Universidade do Minho
Escola de Psicologia

Rita Isabel Curto Baião

The Role of a Maternal Oxytocin Receptor Gene Polymorphism in Maternal Interactive Behavior and its Consequent Impact on Children's Neuroendocrine and Socio-Emotional Functioning

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Tese de Doutoramento em Psicologia Aplicada

Trabalho efetuado sob a orientação da
Doutora Ana Mesquita
e do
Professor Doutor Pasco Fearon

fevereiro de 2018

STATEMENT OF INTEGRITY

I hereby declare having conducted my thesis with integrity. I confirm that I have not used plagiarism or any form of falsification of results in the process of the thesis elaboration. I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

University of Minho, 01st February 2018

Full name:

RITA ISABEL CURTO BAIÃO

Signature:

Rita Isabel Curto Baião

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THE ROLE OF A MATERNAL OXYTOCIN RECEPTOR GENE POLYMORPHISM IN MATERNAL INTERACTIVE BEHAVIOUR AND ITS CONSEQUENT IMPACT ON CHILDREN'S NEUROENDOCRINE AND SOCIO-EMOTIONAL FUNCTIONING

ABSTRACT

Maternal behaviour, particularly sensitive parenting, is fundamental for child development and functioning. Is has been vastly studied, with recent considerable interest on its determinants, expanding the research from environmental (eg. maternal variables and contextual experience), to genetic predictors (eg. polymorphisms in the oxytocinergic system, given its link to affiliation). Despite this growing interest, research is still in many ways preliminary and, when reviewing the literature, results prove inconsistent.

Maternal behaviour has indeed been shown to impact on diverse aspects of child's adjustment, including children's behaviour, but also at the physiological level, such as neuroendocrine functioning. Nonetheless, literature on this domain is still scarce.

The goal of the present doctoral dissertation was to contribute to the gaps in this field of research. Its two main aims were: firstly, to investigate genetic and environmental determinants of maternal interactive behaviour; secondly, to assess the impact of such behaviour on child's functioning, particularly at the behavioural and neuroendocrine levels.

Therefore, Chapter 1 introduced the research background behind the proposed field of study. Chapter 2 and 3 then focus the first aim of this dissertation: to examine the determinants of maternal behaviour. Specifically, Chapter 2 analysed the role of a polymorphism (SNP rs53576) on the Oxytocin Receptor Gene (OXTR) on maternal behaviour in two studies. In the first study, based on a sample of 215 Caucasian mothers, a non-significant association was found between the OXTR SNP rs53576 and maternal behaviour. In the second study, results of a meta-analysis on the available literature on the same OXTR SNP and maternal behaviour revealed a statistically significant, small magnitude effect.

Chapter 3 then took into consideration the link between the oxytocinergic and serotonergic systems, and serotonin's role as a modulator of neural circuitry that controls several behavioural and physiological processes, which may be critical for parenting. It

analysed the role of the maternal 5-HTTLPR polymorphism on maternal behaviour, testing the quality of environmental context as a possible moderator of such association. Results proved consistent with the weak differential susceptibility model of person-X-context interaction.

Chapters 4 and 5 then focused the second aim of this dissertation, which relates to child's functioning. Chapter 4 provided an adaptation and validation of the child codings of the Coding System for Mother–Child Interactions (CSMCI, Healey et al., 2010). The codings' inter-rater agreement was confirmed, and convergent and divergent validity established. Its applications for the assessment of preschoolers' interactive behaviour, and its implications to future research and practice are discussed.

Finally, Chapter 5 explored the child's oxytocin (OT) response to mother-child interaction, and found that it was predicted by the interaction between individual (child's OXTR genotype) and environmental (maternal behaviour) factors. Drawing from these analysis, the identified interaction was investigated as a predictor of the child's interactive behaviour, considering the possible mediator role of the child's OT level.

This dissertation underlines the relevance of considering the interaction between individual and environmental factors in shaping both behavioural and neuroendocrine responses. Building on this work, future studies should consider larger scale samples, and follow approaches which include different biological, psychological and environmental aspects for a broader comprehension of affiliative behaviour.

O Papel de um Polimorfismo no Gene Recetor de Oxitocina Materno no Comportamento Interativo Materno, e o seu Consequente Impacto no Funcionamento Neuroedócrino e Socio-Emocional da Criança

Resumo

O comportamento materno, e particularmente a parentalidade sensível, é fundamental para o desenvolvimento e funcionamento da criança. Recentemente, este comportamento tem sido vastamente estudado, com considerável interesse prestado aos seus determinantes, alargando a investigação de preditores ambientais (p.ex., variáveis maternas e experiências relacionadas com o contexto), para preditores genéticos (p.ex., polimorfismos no sistema oxitocinergico, dada a sua relação com a afiliação). Apesar do interesse crescente, a investigação é, ainda, em grande medida, preliminar, e, ao rever a literatura, os resultados demonstram-se inconsistentes.

De facto, o comportamento materno tem demonstrado ter impacto em diversos aspectos do ajustamento da criança, quer a nível comportamental, quer em aspectos mais fisiológicos, como o funcionamento neuroendócrino. No entanto, a literatura neste domínio é ainda escassa.

O propósito desta dissertação de doutoramento é contribuir para as lacunas neste campo de investigação. Os dois objetivos fundamentais são: em primeiro lugar, investigar determinantes genéticos e ambientais do comportamento interativo materno; seguidamente, avaliar o impacto de tal comportamento no funcionamento da criança, particularmente ao nível comportamental e neuroendócrino.

Assim, o Capítulo 1 incide sobre a revisão da literatura da área em estudo. De seguida, os capítulos 2 e 3 focam o primeiro objetivo desta dissertação: o estudo dos determinantes do comportamento materno. Especificamente, o Capítulo 2 analisa o papel de um polimorfismo (SNP rs53576) no Gene Receptor da Oxitocina (OXTR) no comportamento materno, reunindo 2 estudos. No primeiro estudo, com base numa amostra de 215 mães Caucasianas, não é encontrada uma associação significativa entre o OXTR SNP rs53576 e o comportamento materno. No segundo estudo, os resultados de uma meta-análise com base na literatura disponível revelam um efeito de associação

significativo, embora de pequena magnitude, entre o referido polimorfismo e o comportamento materno.

O Capítulo 3 tem em consideração a relação entre os sistemas oxitocinérgico e serotoninérgico, bem como o papel da serotonina como modulador de circuitos neuronais que controlam diversos processos comportamentais e fisiológicos, que podem ser críticos para a parentalidade. Este capítulo analisa o papel do polimorfismo 5-HTTLPR no comportamento materno, e testa a qualidade do contexto ambiental como possível moderador desta associação. Os resultados revelam-se consistentes com a versão fraca do modelo de susceptibilidade diferencial de interação Gene-X-Ambiente.

De seguida, os capítulos 4 e 5 focam-se no segundo objetivo desta dissertação, que se relaciona com o funcionamento da criança. O Capítulo 4 faz a adaptação e validação das escalas da criança do instrumento Coding System for Mother–Child Interactions (CSMCI, Healey et al., 2010). O acordo inter-cotador é estabelecido, bem como a sua validade convergente e discriminante. São discutidos o seu uso para a codificação do comportamento interactivo de crianças em idade pré-escolar, e as suas implicações para a investigação futura e para a prática.

Finalmente, o Capítulo 5 explora a resposta oxitocinérgica da criança numa tarefa de interação mãe-criança. Esta resposta é predita pela interação entre factores individuais (o genótipo OXTR da criança) e ambientais (comportamento materno). Com base nestas análises, o efeito de interação encontrado é investigado como preditor do comportamento interactivo da criança, considerando o possível papel mediador dos níveis de OT da criança.

De forma global, esta dissertação sublinha a relevância de considerar o impacto da interação entre factores individuais e ambientais nas respostas comportamentais e neuroendócrinas. Partindo deste trabalho, estudos futuros deverão considerar amostras de maior escala, e seguir abordagens que incluam diferentes aspectos biológicos, psicológicos e ambientais, para uma compreensão mais alargada do comportamento afiliativo.

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List of Abbreviations

5-HTTLPR	Serotonin Transporter-Linked Polymorphic Region
ADHD	Attention-Deficit/Hyperactivity Disorder
AIC	Akaik Information Criteria
BIC	Bayesian Information Criteria
BSI	Brief Symptom Inventory
CBCL	Child Behaviour Checklist
CBQ	Children's Behaviour Questionnaire
CSMCI	Coding System for Mother–Child Interactions
GWAS	Genome-wide association study
GXE	Gene-X-environment
HPA	Hypothalamic–pituitary–adrenal
ODD	Oppositional Defiant Disorder
OT	Oxytocin
OXTR	Oxytocin Receptor Gene
PCR	Polymerase chain reaction
PSDI	Positive Symptoms Distress Index, Brief Symptom Inventory
SNP	Single Nucleotide Polymorphism

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CHAPTER 1:
RESEARCH BACKGROUND

CHAPTER 1:
RESEARCH BACKGROUND

I. MATERNAL INTERACTIVE BEHAVIOUR AND ITS DETERMINANTS

1. MATERNAL INTERACTIVE BEHAVIOUR

Humans, as other mammals, learn to function within their environment based on experiences occurring within the mother-infant bond (Feldman, 2012). This bond is largely influenced by maternal behaviour. In fact, the view that maternal interactive behaviour, particularly maternal sensitivity, is a critical factor in early child development has a long history within psychology and psychiatry. Bowlby (1969) was one of its early proponents, referring to maternal sensitivity as an important factor in the development of a secure attachment. He postulated the importance of an infant's confidence in the mother's accessibility and responsiveness. This was also later supported by detailed empirical work by Ainsworth, based on her Baltimore study, where 23 mother-child dyads were observed during home visits at three-week intervals over the first year of life. This study provided a substantial amount of observational data (Ainsworth, Blehar, Waters, & Wall, 1978) on parental behaviour and security of attachment. On the basis of this work, Ainsworth, Bell, and Stayton (1974) conceived the now widely accepted and used definition of sensitivity, as the maternal ability and readiness to perceive infant signals, interpret them correctly, and react promptly and adequately in a consistent way. The authors developed a reliable coding system to measure this construct based on observation (Ainsworth, et al., 1974), which included not only sensitivity, but also other dimensions of maternal interactive behaviour. One of these was Cooperation, defined as mother's ability to respect the child's autonomy, while avoiding situations in which she might have to interrupt the child's activity or exert direct control. Maternal sensitivity and cooperation have since been subject to extensive research. Based on these two constructs of maternal interactive behaviour, more recent research has tended to combine them into a single construct of Sensitive Responsiveness (Baptista, et al., 2013; Juffer, Hoksbergen, Riksen-Walraven, & Kohnstamm, 1997; Tharner et al., 2012). Sensitive Responsiveness would reflect a more comprehensive dimension of maternal interactive behaviour, reflecting not only the mother's awareness of the child's signals, and contingent and

appropriate response, but also the ability to respect the child's autonomy. It would therefore include the "contingent responsiveness" and "appropriateness" of the maternal response (Nicholls & Kirkland, 1996).

From their development, Ainsworth's scales have been found to be consistently related to child's attachment (Ainsthworth et al., 1978), findings which have been widely supported in different samples (De Wolff & van Ijzendoorn, 1997) and replicated worldwide (van IJzendoorn & Kroonenberg, 1988). Secure attachment, in turn, has an important role in future child development, having been associated with better outcomes in extended domains of children's functioning, such as emotion regulation, social-cognition, theory of mind, cognitive and linguistic functioning (see Thompson, 2008).

In addition to its well documented role in attachment, sensitive care has been shown to impact numerous aspects of the child's adjustment. For example, a previous study has shown that insecurely attached children who subsequently experienced high-sensitive mothering significantly outperformed (in terms of problem behaviour, social competence, language, and school readiness) secure children who subsequently experienced low-sensitive mothering (Belsky & Fearon, 2002). In another study, insensitive care to infant signals influenced children's biobehavioural regulation, including observed negative affect and elevated salivary cortisol levels (Spanglar et al., 1994). Negative mothering has also been associated with children's poor physiological regulation, less adaptive emotion regulation, and noncompliant behaviour (Calkins, Smith, Gill, & Johnson, 1998). Considering the widely recognised impact of maternal care on child's development and adjustment, understanding the underlying mechanisms involved is a fundamentally important research goal.

2. Oxytocin and Maternal Care

Within the recent efforts to understand maternal behaviour determinants, and taking into account its affiliative nature, the role of the oxytocinergic system has been considered. Oxytocin (OT) is a nine amino-acid peptide hormone synthetized in the hypothalamus, and released into the neurohypophysis in order to enter in the blood circulation, or alternatively into other locations within the central nervous system (Insel, 1992).

This system has a well-established role in labour and the postpartum period, facilitating uterine contraction and milk production (see Insel, Young, & Wang, 1997).

Besides this crucial peripheral action, the role of OT in central neurotransmission has been postulated for decades (Buijs, De Vries, Van Leeuwen, & Swaab, 1983; Insel, 1997). Several studies have demonstrated that OT-producing neurons from the paraventricular nucleus and supra-optic nucleus of the hypothalamus directly project to several brain regions (Ross et al., 2009), which have important roles in a range of social behaviours, particularly empathy and social affiliation behaviour (Takayanagi et al., 2005; Winslow & Insel, 2002).

Many of the studies in this field of OT and social and affiliative behaviour are based in rodents, not only due to ethical issues, but also to the similarity in parental care they display (Insel, 1992). Such is the case of the research conducted on prairie voles and montane voles, two species which display similar non-social, but not social, behaviour. Investigations have suggested that these differences in social behaviour may be linked to the neural distribution of OT and vasopressin receptors. In the case of prairie voles, they are located in areas associated with reward (nucleus accumbens and prelimbic cortex) and in the montane voles in areas possibly associated with self-grooming behaviour (Lateral Septum) (Insel, 1997). Studies with rats have also informed about the role of OT in parental behaviour and bond formation. For instance, as reviewed by Insel (1992), blocking OT neurotransmission (through an antagonist or antiserum or by lesions of oxytocin-producing cells in the hypothalamus) resulted in a significant inhibition of maternal behaviour, affecting only the onset and not the maintenance of maternal behaviour. Also, a recent study from Marlin and collaborators (2015) found that virgin mice with induced OT presented maternal-like responses, with better responses in helping isolated pups.

The action of OT is mediated by the Oxytocin Receptor, which is encoded by the oxytocin receptor gene (OXTR). The OXTR gene is present in a single copy in the human genome and was mapped to the locus 3p25–3p26.2 (Michelini, Urbanek, Dean, & Goldman, 1995). Animal studies have linked this gene to parental behavior. For example, Takayanagi et al. (2005) using knockout mice for OXTR found deficits on lactation and maternal care, and fewer vocalizations of the offspring in stressful situations. The above-mentioned study by Marlin and collaborators (2015) also found a link between OXTR and the left auditory cortex, which is specialized in recognizing infant distress calls, necessary for maternal retrieval of isolated pups. In humans, Several Single Nucleotide Polymorphisms (SNP) have been identified in this gene. SNPs, or polymorphisms, as they are commonly referred to, are the most frequent type of genetic variation among humans.

Each SNP represents a variation in a single nucleotide that occurs at a specific position in the genome. Nucleotides are a group of molecules that constitute the DNA building blocks. They are composed by a five-carbon sugar, phosphate group and a nitrogenous base: adenine (A), cytosine (C), guanine (G), and thymine (T). These SNPs have been identified in some appreciable degree within a population, and are responsible for the diversity among individuals. Each SNP represents a variation in a single nucleotide that occurs at a specific position in the genome (i.e. in a given individual, in a specific position of the DNA chain, A could be replaced by an G, for example). The identification of numerous of these variations in genes and the analysis of their effects may lead to a better understanding of their impact on gene function and health of an individual.

Regarding OXTR SNPs, little is known about their biological functionality, and recent studies have addressed this issue. For example, Feldman et al. (2012) focused on plasma OT levels and the OXTR rs2254298 and rs1042778 SNPs, finding that higher OT levels were evident in carriers of the minor allele (i.e., A allele of rs2254298 and T allele of rs1042778). However, Parker et al.'s (2014) study indicated that plasma OT levels did not differ as a function of allelic variation in OXTR SNPs rs2254928 and rs53576 but that these SNPs exerted independent, additive, and heritable influences on the children's social functioning. Yet another study found that the presence of the A allele on the rs53576 SNP was associated with less efficacious OT binding (Tost et al., 2010). Therefore, although the functional implications of these polymorphisms have not been established yet, research suggests patterns of associations between different SNPs and specific behavioural phenotypes.

In what concerns maternal behaviour, research has focused mainly in four OXTR SNPs: rs237885 (Mileva-Seitz et al., 2013), rs53576 (Bakermans-Kranenburg & van Ijzendoorn, 2008; Klahr, Klump & Burt 2014; Michalska et al., 2014; Sturge-Apple, Cicchetti, Davies, & Suor, 2012; Tharner et al., 2012), rs2254298 (Feldman et al., 2012; Feldman, Gordon, Influs, Gutbir, & Ebstein, 2013; Tharner et al., 2012), rs1042778 (Feldman et al., 2012; Feldman et al., 2013; Michalska et al., 2014). One of the most studied SNPs in relation to maternal care is the OXTR SNP rs53576. This polymorphism involves a change in the DNA sequence in the third intron of OXTR gene from guanine (G) to adenine (A) (Meyer-Lindenberg, Domes, Kirsch & Heinrichs, 2011), resulting in 3 possible genotypes: GG, AG, AA. Empirical studies have yielded inconsistent results regarding the role of this SNP on maternal behaviour. While some demonstrated that GG mothers were more sensitive when interacting with their children than AA/AG mothers

(Bakermans-Kranenburg & van Ijzendoorn, 2008); others show no main effects of this SNP on multiple parenting behaviours (i.e., harsh/punitive parenting, sensitive parenting) (Sturge-Apple et al., 2012). Moreover, while some demonstrated that A allele carriers - not the G allele carriers - engage in more positive parenting (Michalska et al., 2014); others found that AA mothers exhibit significantly more warmth when interacting with their children (Klahr, et al., 2014). The absence of a clear picture raises the possibility that other factors should be considered when addressing the role of OXTR genetics on maternal behaviour. Samples have varied in number and socioeconomic background, and therefore accounting for these variables is likely necessary to deeper exploring the role of these SNPs. A critical approach for testing and elucidating plausible mechanisms is explored in further detail in Chapter 2.

Also relevant and worth considering is fact that maternal behaviour can be linked to other biological pathways, such is the case of the serotonergic system. For example, Galfi et al. (2005) found that OT secretion was directly influenced by the serotonergic system in rats (Jorgensen, Riis, Knigge, Kjaer, & Warberg, 2003); Lee, Garcia, Van de Kar, Hauger, and Coccaro (2003) have shown that stimulation of the hypothalamus by serotonin leads to the release of OT. Indeed, Serotonin has an important role as a modulator of neural circuitry that controls several behavioural and physiological processes. Specifically, it has widespread effects on cognition and mood (cf. Homberg & Lesch, 2011), which may be critical for parenting quality. Therefore, recent studies on parenting have also included this path, along with OT, to enable a broader comprehension of parenting behaviours (Bakermans-Kranenburg & van IJzendoorn, 2008; Sturge-Apple et al., 2012). Specifically, two studies considered the role of the oxytocin receptor and the serotonin transporter on parenting in the same piece of research, both with mothers and their 2-year-old toddlers. In one of them, Bakermans-Kranenburg and van IJzendoorn (2008) examined 159 dyads and found independent genetic effects of the 2 genes on observed maternal sensitivity. In the second study, Sturge-Apple et al. (2012) inspected 201 dyads and found that in both genes there was an interaction between genotype and interparental conflict in the explanation of parenting behaviour. These two studies are discussed in more detail in Chapters 2 and 3.

3. Oxytocin and Environment as Determinants For Maternal Interactive Behaviour

Results regarding the link between genetics, particularly regarding the OT system, and maternal behaviour have, as stated, been mixed. One possible explanation for these inconsistencies is the variability in the environmental context. In fact, OT has been considered one of the systems most sensitive to environmental influence (Brunton & Russell, 2010; Feldman, 2012). Looking back at prior studies, a wide range of economic and psychosocial backgrounds have been analysed, including middle-class (Bakermans-Kranenburg & van Ijzendoorn, 2008) and high-risk samples (Sturge-Apple et al., 2012); and extremes of positive and negative parenting scores (Michalska et al., 2014). Therefore, these environmental differences may explain some of the variability in the effects of OXTR genes described. Some studies have, in fact, considered the moderating impact of the environment. Sturge-Apple et al. (2012) in the above-mentioned study, gathered data from 201 mothers and their 2-year-old children recruited from a high-risk background. In this study, although no main effect was found for the rs53576 SNP on parenting behaviour, an interaction effect between genotype and interparental conflict was observed. Specifically, interparental conflict differentially affected maternal sensitivity in the case of G homozygotes (i.e. G homozygotes were the least and the most sensitive mothers when experiencing, respectively, higher and lower levels of interparental conflict).

Gene-X-environment interactions have indeed been increasingly considered by recent research. Two frameworks for the understanding of such interactions have been proposed: the diathesis–stress and the differential susceptibility models. The first one, the diathesis–stress model, postulates that stress may activate a diathesis or vulnerability, transforming a predisposition into psychopathology (Monroe & Simons, 1991). In this case, this would mean that there is a genetically driven sensitivity to adverse environments, such that some individuals would carry 'vulnerability genes' or 'risk alleles' that would predispose them to worse outcomes. However, results from different studies show that individuals with the same genetic makeup can simultaneously be the most susceptible to adversity, and the most likely to benefit from supportive experiences – in a 'for better and for worse' manner (Belsky & Pluess, 2009). The recognition of this fact gave rise to the concept of "plasticity to the environment", advocated by proponents of the so-called 'differential susceptibility' framework. It challenges the previous conception of "risk genes", theorized by the diathesis–stress model, and introduces the

concept of "plastic genes" (Belsky et al., 2009). According to Ellis, Boyce, Belsky, Bakermans-Kranenburg & van Ijzendoorn (2011), this model would in fact fit an evolutionary perspective better: as the future is uncertain, to vary in plasticity would make the species better prepared. This plasticity would then moderate the effect of environmental exposure on development, thus being evolutionarily adaptive and advantageous. This would be why these "risk alleles" would be maintained by natural selection – they would confer vulnerability in the face of adversity, but also lead to the best outcomes in protective environments (Belsky & Pluess, 2009). In the case of maternal interactive behaviour, this would mean that carrying a particular genotype would make mothers more susceptible to the environment, displaying better or worse levels of caregiving depending on the quality of the environmental context being, respectively, higher or lower. Exploring Gene-X-environment interactions in this field, and these different approaches to them, may help clarify some of the mixed results identified in previous research.

II. Maternal Interactive Behaviour and children's Socio-Emotional and Neuroendocrine Functioning

1. Preschool Children's Socio-Emotional Functioning

As reviewed above, maternal care has a crucial impact on children's development and functioning. During the preschool years, in particular, it can prompt the many developmental achievements taking place. As described by Marvin and Britner (2008), children at this age are becoming more autonomous, more able to explore the environment, and, at the same time, their communicative and representational abilities are improving, which allows them to engage in more complex interactions. Self-regulation, understanding of causality and acceptance of other's perspective are other achievements taking place during this time. All of these gains have an important impact on the child's interactive behaviour, and the relevance of positive parental care in facilitating these achievements is evident.

In addition, and as stated by Crittenden (1981), these sensitive maternal behaviours are also likely to be accompanied by the child's enjoyment of the interaction, and their expression of their own feelings. Sensitive and responsive parenting have, in fact, been shown to relate to child's cooperative and enthusiastic behaviour and positive emotion

(Eisenberg et al., 2001; Kochanska & Murray, 2000). This behavioural and emotional feedback, in turn, allows mothers to adjust their behaviour – and sensitive mothers would be flexible in their response (Nicholls & Kirkland, 1996). In the same way, social support-seeking behaviour and reactions to receiving social support are likely to be related through a feedback loop (Chen, et al. 2011). This feedback mechanism underlines the dyadic aspect of mother-child interaction, where behaviours have their own and specific meanings (Crittenden, 1981).

Considering the relevance of these interactive behaviours, accessing not only maternal behaviour, but also the child's behaviour is of relevance. Assessment of child behaviour is, indeed, a vital tool in understanding and supporting young children's development. It is therefore essential to document and evaluate different and specific behaviours, using reasonable and valid methods. These methods can be varied, and adjusted to the child's age and setting. In child's assessment, they are usually based on parent or teachers reports, which can be biased in different ways (Lindhal & Malik, 2001). Self-reports, and particularly at preschool age also have important limitations, as they require an appropriate level of cognitive and linguistic skills that would not be developed sufficiently at this age. An important alternative - or complement - are observational measures, particularly those focused on mother-child interaction. When the coding systems used are robust and validated, these are valuable methods, which provide a rich insight into interactive behaviours (Gardner, 1997). They enable assessment of a wide range of behaviours, from global aspects of parent-child relationship quality (e.g. responsiveness, affection), to quantifiable and specified behaviours (e.g. commands, questions), and/or behavioural contingencies (e.g. compliance to previous command) (Aspland & Gardner, 2003).

Despite being a clearly useful tool, few observational scales are widely available to assess the child's interactive behaviour, which is an important gap to address, as detailed in Chapter 4. One of the available scales is the Coding System for Mother-Child Interactions, developed by Healey and colleagues (2010). The child scales from this coding system assess three different dimensions of child behaviour, during a structured mother-child interactive task: Cooperation, Enthusiasm and Negativity. These behaviours give a sense of how the child responds to maternal suggestions and commands quickly/cheerfully (Cooperation); invests in the task and approaches it with energy and excitement (Enthusiasm); and forcefully rejects the mother's ideas or is unreasonably

demanding (Negativity and Hostility). Assessing these dimensions provides valuable information on the child's behaviour towards the mother and the task.

Child's neuroendocrine mechanisms are probably underlying these interactive behaviours. The child's OT response might be of particular interest here, considering our focus on the mother-child interaction, and will therefore be explored in the next section.

2. Children's Neuroendocrine Functioning

Oxytocin Response and Affiliative Behaviour

OT has been associated with prosocial effects, such as social reciprocity, social motivation, stress reduction (Champagne, 2008). Some of the prosocial effects of OT have been linked to affiliative motivations, i.e., to the provision of an impetus affiliation, which is vital for the survival of human beings. According to this framework, when positive affiliative contacts are available, OT attenuates psychological and biological stress responses; however, in the presence of hostile and unsupportive contacts, OT is released in response to stressors, especially those that may trigger affiliative needs, prompting affiliative behaviour (see Figure 1.1). At that point, if positive social contact is provided, stress is reduced; if negative contact follows the need, stress is exacerbated (Taylor, 2006). As stated by this model, the quality of social contacts during stressful times is a pivotal variable for understanding the role of OT in stress responses. Support for this approach comes from studies where OT seems to signal relationship distress (Taylor, Saphire-Bernstein, & Seeman, 2010) and not general psychological distress (Turner et al., 1999), which underlines the specificity in the relationship between OT and social/affiliative behaviour.

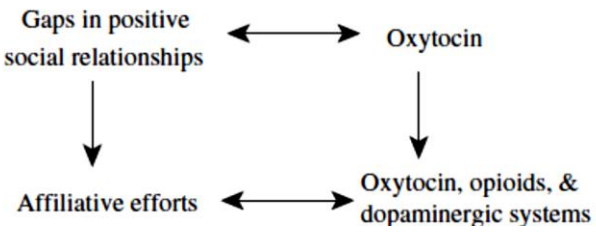


Figure 1.1. Model of the link between OT and affiliative responses (adapted from Taylor, 2006)

OT has also been linked to the alteration in the perceptual salience and/or processing of social cues (Bartz, Zaki, Bolger, & Ochsner, 2011). Studies showing increased gaze in the eye region support the idea that OT is involved in allocating attentional resources to social stimuli (Guastella, Mitchell, & Mathews, 2008; Andari et al., 2010). This would improve the capacity to read non-verbal signals, which would enable individuals to better understand the other's intentions and behaviours (Bartz et al., 2011). An increased social salience/social motivation would be expected to result in more prosocial behaviour but, at the same time, in increased awareness of situations where the social needs are not met (Taylor, 2006). Specifically, as OT increases attention to social cues, it should be especially helpful for those who are less attuned to such cues; in contrast, it could be detrimental to individuals who are generally hypersensitive to social cues and are biased towards interpreting them negatively (Bartz et al., 2011). In line with this, previous studies have found that the effects of OT administration are greater in individuals with lower social functioning (Groppe et al., 2013). Interestingly, authors have argued that affiliative and social motivations are mechanisms that can indeed work together: activating affiliative goals should increase one's attention to and processing of socially relevant information (Bartz et al., 2011).

Despite these efforts to understand OT and its mechanisms, the links between OT and social behaviour are still not completely clear. For example, studies have shown that higher plasma OT levels are linked to trust (Van IJzendoorn & Bakermans-Kranenburg, 2012); supportive communication (Gouin et al., 2010); but also interpersonal distress (Taylor et al., 2010), depression (Parker et al., 2010; Scantamburlo et al., 2007) and anxiety (Feldman, et al., 2009; Scantamburlo et al., 2007). Lower plasma OT has been associated with autism (Husarova et al., 2016); schizophrenia symptom severity, poorer social performance (Rubin et al., 2010), lower empathy levels, and disorders of social dysfunctions (Hirosawa et al., 2012), and also depression (Feldman, et al 2009). In line with the mechanistic approaches presented, it might be that other moderators play a role in the OT response and explain the mixed results found, such as variables related to the environment or to genetics.

Child's OT Response and Parental Environment

The OT system has been shown to be particularly susceptible to environmental changes (Brunton & Russell, 2010), and therefore literature has started to consider it as a possible moderator in OT-related studies. Importantly to the present dissertation, researchers have found that the oxytocinergic system is associated with the quality of parental environments. Animal studies have shown that the neurochemical organization of infant brain OT is shaped in early life through patterns of maternal behaviour, such as licking and grooming (Kappeler & Meaney, 2010), and that OT system plays a critical role in shaping the child's HPA axis via early caregiving experiences (Hostinar, Sullivan & Gunnar, 2014). Adverse early parental environments have been associated with lower OT concentrations in cerebrospinal fluid (Heim et al., 2008; Winslow et al., 2003) and disruption of the oxytocinergic system in regulating stress-related cortisol responses in adult men (Meinlschmidt & Heim, 2007) and to decreased OT receptor binding in several brain regions (Francis et al., 2000). These findings suggest that disruption of oxytocinergic system is one mechanism through which early adverse environments lead to greater psychosocial vulnerability. Relevant evidence of this link between care environment and OT system comes also from studies of early social deprivation, such as institutionalization. Compared with children raised with their biological family, institutionally reared children showed marginally lower levels of OT (Wismer-Fries et al., 2005). In the same way, women with a history of childhood abuse had lower OT concentrations (Heim et al., 2009). Also relevant to parent-child interaction, OT levels have been shown to be increased by socially pleasant sensory experiences, such as comforting touches and smells. For example, mothers and fathers who provided high levels of tactile contact to their infants showed an increase in salivary OT following parent–infant interactions (Feldman et al., 2010). Of particular relevance, OT has been shown to increase after a session of parent–infant contact for both child and parent (Feldman, Gordon, & Zagoory-Sharon, 2010). In this same study, the authors report that infant's OT levels were related to the degree of contingent parenting and to the infant's social engagement with the parent (Feldman et al., 2010), which is in line with previous animal research (see Champagne, 2008; Meaney, 2001). Of note, the infant social engagement construct includes the earliest set of signals of the infant's involvement in a social interaction, and has been shown to predict children's cognitive, social, and emotional growth (Marshal & Fox, 2006). Taking together this evidence, such an environment-sensitive system is likely to be affected by maternal behaviour. Nonetheless,

there is a clear lack of studies focusing on the child's OT response to such interaction, and maternal care.

Child's OT Response and Child's Genetic

Importantly, not only maternal behaviour, but also the child's own characteristics might impact on child's OT response. In fact, numerous studies have suggested that OT effects depend on individual factors. For example, they have been shown to be especially notable among individuals with lower social skills or poorer attachment histories. In contrast, among subjects who report more optimal parent–child relationships or better social abilities, the OT system may be sufficiently adaptive, and OT may encounter a ceiling effect and not have the same pro-social effects (Weisman, 2013). In line with that, in one study OT administration was linked to a decrease in cortisol response, but the effects depended on individuals' emotion regulation capacities (Quirin, Kuhl & Dusing, 2011). Therefore, there seems to be evidence of individual factors impacting on OT response. One possible individual factor explaining OT response is the child's OXTR SNP. Prior research has indeed addressed the role of OXTR and psychological functioning and behavioural phenotypes related to social interaction. In fact, in the case of the OXTR SNP rs53576, A-carriers have shown lower positive affect scores (Lucht et al., 2009), lower levels of optimism, and lower self-esteem (Saphire-Bernstein, Way, Kim, Sherman & Taylor, 2011). A-carriers have also been shown to physiologically and psychologically benefit less from social support than G-carriers, despite showing similar cortisol and subjective stress responses (Chen, Kumsta, von Dawans et al., 2011). In line with these results, adult A-carriers were less likely to seek social support (Kim et al., 2010). On the other hand, in a sample which included children with autism spectrum disorder, unaffected siblings and unrelated neurotypical controls, A-carriers showed better affect recognition performance in all groups (Parker et al., 2014). Considering this evidence of a link between OXTR and social interaction, child OXTR might indeed be an individual factor influencing variation in OT response.

The interest in considering both situational and individual factors is in line with previous research, as reviewed by Bartz et al. (2011). The authors propose that this interactionist approach would benefit the literature on the effects of OT, improving the understanding of current research, and suggesting new mechanisms. A similar position is reiterated by Olff et al. (2013), who reviewed studies in animal, healthy humans and

clinical samples, and underlined the importance of considering both contextual and interindividual factors when trying to understand the role of OT on social bonding and stress regulatory processes.

III. THE PRESENT DOCTORAL DISSERTATION

Taken together, the findings summarised here suggest a critical role for early experience, particularly sensitive parenting, in the development of basic aspects of human functioning. They also suggest that not only early experience, but also genetic factors should be accounted for in this research field, possibly in an interactive manner. Therefore, the aim of the present thesis is two-folded, as depicted in Figure 1.2. The first aim is to investigate the determinants of maternal interactive behaviour, including genetic and environmental factors; the second aim is then to analyse how maternal interactive behaviour relates to the child's functioning, considering the role of child's individual factors, as well as of the environment in this equation.

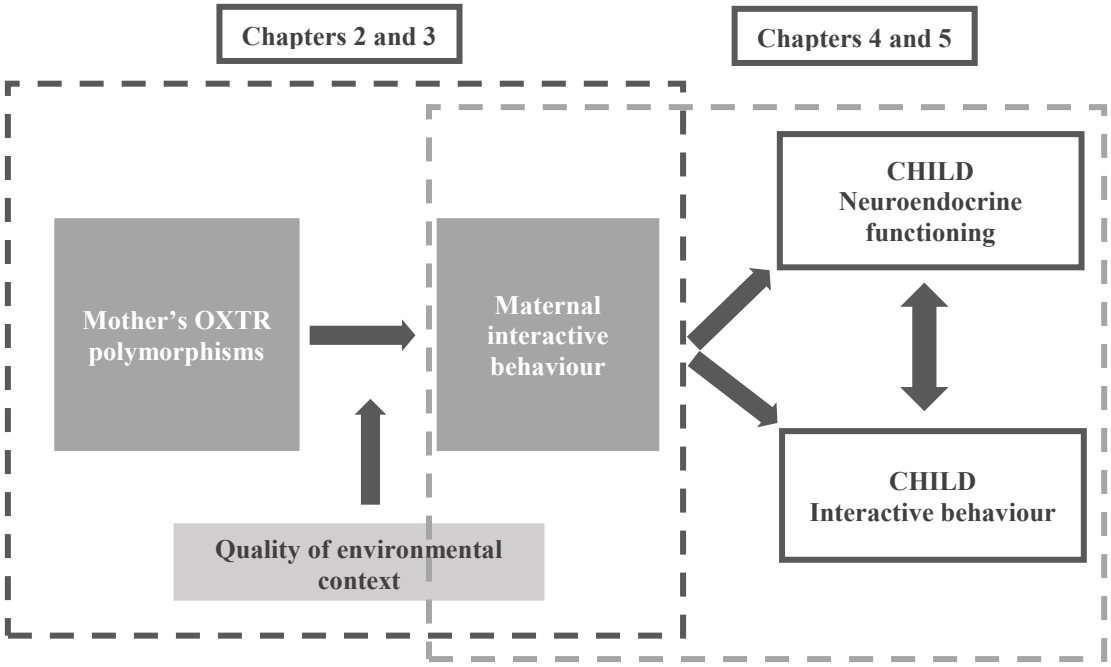


Figure 1.2. Summary of the thesis' chapters.

Specifically, regarding the first aim, and taking into consideration the link between the oxytocinergic system and maternal behaviour identified by prior investigation, Chapter 2 examines the association between the OXTR SNP rs53576and maternal behaviour in two separate studies. In Study 1 of that chapter, the link between the SNP and maternal behaviour is investigated in a sample of 215 Caucasian mothers observed during a structured interactive task with their preschool children. In Study 2, a meta-analysis of the studies linking the OXTR SNP and maternal behaviour is provided, drawing on results of five studies, which included 2,187 participants.

In Chapter 3, based on the above-mentioned link between the oxytocinergic and serotonergic systems, and the unclear picture depicted by the results from the Chapter 2, the role of the serotonin transporter on maternal behaviour is explored. Here, the quality of environmental context is considered as a possible moderator, based on a competitive model-testing analysis designed to contrast diathesis-stress and differential-susceptibility models of person-X-environment interaction.

Chapter 4 moves on to the second outcome, the child's functioning. Taking into account the need for valid, reliable and accessible observational measures of child's interactive behaviour, an adaptation and validation of the child's coding of the Coding System for Mother–Child Interactions (CSMCI, Healey et al., 2010) is conducted in a sample of 220 preschoolers. Its applications and implications in future research and practice are discussed.

Finally, in Chapter 5, given the relevance of OT to affiliative interaction, the child's OT response to a mother child interaction is analysed. Guided by previous research, individual (child's OXTR genotype and child's interactive behaviour) and environmental (maternal interactive behaviour) factors are considered as possible moderators of that response.

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CHAPTER 2:
MATERNAL OXYTOCIN RECEPTOR GENE AND MATERNAL BEHAVIOUR:
AN EMPIRICAL AND META-ANALYTIC STUDY

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I. INTRODUCTION

Oxytocin (OT) is a nine amino-acid peptide hormone synthesized in the hypothalamus and stored in the neurohypophysis for further release into blood circulation. The main peripheral target of this hormone is presumed to be the female reproductive system, thereby facilitating uterine contraction and milk production (see Insel, Young, & Wang, 1997); OT has also been implicated in central neurotransmission (Buijs, De Vries, Van Leeuwen, & Swaab, 1983; Insel, 1997), thus being associated with social behaviour, empathy and social affiliation (Takaynagi et al., 2005; Winslow & Insel, 2002). Of particular interest for the present inquiry is the role of the oxytocinergic system with respect to parenting, a special and developmentally important form of social affiliation (see Galbally, Lewis, van Ijzendoorn, & Permezel, 2011).

In humans, the action of OT is mediated by the Oxytocin Receptor, which is encoded by the oxytocin receptor gene (OXTR) located on chromosome 3. This gene is highly polymorphic, with several Single Nucleotide Polymorphisms (SNP) already identified. Although little is known about their functioning, two recent studies merit attention. One focused on plasma OT levels and the OXTR SNPs rs2254298 and rs1042778, finding, perhaps surprisingly, that higher OT levels were evident in carriers of the minor allele (i.e., A allele of rs2254298 and T allele of rs1042778) in the case of mothers, fathers, and non-parents (Feldman et al., 2012). Results of a second study indicated that plasma OT levels did not differ as a function of allelic variation in OXTR SNPs rs2254928 and rs53576 but that the SNPs exerted independent, additive, and heritable influences on the children's social functioning (Parker et al., 2014). Even though, the function of these OXTR SNPs has yet to be established, they may be associated with specific behavioural phenotypes.

Research in this area has mainly focused on four SNPs in relation to several maternal behaviours: maternal sensitivity and other related maternal behaviours, including orienting away from infant, infant-directed vocalizing, and instrumental care

(Mileva-Seitz et al., 2013) in the case of rs237885; parental gaze and touch (Feldman et al., 2012), early parental care (Feldman, Gordon, Influs, Gutbir, & Ebstein, 2013) and maternal sensitivity (Tharner et al., 2012) in the case of rs2254298; and parental gaze and touch (Feldman et al., 2012), early parental care (Feldman et al., 2013) and positive and negative parenting (Michalska et al., 2014) in the case of rs1042778. Of note is a larger group of studies focused on the OXTR SNP rs53576 and its association with parenting, specifically, maternal sensitivity (Bakermans-Kranenburg & van Ijzendoorn, 2008; Sturge-Apple, Cicchetti, Davies, & Suor, 2012; Tharner et al., 2012), harsh/punitive parenting (Sturge-Apple et al., 2012), positive and negative parenting (Michalska et al., 2014) and parental warmth, control, and negativity (Klahr, Klump & Burt 2014). Nonetheless, the results of studies focusing on parenting and OXTR related SNPS have been quite mixed.

The OXTR SNP rs53576 is indeed one of the most studied polymorphisms. It involves a change in the DNA sequence in the third intron of OXTR gene from guanine (G) to adenine (A). The first report on parenting involving this SNP focused on a sample of 159 middle-class mothers and their two-year-old children at risk for externalizing behaviour problems. Results indicated that GG mothers were more sensitive in interacting with their children than AA/AG mothers (Bakermans-Kranenburg & van Ijzendoorn, 2008). Guided by these findings, Sturge-Apple et al. (2012) conducted a study with 201 high-risk mothers (i.e., with high levels of interparental aggression and sociodemographic adversity) and their two-year old children. Although no main effects of OXTR SNP rs53576 on multiple parenting behaviours (i.e., harsh/punitive parenting, sensitive parenting) proved significant, a Gene-X-Environment (GXE) interaction involving interparental conflict did: higher and lower levels of interparental conflict predicted, respectively, less and more sensitive mothering, but only in the case of GG mothers. More recently, in a sample of 35 mothers and their 4-6-year-old children selected for having extreme positive and negative parenting scores, Michalska et al. (2014) documented an additive association between genotype and parental behaviour, with A allele carriers (not the G allele carriers, as reported in previous research) engaging in more positive parenting. Finally, Klahr, et al. (2014), studying 500 mothers and their 6-10-year-old children, observed that mothers homozygous for the A allele evinced significantly more warmth when interacting with their children than other mothers. It should be evident on the basis of these results that even in the case of investigations chronicling parenting-

OXT associations, results have proved to be quite varied. Not only do some detect main or interaction effects, but even the allelic variants associated with more and less supportive parenting are not consistent across investigations.

Taking into account the increasing interest in the role of OT and OXTR in social behaviour and especially parenting, as well as the inconsistency in results just highlighted, we present herein two separate investigations. Study 1 is an original empirical inquiry involving OXTR SNP rs53576 (the most consistently studied OXTR SNP to date) in relation to parenting in a sample of 215 Caucasian mothers. Study 2 is a meta-analysis of existing OXTR-parenting research, focusing on the same OXTR SNP.

STUDY 1: The OXTR SNP rs53576 and Maternal Behaviour

In this study we investigate the association linking the presence/absence of the A allele on the SNP rs53576 with maternal sensitive responsiveness. Given that the oxytocinergic system has been regarded as especially sensitive to environmental conditions (Feldman, 2012), and therefore the environment might be moderating the effect of maternal genotype, a GXE interaction involving rs53576 and quality of environmental context was also evaluated.

II. METHODS

PARTICIPANTS

The sample consists of 215 Caucasian mothers and their preschool children. Recruitment took place in Portuguese preschools that included children from families with varying levels of psychosocial risk. Maternal age ranged from 20-48 years (M = 33.33, SD = 5.62); 24.2% of mothers had less than nine years of education. Child age ranged from 40-77 months (M = 58.13, SD = 7.63); 117 (54.4%) were girls. The study was approved by the Portuguese National Committee for Data Protection and the Ethical Committee of the University of Minho. Informed consent was obtained from mothers.

Measures

Quality of environmental context and maternal psychopathology

Sociodemographic information was collected in line with Weitzman, Edmonds, Davagnino, and Briggs-Gowan (2013) to derive a quality of environmental context variable based on the presence vs absence of nine factors: teenage pregnancy; single parenthood; parental (low) educational level (i.e., one of the parents had under nine years of education); parental unemployment (i.e., one of the parents was unemployed at the time of the study); economic difficulties; social support; family conflict; maternal psychopathology (see below); chronic health conditions in the family living with the child. The factors were analyzed using item response theory modelling. A two-parameter (2PL) model was estimated (estimating difficulty and discrimination parameters for each question). Two items showed low discrimination (single parenthood and social support) but excluding them did not significantly improve the overall test information function, and hence they were maintained in the composite family-environment measure. A standardized score was calculated based on the presence/absence of the aforementioned factors, by summing and reversing those considered "negative", with higher scores reflecting better environmental context quality.

To assess maternal psychopathology, mothers completed the 53-item of the Brief Symptom Inventory (BSI; Derogatis, 1982; Portuguese version, Canavarro, 1999), which assesses the presence of various symptoms experienced in the past week (Chronbach alpha = .96). A binomial variable (low/high Maternal Psychopathology) was computed based on the Portuguese normative mean and standard deviation for the Positive Symptoms Distress Index, a measure of symptom intensity corrected for the number of symptoms.

Maternal behaviour

Mother-child interaction was videotaped across three 5-minute episodes involving (a) child play with a challenging toy under mother's guidance; (b) maternal completion of a sham questionnaire while the child has only an uninteresting toy to play with, after being instructed by mother not to touch more interesting, but difficult-to-reach toys; and (c) mother and child engage in free play for half the period followed by mother-directed child clean-up. Videotaping occurred in quiet rooms at the family home or at preschools

depending on participants' availability (no differences were found across the two settings in maternal behaviour or on the other variables included herein).

Maternal behaviour was assessed using Ainsworth's scales for rating maternal sensitivity and cooperation (Ainsworth, Bell, & Stayton, 1974), with each dimension rated on a 9-point scale and higher scores indicating more caring behaviour. The videos were coded by a team of trained researchers, who were blind to the sample characteristics. Coding achieved good interrater reliability (sensitivity: *ICC* = .93, *n* = 87; cooperation: *ICC* = .86, *n* = 84). In line with previous studies (Baptista, et al., 2013; Juffer, Hoksbergen, Riksen-Walraven, & Kohnstamm, 1997; Tharner et al., 2012), "sensitive responsiveness" was computed by averaging the two highly correlated scales (r = .73, p < .001).

Genetic assessment

Mother's saliva was collected using OraGene OG-500 (DNA Genotek, Inc., Ottawa, Ontario, Canada) and stored at room temperature. Genomic DNA was isolated as instructed by the manufacturers, using the standard protocol from PrepIT L2P (DNA Genotek) and samples concentration was accessed using Nanodrop technology. Analysis of the SNP rs53576 was performed using 5ng of DNA along with the corresponding KASPar assay (LGC Genomics, UK), for a final volume of 8µL. The thermal profile was performed as instructed be the manufacturers, in a 7500 Fast Real-Time PCR System (Applied Biosystems by Life Technology, USA). Results were validated by Sanger Sequencing of representative samples for each genotype (AA, AG or GG). Allelic frequencies were in Hardy-Weinberg Equilibrium ($\chi 2$ (1) = 0,211, p = 0.646). Primary statistical analysis contrasted A-carriers (AA/AG) and G homozygotes (GG).

STATISTICAL ANALYSIS

Descriptive data are presented first. Next, Pearson or point-biserial correlations among study variables are reported, in order to determine which, if any, should be included as covariates. Finally, hierarchical regression analysis was used to predict maternal sensitive responsiveness, with children's sex entered first, maternal educational level and psychopathology entered in the second block, followed finally by maternal genotype (i.e., presence vs absence of the A allele).

Additional analyses were also conducted evaluating a GXE effect involving the OXTR SNP and the composite index of quality of environmental context. Children's sex was entered in the first block (the other possible covariates - maternal educational level and psychopathology - were already part of the quality of environmental context composite, and therefore were not entered separately in this block). The environmental variable was entered along with maternal OXTR SNP in the second block, and their interaction was included in the third block. The order of inclusion of the variables in the blocks intends to control covariates (entered first), and then to test the effect of the variables of interest (separately, and then in interaction, when that was the case).

III. RESULTS

The mean sensitive responsiveness score was 4.59 (*SD* = 1.54, range 1-8). Concerning genotyping, 97 participants (45.1%) were homozygous for the ancestral allele (G) and 97 (45.1%) were heterozygous. Minor allele (A) frequency was 0.32.

Table 2.1 displays the intercorrelation of study variables. Because maternal education and psychopathology, as well as child sex were significantly associated with maternal sensitive responsiveness, these three variables were included as covariates in the primary regression analyses.

Table 2.1. Correlations between study variables.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1. Child's Sex	-						
2. Child's Age	.094	-					
3. Mother's Age	-.062	-.006	-				
4. Maternal Educational Level	.069	-.031	.153*	-			
5. Maternal Psychopathology	.072	.018	.025	-.100	-		
6. Quality of environment context	-.126	.071	.123	.388**	-.407**	-	
7. Maternal Sensitive Responsiveness	-.158*	.016	.112	.187**	-.209**	.368**	-
8. Maternal OXTR	.071	.026	-.014	.050	-.047	-.011	-.055

Note. Pearson or point-biserial correlations (two-tailed); $N = 215$; *$p < .05$, **$p < .01$. Child's Sex: 0 girls; 1 boys. Maternal OXTR: 0 GG; 1 AG/AA

Inspection of Table 2.2 reveals that, with covariates included in the regression model, maternal genotype failed to predict parenting (*B* = -.124, *SE* = .132, *t* = -.939, *p* = .349). No association between genotype and mothering was detected either when covariates were dropped from the model.

Table 2.2. Prediction model for maternal sensitive responsiveness, considering maternal OXTR SNP rs53576 as a predictor, and children's sex, maternal educational level and maternal psychopathology as covariates.

Model; Predictors	R^2 (R^2 adj)	*F* (df)	Unstandard. coefficient		*t*	*p*	95% CI	
			B	*SE*			Lower	Upper
1								
Child sex	.025 (.020)	5.434 (1,213)*	-.316	.136	-2.331	.021	-.583	-.049
2								
Child sex	.096 (.083)	7.462 (3,211)**	-.315	.132	-2.388	.018	-.575	-.055
Maternal education			.083	.031	2.722	.007	.023	.143
Maternal psychopathology			-.383	.140	-2.725	.007	-.660	-.106
3								
Child sex	.100 (.083)	5.814 (4,210) ***	-.306	.132	-2.314	.022	-.567	-.045
Maternal education			.084	.031	2.757	.006	.024	.144
Maternal psychopathology			-.389	.141	-2.766	.006	-.666	-.112
Maternal OXTR			-.124	.132	-.939	.349	-.384	.136

Note. *N* = 215; *p < .05, **p < .01, ***p < .001.

To further check the absence of the genotype-phenotype association, multiple regressions were rerun separating the two mothering components, sensitivity and cooperation, making each the outcome of separate regression equations. In no case did a significant effect emerged. And, once again, this was true even with covariates dropped from the model.

Finally, given the possibility that the effect of maternal genotype might be moderated by other factors, a GXE interaction involving OXTR SNP rs53576 and quality

of environmental context was evaluated, with children's sex as covariate. As presented on Table 2.3, no GXE interaction effects were discerned (B = -.418, SE = .542, t = -.771, p = .442).

Table 2.3. Prediction model for maternal sensitive responsiveness, considering maternal OXTR SNP rs53576, quality of environmental context and their interaction as predictors, and children's sex as a covariate.

Model; Predictors	R^2 (R^2 adj)	F (df)	Unstandard. coefficient		t	p	95% CI	
			B	SE			Lower	Upper
1								
Child sex	.023 (.019)	5.100 (1,213)*	-.307	.136	-2.258	.025	-.575	-.039
2								
Child sex			-.216	.129	-1.678	.095	-.471	.038
Maternal OXTR			-.083	.128	-.646	.519	-.335	.170
Quality of environmental context	.147 (.135)	12.072 (3,210)***	1.463	.267	5.479	.000	.937	1.990
3								
Child sex			-.224	.129	-1.728	.085	-.479	.031
Maternal OXTR			-.260	.263	-.988	.324	-.779	.259
Quality of environmental context	.150 (.133)	9.185 (4,209) ***	2.129	.904	2.356	.019	.347	3.910
GxE			-.418	.542	-.771	.442	-1.487	.651

Note. N = 215; *p < .05, **p < .01, ***p < .001.

IV. DISCUSSION

In this study, we examined the association between variation in the maternal OXTR SNP rs53576 and their sensitive responsiveness. Unlike some prior work chronicling such associations (Bakermans-Kranenburg and van Ijzendoorn, 2008; Klahr et al., 2014, Michalska et al., 2014), the current investigation of 215 demographically diverse Portuguese mothers of preschoolers failed to discern any reliable association between rs53576 and observed sensitive responsive parenting, consistent with yet other investigations (Sturge-Apple et al., 2012); and this was so whether we relied on a composite measure of the parenting outcome (i.e., sensitive responsiveness) or each of its

two constituent components (i.e., sensitivity, cooperation), or whether we included covariates or moderation of OXTR by the quality of environmental context or maternal psychopathology.

Although this null result could be a function of the sample size of 215 or reliance on observations of mothers and their children for a short period of time in a series of structured situations, this seems unlikely as other work chronicling OXTR-parenting relations employed similar procedures (Bakermans-Kranenburg and van Ijzendoorn, 2008; Michalska et al., 2014). Also, we relied on a well-established measure of maternal behaviour (Ainsworth et al., 1974), one which has proven highly reliable and valid in a wide range of studies.

The null result is also unlikely to be the result of our method of parameterizing allelic variation (AG/AA vs GG), as it has also been employed in prior work that has documented associations with maternal behaviour (Bakermans-Kranenburg and van Ijzendoorn, 2008; Sturge-Apple et al., 2012). Moreover, we regarded it as problematical to separately consider all three allelic variants given that only 21 cases, representing under 10% of the sample, proved homozygous for the A allele. Nevertheless, when analyses were rerun using all three genotypes (AA, AG, GG), maternal behaviour was still not associated with the OXTR SNP.

Ethnic variation is not expected to be a confounding factor in this study either. Even though the frequency of the A allele varies across ethnicities, being significantly more common among those of Asian than European ancestry (Bakermans-Kranenburg and van Ijzendoorn, 2014; Chelala, Khan, & Lemoine, 2009; Sasaki, Kim, & Xu, 2011), all participants in the present study were Caucasian.

The possibility remains that child age may have played a role in the null findings which emerged herein. After all, previous studies have focused on children ranging in age from 2-10 years, and parenting behaviour may vary as a function of child age; there is also the possibility that effects of genetic and /or environmental factors may vary across age (Klahr & Burt, 2014). Such age-related variation must be considered, given the suggestion that oxytocin may be more important immediately after birth for establishing the bond between mother and offspring than for maintaining this bond later (Insel, 1997; Bick & Dozier, 2010). There is only one OXTR-parenting study with children within the same age range as the current inquiry (Michalska et al., 2014). There, investigators did detect an association between maternal genotype and (positive) parenting. Nonetheless,

the sample included only 35 participants, and they were selected for having extreme parenting scores, which may limit the generalizability of the findings.

We may also have failed to detect a direct genotype-phenotype association linking OXTR with parenting because it is moderated by contextual conditions (i.e., GXE interaction). This might seem especially so given that the oxytocinergic system has been regarded as especially sensitive to environmental conditions (Feldman, 2012). And, indeed, as noted in the Introduction, prior research has documented such environmental moderation (Sturge-Apple et al., 2012). Recall, however, that when we considered this possibility using a multi-dimensional index of the quality of the family environment and maternal psychopathology, we failed to find support for any interaction. It is possible, however, that other moderators might have revealed evidence of such; therefore, caution is warranted in embracing our null findings. It should be recalled that absence of evidence cannot be taken as evidence of absence.

In any event, it is well appreciated today that even when genotype-phenotype associations are detected, they tend to be very small in magnitude. This raises the possibility, advanced by some, that very large samples will be required to detect them (NCI-NHGRI, 2007). As our own sample was limited in size, it seemed reasonable to explore this issue in a second study by conducting a meta-analysis of all published research linking OXTR SNP rs53576 with measures of parenting, including those emanating from Study 1.

Study 2: Meta-Analytic Study on the OXTR Rs53576 SNP and Maternal Behaviour

As already noted, the role of the oxytocinergic system in influencing social affiliation and parenting has been widely investigated in recent years. Despite this growing interest, studies addressing OXTR polymorphisms and their association with parental behaviour remain few in number and present seemingly inconsistent results. Therefore, Study 2 sought to synthesize the available literature and examine the convergence of findings regarding the OXTR SNP rs53576 and maternal behaviour across all existing studies by means of meta-analysis.

V. METHODS

LITERATURE SEARCH

The search strategy followed the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher, Liberati, Tetzlaff, & Altman, 2009). A systematic search was undertaken on PubMed and PsycArticles electronic databases. Key search terms included parenting behaviour and related terms ("interactive behaviour" OR "mother–infant" OR "parent-infant" OR "parenting quality" OR "parenting" OR "parental behaviour" OR "maternal behaviour" OR “sensitivity" OR "sensitive parenting") and “Oxytocin Receptor Gene” or “OXTR”. Reference lists of relevant papers identified in the literature search were also examined for pertinent references. Study 1 of the present report was also included.

STUDY SELECTION

In the search, 476 articles were identified, which were reviewed as presented on Figure 2.1. Articles were analysed in order to include those which investigated maternal OXTR SNP rs53576 and maternal behaviour in humans. Literature reviews, theoretical discussions and studies using animal models were excluded. Study selection based on inclusion/exclusion criteria started with title inspection (which led to the exclusion of 443 articles), abstract review of the possibly relevant articles (leading to the exclusion of 10 more articles) and then full text analysis of the pertinent reports. Final selection was based on the availability of the statistical information required to carry out the meta-analysis, which is detailed below.

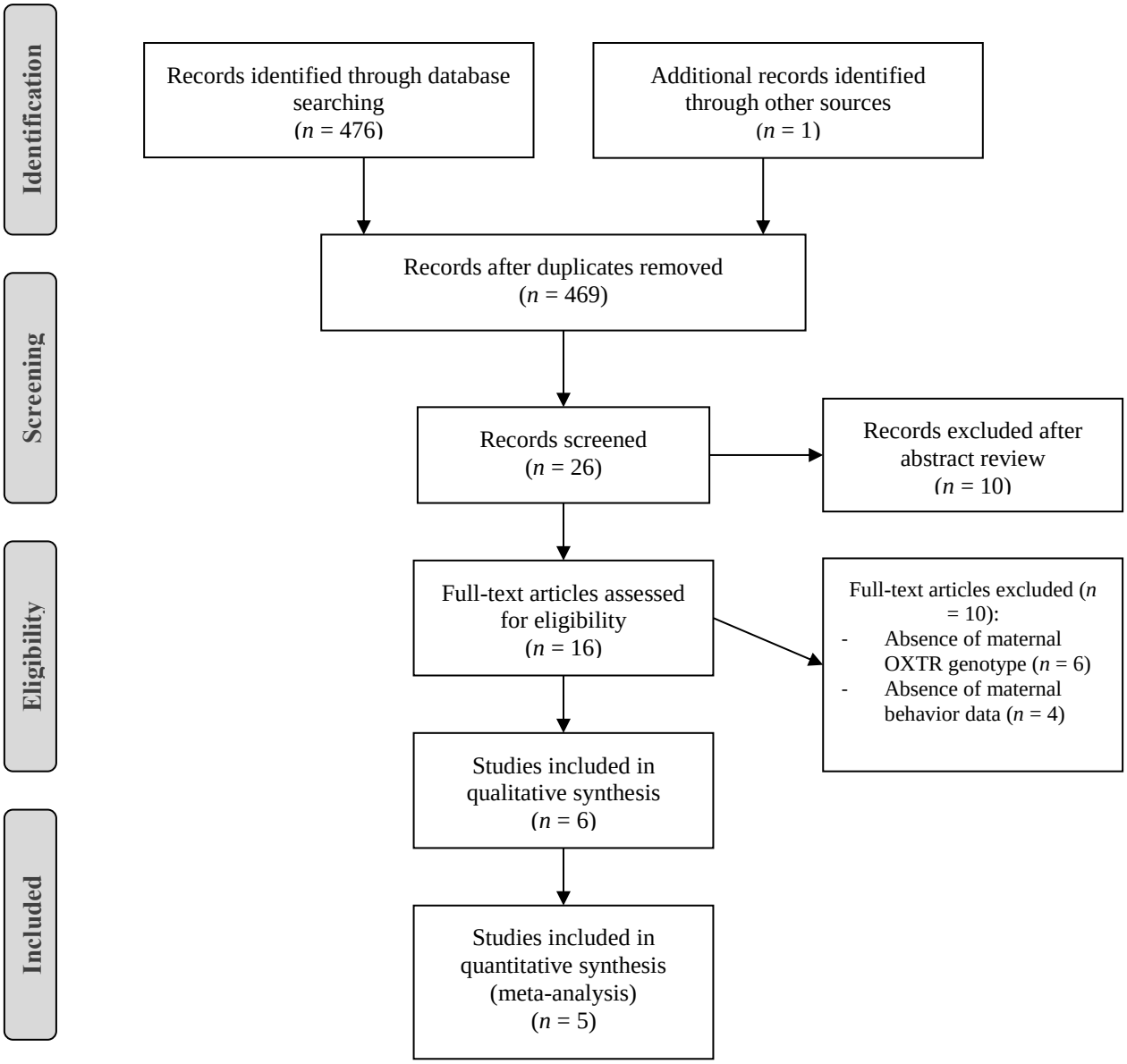


Figure 2.1. Flow chart of study selection.

DATA EXTRACTION

After applying all the eligibility criteria, six studies initially met criteria for inclusion in the meta-analysis. Relevant statistics were extracted from the empirical reports in the form of means and standard deviations, and correlations, based on the presence vs. absence of the susceptibility allele in the OXTR SNP rs53576 (i.e., presence vs. absence of the derived allele (A), instead of the ancestral allele (G) in that locus).

Two of these six reports included all the statistical information desired; in the remainder, authors were contacted to obtain the additional necessary information missing from the published reports. This procedure led to inclusion of three additional studies in

the meta-analysis (Klahr, et al., 2014; Sturge-Apple et al., 2012; Tharner et al., 2012), resulting in a meta-analysis of a total of five research reports whose combined sample size was 2,187.

One effect size was computed for each report. When it was possible to derive an effect for "positive" and "negative" parenting variables (Klahr, Klump & Burt, 2014; Sturge-Apple et al 2012), only the positive ones were derived in order to homogenize the data and facilitate the interpretation of the results.

Data analysis

Given the small set of studies, a prospective power analysis was run (Ellis, 2010). To be able to detect a minimum effect size of $r = .10$ (a small effect size) with a minimum power of .80 (Cohen, 1988), the number of subjects included would have to be higher than 782. This number is surpassed in the present meta-analysis.

A common metric effect size was derived for every study based on the available analysis, using the Comprehensive Meta-Analysis Program (Borenstein, Hedges, Higgins, & Rothstein, 2005). A combined effect size and its statistical significance were computed. Homogeneity in the distribution was assessed using Q-and I^2 statistics. All analyses were conducted based on random-effects models, commonly considered to be most appropriate as there was methodological diversity in the included studies (Borenstein, Hedges, Higgins, & Rothstein, 2009; Ellis, 2010; Field & Wright, 2006; Fu et al. 2011). Publication bias was considered using a funnel plot; and fail-safe analysis was also performed. Despite the interest in the analysis of moderators for explaining between-study variation in effect sizes, such analysis was not conducted due to the small number of studies (Borenstein et al., 2009).

VI. Results

Table 2.4 presents the five studies included in the meta-analyses (Baiao et al., 2015, Study 1; Bakermans-Kranenburg & van Ijzendoorn, 2008; Klahr, et al., 2014; Sturge-Apple et al., 2012; Tharner et al., 2012), involving a total of 2,187 participants. There was wide variation in sample sizes, ranging from 159 to 929 participants. Most participants were Caucasian.

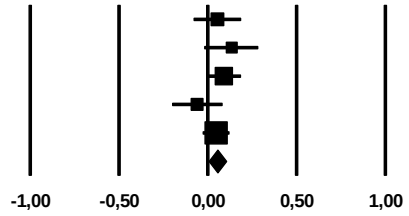
Table 2.4. Original studies included in the meta-analysis

Study	N	Ethnicity	OXTR SNP rs53576 (distribution)	Parental Behavior Measure	Observation context	Age of children
Baiao et al., 2015, study 1	215 mothers	Caucasian	AA 21; AG 97; GG 97	Maternal Sensitive Responsiveness (averaged Sensitivity and Cooperation based on Ainsworth et al., 1974)	Laboratory/home structured	3-6 years (M = 58.13, SD = 7.63, range 40-77 months)
Bakermans-Kranenburg and van Ijzendoorn, 2008	159 mothers	Caucasian	AA 17; AG 71; GG 89	Maternal Sensitivity – Egeland et al. (1990) observational scales	Laboratory structured	2-3 years (M =23.30; SD = 10.14 months)
Klahr, et al., 2014	929 mothers	86.4% Caucasian	AA 39; AG 190; GG 182	Warmth measured with joystick coding system (Burt, Klahr, Neale, & Klump 2013), based on a videotaped interaction	Laboratory/home structured	6-10 years (M = 8.23, SD = 1.44)
Sturge-Apple et al.,2012	193 mothers	23% European American; 77% non-European American	AA 11; AG 64; GG 118	Maternal Sensitivity - Iowa Family Interaction Rating Scales (IFIRS; Melby & Conger, 2001)	Laboratory structured	2 years (M = 25.4; SD = 1.55; range 22-32 months)
Tharner et al., 2012	691 mothers	Caucasian	AA 86; AG 292; GG 313	Ainsworth Maternal Sensitivity Scales (Ainsworth et al., 1974)	Laboratory structured	14 months

Common metric effect sizes (*r*) and confidence intervals (CI) for each study are presented on Table 2.5. The choice for the *r* metric is in line with other meta-analytic researchers (Bakermans-Kranenburg & van IJzendoorn, 2014; Ellis, 2010; Field & Wright, 2006). All data were *z*-transformed before computing the final effect size and back-transformed to *r* for presentational purposes.

Table 2.5. Original studies' and combined effect sizes and forest plot.

Study name	Correlation	Lower limit	Upper limit	p-Value
Baiao et al., 2015	0,054	-0,079	0,186	0,424
Bakermans-Kranenburg & vanIjzendoorn, 2008	0,135	-0,019	0,283	0,085
Klahr, Klump & Burt, 2014	0,091	-0,005	0,185	0,064
Sturge-Apple et al.,2012	-0,060	-0,200	0,082	0,408
Tharner et al., 2012	0,047	-0,028	0,121	0,217
	0,055	0,006	0,104	0,028



As displayed on Table 2.5, original studies' effect sizes ranged from -0.06 and 0.14. The combined effect size (k = 5, N = 2187) was r = 0.06 (p = .03). To ascertain the statistical significance of the effect, z scores and confidence intervals were computed (see Ellis, 2010). Both *z* score (z = 2.19, p =.03) and confidence interval (95% CI = .01 – 0.10) confirmed the significant result. In sum, in the case of the five studies here included, a relation emerged between the OXTR rs53576 SNP and parenting, which suggested a tendency for G allele homozygotes to display more supportive parenting than A allele carriers. Homogeneity of the distribution was tested and ascertained (Q = 4.15, p = .39, I^2 = 3.68%). No outliers were found.

To determine whether any publication bias was present, the funnel plot was analyzed. Visual inspection showed that studies fell within the funnel, with no significant asymmetry in the plot, thus providing no clear indication of publication bias. Nonetheless, considering the small number of studies included, we acknowledge the limitations of this analysis, and the difficulty in distinguishing chance from real asymmetry (Higgins & Green, 2011). Also, it seems possible that studies yielding positive results would be more likely to make their way into the published literature than those yielding null findings. The fail-safe analysis addressed this issue; it revealed that the inclusion of just one study

documenting a null result would reduce meta-analytic significance documented herein to insignificance. As this number is clearly below the Rosenthal (1991) criterion of $5k + 10$ (k = number of studies included), the result of the current meta-analysis cannot be embraced with certainty.

VII. DISCUSSION

Study 2 sought to synthesize the limited available literature on the association of the OXTR SNP rs53576 and maternal behaviour, in an effort to estimate its "true effect". The results of this meta-analytic study suggest the existence of such effect, in a homogeneous distribution, both considering Q statistics and I^2, the latter statistic being not dependent on the number of studies as is the former (Littell, Corcoran, & Pillai, 2008). However, as the magnitude of the association proved to be small ($r = 0.06$, $p = .03$) and the fail-safe analysis indicated that a single additional study yielding a null result would nullify this meta-analytic finding, it cannot be considered to be robust.

Two previous meta-analyses have focused on the OXTR SNP rs53576 and social behaviour. One of them focused on five potential correlates of sociality—biology, personality, social behaviour, psychopathology or autism—finding that none of the individual effect sizes or even their collective effect differed from zero (Bakermans-Kranenburg & van IJzendoorn, 2014). In response to these findings, Li et al. (2015) performed a second meta-analysis distinguishing two types of sociality: general sociality (i.e., responses to other people in general) and close relationships (i.e., responses to individuals with close connections, such as parent-child or romantic relationships). Results revealed a significant association between OXTR SNP rs53576 and general sociality, with A allele carriers scoring lower on general sociality, but not between this SNP and measures of close relationships. These latter results add to the unclear picture of associations between OXTR SNP rs53576 and social functioning. After all, parenting, which we found to be significantly related to the SNP, even if ever-so-modestly, would qualify as a kind of close relationship. Of note, our meta-analysis overlaps only to a limited extent with the previous meta-analyses, having only 2 studies in common with them.

The meta-analysis presented herein was limited in a number of aspects. Most importantly, the number of studies which could be included was small. Despite this, the cumulative sample size proved to be rather substantial (N = 2,187). Moreover, the fact that all of the studies were published in indexed journals in the last seven years means that they reflected the current (published) state of the art in this area—as well as the relative novelty of the topic. Therefore it seems likely that more empirical reports will soon be forthcoming, thereby enabling further meta-analytic evaluation. This would seem ever more likely given the unclear results depicted here.

Another limit—or perhaps strength—of our meta-analysis was the diversity of parenting measurements used in the five studies which were analyzed. Such variation in measurement affords a more general and less measure-specific evaluation of the question at hand—the association between OXTR SNP rs53576 and parenting. This, of course, is the very reason for meta-analysis—to determine whether any documented effects are tolerably general in character. Also, even if diverse, all of the studies relied on observational measures. Nevertheless, such methodological variation may have added noise and reduced the meta-analytic effect size.

A final limit of the current work has to do with the fact no analysis of the effect of study quality was undertaken. To be noted, however, is that all five studies included in the meta-analysis reported genotype frequencies consistent with what was expected for the population. This has been considered a potential indicator of quality (Bakermans-Kranenburg & van Ijzendoorn, 2014), as would be the fact that all were published in indexed journals. In other words, variation in study quality seems unlikely to have affected the results discerned.

In conclusion, the present inquiry aimed at responding, for the first time, to the rising interest found in literature, and compiling the available data on the most commonly studied OXTR SNP on maternal behaviour. Here, we failed to detect a link between the rs53576 and parenting in Study 1 but, despite this, we did document an overall effect in the 5-study meta-analysis (i.e., Study 2). Nonetheless, because the effect was small and would be nullified by a single additional study failing to document the association in question, it seems that caution should be placed in a conclusion that there is real genotype-phenotype relation involving OXTR SNP rs53576 and parenting. This state of affairs might be considered surprising given the widely embraced view that the oxytocinergic

system plays a role in social and affiliative behaviour. Nonetheless, it is in line with mixed results evident in the published literature.

Exactly why the empirical situation remains as it does have been addressed by Wray et al. (2013) in their discussion of the difficulties in documenting genotype-phenotype associations when focused on single candidate genes. Not only are most traits likely to be a function of many genes, with each probably exerting a small effect and possibly doing so in interaction with one another (i.e., epistasis), but gene-environment interaction may also undermine the ability to discern main effects of candidate genes (Robinson, Wray, & Visscher, 2014). These observations must be kept in mind when efforts are made to directly link individual SNPs to phenotypes, and future studies, carried out in larger scale samples, should follow a broader approach, including different biological, psychological and environmental aspects of the individual's context.

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CHAPTER 3:
DOES 5-HTTLPR MODERATE THE EFFECT OF CONTEXT ON MATERNAL SENSITIVITY?
TESTING THE DIFFERENTIAL-SUSCEPTIBILITY HYPOTHESIS

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I. INTRODUCTION

Different biological systems have been implicated on parenting, in both animal and human studies. One of the most studied has been the oxytocinergic system given its well-established role on parturition and lactation but also in affiliation and social interactions. However, other systems linked to oxytocin, may also play an important role, such is the case of serotonin. For example, Galfi et al. (2005) observed that oxytocin secretion was directly influenced by the serotonergic system in rats (Jorgensen, Riis, Knigge, Kjaer, & Warberg, 2003); and Lee, Garcia, Van de Kar, Hauger, and Coccaro, (2003) found that stimulation of the hypothalamus by serotonin resulted in the release of oxytocin as a precursor molecule. Other than its link to oxytocin, as a modulator of neural circuitry regulating several physiological and behavioural processes, serotonin has widespread effects on cognition and mood (cf. Homberg & Lesch, 2011), conceivably affecting parenting, the focus of this report. For these reasons, interest in the relation between serotonin and parenting has been increasing (Bakermans-Kranenburg & van IJzendoorn, 2008; Mileva-Seitz et al., 2011; Cents et al., 2014; Sturge-Apple, Cicchetti, Davies, & Suor, 2012).

Within the serotonergic system, the gene encoding the serotonin transporter, and in particular the Serotonin Transporter-Linked Polymorphic Region (5-HTTLPR), has been one of the most extensively studied polymorphisms in research on mood, cognition and parental behaviour. It consists of two functional alleles, long (L) and short (s), with the s allele associated with a decrease in the transcription of the serotonin transporter gene, resulting in increased levels of serotonin in the synaptic cleft. The presence of this s allele has been associated with increased anxiety (Gunthert et al., 2007), depression and suicidality (Caspi et al., 2003), negative emotion processing (for a review Jonassen & Landrø, 2014) and improved social cognition (Homberg & Lesch, 2011). Nonetheless, these, like many other genotype-phenotype associations, have proven inconsistent across

studies (e.g., Mesquita et al., 2015; Taylor et al., 2006; Wilhelm et al., 2006 for combined ss vs sL/LL genotypes; Brummett et al., 2008; Caspi et al., 2003; Gunthert et al., 2007 for ss vs. sL/LL combined genotypes).

This seems to be so also in the case of research on parenting. In the first relevant study focused on middle-class mothers whose toddlers were at risk for externalizing behaviour problems, Bakermans-Kranenburg and van IJzendoorn (2008) found that mothers homozygous for the s allele provided less sensitive care than other mothers. Somewhat similar results were reported by Morgan, Hammen and Lee (2016): Carriers of the s allele exhibited less positive parenting when interacting with their 6 to 9 year-old children than LL homozygotes. Yet in a third study, mothers carrying the s allele displayed *greater* sensitivity than others when observed interacting with their six-month olds (Mileva-Seitz et al., 2011). Results similar to these emerged when Cents and associates (2014) examined effects of 5-HTTLPR on sensitivity in a large cohort study in which mother-child dyads were observed at three time points—when children were 14, 36 and 48 months of age. Another study reports on the failure to detect any 5-HTTLPR-parenting association when high-risk mothers were observed interacting with their two-year old children (Sturge-Apple et al., 2012).

Regarding genotype-phenotype inconsistency of the kind just outlined, Caspi and associates (2002) based their pioneering work on the proposition that it might result from the interplay of genes (G) and environment (E). Especially notable in this regard is that three of the just-cited studies discerned Gene-X-Environment (GXE) effects. Mileva-Seitz et al. (2011) found that mothers carrying the s allele provided higher quality care than other mothers when they had reported experiencing positive parenting in their own childhoods. Morgan et al. (2016) observed that s-carrying parents engaged in more negative and less positive parenting than other parents when experiencing disruptive child behaviour. And Sturge-Apple et al. (2012) reported that s-carrying mothers displayed the most and least supportive parenting depending, respectively, on whether they experienced low or high levels of interparental conflict. The latter findings are particularly noteworthy in that they suggest that the s allele may be associated with heightened sensitivity to both supportive and adverse environmental conditions, thus being consistent with the differential-susceptibility hypothesis (Belsky et al., 2009; Belsky & Pluess, 2009; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011).

Just as notable is that GXE research involving 5-HTTLPR and other non-parenting phenotypes also provides support for the differential-susceptibility model of person-X-environment interaction. This includes work predicting depression (Brummett et al., 2008; Caspi et al., 2003; Taylor et al., 2006; Wilhelm et al., 2006; Zalsman et al., 2006), anxiety (Gunthert et al., 2007), and ADHD (Retz et al., 2008). Indeed, all these studies found that s-carriers (i.e., either ss or sL) proved most responsive to both positive and negative contextual conditions (with positive conditions often operationalized as low levels of negative ones); and this was so even as they focused on diverse contextual factors, including parental education (Brummett et al., 2008), daily stressors (Gunthert et al., 2007), childhood environment (Retz et al., 2008), and positive life events (Wilhelm et al., 2006).

To be noted, however is that none of this GXE work, including the three aforementioned studies on parenting (Mileva-Seitz et al., 2011; Morgan et al., 2016; Sturge-Apple et al., 2012), formally tested the differential-susceptibility hypothesis, especially against the competing and prevailing diathesis-stress model of person-X-environment interaction. Whereas the differential-susceptibility model stipulates that susceptible individuals will be more affected than others by both positive and negative contextual conditions, the diathesis-stress model only postulates that some "vulnerable" individuals will be more susceptible to the negative effects of adversity than will others (i.e., there will be no difference between more and less "vulnerable" individuals under benign or supportive conditions). In fact, it was the failure of investigations to formally evaluate how well GXE findings fit competing models of person-X-environment interaction that led Widaman and associates (2011; Belsky et al., 2012; Belsky & Widaman, in press) to develop the competitive model-testing approach that is employed in the GXE research reported herein.

Thus, in the present inquiry examining the interaction of 5-HTTLPR and family contextual conditions in predicting observed parenting, we formally test alternative models of person-X-environment interaction by directly contrasting the differential-susceptibility and diathesis-stress models, while evaluating weak vs. strong versions of each. Strong versions posit that only one genotypic subgroup is affected by the contextual condition under investigation (i.e., a zero correlation between context and outcome for the non-susceptible allelic subgroup); the weak version, in contrast, posits that the two genotypic groups are affected by the environment, but one more strongly

than the other. As displayed on Figure 3.1, the strong version assumes that the non-susceptible/non-vulnerable group (sL/LL) would not be influenced by the quality of environmental context. Conversely, the weak version allows that the family environment may impact the non-susceptible/non-vulnerable group, but to a lesser extent than the susceptible/vulnerable group (ss).

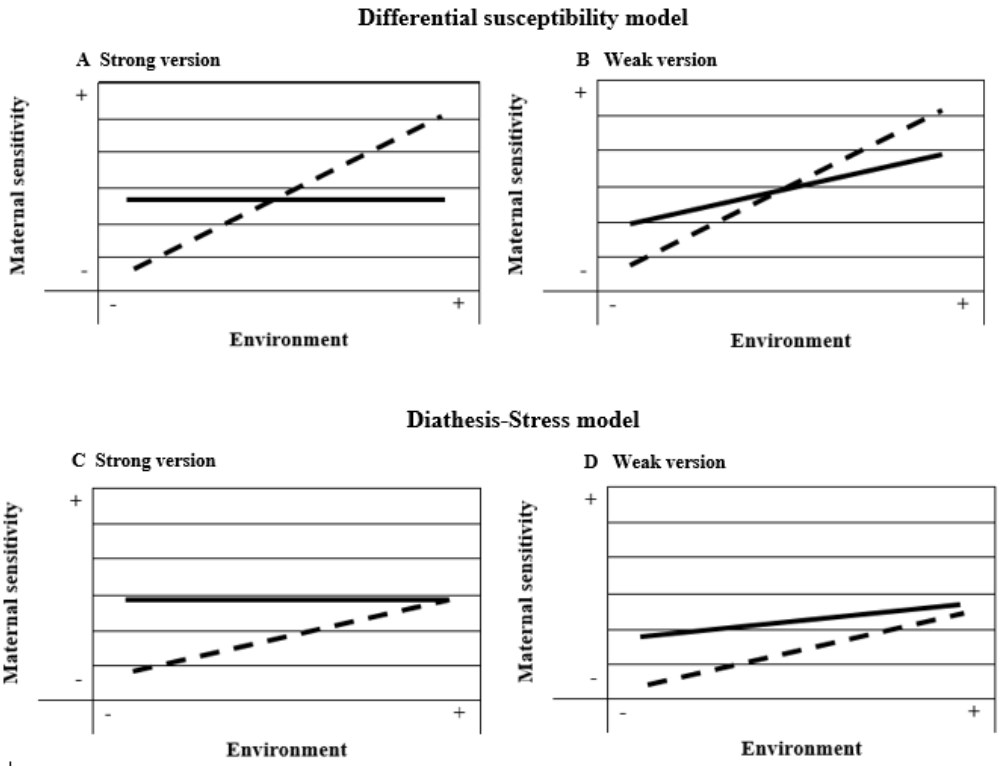


Figure 3.1. Graphical display of strong and weak versions of differential susceptibility (A and B) and diathesis-stress (C and D) models. The x-axis represents variation in the quality of environmental context, from negative to positive; the y-axis represents the maternal sensitivity scores, from negative to positive. The lines depict the two genotypes (ss vs. sL/LL).

In the present study we evaluated whether mothers carrying the ss vs. sL/LL genotypes were differentially affected by the quality of environmental context, while considering both strong and weak versions of the differential-susceptibility and diathesis-stress models of person-X-environment interaction. It was predicted that the parenting of mothers homozygous for the s allele would be more strongly affected by the quality of environmental context — in a differential-susceptibility-related manner — though no

hypothesis was advanced as to whether the strong or weak version of the model would be supported.

Although some authors have distinguished between s-carriers (ss/sL) and L homozygotes, we compared s homozygotes and L-carriers (LL/Ls) in our analysis. We have done so because the ss genotype is believed to be the most affected in terms of the serotonin transporter activity, as the s allele is associated with lower levels of transcription (Lesch et al., 1996). This parametrization has been used in previous research that have investigated 5-HTTLPR moderation of environmental influences (e.g. Bakermans-Kranenburg & van IJzendoorn, 2008; Hayden et al., 2007; Mesquita et al., 2015; Taylor et al., 2006; Wilhelm et al., 2006; Young et al., 2007), and also in meta-analytic work (Crawford, Lewis, Lewis, & Munafò, 2013). With regard to environmental influences, we considered, in aggregate, nine well-established family risk factors (including teenage pregnancy, single parenthood, economic disadvantage, lack of social support) to create a composite index of quality of environmental context (as detailed in the Methods section). We proceed this way for two reasons. First, the environmental context indicators we composited are associated with parenting (Belsky, 1984; Belsky & Jaffee, 2007); and second, composite measures of risk and support, including just the relative absence of risk, prove more powerful than single risk-support indicators when predicting many phenotypes (Evans, Li & Whipple, 2013).

II. METHODS

PARTICIPANTS

The sample consists of 210 Caucasian mothers and their preschool children. Recruitment took place in Portuguese preschools with children from families that varied in terms of psychosocial risk. Mother's age ranged from 20-48 years (M = 33.36, SD = 5.60); 23.3% had less than nine years of education. Children's ages ranged from 40-77 months (M = 58.26, SD = 7.63); 114 (54.3%) were girls. The study was approved by the Portuguese National Committee for Data Protection and the Ethical Committee of the University of Minho. Informed consent was obtained from mothers.

MEASURES

Quality of Environmental Context Composite

To create a summary measure reflecting the developmental supportiveness, or lack thereof, of the environmental context, multiple measurements were composited. Following Weitzman, Edmonds, Davagnino, and Briggs-Gowan (2013), we assessed presence vs. absence of nine factors, including (1) teenage pregnancy (9, 4.3%); (2) single parenthood (37, 17.6%); (3) (low) parental educational level (i.e., one of the parents had under nine years of education) (88, 41.9%); (4) parental unemployment (i.e., one of the parents was unemployed at the time of the study) (74, 35.2%); (5) economic difficulties (89, 42.4%); (6) absence of social support (18, 8.6%); (7) family conflict (64, 30.5%); (8) maternal psychopathology (47, 22.0%); (9) and chronic health conditions in the family living with the child (128, 59.8%). To assess presence/absence of (8) maternal psychopathology, mothers completed the 53-item of the *Brief Symptom Inventory* (BSI; Derogatis, 1982; Portuguese version, Canavarro, 1999), based on a 5-point scale (0 ="not at all" to 4="extremely"), in terms of the presence of various symptoms experienced in the past week (Chronbach alpha = .96). A binary variable (presence/absence of Maternal Psychopathology) was computed based on the Portuguese normative mean and standard deviation for the Positive Symptoms Distress Index; a score greater than 1.96 qualified as evidence of maternal psychopathology. The nine factors were analysed with Item Response Theory (Bolt, 2005). A standardized score of the quality of environmental context was calculated by summing and reversing those considered “negative”, with higher scores reflecting better environmental context quality. Thus, higher scores reflect a more developmentally supportive environmental context (M = 2.11; SD = 1.45, range 0-6).

Genetic assessment

Mother’s saliva was collected using OraGene OG-500 (DNA Genotek, Inc., Ottawa, Ontario, Canada) and stored at room temperature. Genomic DNA was isolated as instructed by the manufacturers, using the standard protocol from PrepIT L2P (DNA Genotek) and sample concentrations were assessed using Nanodrop technology. The *5-HTTLPR* allelic assay was performed by polymerase chain reaction (PCR). The amplification products were separated on a 3% agarose gel and visualized using Gel Doc EZ system (Bio-Rad, USA), in order to identify the short (s) and long (L) alleles. Results

were also validated using Sanger Sequencing of representative samples of each genotype (ss, sL and LL). The genotypes were in Hardy-Weinberg Equilibrium ($\chi 2$ (1) = 1.178, p = 0.278). The majority of the participants were heterozygous (sL) (n = 111, 52.9%), followed by homozygous for the Long allele (LL) (n = 63, 30%). Short allele (s) frequency was 0.44. Primary statistical analysis contrasted the ss-genotype with LL/sL genotypes.

Maternal behaviour

Mother-child interaction was videotaped in a quiet room (at the family home or at the preschool) across three 5-minute episodes involving (a) child play with a challenging toy under mother's guidance; (b) maternal completion of a sham questionnaire while the child had only an uninteresting toy to play with, after being instructed not to touch more interesting, but difficult-to-reach toys; and (c) mother and child engage in free play for half the period followed by mother-directed child clean-up.

Mother's ability to accurately perceive the child's signals and to respond to them promptly, contingently and appropriately was rated — based on behaviour observed across all three videotaped episodes — using Ainsworth, Bell and Stayton's (1974) 9-point, maternal sensitivity scale; higher scores reflect greater sensitivity. Inter-rater reliability proved high (sensitivity: ICC = .93, n = 87; M = 4.53; SD = 1.70, range 1-8).

STATISTICAL ANALYSIS

The statistical analysis proceeded in two stages. First we evaluated in a traditional regression analysis whether the GXE effect involving 5-HTTLPR and environmental context predicted parenting; following Belsky and Widaman (in press), this was done not to see whether the interaction was significant, but whether the F value was sufficiently large — greater than 1.0 — to permit formal, competitive model testing.

After establishing that the F ratio of the GXE effect exceeded 1.0, we proceeded to the second stage in which we evaluated competing models of person-X-environment interaction. To test whether ss carriers were vulnerable (diathesis-stress) or susceptible for better and for worse (differential susceptibility) to the quality of the environmental context, a reparametrized equation was used, following Widaman et al. (2012):

$$Y: \begin{cases} D = 0 : Y = B0 + B1\ (X - C) + E \\ D = 1 : Y = B0 + B3\ (X - C) + E \end{cases}$$

Where variable D are the genotypes (0 = sL/LL; 1 = ss), B_0 is the intercept, B_1 the slope for sL/LL genotype, B_3 the slope for ss genotype, and C is the cross over point between the two slopes. The magnitude of the crossover point (C) distinguishes a diathesis-stress from a differential susceptibility model: if the magnitude of C is zero, then the two lines meet at the left of the graph without crossing-over, and the ss genotype cannot have a better outcome than the sL/LL genotype — which would be in accordance to diathesis-stress model. If the magnitude of C is not zero, then the two lines cross over in the middle of the graph and the ss genotype can have a better outcome than sL/LL genotype — which would be in accordance to the differential susceptibility model. Using this comparative approach, we simultaneously tested for two versions (strong and weak) of the differential susceptibility and diathesis-stress models (see Figure 3.1).

Following Widaman et al. (2012), the four possible models were tested and compared with each other in terms of fit to the data based on explained variance (R^2) and Akaik and Bayesian Information Criteria (AIC and BIC, respectively). Models that fit the data better should explain more variance (higher R^2), and those with lower values of AIC and BIC are preferred as showing better fit to the data. For detailed statistical procedures, see Belsky, Pluess and Widaman (2013) and Widaman et al. (2012).

III. RESULTS

Table 3.1 presents the prediction model for maternal sensitivity including maternal 5-HTTLPR and family context as predictors (independently and in interaction). As displayed, the *F* value of the GXE interaction term greatly exceeded 1.0 ($F_{(3,205)}$= 12.272), thereby allowing us to test competing GXE models.

Table 3.1. Prediction model for maternal sensitivity, considering maternal 5-HTTLPR and quality of environmental context.

Model; Predictors	R^2 (R^2_{adj})	*F* (df)	Unstandard. coefficient		*t*	*p*	95% CI	
			B	*SE*			Lower	Upper
1								
5-HTTLPR (ss)	.130 (.122)	15.411 (2,206)	-.120	.172	-.698	.486	-.458	.219
Environmental context			.452	.083	5.439	<.001	-.616	-.288
2								
5-HTTLPR (ss)	.152 (.140)	12.272 (3,205)	-.070	.171	-.408	.684	-.407	.268
Environmental context			-.365	.090	-4.030	<.001	-.543	-.186
GXE			-.501	.217	-2.311	.022	-.929	-.074

Note. *N* = 210

Weak and strong versions of differential-susceptibility and diathesis-stress models were simultaneously tested. Inspection of Table 3.2 indicates, upon considering both R^2 and AIC and BIC criteria, that the best fitting model (i.e., the one explaining more of the variance and having lower values of AIC and BIC) proved to be the weak version of differential susceptibility (i.e., Model B) (R^2 = .152, $F_{(3,204)}$=8.728, p <.001; AIC = 841,467, BIC = 846.890). Despite the non-significant difference between Models B and D ($F_{(2,205)}$= 0.16, p = .85), the actual difference in BIC between Model B and the three other ones is higher than 10, which, according to Raftery (1995), is considered "very strong" evidence in favour of the model with the more negative BIC value – in this case, the weak differential susceptibility model.

Table 3.2. Results for alternate regression models for maternal sensitivity

	Re-parameterized regression equation			
	Differential susceptibility		**Diathesis-Stress**	
Parameter	**Model A Strong**	**Model B Weak**	**Model C Strong**	**Model D Weak**
B_0	4.601 (0.12)	4.669 (0.29)	4.683 (0.12)	5.517 (0.16)
B_1	0 (-)	.484 (0.12)	0 (-)	.550 (0.00)
C	.116 (.00)	.175 (0.45)	10.0 (0.00)	10.0 (0.00)
B_3	1.149 (.27)	1.149 (0.26)	.445 (0.15)	.800 (0.21)
R^2	.085	.152	.044	.141
F	8.014	8.728	7.625	8.559
Df	2,205	3,204	1,206	2,205
P	< .001	< .001	< .001	< .001
F vs. a	-	1.88	1.16	-
Df	-	1,206	1,206	-
P	-	.17	.28	-
F vs. b	1.88	-	3.06	0.16
Df	1,206	-	1,206	2,205
P	.17	-	.08	.85
AIC	910.341	841.467	930.018	853.513
BIC	916.427	846.890	935.008	859.053

Note. Tabled values are parameter estimates, with their standard errors in parentheses.

F vs. a and *F* vs. b stand for *F* tests of the difference in R^2 for a given model versus Model A and Model B, respectively.

C, Cross-over point; AIC, Akaike information criterion; BIC, Bayesian information criterion.

The graphic depiction of findings in Figure 3.2 also proves highly consistent with the weak differential susceptibility model in that (a) mothers of both genetic subgroups evinced greater sensitivity under more positive environmental conditions and less sensitivity under more negative environmental conditions, though (b) the strength of this for-better-and-for-worse pattern of association was greater for mothers homozygous for the s allele than for those who were L-carriers.

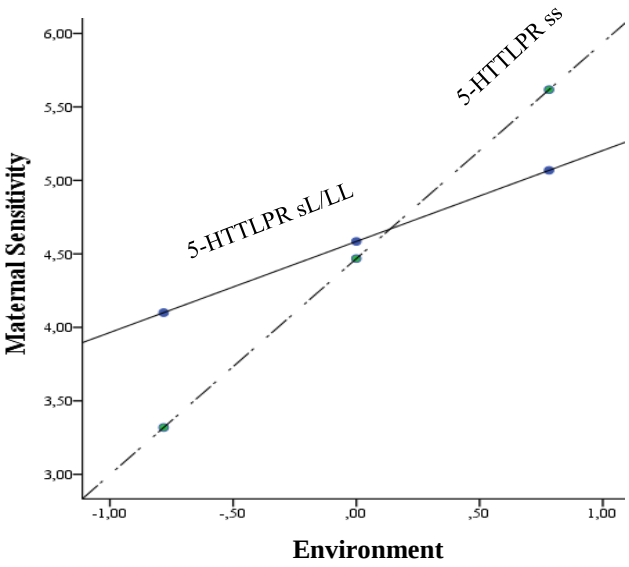


Figure 3.2. Plot of the interaction between the 5-HTTLPR genotype (LL/sL vs. ss) and quality of environmental context on the explanation of maternal sensitivity.

IV. DISCUSSION

The aim of this study was to determine whether—and how—the serotonin-transporter gene, 5-HTTLPR, moderated the effect of quality of environmental context on parenting, thereby extending research on the determinants of parenting and on GXE interaction. We first ascertained the gene-X-environment (GXE) interaction in the prediction of maternal sensitivity. Then our competitive model-testing analysis designed to contrast diathesis-stress and differential-susceptibility models of person-X-environment interaction indicated that the weak differential-susceptibility model fitted the data best. Thus, even though both mothers homozygous for the s allele and those carrying the L allele proved more sensitive in parenting when the environmental context was supportive and less sensitive when such context posed risk, this context-parenting association proved stronger in the case of ss mothers than those carrying L alleles. It must be acknowledged, however, that despite meeting statistical criteria – namely, explaining more of the variance, having lower values of AIC, and, particularly, having a difference in BIC which was enough to be considered "very strong" evidence in favour of the model (Raftery, 1995) – this best-fitting model did not prove significantly different from the

weak diathesis-stress one. Thus, there is a need to replicate these results, ideally using larger samples or employing meta-analysis to multiple samples.

Given the fact that different allelic subgroups have been tested in previous GXE literature involving 5-HTTLPR, we reran the analysis in order to contrast the presence vs. absence of the s allele (i.e., sL/ss vs. LL); proceeding in this alternative GXE manner yielded results consistent with those already reported. Notable, then, is that the results presented herein are generally in line with those of a meta-analysis showing that 5-HTTLPR is a genetic marker of differential susceptibility in Caucasian children and adolescents (van IJzendoorn, Belsky, & Bakermans-Kranenburg, 2012).

Therefore, this study underlines the importance of considering both genetic and environmental sources of influence when investigating the determinants of parenting. Recall in this regard how inconsistent genotype-phenotype results reviewed in this chapter's Introduction proved to be when 5-HTTLPR was only directly related to parenting (i.e., as a main effect). The findings reported herein raise the possibility that one reason why the data proved so inconsistent across prior studies was because samples differed in fundamental ways in terms of family conditions that were not taken into account by considering GXE interaction.

Despite the strengths of this study, most notably its GXE focus on parenting and reliance on competitive model testing, this work has some limitations that should be highlighted. First, the sample size was limited to 210 dyads. Second, as the study involved a community sample, and even if some degree of risk was captured, the number of families at very high risk was limited. A third limitation, involved the need to create a quality of environmental context composite and the resultant inability to decompose it in order to illuminate, perhaps, which components proved most predictive in a GXE analysis. A final limitation might be that the study was not designed to illuminate endophenotypic processes that could explain why ss mothers appear more affected, for better and for worse, by environmental context when it comes to their parenting.

Future research could extend this study through the use of experiments. Do mothers with the ss genotype benefit more from parenting intervention than mothers carrying the L allele? Notably, previous experimental research has shown that putative "risk" factors actually operate as "opportunity" factors in that those carrying them benefit more from interventions than those not carrying them (Cassidy et al., 2011; Klein Velderman, Bakermans-Kranenburg, Juffer, & Van IJzendoorn, 2006). Indeed, a meta-analysis of

such gene-X-intervention work revealed greater effects of a variety of experimental manipulations for hypothesized susceptible genotypes than nonsusceptible genotypes (Bakermans-Kranenburg & van IJzendoorn, 2015). Further investigation on this subject would provide insights into "what works for whom", better matching intervention to mother–infant dyads (Belsky & van IJzendoorn, 2015).

Also, research on mechanisms underlying differential susceptibility is clearly called for (Moore & Dupre, 2016). Such work should consider in particular possible mechanisms by which the polymorphism comes to affect sensitivity to context and, thereby how family conditions come to influence parenting. Possible mechanisms include maternal cognitive functioning (cf. Homberg & Lesch, 2011) and amygdala activation (Furman, Hamilton, Joormann & Gotlib, 2011).

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CHAPTER 4:
ASSESSING PRESCHOOLERS INTERACTIVE BEHAVIOUR: A VALIDATION STUDY OF THE "CODING SYSTEM FOR MOTHER-CHILD INTERACTION"

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ASSESSING PRESCHOOLERS INTERACTIVE BEHAVIOUR: A VALIDATION STUDY OF THE "CODING SYSTEM FOR MOTHER-CHILD INTERACTION"

I. INTRODUCTION

Reliable and valid behavioural assessment is an essential tool for understanding child development. Most assessments of child behaviour are based on parents' or teachers' reports, which are relatively easy to acquire, but rely on the individual's ability to describe or quantify complex behavioural and relational characteristics (Lindhal & Malik, 2001) and are potentially subject to bias. Self-reports have obvious limited utility in young children, as they require an appropriate level of cognitive and linguistic skills that is not be sufficiently developed. Therefore, an important alternative (or complement) are observational measures, which provide an objective window on real processes and outcomes of interest.

One of the domains where observational measures have been widely used is parent-child interaction. They allow detailed measurement of the quality of such interaction (Gardner, 1997), with behaviours being defined consistently and reliably by the researcher, and not by the parent – being thus less likely to be biased by their judgements, expectations, and/or mood. Observation enables the assessment of a wide range of behaviours, from global aspects of parent-child relationship quality (e.g., cooperation or negativity), to quantifiable and specified behaviours (e.g., commands, questions), or behavioural contingencies (e.g., acknowledgement following cooperative behaviour) (Aspland & Gardner, 2003).

Specifically in the preschool years, observing interactive behaviour is critical due to the major developmental achievements taking place during this period. At this time, children become more autonomous, with growing capacity for exploring the environment. As described by Marvin and Britner (2008), their representational and communicative abilities increase, and allow them to practice many social behaviours. In fact, by this age most children are able to reason in a gradually more sophisticated and accurate manner (i.e., nonegocentrically) about the causal relations between their own and others' behaviour and internal experiences (Marvin, Greenberg, & Mossler, 1976), to

communicate about events and emotional states, and to relate logical sequences (e.g., Dunn, 1994). These achievements allow children to recognize and talk about the feelings, goals and behaviours of self and others, and to distinguish between their own and others' perspectives. They are able to organize a plan, to construct a shared perspective (see Lewis & Mitchell, 1994), and to start to understand and apply complex implicit and explicit rules for social interaction (see Eisenberg, 2006).

The preschool years are also a period of progress in emotion regulation, with children gradually developing the ability to attune their cognitions, behaviours, and emotions to respond to situational demands (Kopp, 1989), and going from other-reliant strategies to increasingly active and autonomous ones (Grolnick, McMenamy & Kurowski, 2006). All these socio-cognitive and emotional capacities contribute in important ways to children's capacity to engage in complex interactions with others (Marvin & Britner, 2008). Nonetheless, despite the relevance of observation as an assessment tool for child's interactive behaviour, remarkably few measures exist focusing on the preschool period, and even less are accessible for professionals and researchers. Specifically, some of the existing scales have significant costs or training burden that impedes their broad use. Therefore, it is crucial to have reliable and valid observational measures available to both researchers and clinicians at low cost and without extensive training requirements, that have proven reliability and validity.

The present study aimed to adapt and validate an observational measure for assessing child interactive behaviour for preschool age children, during a structured mother-child interactive task. The coding system was developed by Healey and colleagues (2010), based on previous measures: the NICHD Study of Early Child Care (NICHD Early Child Care Research Network [ECCRN], 1999, 2003, 2006); Campbell, Pierce, March, Ewing, and Szumowski (1994); and Eyberg, Bessmer, Newcomb, Edwards, and Robinson (1994). These previous coding systems, which were combined in order to assess a wider array of characteristics, have been extensively used in research on mother–child interactions and found to be valid and reliable measures of both individual and dyadic behaviours. The new Coding System for Mother-Child Interactions (Healey, Gopin, Grossman, Campbell, & Halperin, 2010) has recently been evaluated in research studies, with promising findings. In the first study reporting on this measure, in a sample of 126 preschoolers with symptoms of hyperactivity/inattention, Healey et al. (2010) found that dyadic synchrony between mother and child was reliably associated with

children's functioning. A subsequent study found that Attention-Deficit/Hyperactivity Disorder (ADHD) and Oppositional Defiant Disorder (ODD) were associated with more negative behaviours during the mother–child interaction, and that ADHD + ODD comorbidity was associated with greater impairment during relatively repetitive tasks requiring sustained attention (Gopin, Healey, Grossman, Campbel & Halperin, 2011). The most recent work is a prospective study by Rajendran, Kruszewski, and Halperin (2016) with 162 children, reporting on a link between greater parent support for child autonomy at age 4 to 5 years and reduced bullying. All these studies report good intercoder reliability, and therefore underline the value of this new coding system.

In the current inquiry, we focused on the child behaviour codings, which assess the dimensions of Cooperation, Enthusiasm and Negativity, during the structured mother-child interactive task. Our aim was to conduct the first reliability study of these child codings in a preschoolers sample. For that propose, we assessed the validity of the translated coding system, adapted to a 10-min mother-child interactive task, in a sample of 220 Portuguese preschoolers. We then reported on its descriptive data, and its convergent and discriminant validity, considering other relevant measures of children development and adjustment. Finally, we tested the association between child interactive behaviour and other relevant maternal and environmental factors. We ended with a discussion on its implications for future research and practice.

II. METHODS

PARTICIPANTS

The sample consists of 220 Portuguese preschoolers and their mothers. Children's ages ranged from 40 to 77 months (M = 58.23, SD = 7.66); 120 (54.5%) were girls. 82 (37.3%) of the children were only children, and the mean number of children per family was 1.88 (SD = .90, range = 1-6).

Mothers' ages ranged from 20 to 48 years (M = 33.35, SD = 5.66). Regarding education level, 54 mothers (24.5%) had less than nine years of education, 66 had completed 9 years of education (30%), 63 (28.6%) completed high school, and 37 (16.8%) had a higher education degree. With respect to marital status, 43 (19.5%) of the mothers

were single, 41 (18.5%) were living with partners, 114 (51.8%) were married, 19 (8.6%) were divorced and 3 (1.4%) were widowed.

Procedure

Ethical approval was obtained from University of Minho, Portuguese Ministry of Education, and National Commission for Data Protection. Following the recruitment of dyads from Portuguese preschools, the assessment was conducted either in participants' homes or preschools, depending on participants' availability. When tested, no group differences according to settings were found regarding the variables of interest. The visit started with the study description. Then, informed consents were obtained from the mothers. Mothers were then asked to fill in the questionnaires described below and to join the child in an interactive task. The interaction was videotaped across three 5-minute episodes involving: (a) child playing with a challenging toy under mother's guidance; (b) child playing exclusively with an uninteresting toy, after being instructed not to touch more interesting, but difficult-to-reach toys, while mother completes a sham questionnaire; and (c) child and mother engaging in free play for half the period, followed by mother-directed child clean-up.

Measures

Child interactive behaviour

The child codings of the Coding System for Mother–Child Interactions (CSMCI, Healey et al., 2010) aim to assess a child's behaviour based on observation of an interactive task. It consists of three subscales: (a) Cooperation-Compliance, which measures whether the child responds to maternal suggestions and commands quickly/cheerfully; (b) Enthusiasm, an assessment of whether the child invests in the task and approaches it with energy and excitement; and (c) Negativity and Hostility, a measure of whether the child forcefully rejects the mother's ideas or is unreasonably demanding.

For the present validation, child behaviour was assessed based on the videotaped mother-child interactive task. Following the work of Haley et al. (2010), two episodes of that interaction were considered (episodes (a) and (c) described in the section "Procedure"), in order to include three tasks: 1) child playing with a demanding toy under

mother's guidance (demanding toy task, 5min); 2) free play with the mother (free play task, 2,5min); and 3) clean up (clean up task, 2,5min). The coding system was requested from the team who developed it. It was then adapted to the Portuguese language and learned by two coders experienced in other observational measures, without direct training from the developers. Afterwards, those two coders introduced the system to a team of naïve coders during 3 half days of training (12h total). The videos were coded individually and in group during the training. Ten videos were then coded individually for the reliability analysis.

The coding of the subscales (Cooperation, Enthusiasm and Negativity) is based on an anchored, 5-point Likert scale (1 = very low to 5 = very high). Negativity scores were inverted so that higher scores in the three subscales represent better (more adaptive) behaviour. Ratings reflect qualities of the child interactive behaviour occurring through the observational period, as exhibited in the observed interaction with the mother. Following the original study reporting to this measure (Healey et al., 2010), a minimum interrater reliability of .70 was ensured for each subscale, in the present report.

Child Emotional and Behavioural Problems

The Child Behaviour Checklist for ages 1 1⁄2-5 (CBCL 1 1⁄2-5; Achenbach & Rescorla, 2000; Portuguese version Dias, Sousa Lima, Machado, Carneiro & Campos, 2017) was used to assess child emotional and behavioural problems. The 100-item questionnaire was completed by mothers in a 3-point Likert scale (0 = Not true to 2 = Very/Often True). It assesses empirically based subscales, DSM-oriented subscales and two second order subscales: internalizing and externalizing problems. A total score is also computed – i.e., Total Problems Scale - which compiles all the child's emotional and behavioural symptoms reported by the parent. In the present study, the subscales of Externalization and Internalization problems were used, as well as the Total Problems Scale. Good validity has been reported for the Portuguese version (Dias et al., 2017).

Child Temperament

The Children's Behaviour Questionnaire - Short Form Version (CBQ-SF, Rothbart, 2000; Portuguese version by Lopes, 2011) was used to assess mother's perspective of child's self-regulation strategies and emotional reactions. This instrument is composed of 94 items that assess 15 domains of temperament in children aged 3 to 7 years, and three

broad dimensions of temperament (Extraversion/Surgency, Negative Affectivity, and Effortful Control). Mothers were asked to rate how much the items described the children's behaviour in the past six months, on a 7-point Likert scale (1 - Extremely Untrue to 7 - Extremely True). In this study, the three broad dimensions were used. The validity of the Portuguese scale has been established and reported by Lopes (2011).

Child Mental Development

The children's mental development was assessed using the Griffiths Mental Development Scales (2-8 years) (Griffiths, 1984, partial Portuguese version Castro & Gomes, 1996). The scales evaluate six domains: locomotor, personal-social, language, eye and hand co-ordination, performance, and practical reasoning. The domains can be considered separately as subscales or can be averaged into a global mental development score. In the present study, the global mental development score was used.

Maternal behaviour

Maternal behaviour was assessed based on the 15-minutes videotaped interaction task, using Ainsworth's scales (Ainsworth, Bell, & Stayton, 1974) for rating maternal sensitivity, meaning the mother's ability to accurately perceive the child's signals and to respond to them promptly, contingently and appropriately; and cooperation, defined as mother's ability to respect the child's autonomy while avoiding situations in which she might have to interrupt the child's activity or exert direct control. The coding is based on a 9-point scale with higher scores indicating more sensitive/cooperative behaviour. Coding achieved good interrater reliability (sensitivity: ICC = .93, n= 87; cooperation: ICC = .86, n = 84). In line with previous studies (Baptista, et al., 2013; Juffer, Hoksbergen, Riksen-Walraven, & Kohnstamm, 1997; Tharner et al., 2012), a dimension of *sensitive responsiveness* was considered. The score was computed by averaging the two highly correlated scales of sensitivity and cooperation (r = .73, p < .001).

Maternal Psychopathology

Mothers completed the 53-item of the Brief Symptom Inventory (BSI; Derogatis, 1982; Portuguese version, Canavarro, 1999), based on a 5-point scale (0 = not at all to 4 = extremely), in terms of the presence of various symptoms experienced in the past week. The questionnaire assesses nine basic psychopathological dimensions and three global

scores. The Positive Symptoms Distress Index (PSDI), one of the global scores, was used in this study, as a measure of symptom intensity corrected for the number of symptoms. The Portuguese validation study reports on the good psychometric qualities of the inventory, and states the validity of the PSDI as a separate scale (Canavarro, 2007).

Quality of the environmental context

Sociodemographic information was collected in line with Weitzman, Edmonds, Davagnino, and Briggs-Gowan (2013) to derive a quality of the environmental context variable, based on the presence (vs. absence) of nine factors: teenage pregnancy (10, 4.5%); single parenthood (40, 18.2%); (low) parental educational level (i.e., one of the parents had under nine years of education) (95, 43.2%); parental unemployment (i.e., one of the parents was unemployed at the time of the study) (74, 33.6%); economic difficulties (93, 42.3%); absence of social support (20, 9.1%); family conflict (67, 30.5%); maternal psychopathology (see above) (49, 22.3%); and chronic health conditions in the family living with the child (133, 60.5%). The factors were analysed based on Item Response Theory (Bolt, 2005). A standardized score was calculated based on the presence/absence of the aforementioned factors, by summing and reversing those considered "negative", with higher scores reflecting better environmental context quality.

STATISTICAL ANALYSIS

Firstly, inter-coder reliability was calculated for the three subscales of the coding system, based on Intraclass Correlation (ICC) (Winer, 1971). Descriptive statistics were then computed for the variables of interest, as well as cross-situational consistency. A series of correlations were performed to assess the validity of the coding system. Finally, bivariate correlations were run to test for associations between variables of interest, regarding child, mother and environmental context.

III. RESULTS

RELIABILITY

Inter-coder reliability was determined by randomly assigning 62 cases (28.18%) to 3 pairs of coders. Coders were blind to participant's information. Between the pairs of coders, reliability ranged from ICC = .88 to .93 for Cooperation; ICC =.91 to .93 for Enthusiasm; and ICC =.89 to .94 for Negativity. These values are indicative of high inter-coder agreement.

DESCRIPTIVE STATISTICS AND CORRELATION ANALYSIS OF THE CHILD CODINGS

First of all, to examine the consistency of Cooperation, Enthusiasm and Negativity scores, we tested their associations across the three tasks (i.e., demanding toy, free play and clean up).

Table 4.1. Associations between Cooperation, Enthusiasm and Negativity scores across the three tasks.

	Cooperation		**Enthusiasm**		**Negativity**	
	1.	**2.**	**1.**	**2.**	**1.**	**2.**
1. Demanding toy						
2. Free play	.523**		.282**		.590**	
3. Clean Up	.398**	.476**	.208**	.301**	.459**	.580**

Note. Pearson correlations. *$p < 0.05$; **$p < 0.01$.

As presented on Table 4.1, Cooperation scores were significantly associated across the different tasks. The same was the case for Enthusiasm and Negativity. The magnitude of the correlations was moderate to high (r >.30) in the case of Cooperation and Negativity; and small to moderate for Enthusiasm (r > .21) (Cohen, 1988). Given the significant associations, Cooperation, Enthusiasm and Negativity scores across the three tasks were averaged and considered as subscale scores for the entire interaction.

Table 4.2 presents descriptive data and associations between the three subscales, and child age and sex. Subscales scores were rated from 1 to 5, with means ranging from 3.46 (*SD* = .73) for Enthusiasm and 3.96 (*SD* = .79) for Negativity. Moderate to high correlations among them were found ($r > .397$, $p < .001$), and for that reason the subscales were standardized and averaged into a global *Child Interactive Behaviour* variable. Child age was positively associated with Cooperation and Enthusiasm, but not with Negativity. Sex was not significantly associated with the other variables.

Table 4.2. Descriptive data and associations between child interactive behaviour and child age and sex.

	Range	***M (SD)***	**1.**	**2.**	**3.**	**4.**
1. Cooperation	1.33-5	3.75 (.75)	-			
2. Enthusiasm	1.33-4.67	3.46 (.73)	.636**	-		.
3. Negativity	1-5	3.96 (.79)	.779**	.397**	-	
4. Age (months)	40-77	58.23 (7.66)	.185**	. 239**	.130	-
5. Sex	-	-	-.131	.011	-.070	.104

Note. Pearson and point-biserial correlations. **$p < 0.01$.

CONVERGENT AND DISCRIMINANT VALIDITY

Due to the expected overlap between the child interactive behaviour and emotional and behavioural problems, CBCL 1 1⁄2-5 scores were included in the analysis for convergent validity. As presented on Table 4.3, child's interactive behaviour was inversely associated with emotional and behavioural problems, meaning that higher scores in child interactive behaviour were related to lower levels of reported behavioural and emotional problems. This was the pattern of associations for all of the subscales of both measures, as well as for the global scores. Despite being significant, the magnitude of those associations was small ($r < -.237$).

Table 4.3. Associations between the child's interactive behaviour, emotional and behavioural problems, temperament and mental development.

	Convergent validity			**Divergent validity**			
	CBCL Internalizing	CBCL Externalizing	CBCL Total problems	CBQ Surgency-Extraversion	CBQ Effortful control	CBQ Negative affect	Griffiths
Cooperation	-.141*	-.229**	-.186**	-.028	.140*	-.089	.196**
Enthusiasm	-.143*	-.135*	-.155*	-.019	.111	-.075	.120
Negativity	-.151*	-.237**	-.192**	-.049	.050	-.098	.137*
Child's Interactive Behaviour	-.169*	-.234**	-.207**	-.037	.117	-.102	.176**

Note. Pearson correlations. *$p < 0.05$; **$p < 0.01$

To investigate the discriminant validity of the coding scales, we examined its associations to measures of temperament and mental development. According to Table 4.3, regarding the temperament measure, there was only one significant and positive association between Cooperation and Effortful Control ($r = .140$, $p = .04$), which was of small magnitude. Regarding the mental development measure, positive associations with Cooperation and Negativity were found, again of small magnitude ($r < .20$).

Associations between child interactive behaviour and maternal and environmental factors

Correlation analyses were also performed regarding child interactive behaviour and maternal and environmental factors. As displayed on Table 4.4, child Cooperation, Enthusiasm and Negativity were inversely associated with maternal psychopathology ($r > -.135$, $p < .05$), meaning that lower maternal psychopathology scores were associated with better outcomes in child interactive behaviour.

Finally, we analysed the associations between maternal and child interactive behaviour – considering both the subscales and global measures. As presented on Table 4.4, all interactive behaviour constructs were positively correlated.

Non-significant associations were found between the other variables.

Table 4.4. Correlations between the child interactive behaviour and maternal and environmental factors.

	Maternal age	Maternal education	Maternal psychopathology	Quality of the environmental context	Maternal sensitive responsiveness
Cooperation	.076	-.004	-.147*	-.094	.288**
Enthusiasm	.075	.088	-.135*	-.087	.270**
Negativity	.126	-.020	-.156*	-.096	.275**
Child Interactive Behaviour	.108	.025	-.170*	-.108	.324**

Note. Maternal sensitive responsiveness – average of maternal sensitivity and cooperation. *$p < 0.05$; **$p < 0.01$

IV. DISCUSSION

The present inquiry focused on an observational measure of child interactive behaviour and had three aims. The primary one was to conduct the first reliability study of the child codings of the Coding System for Mother-Child Interactions in a preschoolers sample. Our results confirm high levels of interrater agreement, which support the reliability of the coding system for assessing child's Cooperation, Enthusiasm and Negativity in an interactive task with the mother. In fact, the high values of reliability suggest that the content of the subscales was based on well operationalized definitions which were clear for the coders. This is of relevance since observational coding of behaviour is a challenging task, and it is therefore crucial that constructs are conceptually well defined in order to derive reliable and consistent data (Furr & Funder, 2007).

The second aim of this study was to report on the descriptive data of the coding system and analyse its validity. First of all, associations of Cooperation, Enthusiasm and Negativity scores across tasks suggest the stability of the constructs, which do not seem to be task dependent, and support their use as subscales. Of note, we found a lower magnitude of associations regarding Enthusiasm. Nonetheless, the existence of some task-specificity in this domain (Enthusiasm) is conceivable, given the content of the episodes (demanding toy, free play and clean up). The three subscales were positively associated among themselves, which supports the use of a global score for child interactive behaviour.

Regarding child's variables, age was positively associated with Cooperation and Enthusiasm, such that older children tended to score higher on those subscales. As mentioned in the introduction, the preschool years are a period in which the child acquires many critical social abilities and skills related to a non-egocentrical relation with the other, recognition of different perspectives and apprehension and use of rules for social interaction (Marvin & Britner, 2008), which are likely to facilitate cooperation. The same is true regarding the Enthusiasm subscale, as it relates to the ability to be involved in the task, and being confident and interested (Healey et al. 2010), which can benefit from the growing autonomy and self-reliance of the preschool years. The absence of an age-association for the Negativity subscale might seem surprising since this period is also characterized by achievements in emotion regulation. Nonetheless, it should be noted that, in this particular scale, negativity refers specifically to the child-mother interaction, and not the general ability to regulate emotions.

Concerning convergent validity, it was supported by the association between children's emotional and behavioural problems and their interactive behaviour. The strongest associations found related to externalizing behavioural problems. This finding may not be surprising, as externalizing behaviour is expressed through clearly outwardly observable behaviour, which may be more easily captured by the child behaviour codings - particularly the subscales of Cooperation-compliance (i.e. the child's capacity of responding quickly and cheerfully to the mother suggestions/commands) and Enthusiasm (particularly in the child's ability to invest in the task).

In the case of divergent validity, child's temperament and mental developmental measures were considered. Regarding temperament, the absence of correlations between the measures suggests that they do assess different constructs, being the coding system related to interactive behaviour in a dyadic situation, and temperament based on general individual differences in reactivity and self-regulation (Rothbart, Posner, Kiers, 2006). A small, although significant, association was found between the CBQ-SF dimension of Effortful Control and the Cooperation subscale. As effortful control relates to the capacity to organize attention and regulate emotions and behaviours according to immediate and long-term goals (Posner & Rothbart, 2000), this (weak) association with cooperation is reasonable. Of note, both methods include subscales of Negativity (in the CBQ-SF called "negative affect") which were not associated. In fact, in the child codings Negativity relates to the child's demanding behaviour or rejection of the mother's ideas (Healey et

al. 2010); whereas the CBQ-SF Negative Affect is a broader dimension, which relates to the presence of negative emotions as fear, anger, sadness, and difficulty in controlling them.

Concerning the mental development measure, it was associated with Cooperation and Negativity. We acknowledge that mental development may impact on child behaviour, and therefore a weak correlation seems reasonable. However, mental development was not associated with Enthusiasm. This absence of association may relate to the fact the Enthusiasm subscale includes a more emotional dimension, being defined as expressing energy and excitement towards the task (Healey et al. 2010).

The third aim of this research was to test the association between child interactive behaviour and relevant maternal and environmental variables. As expected, we found a negative association with maternal psychopathology. This result is in line with a vast literature on the impact of maternal psychopathology on child development and behaviour (Cicchetti, Rogosch, & Toth, 1998; Cummings & Davies, 1994; Goodman & Gotlib, 1999). Child behaviour was, perhaps surprisingly, not associated with maternal education or quality of the environmental context. The presence of these factors, particularly low quality of the environmental context, documents that the families in this sample were facing multiple chronic stressors known to impact on the parent-child relationship, supportive parenting and the child's healthy socio-emotional development (Cicchetti, Rogosch, & Toth, 1998). They have also been documented to contribute to the early development of behavioural problems in children (Cicchetti, Rogosch, & Toth, 1998; Gardner et al., 2004). In this case, it is possible that maternal education and the quality of the environmental context do not impact specifically on the child's behaviour when interacting with the mother, but would (and in our sample does) impact on other more general and less context specific measures of child adjustment, such as emotional and behavioural problems and mental development (unpublished data). On the other hand, although we did not find an association between cumulative home environmental quality and child behaviour, it is also possible that the individual factors included in our composite measure of the quality of environment had, on their own, independent associations with child behaviour. This is in line with the evidence that maternal psychopathology – one of the factors comprised in the composite - is in itself associated with the child interactive behaviour, despite the composite not being. It should also be acknowledged that other environmental variables, besides the ones captured by our

composite, may be impacting on child interactive behaviour. Future studies should, therefore, include different environmental variables and test them separately and composited to access their association with child interactive behaviour.

Regarding the associations between mother-child interactive behaviour, a consistent pattern was found, which implies an agreement between ratings of maternal and child behaviour, both in the subscales, and in global behaviour indices. This pattern may illustrate how interactive behaviour is being reciprocated within the dyad. This is in accordance with research underlining the role of sensitive parenting in facilitating positive exchanges and promoting the establishment of a mutually responsive relationship (e.g., Kochanska, 1997). These findings are also in line with studies linking sensitive and responsive parenting to a range of adaptive outcomes in children, in this case child's cooperative and enthusiastic behaviour and positive emotion (Eisenberg et al., 2001; Kochanska & Murray, 2000). That said, as this study is cross-sectional, a direction or causality should not be implied.

Despite the relevance of these findings, this study has some limitations that should be addressed. Methodologically, it should be noted that this validation study included two different methods (observation and questionnaires), with information provided by two different informants (observer/researcher and mother), and therefore the possibility of method effects should be acknowledged (Podsakoff, MacKenzie, Lee, & Podsakoff, 2003). Also, it would be informative to test for the convergent validity of the child codings using a different observational measure of child behaviour; and to include more informants of the child behaviour, besides the mother – for example, the father. These issues should be addressed by future research.

Moreover, despite the advantages regarding the operationalization of constructs and the definition of an observational paradigm that elicits the behaviours of interest (Aspland & Gardner, 2003), the use of an observational measure entails limitations. One of them is observer bias; nonetheless, the high values of reliability found in this study reduce that possibility. Reactivity effects could have also accounted for the results (Kazdin, 2003), resulting in more mild behaviour, particularly from parents. To minimize this effect, researchers allowed time for participants to become familiarised with them and with the procedures, and reduced as possible the intrusiveness of the recording equipment. The fact that both maternal and child interactive behaviour scores have a large range suggests that a wide variety of behavioural performances were captured.

In conclusion, this work contributes to the existing literature on the assessment of child's interactive behaviour, by providing data on the reliability of an observational method. The coding system validated here can be requested from the developing team without any cost, and trained by in-house coders who, despite being experienced in other observational measures, have no formal training in this system. It therefore represents a low-cost investment, with more flexible training requirements than some other systems currently available, and overcomes some of the difficulties faced by research teams or practitioners when learning and accessing other observational measures. Its use is recommended as a part of a multi-method approach of the study of child interactive behaviour, in which the strengths of different methods complement each other. Future research should expand this work to clinical populations and possibly other age groups.

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CHAPTER 5:
THE CONTRIBUTION OF CHILD GENETICS AND MATERNAL BEHAVIOUR TO CHILD'S OXYTOCIN RESPONSE AND INTERACTIVE BEHAVIOUR

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I. INTRODUCTION

The oxytocinergic system is a key biological system underlying the need for bonding and protection from threats (Insel, 2010), with recognized associations with social behaviours, particularly social affiliation behaviour (Winslow & Insel, 2002). In particular, it has been shown to respond to close social interactions. For example, Feldman, Gordon, Schneiderman, Weisman, and Zagoory-Sharon (2010) have shown that mothers and fathers who provided high levels of tactile contact to their infants presented an increase in salivary oxytocin (OT) following parent–infant interaction. Of particular relevance to this inquiry, the increase in OT after such interaction has been shown to happen not only in the parents, but also in the children (Feldman, Gordon, & Zagoory-Sharon, 2010). In this last study, Feldman, Gordon, and Zagoory-Sharon (2010) report that infant's OT levels were related to the degree of contingent parenting and to the infant's social engagement with the parent. Of note, infant's social engagement represents the earliest set of signals of involvement in a social interaction, and has been shown to predict cognitive, social, and emotional growth (Marshal & Fox, 2006). However, research reporting on children's OT responses to close interactions, particularly in the context of the mother–infant relationship, remains limited.

According to recent research, one individual factor that might impact on the OT response is the oxytocin receptor gene (OXTR). The OXTR gene is present in a single copy in the human genome and was mapped to the locus 3p25–3p26.2 (Michelini, Urbanek, Dean, & Goldman, 1995). A known single-nucleotide polymorphism (SNP) on that gene is the rs53576, which involves a change between a guanine (G) to adenine (A) (Meyer-Lindenbert, Domes, Kirsch & Heinrichs, 2011). Although the biological consequences of this variation remain largely unknown, research suggests some mechanistic differences between carriers vs. non-carriers of the A allele. For example, the presence of the A allele has been shown to be associated with less efficacious OT

binding (Tost et al., 2010). Specifically on research with children, however, Parker et al. (2014) report no differences in plasma OT levels as a function of allelic variation.

OXTR has, in turn, been implicated in psychological functioning and behavioural phenotypes related to social interaction. In that regard, A-carriers of the OXTR SNP rs53576 have shown lower positive affect scores (Lucht et al., 2009), lower levels of optimism, and self-esteem (Saphire-Bernstein, Way, Kim, Sherman & Taylor, 2011). Interestingly, in the context of psychosocial stress exposure, A-carriers have also been shown to benefit less from social support than G-carriers, showing similar cortisol and subjective stress responses in both support and no-support conditions (Chen, Kumsta, von Dawans et al., 2011). In line with these results, adult A-carriers have been found to be less likely to seek social support (Kim et al., 2010). In contrast to these findings though, Parker et al. (2014) found that A-carriers showed better affect recognition performance in a sample which included children with autism spectrum disorder, unaffected siblings and unrelated neurotypical controls. Although little is known about how OXTR affects these social and social-cognitive processes, a plausible explanation is that OXTR affects OT levels, which in turn influence social behaviour.

The oxytocinergic system has also been shown to be particularly susceptible to environmental influences (Brunton & Russell, 2010; Feldman, 2012). Importantly to the present inquiry, researchers have found that the OT biology is associated with the quality of early caregiving. In animal research, studies have shown that the OT system plays a critical role in shaping the child's hypothalamic–pituitary–adrenal (HPA) axis via early caregiving experiences (Hostinar, Sullivan, & Gunnar, 2013), and that the neurochemical organization of infant brain OT is shaped in early life through patterns of maternal behaviour, such as licking and grooming (Kappeler & Meaney, 2010). Specifically, adult female rats that received lower levels of maternal licking and grooming as pups subsequently exhibited decreased OT receptor binding in several brain regions (Francis, Champagne, & Meaney, 2000). Furthermore, in humans, adverse early parental environments have been associated with lower OT concentrations in cerebrospinal fluid (Heim et al., 2008) and disruption of OT biology in regulating stress-related cortisol responses (Meinlschmidt & Heim, 2007). Studies on early social deprivation, such as institutionalization, also add to this link between environmental care and OT. Compared with children raised with their biological family, institutionally reared children have shown marginally lower levels of OT (Wismer-Fries, Ziegler, Kurian, Jacoris, & Pollak,

2005). Despite recognizing this link between early life adversity and child's oxytocinergic functioning, few studies investigate the role of the quality of proximal interaction, namely maternal interactive behaviour, on child's OT response.

In line with this literature, in the present study we focused on child's OT, and explored how it responds to a mother-child interaction. For that purpose, we followed Bartz, Zaki, Bolger, and Ochsner (2011) position regarding OT and social behaviour, according to which literature should be informed by an interactionist approach in which the effects of OT are constrained by features of individuals and situations. Specifically, we examined the role of child's OXTR SNP rs53576 and maternal interactive behaviour as, respectively, individual and environmental determinants of the child's OT response to a mother-child interaction task. The two factors were considered separately and in interaction.

Then, drawing from these analysis, and from prior research showing that child's OT levels were related to the child's social engagement with the parent (Feldman, Gordon, & Zagoory-Sharon, 2010), we also analysed if the interaction between the individual and environmental factors identified (child's OXTR SNP and maternal interactive behaviour) in itself predicted the child's behaviour when interacting with the mother, considering the possible mediating role of the child's OT response.

II. METHODS

PARTICIPANTS

The sample consisted of 88 Portuguese preschool children and their mothers. The children were all Caucasian and were aged from 40 to 76 months (M = 57.70, SD = 7.25); 45 (51.1%) were girls. Mothers' age ranged from 21-48 years (M = 33.67, SD = 5.43). Regarding education level, 14 mothers (15.9%) had less than nine years of education, 32 completed 9 years of education (36.4%), 28 (31.8%) completed high school, and 14 (15.8%) had a higher education degree. With respect to marital status, 18 (20.5%) of the mothers were single, 11 (12.5%) were living with their partners, 49 (55.7%) were married, 8 (9.1%) were divorced, and 2 (2.3%) were widowed.

Procedure

Ethical approval was obtained from University of Minho, Portuguese Ministry of Education, and National Commission for Data Protection. Following the recruitment of dyads from Portuguese preschools, the assessment was conducted either in participants' homes or preschools, depending on participants' availability. When tested, no group differences according to settings were found regarding the variables of interest. The visit started with the study description. Then, informed consent was obtained from the mothers. Mothers were then asked to join the child in an interactive task. The interaction was videotaped across three 5-minute episodes involving: (a) child play with a challenging toy under mother's guidance; (b) maternal completion of a sham questionnaire while the child has only an uninteresting toy to play with, after being instructed by the mother not to touch more interesting, but difficult-to-reach toys; and (c) mother and child engage in free play for half the period followed by mother-directed child clean-up. Child's saliva for OT concentration analysis was collected in two different time points: 1) before the mother-child interaction task, following a 10-min familiarization period with the researchers (with no contact with the mother); 2) immediately after the end of the mother-child interaction task. Finally, a separate saliva sample was collected for genetic analysis. To control for hormonal variability, data collection was done during the afternoon (between 2 p.m. and 5 p.m.).

Measures

Maternal interactive behaviour

Maternal behaviour was assessed based on the 15-minute videotaped interaction, using Ainsworth's scales for rating maternal sensitivity and cooperation (Ainsworth, Bell, & Stayton, 1974). Each dimension was rated on a 9-point scale, with higher scores indicating more caring behaviour. The videos were coded by a team of trained researchers, who were blind to the sample characteristics; 32 (36%) of them were coded in pairs. Coding achieved good interrater reliability (sensitivity: ICC = .93; cooperation: ICC = .87). In line with previous studies (Baptista, et al., 2013; Juffer, Hoksbergen, Riksen-Walraven, & Kohnstamm, 1997; Tharner et al., 2012), a "sensitive responsiveness" score was computed based on the two highly correlated scales (r = .73, p < .001), by averaging the two z scores.

Child interactive behaviour

The child codings of the Coding System for Mother–Child Interactions (CSMCI, Healey, Gopin, Grossman, Campbell, & Halperin, 2010) were used to assess the child's behaviour. The three subscales of Cooperation/Compliance, Enthusiasm and Negativity/Hostility were rated on an anchored, 5-point Likert scale (1 = very low to 5 = very high). Following the work of Haley et al. (2010), two episodes of the mother-child interaction were considered (episodes (a) and (c) described in the section "Procedure"), in order to include three tasks: 1) child playing with a demanding toy under mother's guidance; 2) free play with the mother; and 3) clean up. Negativity scores were inverted so that higher scores in the three subscales represented better (more adaptive) behaviour. Thirteen (15%) were coded in pairs. Coding achieved good interrater reliability (Cooperation: ICC = .90; Enthusiasm: ICC = .89; Negativity: ICC = .92). In line with Chapter 4, a "child interactive behaviour" score was computed based on the three correlated scales ($r > .40$, $p < .01$), by averaging the three z scores.

Child's Salivary OT Concentrations

To assess child's OT levels, saliva samples were collected using Salivette devices (Sarstedt, Rommelsdorft, Germany). Children were instructed to place a cotton swab in the mouth and chew for a minute. Salivettes were kept chilled before being centrifuged at 4 °C at 3200rpm for 10min. Samples were then stored at −80 °C. Determination of OT was performed using a commercial Oxytocin ELISA kit (Enzo Life Sciences, NY, USA) according to the manufacturer's instructions.

Child's OXTR Genotype

For genotyping, child's saliva was collected using OraGene OG-500 (DNA Genotek, Inc., Ottawa, Ontario, Canada) and stored at room temperature. Genomic DNA was isolated as instructed by the manufacturers, using the standard protocol from PrepIT L2P (DNA Genotek) and sample concentrations were assessed using Nanodrop technology. Analysis of the OXTR SNP rs53576 was performed using 5ng of DNA along with the corresponding KASPar assay (LGC Genomics, UK), for a final volume of 8µL. The thermal profile was performed as instructed by the manufacturers, in a 7500 Fast Real-Time PCR System (Applied Biosystems by Life Technology, USA). Results were

validated by Sanger Sequencing of representative samples for each genotype (AA, AG or GG). Allelic frequencies were in Hardy-Weinberg Equilibrium (GG 50, GA 31, AA 7, χ^2 (1) = 0.488, p = 0.485). Primary statistical analysis contrasted A-carriers (AA/AG) and G homozygotes (GG).

Statistical analysis

Descriptive data and pearson or point-biserial correlations among study variables are presented first. Next, hierarchical regression analysis was used to predict changes in child's OT response. In this analysis, child's genotype (i.e., presence vs absence of the A allele) and maternal sensitive responsiveness were entered separately in the first step, and their 2-way interaction in the second step. Finally, follow-up analysis in the form of *t*-tests were performed.

Drawing from these analyses, a moderation-mediation analysis was conducted whereby the effect of the interaction found (between child's OXTR genotype and maternal sensitive responsiveness) on child's interactive behaviour would be mediated by the child's OT response. To that end, we analysed the mediation's assumptions, and tested the effect of the child's OXTR SNP rs535765 and maternal sensitive responsiveness on child's interactive behaviour, in a 2-step regression model: first we included them independently; and then in interaction.

III. Results

The mean score for sensitive responsiveness was 4.75 (*SD* = 1.57, range 1.5-7.5), and for child interactive behaviour was 3.74 (*SD* = 0.69, range 1.56-4.78).

Regarding child's salivary OT concentrations, the mean score for the baseline was 23.60 pg/ml (*SD* = 14.92); and for the post-interaction was 24.96 pg/ml (*SD* = 23.70). An OT response variable was calculated based on the difference between the OT values post- and pre-interaction task, each of them previously log-transformed.

Concerning genotyping, 50 children (56.8%) were homozygous for the G allele and 31 (35.2%) were heterozygous. Minor allele (A) frequency was 0.26.

Table 5.1 displays the bivariate association of study variables. No association was found between the child's OT response and child's sex, age, interactive behaviour or

OXTR genotype. Likewise, child's OT response was not associated with maternal age, level of education or sensitive responsiveness.

Table 5.1. Correlations between study variables.

	1.	2.	3.	4.	5.	6.	7.
1. Child's Sex	-						
2. Child's Age	-.001	-					
3. Child's Interactive Behaviour	-.042	.280**	-				
4. Child's OXTR	-.026	-.143	.114	-			
5. Mother's Age	-.041	.139	.061	-.138	-		
6. Maternal Educational Level	.077	-.042	.019	.018	.150	-	
7. Maternal Sensitive Responsiveness	-.168	.115	.320**	-.060	.097	.407**	-
8. Child's OT response	.074	.067	.074	-.068	.166	.100	.040

Note. $N = 88$; ** $p < .01$. Child's Sex: 0 girls; 1 boys. Child OXTR: 0 GG; 1 AA/AG.

Regarding the hierarchical regression analysis, inspection of Table 5.2 reveals that maternal sensitive responsiveness and child genotype did not independently predict the child's OT response, but their interaction did predict such response ($B = .084$, $SE = .037$, $t = 2.277$, $p = .025$).

Table 5.2. Prediction model for OT change, considering child OXTR SNP rs53576 and maternal sensitive responsiveness as predictors.

Model; Predictors	R^2 (R^2_{adj})	F (df)	Unstandard. Coefficient		T	p	95% CI	
			B	SE			Lower	Upper
1								
Maternal sensitive responsiveness	.015 (-.008)	.652 (2,86)	.005	.018	.299	.765	-.030	.041
Child OXTR			-.037	.034	-1.082	.282	-.105	.031
2								
Maternal sensitive responsiveness			-.024	.022	-1.108	.271	-.068	.019
Child OXTR	.073 (.040)	2.184+ (3,86)	-.043	.034	-1.293	.200	-.110	.023
Maternal sensitive responsiveness X Child OXTR			.084	.037	2.277	.025	.011	.157

Note. $N = 88$; + $p < 1$

Follow-up analysis showed that, in the presence of lower scores in maternal sensitive responsiveness, the two genotypes had significantly different scores in OT response ($t_{(36)}$ = 3.007, p =.005). More specifically, in the presence of lower quality maternal behaviour, OT levels tended to increase during the interaction only for the G homozygotes (M = .08, SD = .18), and the inverse for A-carriers (M = -.10, SD = .164). In the case of children whose mothers scored more highly in sensitive responsiveness, the two genotypes did not significantly differ in OT change scores. This result is depicted in Figure 5.1.

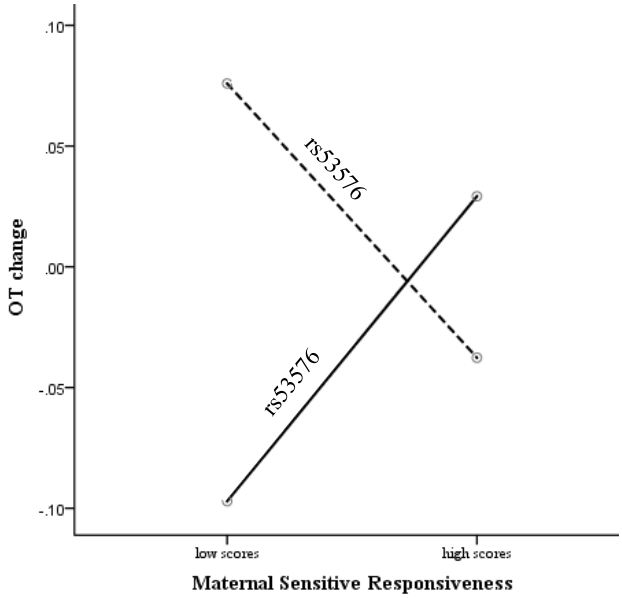


Figure 5.1. Plot of the interaction between child OXTR SNP rs535765 (AG/AA vs GG) and maternal sensitive responsiveness in relation to child OT change during an interactive task with the mother.

Finally, we tested whether differences in the child's behaviour were explained by the interaction effect found (between child's OXTR genotype and maternal sensitive responsiveness), and if child's OT levels mediated this link. We started by analysing the mediation's assumptions. As presented in Table 5.3, we failed to find support for the effect of the predictor (interaction between child's OXTR SNP rs535765 and maternal sensitive responsiveness) on child's interactive behaviour. Only maternal behaviour had

a main effect (B = .315, SE = .100, t = 3.162, p = .002) on child's behaviour. Considering that this assumption was not met, we did not proceed with the analysis.

Table 5.3. Prediction model for child interactive behaviour, considering child OXTR rs53576 (AG/AA vs GG) and maternal sensitive responsiveness as predictors.

Model; Predictors	R^2 (R^2_{adj})	F (df)	Unstandard. Coefficient		t	p	95% CI	
			B	SE			Lower	Upper
1								
Maternal sensitive responsiveness	.119 (.098)	5.617 (2,85)*	.315	.100	3.162	.002	.117	.514
Child OXTR			.239	.190	1.255	.213	-.140	.617
2								
Maternal sensitive responsiveness			.345	.124	2.787	.007	.099	.592
Child OXTR	.121 (.089)	3.763 (3,86)*	.247	.192	1.284	.203	-.136	.629
Maternal sensitive responsiveness X Child OXTR			-.087	.211	-.411	.682	-.506	.333

Note. N = 88; * p <.05

IV. DISCUSSION

In an effort to identify individual and environmental determinants of the variation in the child's OT response during a mother-child interaction, we examined the role of child OXTR genotype and maternal behaviour. Our results indicate that, despite not being independent predictors of the child's OT change, child's OXTR SNP rs53576 and maternal behaviour interact to predict it. Specifically, in the presence of lower quality scores of maternal sensitive responsiveness, child's OT levels tend to increase during the interaction only for the G homozygotes, and the opposite trend was found for A-carriers.

Regarding these two patterns of OT response to maternal behaviour, the fact that, for a group of children (A-carriers) OT seemed to be lower after their interaction with less responsive mothers might be explained by the fact that it would not be perceived as pleasant, rewarding or engaging to the child. Conversely, there was a group of children (G homozygotes) where there was an increase in OT levels during the interaction with less responsive mothers, which is more intriguing, as we would possibly expect a lower

level of engagement and pleasantness in the interaction with a mother who tends to be less sensitive and cooperative. It is, nonetheless, in agreement with the research line that considers OT as a biomarker of social distress. This approach proposes that OT production accompanies gaps or problems with social relationships, providing an impetus for affiliation in situations where such support is not present (cf. Taylor, 2006). Specifically, based on the need to maintain an adequate level of protective and rewarding social relationships, OT would be released to seek affiliative contact.

Interestingly, our results show that these different patterns of response depend on the child's genotype. This difference seems in line with previous findings showing that G-carriers are more likely to seek social support (Kim et al., 2010), and benefit more from that support (Chen et al., 2011), which would conceivably make this genotype more prone to engage in affiliative efforts. G-carriers have also been shown to be more optimistic and with higher levels of self-esteem (Saphire-Bernstein et al., 2011), which is also seemingly consistent with this view.

The interaction effect we found is in line with Bartz et al. (2011), who reflected on the importance of considering an interactionist approach including individual and situational factors when looking at the oxytocinergic function. The authors revised 53 studies on the social effects of OT administration and found that, in the majority of the studies, the effect was person- and context-dependent. Our results, which focus on OT production, support this perspective.

We then tested if the interaction between child's genetics and maternal behaviour predicted the child's interactive behaviour and we did not find such effect. Only maternal behaviour was linked to the child's behaviour, which is perhaps not surprising as both behaviours are interactive and measured simultaneously. The fact that child's OXTR genotype showed a null effect on the child's interactive behaviour is in line with recent discussions on single candidate genes studies, and the difficulties in documenting genotype-phenotype associations based on them (Wray et al., 2013). As most human traits are complex and are likely to be determined by mutations at multiple loci, with independent and/or interaction effects between them (i.e., epistasis) (Robinson, Wray, & Visscher, 2014), future studies should focus on children's interactive behaviour and different correlates from the OT and related systems. In light of the arguments advanced above, it may also be valuable in future research to specifically employ measures that

capture children's affiliative behaviour, as it may be that oxytocinergic effects are restricted to this class of behaviour.

Future research should also assess other constructs related to social interaction, such as emotion regulation (Quirin, Kuhl, & Dusing, 2011), psychopathology (Prenoveau et al. 2013), tactile contact (Feldman, Gordon, Schneiderman, et al., 2010) or synchronous parenting (Feldman, Gordon, Zagoory-Sharon, 2010). All of these constructs have previously shown links to OT. Importantly, we did not assess attachment either. Given our findings regarding the link between maternal behaviour and child's OT response, it would also be informative to investigate if the child's attachment style is contributing to the child's OT function in future studies.

Moreover, it would be interesting and informative to explore the seemingly paradoxical fact that high levels of OT can be associated both with relationship distress and with reduced stress responses. In this regard, Taylor (2006) suggests that this may relate to different patterns of OT activation – where bursts of OT (for example, related to exogenous administration, or in response to anticipated/actual social contact) would reduce stress responses; but persistent elevated OT would associate with relationship distress. This topic should be further examined by future research.

Limitations of the present study should be taken into account in the interpretation of these findings. Firstly, it has a limited sample size, especially as it includes a genetic variable. Nonetheless, the allelic frequencies were in Hardy-Weinberg Equilibrium (HWE), which has been considered a potential indicator of quality (Bakermans-Kranenburg & van Ijzendoorn, 2014; Namipashaki, Razaghi-Moghadam & Ansari-Pour, 2015). As stated by Namipashaki et al., (2015), deviations from HWE may reflect important problems including selection bias, population stratification and genotyping errors, which are not expected to occur in the present study.

Another limitation, which is unavoidable in human research, is the reliance on a peripheral assessment of OT. Although the relation between central and peripheral OT is not fully understood, studies in animals (Wotjak et al., 1998; Carter et al., 2007; Ross & Young, 2009) and humans (eg. Strathearn, Fonagy, Amico, & Montague, 2009) suggest that central and peripheral activities of the OT system are likely to be coordinated. Regarding salivary OT in particular, Carter et al. (2007) demonstrated that it is a reliable biomarker of peripheral OT, showing moderate correlations between plasma and salivary

OT concentrations. This finding was supported by subsequent research (Feldman, Gordon, & Zagoory-Sharon, 2011; Grewen, Davenport, & Light, 2010). A recent meta-analysis of central and peripheral OT concentrations also found this link, but only for exogenous OT administration, not for OT baseline conditions (with no experimental intervention) (Valstad et al, 2017). In our study, the salivary OT measure is based on two time points of a structured interaction, and therefore it would not be considered baseline, but an experimental situation. Nonetheless, further research comparing salivary and plasma OT in different settings and measured simultaneously is needed, as well as further studies on central and peripheral OT.

An additional limitation has to do with the nature of the interaction task used in this research. In fact, unlike Feldman, Gordon, Zagoory-Sharon et al., (2010), we did not find an increase in OT during the interaction, which may be linked to a weak experimental manipulation. Also, not all the episodes included in the 15-min interaction task were supportive/pleasant/rewarding; in the second episode, the child had only an uninteresting toy to play with, after being instructed by the mother not to touch more interesting, but difficult-to-reach toys. This "don't touch" episode might potentially introduce some negative affect or stress. Future studies using different tasks are needed to further analyse the results reported herein.

Despite the limitations, these findings are overall consistent with research indicating that the OT response/oxytocinergic function is not uniform and depends on both individual and situational factors (Bartz et al., 2010). Our results suggest an interplay between genetics and quality of care, which could be pivotal to the understanding of the OT-based foundations of social behaviour and close relationships in young children.

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CHAPTER 6:
GENERAL DISCUSSION

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I. SUMMARY OF RESEARCH FINDINGS

A large body of evidence has focussed on early experience, particularly sensitive parenting, and its crucial role in human development. Nonetheless, what the genetic and environmental basis for this sensitive parenting are and what its consequent impact on children behavioural and neuroendocrine functioning is, has not been fully addressed or understood, and that was the goal of the present thesis. Drawing on previous research, four investigations were carried out aiming at contributing to the incomplete picture found in the existing literature.

The first two experimental chapters (Chapters 2 and 3) focused on the genetic and environmental determinants of maternal behaviour. Specifically, Chapter 2 considered a single-nucleotide polymorphism (SNP) on maternal Oxytocin Receptor Gene (OXTR) as a predictor of maternal care in two separate studies. The first study relied on a sample of 215 Caucasian mothers observed during a structured interactive task with their preschool children, and yielded a non-significant association between the OXTR SNP rs53576 and maternal behaviour. This was so even when the quality of the families' environmental context was addressed, and gene-X-environment (GXE) interaction was considered. The second study looked back on previous literature, and meta-analysed the available literature on the same OXTR SNP and maternal behaviour. A statistically significant effect was found in a homogeneous distribution, which suggested a tendency for G allele homozygotes to display more supportive parenting than A allele carriers. Nonetheless, that effect found was of small magnitude, and the fail-safe analysis indicated that a single additional study with a null result would nullify this finding. The results from both studies highlighted the need for further, larger and more comprehensive inquiries in order to achieve more conclusive results.

Building on previous research reporting on the role of serotonin on oxytocin's (OT) stimulation and release (Jorgensen, Riis, Knigge, Kjaer, & Warberg, 2009; Lee, Garcia, Van de Kar, Hauger, & Coccaro, 2003), as well as on serotonin's role as a modulator of neural circuitry that controls several behavioural and physiological processes which may

be critical for parenting quality, such as cognition and mood (cf. Homberg & Lesch, 2011), Chapter 3 tried to extend research on the genetic determinants of parenting by focusing on the gene encoding the serotonin transporter as a predictor. In a sample of 210 Caucasian mothers and their preschool children, results revealed a gene-X-environment interaction, such that short-allele homozygotes mothers proved more susceptible to the family context than long allele carriers (i.e., sL/LL), displaying the highest and lowest levels of maternal sensitivity, depending on, respectively, low and high levels of environmental adversity. As mothers carrying the long allele evinced similar responsiveness to the environmental context, but to a lesser extent, findings of our competitive model-testing analysis proved consistent with the weak differential susceptibility model of person-X-context interaction. This result supported the importance of considering both genetic and environmental sources of influence when investigating the determinants of parenting.

This doctoral dissertation then focused on the children's functioning. In light of the need for valid, reliable and accessible measures to assess interactive behaviour during preschool age, Chapter 4 provided an adaptation and validation of the child coding of the Coding System for Mother–Child Interactions (CSMCI, Healey et al., 2010), and discussed its applications and implications in future research and practice. In a sample of 220 preschoolers and their mothers, the coding's inter-rater agreement was confirmed, and the use of child Cooperation, Enthusiasm and Negativity as subscales was supported by their correlations across tasks. Moreover, the 3 subscales were correlated with each other, which supported the use of a global child interactive behaviour score. Convergent validity with a measure of emotional and behavioural problems (Child Behaviour Checklist for ages 1 1⁄2-5 (CBCL 1 1⁄2-5; Achenbach & Rescorla, 2000; Portuguese version Dias, Sousa Lima, Machado, Carneiro & Campos, 2017) was established, as well as divergent validity with measures of temperament (Children's Behaviour Questionnaire - Short Form Version (CBQ-SF, Rothbart, 2000; Portuguese version by Lopes, 2011) and mental development (Griffiths Mental Development Scales 2-8 years, Griffiths, 1984; partial Portuguese version Castro & Gomes, 1996). Regarding associations with family variables, child interactive behaviour was only associated with maternal behaviour and psychopathology and not with maternal age or education, nor with the quality of the environmental context. Findings suggest that this coding system is a valid and reliable measure for assessing child interactive behaviour in preschool age children.

As this work focused on mother-child interaction, which has been consistently related to the OT system, Chapter 5 looked at the child's OT response to mother-child interaction, by exploring possible individual and environmental factors (child's OXTR genotype, and maternal behaviour, respectively) affecting such response. An interaction effect of child's OXTR SNP rs53576 by maternal sensitive responsiveness in relation to variation in salivary child OT concentrations was found, such that only in the presence of lower scores in maternal responsiveness, did the two genotypes significantly differ. Specifically, when interacting with low responsive mothers, OT levels increased during the interactive task, for the G allele homozygotes, and diminished for A allele carriers. We then tested if the interaction between the individual and environmental factors identified (child's OXTR SNP and maternal interactive behaviour) in itself predicted the child's behaviour, and whether the child's OT response might mediate such an association. However, such interaction did not significantly predict the child's behaviour, and we therefore did not proceed with the analysis of mediation. Findings from this chapter provided preliminary support for the notion that children's OT and close relationships involves both individual and environmental factors.

In the following sections, these findings and their implications will be discussed in more detail. Some methodological aspects will also be considered, as well as directions for future research.

II. Genetic Determinants of Maternal Behaviour

1. Oxytocin Receptor Gene

Following the recent interest found in literature, in Chapter 2 we analysed the impact of the OXTR on maternal behaviour in a new set of data (Study 1) and made an analytical summary of former research (Study 2). In Study 1, we failed to detect a link between the OXTR SNP rs53576 and parenting; and in Study 2, we found an effect of small magnitude. These results might seem surprising given the widely accepted link between the oxytocinergic system and social and affiliative behaviour. This is the case, for example, of a systematic (but not meta-analytic) review of OT and mother-infant relation, where Galbally, Lewis, van Ijzendoorn, and Permezel (2011) postulated their crucial link. Nonetheless, in the case of the present studies, we focused on a single OXTR

SNP, whose biological function has not been fully established, and with mixed results in previous research.

Specifically regarding this OXTR SNP, two previous meta-analyses have investigated its role on social behaviour. One found no effect (Bakermans-Kranenburg & van IJzendoorn, 2014), whilst the other, which distinguished between general sociality (i.e., responses to other people in general) and close relationships (i.e., responses to individuals with close connections, such as parent-child or romantic relationships), found a specific association between this SNP and general sociality (Li et al., 2015). This specificity is, however, not in accordance with the significant association we found between OXTR SNP rs53576 and maternal behaviour, which is a form of close relationship. Nonetheless, Li et al.'s (2015) meta-analysis, which considers 29 studies and a total of 41 samples, did not distinguish between types of close relationship (i.e., parent-child and romantic/marital), which raises the possibility of this polymorphism being differentially associated with different forms of close relationship. This hypothesis needs further clarification.

Also relevant to consider, is the possibility of an environmental moderator explaining the failure to detect a direct genotype-phenotype association linking OXTR with parenting in previous studies. In our study, we did explore that possibility, based on a multi-dimensional composite of the quality of the family environment, which included: teenage pregnancy; single parenthood; (low) parental educational level (i.e., one of the parents had under nine years of education); parental unemployment (i.e., one of the parents was unemployed at the time of the study); economic difficulties; absence of social support; family conflict; maternal psychopathology; and chronic health conditions in the family living with the child. This composite is in line with extensive literature showing these factors put strain on the parent-child relationship and constitute risk factors for supportive parenting, and child's healthy socio-emotional development (Cicchetti, Rogosch, & Toth, 1998; Gardner et al., 2004; Webster-Stratton & Hooven, 1998). However, despite the oxytocinergic system being regarded as especially sensitive to environmental conditions (Feldman, 2012), our findings failed to find support for any GXE interaction in relation to maternal sensitive responsiveness. Even if some degree of risk was captured in our study, the fact that our research involved a community sample with a limited number of families at very high risk might have contributed to this null finding. Moreover, it is also possible that other environmental moderators might have

revealed evidence of such GXE effect, as was true for the moderating effect of interparental conflict documented by Sturge-Apple, Cicchetti, Davies, and Suor (2012). Therefore, caution is warranted in embracing null findings as the absence of evidence cannot be taken as evidence of absence.

2. Serotonin Transporter Gene

Chapter 3 than proceeded to the analysis of the relation between the serotonergic system and maternal behaviour. Results were in favour of the weak differential susceptibility model, considering both Akaik and Bayesian Information Criteria (AIC and BIC, respectively) – even if this model did not prove significantly different from the weak diathesis-stress one, and results would thus benefit from replication. This is generally in line with a meta-analysis showing that 5-HTTLPR is a genetic marker of differential susceptibility in Caucasian children and adolescents (van IJzendoorn, Belsky, & Bakermans-Kranenburg, 2012). According to this result, the same genotype that increases vulnerability to poor outcomes under adverse environmental conditions, has the potential to be advantageous when the environment is supportive or enriched (Belsky et al., 2009). As stated by Brüne (2012), this "for better or for worse" susceptibility would better explain why these allelic variants were maintained by natural selection through evolution – since it would be implausible to assume that natural selection would favour allelic variants that exclusively increase vulnerability to adversity.

A relevant issue to address in differential susceptibility studies is the conceptualization of the environmental variable. Specifically, authors have argued that it is crucial to take into account not only the presence vs absence of adversity, but also what could be seen as the "bright side" of the environmental continuum, meaning a protective and beneficial environment (Belsky & Pluess, 2009). According to Belsky et al. (2009), this is indeed a methodological problem that is prevalent in most GXE interaction studies, whereby the absence of adversity is treated as the "good" end of the environmental continuum. This may lead to the under-detection of differential susceptibility findings and an overrepresentation of vulnerability ones. Despite acknowledging this matter, in the experimental work presented here it proved challenging to include a distinct "bright side", as there was no clear discrepancy between what would be protective or risk factors (for example, "economic difficulties" could either be considered a protective or a risk

factor, depending on his absence/presence respectively). Nonetheless, an effort was made to include a broad range of psychosocial variables in order to capture a greater spectrum of environmental diversity. As mentioned in the previous subheading, exploring other environmental factors might provide more information on the specificity of this GXE effect.

The interaction effect found in Chapter 3 contrasts with the null finding regarding the OXTR SNP. In the case of the 5-HTTLPR polymorphism, our results underline the importance of considering both genetic and environmental sources of influence when investigating the determinants of parenting. In fact, this GXE effect raises the possibility that inconsistencies across prior studies – namely the s allele being related to either *lower* (Bakermans-Kranenburg & van IJzendoorn, 2008; Morgan, Hammen, & Lee, 2016) or *greater* sensitivity (Cents et al., 2014; Mileva-Seitz et al., 2011); or even to the absence of a genetic main effect (Sturge-Apple et al., 2012) – might partially relate to differences in the samples' environmental context, that was not taken into account by considering GXE interaction.

3. Comment on Genetic Effects on Maternal Behaviour

Chapters 2 and 3 focused on genotype-phenotype associations. Even if results of this type of associations are not always clear, there are benefits in the identification of specific genomic variants that are the basis for individual differences. As stated by Robinson, Wray, and Visscher (2014) these studies enable prediction, risk profiling, and personalized medicine; identification of pathways; and the understanding of the influence of selection and the maintenance of diversity through time.

At the forefront of genotype-phenotype association studies was the candidate gene approach, used in Chapters 2 and 3. Studies based on this approach have an *a priori* hypothesis, which is grounded on prior scientific evidence (Foulkes, 2009), and are easy to implement, as they are relatively cheap and quick to perform (Patnala, Clements, & Batra, 2013). They are valuable and clinically relevant, but are criticised on some aspects. A major criticism relates to its reductionism, as most human traits are genetically complex and are likely to be determined by variations at multiple – not just one – loci, with each exerting a small effect, and possibly doing so in interaction with multiple genetic variants (i.e., epistasis) (Robinson, et al., 2014). Additionally, non-genetic factors also play a

crucial part in these traits, and GXE interaction may also undermine the ability to discern consistent main effects (Robinson, et al., 2014). Evidence of the impact of multiple loci and GXE interaction comes, for example, from Belsky and Beaver (2011). The authors examined five loci (dopamine receptors D2 and D4, 5-HTTLPR, dopamine transporter and monoamine oxidase A), in adolescents and reported that each of the alleles can exert an additive effect on behaviour: the more plasticity alleles carried, the more and less self-regulation was manifested under, respectively, supportive and unsupportive parenting conditions (Belsky & Beaver, 2011).

Regarding maternal behaviour in particular, a previous study has considered simultaneously the oxytocinergic and serotonergic systems. In this study, the OXTR SNP rs53576 was shown to have a similar but independent effect to the 5-HTTLPR on maternal care (Bakermans-Kranenburg & van Ijzendoorn, 2008). Concretely, the authors found that the presence of the A allele of the OXTR SNP rs53576 or the s allele of 5-HTTLPR both explained 3% of the variance of maternal sensitivity, yet no interaction or potentiating effect of the two SNPs was found.

These studies have partially addressed single candidate approach's limitations, by including more than one gene. Another and more comprehensive way of addressing this issue is genome-wide association studies (GWAS), which involve whole and partial genome-wide scans. Despite sharing similar underlying goals with candidate gene studies (to identify associations between SNPs and a trait), GWAS tend to be less hypothesis driven and involve the characterization of a much larger number of SNPs across the genome (Foulkes, 2009). It therefore represents a promising way of revealing possible molecular pathways involved in complex phenomena. Nonetheless, these studies involve extensive amounts of data and computational burden (Patnala, et al., 2013), which reduce their applicability in smaller settings. As argued by Foulkes (2009), GWAS do not deny the need for candidate gene studies, as these can validate findings from GWAS, and further explore the interaction between genes and traits.

Therefore, despite recognizing the limitations of the candidate gene approach used in this dissertation, this work was based on hypothesis already rose in literature, and intended to contribute to the available knowledge on this topic, using statistically adequate measures.

III. CHILDREN'S INTERACTIVE BEHAVIOUR AND CHILD'S OT RESPONSE

1. CHILDREN'S INTERACTIVE BEHAVIOUR

Chapter 4 tried to address the gap in validated, reliable and widely available observational measures regarding preschoolers interactive behaviour. Findings from this study indeed suggest that this coding system is a valid and reliable measure for assessing child interactive behaviour in preschool age children. It therefore represents an important alternative for this area of research and practice, with reduced costs and with more flexible training requirements compared to several existing alternatives (eg. Coding Interactive Behavior, CIB, Feldman, 1998; Emotional Availability Scales, EA Scales – 4th Ed, Biringen, 2008).

In fact, the high values of reliability suggest that the content of the subscales was well operationalized and clear for the coders. Moreover, support was found for the use of the subscales individually, or combined into a global score for child interactive behaviour.

Regarding associations with other relevant constructs, we found a negative association with maternal psychopathology, in line with a vast literature on its impact on child development and behaviour (Cicchetti, et al., 1998; Cummings & Davies, 1994; Goodman & Gotlib, 1999). Child behaviour was, perhaps surprisingly, not associated with maternal education, nor with the quality of the environmental context. Different factors might account for this last result, for e.g., other environmental factors than the ones included in our environmental composite may play a role and should be considered in future research; also, addressing the individual – not composited – role of each of the factors included in the composite might provide additional insights on how they independently relate to child behaviour.

Concerning the positive associations between mother and child's interactive behaviour, the consistent pattern found implies a close relation between ratings of maternal and child behaviour, both in the subscales, and in the global behaviour indices. This pattern may illustrate how attachment-related maternal care and indices of adaptive child behaviour are being reciprocated within the dyad. It is also in line with research emphasising the role of sensitive parenting in facilitating positive exchanges and the establishment of a mutually responsive relationship (e.g., Kochanska, 1997), as well as promoting a range of adaptive outcomes in children, such as children's cooperative and enthusiastic behaviour and positive emotion (Eisenberg et al., 2001; Kochanska &

Murray, 2000). That said, both directions (mother-child; child-mother) have been documented by previous literature and, as this study is cross-sectional, a direction of causality should not be implied.

2. Children's OT Response

Chapter 5 reported on the child's OT response to mother-child interaction. Different patterns of response to maternal behaviour were found for the two OXTR SNP rs53576 genotypes. For A allele carriers, OT seemed to be lower after the child's interaction with less responsive mothers - perhaps because the interaction was not perceived as pleasant, rewarding or engaging to the child. Conversely, for G allele homozygotes there was an increase in OT levels during the interaction with less responsive mothers. Despite being intriguing, as we would possibly expect a lower level of engagement and pleasantness in the child's interaction with a mother who tends to be less sensitive and cooperative, this result might reflect previous findings on this SNP. Specifically, G-carriers have been shown to be more likely to seek social support (Kim et al., 2010), to benefit more from that support (Chen et al., 2011), and to be more optimistic and with higher levels of self-esteem (Saphire-Bernstein, Way, Kim, Sherman, & Taylor, 2011). This would possibly make G-carriers more prone to make affiliative efforts in situations where such support is not present, in order to maintain an adequate level of protective and rewarding social relationships. This would be in line with the perspective of OT as a biomarker of social distress, where OT production accompanies gaps or problems with social relationships, providing an impetus for affiliation even in situations where such support is not present (cf. Taylor, 2006). Nonetheless, this was not directly measured in this chapter, and it would be valuable to do so in future studies.

As suggested, it would be informative to explore this seemingly paradoxical fact, where OT can relate to both reduced stress responses (as, would be the case for A allele carriers in our study), but also to relationship distress (as would happen for G allele homozygotes). Authors have suggested that it relates to different patterns of OT activation – where bursts of OT (for example, related to exogenous administration, or in response to anticipated/actual social contact) would reduce stress responses; but persistent elevated OT would associate with relationship distress (Taylor, 2006; Taylor et al., 2006). This

hypothesis still needs further examination, not only on the pattern but also on the processes behind it.

Nonetheless, our results seem to indicate that the OT response depends on both individual and environmental factors, specifically, on genetic background of the OXTR and maternal sensitive responsiveness.

IV. METHODOLOGICAL CONSIDERATIONS

1. THE RELIABILITY OF OBSERVATIONAL MEASURES

There are clear advantages in the use of observational measures, namely direct observation, the operationalization of constructs and the non-reliance on the parents or children's ability to describe or quantify complex behavioural and relational constructs (Lindahl & Malik, 2001). This dissertation relied on these types of measures for the coding of both maternal and child interactive behaviour. Specifically, for the coding of maternal behaviour, we used the Ainsworth's Maternal Sensitivity Scales (Ainsworth, Bell, & Stayton, 1974), a well-established measure which has proven highly reliable and valid in a wide range of studies, and has been used in the preschool period (Corval et al., 2017; Oliveira, Fearon, Belsky, Fachada, & Soares, 2015; Baptista, Belsky, Mesquita, & Soares, 2017). Regarding the child's interactive behaviour, the Coding System for Mother–Child Interactions (CSMCI, Healey, et al. 2010) was used, which has also proved to be valid and reliable, as reported in Chapter 4.

Despite the validity of both of these coding systems, which in our sample achieved good reliability scores, the use of observational measures also entails limitations that should be acknowledged. One of them is the possibility of observer bias, related to the observer's impact on the coding of observations. Another important limitation has to do with the reactivity effect (Kazdin, 2003), as the process of being observed might affect participants' behaviour and, therefore, the generalisability of the information collected. A third important limitation is the subjective definition of the adequacy of the setting and task(s) observed (Aspland, 2001).

The work reported here tried to address some of these limitations. For example, a structured task was used in order to minimise bias, and to elicit the behaviours of interest. This way, it would be possible to rely on a shorter interaction (compared to an

unstructured home observation, for example), and thus improve data collection and coding, which are time consuming tasks. The choice of the task's episodes was informed by previous research (Bakermans-Kranenburg and van Ijzendoorn, 2008; Michalska et al., 2014).

The setting was also considered, as it has been argued that differences in settings (laboratory vs. home settings), may also impact on results, with laboratory settings possibly being more stressful (Mileva-Seitz, et al., 2013). In our total sample (N = 220), due to constraints in families' and schools' availability, 22.4% of the dyads were observed in their homes, and the rest of them in the children's preschools. No group differences were found in terms of maternal or child interactive behaviour based on the setting, so we do not expect this factor to influence our results.

We also tried to minimise reactivity to the observation procedure, by allowing time for participants to become familiarised with the researchers and with the observational procedures, and minimising the intrusiveness of the recording equipment. The fact that both maternal and child interactive behaviour scores have a large range suggests that a wide variety of behavioural performances were captured.

2. The Validity of Saliva Samples as a Peripheral OT Measure

For the analysis of the child's OT levels, we used a peripheral assessment of OT, which is unavoidable in humans. Although the relations between central and peripheral OT are not fully understood, studies in animals (Wotjak et al., 1998; Carter et al., 2007; Ross & Young, 2009) and humans (eg. Strathearn, Fonagy, Amico & Montague, 2009) suggest that they are likely to be coordinated. This is partially supported by a recent meta-analysis of 17 studies on central and peripheral OT concentrations, which found their positive association. Nonetheless, this study reported a moderation by experimental context, such that this association was not significant for OT baseline (condition with no experimental intervention), but only for exogenous OT administration conditions (Valstad et al., 2017). In our study, however, peripheral OT concentrations were assessed in two time-points of a structured interaction, and therefore it would not classify as a baseline condition, but as an experimental situation.

Regarding the type of peripheral measures used, saliva samples have some advantages, particularly the fact that they are non-invasive (Carter et al., 2007; White-

Traut et al., 2009). This is especially relevant in the case of children, for whom blood or urine collection would be complex, and conceivably more stress inducing – which is debatable for ethical reasons, and also due to the bias that might arrive from the interaction between OT and stress response hormones (White-Traut et al., 2009). Possible studies have shown that saliva samples area reliable biomarker of peripheral OT (Carter et al., 2007; Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Gordon, & Zagoory-Sharon, 2011; Grewen, Davenport, & Light, 2010; White-Traut et al., 2009). With particular relevance for our inquiry, a study with 112 mothers and fathers interacting with their 4-6 months old infants measured plasma and salivary OT simultaneously and found their association to be high (plasma OT and precontact salivary OT $r = .41$, $p < .000$; plasma OT and postcontact salivary OT, $r = .33$, $p < .000$) (Feldman, Gordon, Schneiderman et al, 2010). In this investigation, plasma and salivary OT levels were related to parents' and children's social engagement and affect synchrony, which provides support for our research methods. Nonetheless, further research on central and peripheral OT is needed, as well as studies comparing salivary and plasma OT in different settings and measured simultaneously.

In our study we assessed salivary OT during an interactive task and found child's OT levels within the same range to the ones previously published (Feldman, Gordon, Influs, Gutbir, & Ebstein, 2013; Feldman, Gordon, & Zagoory-Sharon, 2010; Lebowitz, et al., 2016; Lebowitz, et al., 2017; Nishizato, Fujisawa, Kosaka, & Tomoda, 2017). However, we failed to show an OT increase during the interaction. This may be related, for example, to the specific interaction task used in this research. In fact, not all the episodes included in the 15-min interaction were supportive/pleasant/rewarding; in the second episode, the child had only an uninteresting toy to play with, after being instructed by the mother not to touch more interesting, but difficult-to-reach toys. This "don't touch" task might potentially introduce some negative affect or stress that might affect OT increase during the interaction. Future studies using different tasks are needed to further analyse these results.

3. Sample Sizes and Power

The studies reported herein relied on a global sample of 220 Portuguese dyads, and the Chapters 2 and 3 included genetic predictors. As studies based on the candidate gene approach have difficulties in documenting genotype-phenotype associations (Wray et al.,

2013), and even when such associations are detected, they tend to be of small in magnitude, large samples might be required to detect them (NCI-NHGRI, 2007). In fact, as Robinson et al. (2014) discuss, large samples are generally needed to have adequate power to detect many commonly observed genetic effects.

Despite being restricted in sample size, the work presented here addressed one of the factors suggested to undermine the ability to discern genotype-phenotype main effects (Robinson, et al., 2014): the possibility of GXE interaction effects. As discussed by Thomas (2010), the study of GXE interactions can shed light on fundamental biological mechanisms, and for that reason should be considered. Here, in Chapters 2 and 3, the contextual conditions of the families were assessed, particularly those typically associated with maternal behaviour, by means of a quality of the environmental context composite. Nonetheless, as commented on above, even if our sample varied in this environmental quality index, the number of families at very high risk was limited. Results must, thus, be cautiously considered, as these (or other) environmental variables, tested in larger and higher-risk samples, might impact on the findings. As a final remark on this matter, replication studies, performed independently in other samples in the future, would also be of high relevance to validate the genotype–phenotype, and GXE interaction effects found (Chanock et al., 2007).

V. FUTURE STUDIES

The first, more general note from this work for future research is to expand the investigation on maternal interactive behaviour and child functioning, in order to include different individual and environmental aspects. Next, some more specific directions will be explored.

In what concerns the study of parenting, analysing different types of behaviour in the same sample might prove useful, as previous studies have looked at various parenting behaviours and found different patterns of associations (cf. studies reviewed in Chapters 2 and 3).

In the case of the GXE effect found between the 5-HTTLPR and environmental conditions, it would be relevant to consider possible mechanisms – for example, maternal cognitive functioning (cf. Homberg & Lesch, 2011) or amygdala activation (Furman,

Hamilton, Joormann & Gotlib, 2011) - by which the polymorphism affects sensitivity to context and, thereby how family conditions exert influence on parenting. This would provide insights into "what works for whom", and help in matching interventions to mother–infant dyads (Belsky & van IJzendoorn, 2015).

Regarding the child coding of the Coding System for Mother–Child Interactions (CSMCI, Healey et al., 2010) validated here, future research should expand this work to clinical populations, other age groups, and to other informants of child behaviour, beside the mother.

Finally, with respect to research on OT, and particularly OT response, analysing other social interaction constructs, which have previously been related to OT, might provide important insights. Pertinent examples include emotion regulation (Quirin, Kuhl, & Dusing, 2011), psychopathology (Prenoveau et al. 2013), tactile contact (Feldman, Gordon, Schneiderman, et al., 2010) or synchronous parenting (Feldman, Gordon, Zagoory-Sharon, 2010). It would also be informative to explore the above-mentioned paradoxical observation that high levels of OT can be associated both with relationship distress and with reduced stress responses, perhaps looking at the pathways and patterns of activation associated with each of these interlinked outcomes (Taylor, 2006). The assessment of cortisol levels as an indicator of distress during the interaction task, along with OT levels, could prove helpful in this endeavour. Moreover, it would be important to further investigate the relation between OXTR SNPs and OT levels in greater depth, in order to better understand the functional significance of these gene variants. Finally, it would be interesting to look at child and mother's OT levels simultaneously, and analyse if they display the same pattern, and if similar individual-situational variables contribute to them.

VI. CONCLUSIONS

The work carried out in this dissertation generally lends support to the centrality of the interaction between individual and environmental factors, in shaping both behavioural and neuroendocrine responses. One of the major findings from this study is, indeed, the importance of GXE interactions. In fact, our results are in line with Wray et al. (2013) in their discussion of the difficulties in documenting genotype-phenotype associations when

focused on single candidate genes. This is intrinsically a challenging enterprise because most human behavioural phenotypes are likely to depend on the small effect of many genes, possibly in interaction among them (i.e., epistasis), but also because gene-environment interactions may undermine the ability to discern main effects (Robinson et al., 2014). Currently, GXE interactions provide an important framework for an array of research, which has a number of different implications both for research and practice. Indeed, a meta-analysis of such GXE work revealed greater effects of a variety of experimental manipulations for hypothesized susceptible genotypes than non-susceptible genotypes (Bakermans-Kranenburg & van IJzendoorn, 2015). Evidence like this provides relevant information to prepare and provide targeted interventions. Regarding the biological systems analysed, the relevance of such interaction effects seems clear, both for the oxytocinergic and serotonergic systems.

This dissertation also contributes to an important gap in the literature regarding preschool age. In fact, despite the growing interest in OT and affiliation, studies with children are still scarce, both considering genetic and neuroendocrine assessments. By addressing this gap, we hope to contribute to the understanding of typical development, which can consequently enable a better understanding of pathological trajectories.

Research would benefit from further investigation into this field, carried out in larger scale samples, and following broader approaches, which include different biological, psychological and environmental aspects of the individual's functioning.

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