Archetype 2:OCD	0.31 (0.37)	0.624	0.28 (0.36)	0.624
Archetype 3				
Archetype 3	0.32 (0.24)	0.624	0.79 (0.23)	0.020*
OCD	0.12 (0.22)	0.721	0.15 (0.21)	0.624
Archetype 3:OCD	-0.12 (0.33)	0.776	-0.37 (0.31)	0.624

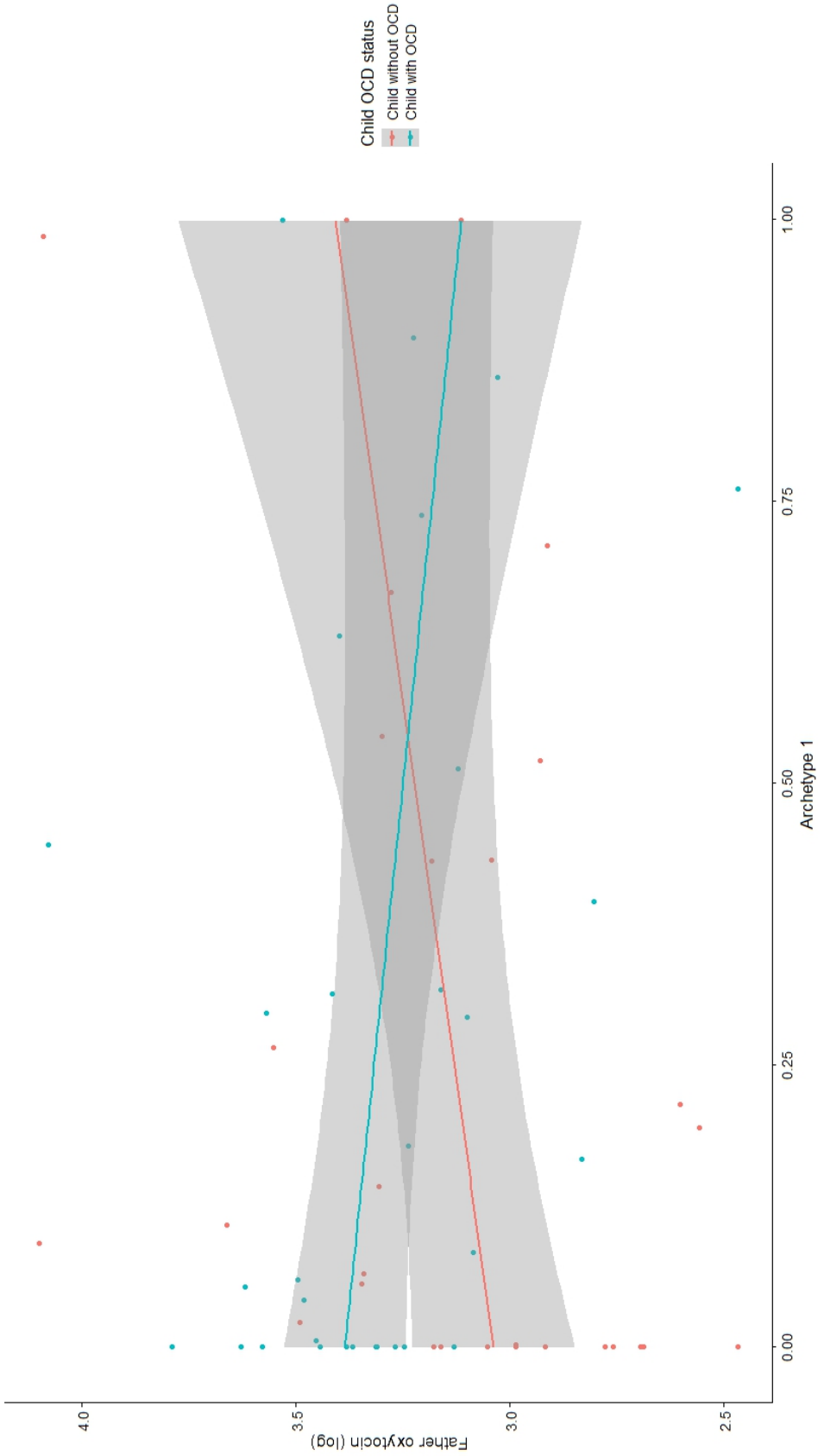
Note: log, logarithmic; OCD, Obsessive Compulsive Disorder; *SE*, standard error; [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2). *Significant with an accepted false discovery rate of 20% (p-value > 0.20).

Table A.4.2 Regression analyses predicting child and father oxytocin from archetype 1-3 and the interaction between archetype 1-3and child OCD status.

	Child log oxytocin[A] (*n*=56)		Father log oxytocin (*n*=60)	
	Estimate (*SE*)	Adjusted *p*-value	Estimate (*SE*)	Adjusted *p*-value
Archetype 1				
Archetype1	0.24 (0.28)	0.713	0.37 (0.20)	0.310
OCD	0.32 (0.17)	0.310	0.35 (0.12)	0.067*
Archetype1:OCD	-0.69 (0.41)	0.360	-0.64 (0.28)	0.226
Archetype 2				
Archetype 2	-0.08 (0.31)	0.903	-0.15 (0.22)	0.713
OCD	0.01 (0.24)	0.983	0.04 (0.17)	0.903
Archetype 2:OCD	0.31 (0.46)	0.713	0.32 (0.31)	0.630
Archetype 3				
Archetype 3	-0.22 (0.33)	0.713	-0.31 (0.23)	0.480
OCD	-0.004 (0.19)	0.983	0.06 (0.13)	0.793
Archetype 3:OCD	0.48 (0.45)	0.630	0.43 (0.30)	0.480

Note: log, logarithmic; OCD, Obsessive Compulsive Disorder; *SE*, standard error; [A]Log oxytocin adjusted for child pubertal stage (See Appendix section 1.2). *Significant with an accepted false discovery rate of 20% (p-value > 0.20).

Figure A.4.5. Scatter plot of father-child archetype 1 and father oxytocin (log) by child OCD status



Note: log, logarithmic; OCD, Obsessive Compulsive Disorder