ALLELIC VARIATION IN THE OXYTOCIN RECEPTOR GENE AND EARLY-EMERGING SOCIAL BEHAVIORS IN BOYS AND GIRLS

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ABSTRACT

The role of neuropeptide oxytocin in human social behavior has been examined in several recent studies. It has been shown that genetic variations in oxytocin function are associated with individual differences in various social behavioral and cognitive processes. One of the most extensively studied genetic variations has been a common guanine (G) to adenine (A) substitution in the oxytocin receptor gene (OXTR, *rs53576*). Accumulated research has shown heightened levels of social behavior in the *rs53576* G/G homozygotes compared to the A-carriers. However, the majority of the existing studies have been conducted in adults, so little is known about how genetic variations in oxytocin function are associated with social behavior in early development, and therefore, about the developmental processes that lead to the adult phenotype. In this thesis, this question was addressed by studying the effects of allelic variation in the OXTR *rs53576* on early-emerging social behaviors in human infants and children. Also, potential interactions between the OXTR *rs53576* genotype and gender on social behaviors were explored.

A group of 79 children were genotyped for the OXTR *rs53576* at 7 months of age. These children were tested for early-emerging social behaviors at 7, 24, and 48 months of age. The final sample sizes at each age varied due to participant attrition. The children were tested for attention to facial expressions of emotion at 7 months of age (n = 66), for empathy, helping behavior, and social referencing at 24 months of age (n = 27), and for emotion recognition and theory of mind at 48 months of age (n = 61).

The results showed that the allelic variation in the OXTR *rs53576* had an effect on some measures of early-emerging social behaviors in boys but not in girls. Compared to the A-carrier boys, boys with the G/G genotype exhibited enhanced attention to fearful and happy facial expressions when they were 7-month-old, and behaved more prosocially when they were 24-month-old. Genotype did not affect emotion recognition or theory of mind when the children were 48-month-old. If confirmed with larger sample sizes, these results suggest that allelic variation in the OXTR *rs53576* interacts with gender to influence early social behavior in human.

Key words: oxytocin, OXTR, gender, development, social behavior

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TIIVISTELMÄ

Oksitosiinineuropeptidin vaikutuksia ihmisen sosiaaliseen käyttäytymiseen on tutkittu useissa viimeaikaisissa tutkimuksissa. Perinnöllinen vaihtelu oksitosiinin toiminnassa liittyy yksilöllisiin eroihin erilaisissa sosiaalisen käyttäytymisen ja tiedonkäsittelyn prosesseissa. Yksi tutkituimmista perinnöllisistä vaihteluista on verrattain yleinen guaniinin (G) korvautuminen adeniinilla (A) oksitosiinireseptorigeenissä (OXTR, *rs53576*). Karttuneen tiedon mukaan G/G-homotsygootit käyttäytyvät sosiaalisemmin kuin A-alleelin kantajat. Toisaalta suurin osa tutkimuksista on tehty aikuisilla, joten siitä miten perinnöllinen vaihtelu oksitosiinin toiminnassa liittyy sosiaaliseen käyttäytymiseen varhaisen kehityksen aikana, ja siitä millaiset kehitykselliset prosessit johtavat aikuisen fenotyyppiin, tiedetään hyvin vähän. Tässä tutkielmassa pyrittiin vastaamaan tähän kysymykseen selvittämällä OXTR-geenin alleelisen vaihtelun (*rs53576)* vaikutusta varhain ilmeneviin sosiaalisen käyttäytymisen muotoihin varhaislapsuudessa. Lisäksi tarkasteltiin mahdollista OXTR *rs53576* -genotyypin ja sukupuolen yhdysvaikutusta sosiaaliseen käyttäytymiseen.

DNA-analyysin avulla selvitettiin 79 vauvan OXTR *rs53576* -genotyyppi seitsemän kuukauden iässä. Näiden lasten varhain ilmeneviä sosiaalisen käyttäytymisen muotoja tutkittiin 7, 24 ja 48 kuukauden iässä. Jokaisen mittausajankohdan lopulliset otoskoot vaihtelivat osallistujakadon vuoksi. Kun lapset olivat seitsemän kuukauden ikäisiä, tutkittiin miten he kiinnittävät tarkkaavaisuutta emotionaalisiin kasvonilmeisiin (n = 66). Kun lapset olivat 24 kuukauden ikäisiä, tutkittiin empatiaa, auttamiskäyttäytymistä ja sosiaalista varmistamista (n = 27). Kun lapset olivat 48 kuukauden ikäisiä, tutkittiin emootioiden tunnistusta ja mielen teoriaa (n = 61).

Tulokseksi saatiin, että OXTR-geenin alleelinen vaihtelu (*rs53576)* vaikutti joihinkin varhain ilmeneviin sosiaalisen käyttäytymisen muotoihin pojilla, mutta ei tytöillä. Verrattuna A-alleelia kantaviin poikiin, G/G-genotyypin omaavat pojat osoittivat tehostuneempaa tarkkaavaisuutta iloisiin ja pelokkaisiin kasvonilmeisiin seitsemän kuukauden iässä ja käyttäytyivät prososiaalisemmin 24 kuukauden iässä. Genotyypillä ei ollut vaikutusta emootioiden tunnistukseen eikä mielen teoriaan 48 kuukauden iässä. Jos nämä tulokset pystytään varmistamaan suurempia otoskokoja käyttäen, ne viittaavat siihen, että OXTR-geenin alleelinen vaihtelu (*rs53576*) vaikuttaa yhdessä sukupuolen kanssa ihmisen varhaiseen sosiaaliseen käyttäytymiseen.

Asiasanat: oksitosiini, OXTR, sukupuoli, kehitys, sosiaalinen käyttäytyminen

CONTENTS

1. INTRODUCTION

Recently, several studies have examined the role of neuropeptide oxytocin in human social behavior and cognition (reviewed in Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011; Zink & Meyer-Lindenberg, 2012). It has been suggested that genetic variations in oxytocin function (especially in the oxytocin receptors through which oxytocin exerts its functions as a neurotransmitter), may provide an important basis for individual differences in social behavior in humans (Breton & Zingg, 1997; Inoue et al., 1994; Insel & Young, 2000). The most consistent evidence for this hypothesis comes from studies linking a common guanine (G) to adenine (A) substitution in the third intron of the oxytocin receptor gene (OXTR, *rs53576*) with neural processing of social cues, empathy, and prosocial behavior in human adults (reviewed in Meyer-Lindenberg et al., 2011). However, little is known about how genetic variations in oxytocin function are expressed in early development and therefore, about the developmental processes that lead to the adult phenotype (e.g. prosocial behavior). In this thesis, this question was addressed by studying the effects of allelic variation in the OXTR *rs53576* on early-emerging social behaviors in human infants and children. Also, the interaction between the OXTR *rs53576* genotype and gender was examined, because a recent study suggests that the effects of OXTR *rs53576* on social behavior might be different for females and males (Tost et al., 2010).

1.1. Oxytocin, oxytocin receptors, and the oxytocin receptor gene (OXTR)

Oxytocin is a neuropeptide that acts both as a hormone and a neurotransmitter (e.g. Gimpl & Fahrenholz, 2001; MacDonald & MacDonald, 2010). Oxytocin consists of nine amino acids (Gimpl & Fahrenholz, 2001), and it is primarily synthesized in the hypothalamus (e.g. Lee, Machbeth, Pagani, & Young^{3rd}, 2009; MacDonald & MacDonald, 2010), but also in smaller amounts in other tissues (Carter, 2007). It is secreted from the hypothalamus through the posterior pituitary to blood circulation (Carter, 2007; Lee et al., 2009). Oxytocin is also released into the central nervous system, where it acts as a neurotransmitter affecting several different areas such as the amygdala, brainstem, hippocampus, hypothalamus, and striatum (Gimpl & Fahrenholz, 2001). It also has functional interactions with hormonal and neurotransmitter systems such as gonadal hormone, dopamine, and serotonin systems (Meyer-Lindenberg et al., 2011). Oxytocin has an important role in typical mammalian functions facilitating reproduction such as uterine contraction during labor

and milk ejection during lactation (reviewed in Gimpl & Fahrenholz, 2001), and it also mediates, for example, attachment (Insel, 1997) and social cognition (e.g. Heinrichs, von Dawans, & Domes, 2009; Meyer-Lindenberg et al., 2011).

Oxytocin concentrations in blood plasma, cerebrospinal fluid, saliva, and urine may not reflect intracerebral oxytocin release (Neumann, 2008; MacDonald & Macdonald, 2010). For this reason, effects of oxytocin on human behavior have been studied mostly via intranasal administration (see MacDonald & Macdonald, 2010), because intranasally given peptides have direct access to cerebrospinal fluid and thus to the brain (Born et al., 2002). Kosfeld, Heinrichs, Zak, Fischbacher, and Fern (2005) were the first to study the effects of intranasally administered oxytocin on human behavior, and they found that oxytocin increased trust in a trust/betrayal game. A study by Rimmele, Hediger, Heinrichs, and Klaver (2009) was the first to show that intranasally administered oxytocin specifically improved the processing of social information (recognition memory of faces) but not of non-social information. These early findings were subsequently corroborated in studies showing that intranasally administered oxytocin modulates activity in brain areas that are involved in social information processing (e.g. the amygdala, Domes, Heinrichs, Michel, Berger, & Herpertz, 2007b; Domes et al., 2010; Kirsch et al., 2005; Petrovic, Kalisch, Singer, & Dolan, 2008; Rilling et al., 2012), increases gaze to the eye region of human face in healthy males (Domes, Steiner, Porges, & Heinrichs, 2013; Guastella, Mitchell, & Dadds, 2008) and patients with high-functioning autism spectrum disorder (Andari et al., 2010), enhances the ability to infer others' mental states from social cues of the eye region (Domes et al., 2007b), enhances recognition of facial expressions of emotions in healthy individuals (Van IJzendoorn & Bakermans-Kranenburg, 2012) and patients with autism spectrum disorders (Guastella et al., 2010), and increases empathy (Bartz et al., 2010; Hurlemann et al., 2010) and prosocial behavior (reviewed in Heinrichs et al., 2009; MacDonald & MacDonald, 2010; Meyer-Lindenberg et al., 2011; Ross & Young, 2009).

Neurotransmitters exert their functions through receptors, and each neurotransmitter fits into the binding site of a specific receptor in a same way as a key fits into its lock (Kandel, & Siegelbaum, 2000). The action of a neurotransmitter in the postsynaptic cell is determined by the properties of the receptors it binds with (Kandel, & Siegelbaum, 2000). Oxytocin is known to have only one kind of receptor (OTr) (Ebstein, Knafo, Mankuta, Chew, & Lai, 2012), through which it exerts its functions as a neurotransmitter (Breton & Zingg, 1997; Inoue et al., 1994). Oxytocin receptors are found throughout the brain (Lee et al., 2009), but also in other parts of the body (Gimpl & Fahrenholz, 2001). Studies examining sex differences in oxytocin function have shown higher oxytocin levels and oxytocin receptor expression in females, as well as indications of differences in

oxytocin and oxytocin receptor distributions between brains of different sexes (reviewed in Carter, 2007). It is suggested that these differences could explain variations in behaviors in which oxytocin plays a central role (Lee et al., 2009). The distribution of oxytocin receptor also differs between different species (Carter, 2007), for example between rodents and humans (Zink & Meyer-Lindenberg, 2012). Also, there are individual differences in the distribution of oxytocin receptors (Carter, 2007). These differences might be due to variation in the receptor gene structure (Insel & Young, 2000).

In humans, oxytocin receptor protein is encoded by the oxytocin receptor gene (OXTR) located on the 3rd chromosome of human genome, and it consists of three introns and four exons (Inoue et al., 1994; Simmons, Clancy, Quan, & Knoll, 1995). There are several relatively common single nucleotide variations in the OXTR gene in humans (e.g. Israel et al., 2009). One of the most extensively studied of these single nucleotide polymorphisms (SNPs) is a guanine (G) to adenine (A) substitution in the third intron of the OXTR gene (*rs53576,* e.g. Bakermans-Kranenburg & van IJzendoorn, 2008; Meyer-Lindenberg et al., 2011). This variation results in three possible *rs53576* genotypes: G/G, G/A, and A/A of which the G/G and G/A are more common than the A/A genotype (e.g. Bakermans-Kranenburg & van IJzendoorn, 2008; Poulin, Holman, & Buffone, 2012).

The significance of the G to A substitution in the OXTR *rs53576* for oxytocin function is not known but there is consistent evidence suggesting that individual with two copies of the G allele (the G/G genotype) exhibit heightened levels of social behavior across a variety of measures. Compared to the A-carriers (G/A and A/A genotypes), individuals with the G/G genotype are more able to infer mental states of others (Rodrigues, Saslow, Garcia, John, & Keltner, 2009), experience higher levels of empathy (Rodrigues et al., 2009), display higher levels of parental sensitivity (Bakermans-Kranenburg & van IJzendoorn, 2008), and show structural and functional differences in the hypothalamic-limbic system (e.g. heightened activity in the amygdala in response to facial expressions) that correlate with increased levels of prosocial behavior, especially in males (Tost et al., 2010). Because hypothalamic-limbic circuits are critical for emotion regulation and sociality in humans, Tost et al. (2010) suggest that males are more at risk for social dysfunction based on the effects of OXTR *rs53576* on these neural circuits. Poulin and his colleagues (2012) have further shown that greater perceived threat assessed by self-report predicted lower engagement in volunteer work or charitable activities and commitment to civic duty for individuals carrying the *rs53576* A-allele, but not for those with the G/G genotype. The authors concluded that the *rs53576* genotype might affect prosocial behavior via processing of threat. Finally, there is evidence linking the *rs53576* genotype with autism (Wu et al., 2005; Ylisaukko-oja et al., 2006) but the results have

been inconsistent (e.g. no association in Jacob et al., 2007; almost significant association in Liu et al., 2010).

1.2. Developmental expression of oxytocin, oxytocin receptor, and the OXTR

There are no data on developmental expression of oxytocin and its receptors in humans, but animal studies suggest that the oxytocin system becomes functional early in development. For example in rats, genes for oxytocin are transcribed as early as the 16th day of the embryonic period, but synthesis of oxytocin is not detected until the second postnatal day (reviewed in Carter, 2003). Tribollet, Charpak, Schmidt, Dubois-Dauphin, and Dreifuss (1989) studied the development of oxytocin receptors in rats' central nervous system using *in vitro* (i.e. tissue sections) autoradiography and electrophysiology. Oxytocin receptor binding was detected first at the embryonic day 14 in a region that later differentiates into the dorsal motor nucleus of the vagus nerve. Binding was detected in many other regions from embryonic day 20 onwards. In the hypothalamus and amygdala, oxytocin receptor binding was clearly visible at postnatal day 5.

Oxytocin receptor expression is highly plastic in the limbic system and particularly sensitive to estrogen, which promotes sex-dependent alterations by up-regulating oxytocin expression, inducing oxytocin receptor binding in the amygdala, and stimulating oxytocin release from the hypothalamic neurons (Gimpl & Fahrenholz, 2001; Lee et al., 2009). It is known that females tend to have higher oxytocin and oxytocin receptor expression, and also differences in oxytocin and oxytocin receptor distributions might exist between brains of different sexes (Carter, 2007). As animal models suggest, gonadal steroids such as estrogen have a role in establishing sex differences in the brain early in development (Cooke, Hegstrom, Villeneuve, & Breedlove, 1998), and this seems to apply for the oxytocin receptor system as well.

Studies done with rats have also shown that oxytocin has a significant role in organizing early interaction between the mother and the pup (reviewed in Pedersen & Boccia, 2002). For example, oxytocin in the mother enhances licking and grooming behaviors towards the pup, which in turn increases oxytocin activity in their female pups (Pedersen & Boccia, 2002). Moreover, female rats who have received more licking and grooming by their mothers during infancy, show higher levels of maternal care and oxytocin receptors as adults (Francis, Young, Meaney, & Insel, 2002; Pedersen & Boccia, 2002). However, in a study by Francis et al. (2002), oxytocin receptor binding was not increased in male rats who had received more licking and grooming by their mothers during infancy, but instead their vasopressin receptor binding was increased. Oxytocin and vasopressin are

peptides that are closely related structurally, act both as hormones and neurotransmitters, and receptors for both peptides are widely distributed in the brain (Insel, 1997). Both oxytocin and vasopressin mediate complex social cognition and behavior in humans (reviewed in Meyer-Lindenberg et al., 2011). Several studies with different species have observed more significant effects of oxytocin in females (reviewed in Insel & Young, 2000).

The evidence from rat studies described above shows that oxytocin and oxytocin receptor systems are functional and regulated by gonadal steroids early in development. Also, these animal studies suggest that early experiences such as interaction with caregivers in infancy modulate oxytocin receptor expression. The early appearance of oxytocin receptor suggests also early expression of OXTR because it encodes protein for oxytocin receptor (Gimpl & Fahrenholz, 2001). Few studies have examined allelic variation in the OXTR in human children. Allelic variation in the OXTR has been associated with attachment at the age of 12–16 months (*rs2254298*, Chen, Barth, Johnson, Gotlib, & Johnson, 2011) and callous-unemotional traits (e.g. lack of guilt and empathy, Frick & White, 2008) at the age of 6–16 years (*rs237885,* Beitchman et al., 2012). Also, Johnson and Chen (2011) found that allelic variation in the OXTR *rs2254298* affected attention-related fear bias at the age of 12 months but variation in the OXTR *rs53576* did not. No other study has examined how the most commonly examined candidate variation in the OXTR *rs53576* is associated with social behaviors in human infants and children.

1.3. Early-emerging social behaviors

The evidence for the early emergence of oxytocin function in development, and the consistent findings showing the role of this neuropeptide in human social behavior led us to examine the potential role of oxytocin in the early development of human social behavior. Because intranasal administration of oxytocin might not be suitable for children (Johnson & Chen, 2011), it was examined whether genetic variation in the OXTR (*rs53576*) is associated with the early development of social behavior in children. Variation in the structure of OXTR might produce individual differences in the distribution of oxytocin receptors (Carter, 2007; Insel & Young, 2000), modulating oxytocin function in the brain (e.g. Rodrigues et al., 2009), and thus leading to observable individual differences in early-emerging social behaviors.

Because oxytocin has been associated with several brain systems and social behaviors (Gimpl & Fahrenholz, 2001; Heinrichs et al., 2009; Insel, 1997; Meyer-Lindenberg et al., 2011), a spectrum of early-emerging social behaviors were examined, including potentially amygdala-mediated attention

biases towards emotional faces in infants, prosocial behaviors in toddlers, and emotion recognition and theory of mind (mentalizing) in preschoolers.

1.3.1. Infants' preference for faces and social signals of emotion

One of the earliest manifestations of human social behavior is infants' natural preference to orient to other people, especially to their faces. Before language development, human face provides important social information for infants (e.g. Nelson, 1987). Even newborns prefer to look at face-like stimuli over other visual stimuli (e.g. Goren, Sarty, & Wu, 1975; Valenza, Simion, Cassia, & Umiltà, 1996), but it seems that a robust preference for human face is not present until 4–6 months of age (DeNicola, Holt, Lambert, & Cashon, 2013; Di Giorgio, Turati, Altoè, & Simion, 2012; Frank, Vul, & Johnson, 2009; Gliga, Elsabbagh, Andravizou, & Johnson, 2009).

Around seven months of age, infants' face preference seems to be especially strong for specific facial expressions, possibly reflecting a rudimentary understanding of the emotional and social significance of some facial expressions. At this age, infants begin to show larger event-related potentials (ERPs) and longer looking times for fearful than happy and neutral faces (e.g. Nelson & Dolgin, 1985; Peltola, Leppänen, Mäki, & Hietanen, 2009a; Peltola, Leppänen, Palokangas, & Hietanen, 2008). Peltola and his colleagues (2008) showed that infants' preference for fear is also reflected in a relatively greater difficulty to disengage attention from fearful as compared to various non-fearful expressions. In this study, infants were shown female models with a fearful, happy, or novel expression, or a non-face control stimulus (a scrambled face). The stimuli were shown in the center of a computer screen for one second. After this, a competing peripheral target stimulus was presented either to the left or to the right of the face or control stimulus. Target stimulus was a black-and-white checkerboard pattern. It was found that infants disengaged their fixation from the centrally-presented stimulus towards the location of the peripheral stimulus less likely when the fearful face was presented compared to the happy face or control stimuli. The novel face did not have a similar effect on disengagement of the infants' attention. In later studies using the same paradigm, the same authors found that 7-month-old infants were slower to disengage their fixation from fearful face compared to the happy and neutral face and a neutral face with fearful eyes (Peltola et al., 2009b). Also, when 7-month-old infants disengaged from the central stimulus to the target stimulus, they moved their eyes back to the central stimulus (i.e. re-engaged) faster when it was a fearful face compared to a happy face (Peltola, Leppänen, & Hietanen, 2011). Because the novel expression or the neutral face with fearful eyes did not have the same effects on infants'

attention as the fearful face, it seems that the preference for fearful faces does not depend merely on widely open eyes or because of the fact that fearful faces are more novel in infants' living environment (Peltola et al., 2008, 2009b). Hoehl and her colleagues (2008, 2010) have found that infants' attention was enhanced when another person was looking at an unfamiliar object with a fearful face compared to a neutral face. These results suggest that infants might show preference for fearful expressions because they are signals of possible threat in the environment (Leppänen, 2011; Leppänen & Nelson, 2012).

The amygdala matures early in life (Leppänen & Nelson, 2009), and may have an important role in mediating attention to faces (particularly to the eyes, Klin, Jones, Schultz, & Volkmar, 2003; Peltola et al., 2009b) and fearful facial expressions (Adolphs et al., 2005; Gamer & Büchel, 2009; Herba & Phillips, 2004). From this perspective, it can be speculated that genetic variation in the neurochemical system that involves the oxytocin and the amygdala (Tost et al., 2010) are also present early in development in humans, and are potentially reflected as variations in attentiveness to facial expressions of emotion.

1.3.2. Social behavior in toddlers

Two noticeable forms of social behavior in toddlers are their tendency to socially refer to adults and the early forms of empathic responding. Young children look at their parents' emotional expressions to regulate their behavior accordingly, especially in ambiguous situations (Hirshberg & Svedja, 1990; Hornik, Risenhoover, & Gunnar, 1987; Mumme, Fernald, & Herrera, 1996; Rosen, Adamson, & Bakeman, 1992; Sorce, Emde, Campos, & Klinnert, 1985). This kind of behavior is called social referencing (Sorce et al., 1985). Empathy is defined as an emotional response that results from recognizing and understanding of another person's emotional state or condition, and this response is similar to what the another person is perceived to experience (Eisenberg & Miller, 1987; Eisenberg et al., 1996).

Social referencing is typically studied by using a paradigm that includes an ambiguous situation in which infants frequently look at their caregivers. Sorce and his colleagues (1985) were one of the first to study social referencing in human infants by using a "visual cliff" paradigm. In the visual cliff paradigm, a transparent plexiglass covers a gap between two tables, and therefore, creates an apparent drop-off (a visual cliff). When infants approached the edge of the cliff, they typically looked at their mother who was advised to express different emotional expressions. The mother's expression significantly influenced infants' behavior: the infants were more likely to cross the

visual cliff when their mothers expressed happy or interest, compared to angry or fearful expressions. When the mothers were expressing fearful facial expressions, none of the infants crossed the cliff. Later, social referencing have been studied using different paradigms. For example, in a novel toy paradigm the child plays with an adult (caregiver or experimenter) when an unusual toy appears, and the adult expresses either negative or a positive affect by facial expressions, voice and/or gestures (e.g. Mumme et al., 1996). The results have shown that perceived positive emotional expressions facilitate 12-month-old infants' approach behavior to novel stimuli (i.e. exploring) whereas perceived negative emotional expressions typically lead to avoidance and inhibition of exploring (Hirshberg & Svedja, 1990; Hornik et al., 1987; Mumme et al., 1996; Rosen et al., 1992). Some studies suggest that gender differences might exist in social referencing behavior (e.g. Mumme et al., 1996; Rosen et al., 1992), but the majority of studies do not.

According to Hoffman (1975), development of empathy begins with so called empathic distress. For example, newborns react by crying to another infant's distress and cry, because very young infants cannot separate themselves from others (Hoffman, 1975). Only after cognitive understanding of others has developed during the second year of life (Zahn-Waxler, Radke-Yarrow, Wagner, & Chapman, 1992), a child starts to show more differentiated empathic reactions to others' distress (Hoffman, 1975). A study by Roth-Hanania, Davidov, and Zahn-Waxler (2011) suggests that children show differentiated empathic responding to others' distress already in the first year of life. They found modest levels of affective and cognitive markers of empathy, that is, expressions of concern for the victim and attempts to explore the distress, and/or comprehend cognitively the victim's situation, already at the ages of 8 and 10 months, which continued to increase gradually into the second year. A study by Vaish, Carpenter, and Tomasello (2009) suggests that at 18 months of age, children are able to feel concern for others experiencing harm even when the other person does not display outward signs of emotions. The authors speculate that children might accomplish this ability to sympathize with a victim experiencing harm through the use of situational cues and some form of affective perspective taking; that is, by making inferences about the victim's affective state (what the victim is feeling), and by taking his or her affective perspective (putting themselves to his or her place).

The ability to recognize others' distress and feelings of empathy may motivate to prosocial behavior (Eisenberg & Miller, 1987; Hoffman, 1975). Prosocial behavior is defined as voluntary, intentional behavior that benefits others (Eisenberg & Miller, 1987). Prosocial behavior is other-oriented, and it refers to actions such as helping, sharing, and comforting others (Dunfield, Kuhlmeier, O'Connel, & Kelley, 2011). Prosocial behavior emerge during the first two years of life (Dunfield et al., 2011; Kärtner, Keller, & Chaudhary, 2010; Svetlova, Nichols, & Brownell, 2010;

Roth-Hanania et al., 2011; Vaish et al., 2009; Warneken & Tomasello, 2006; Zahn-Waxler et al., 1992), and some studies suggest that children are naturally predisposed to be prosocial (e.g. Hoffman, 1975; Warneken & Tomasello, 2009). Children are able to help others to achieve simple goals such as handing over objects that another person cannot reach already at 14 months of age (Warneken & Tomasello, 2007). Warneken and Tomasello (2007) presented various helping tasks and corresponding control tasks to 14-month-old children. In the helping tasks, experimenter was unsuccessfully trying to achieve some goal, for example, he accidentally dropped a marker on the floor and tried to reach for it. The basic situation was the same in the control tasks, except that the experimenter did not indicate that he had problems with the situation, for example, he intentionally threw the marker on the floor. The majority of children helped the experimenter spontaneously and almost always immediately when he could not reach for the object in comparison to the control tasks including reaching. However, the children at this age had difficulties helping the experimenter to achieve more complex goals, for example when the experimenter was trying unsuccessfully to place a book on a stack of books. The first signs of helping in more complex situations have been observed at 18 months of age (Brownell, Iesue, Nichols, & Svetlova, 2013; Brownell, Svetlova, & Nichols, 2009; Dunfield et al., 2011; Svetlova et al., 2010; Vaish et al., 2009).

The results regarding gender differences in empathy and prosocial behavior have been inconsistent (Eisenberg & Lennon, 1983). Some studies have shown that girls are more empathic (e.g. Zahn-Waxler et al., 1992) and prosocial (e.g. Baillargeon et al, 2007; Holmgren, Eisenberg, & Fabes, 1998) than boys. For example, Baillargeon and his colleagues (2007) found that gender differences in prosocial behavior (i.e. girls behaving more prosocially) emerged between 17 and 29 months of age. Other studies have not found significant differences between boys and girls in empathy or prosocial behavior (e.g. Roth-Hanania et al., 2011; Vaish et al., 2009; Warneken & Tomasello, 2007).

1.3.3. Emotion recognition and theory of mind in preschoolers

Although components of the ability to discriminate between different emotional facial expressions are observed in early infancy (Bornstein & Arterberry, 2003; Kobiella, Grossmann, Reid, & Striano, 2008; Kobiella et al., 2008; LaBarbera, Izard, Vietze, & Parisi, 1976; Ludemann & Nelson, 1988; Nelson 1987), the ability to recognize and label emotions from facial expression is not accurate until four years of age (Herba, Landau, Russell, Ecker, & Phillips, 2006). However, four-year-old children are still not as accurate as adults are in discriminating and labeling these expressions

(Widen & Russell, 2003, 2008). Preschoolers are more accurate at recognizing happy expression than in discriminating between negative expressions, for example angry, fearful, and sad expressions (Markham & Adams, 1992; Székely et al., 2011; Widen & Russell, 2003, 2008).

Markham and Adams (1992) presented different kinds of facial emotion recognition tasks to four-, six-, and eight-year-old children. They found that four-year-old children were the least accurate at recognizing all emotions except happiness from facial expressions. The authors suggested that emotion categories might not be fully developed at four years of age. Later studies have shown that children's emotion categories are broad at first, that is, these categories include emotions with same valence (i.e. positive vs. negative emotions) (Székely et al., 2011; Widen & Russell, 2003, 2008). This is demonstrated by systematic errors that children make when labeling emotions: one negative expression is often confused with another negative expression (e.g. fearful expression is labeled as sad or angry), but it also seems that happy expression is confused with fearful expression (Székely et al., 2011; Widen & Russell, 2003, 2008). As these categories narrow over the preschool years (Widen & Russell, 2003, 2008), the ability to discriminate and recognize emotions from facial expressions improves (e.g. Boyatzis, Chazan, & Ting, 1993; Durand, Gallay, Seigneuric, Robichon, & Baudouin, 2007; Herba et al., 2006; Vicari, Reilly, Pasqualetti, Vizzotto, & Caltagirone, 2000).

According to Harris (1989), during development preschoolers learn to understand the relationships between desires, beliefs, and emotions, and this understanding forms a basis for their ability to predict and explain other people's behavior and emotions. This ability to predict and explain other people's behavior by referring to their mental states (e.g. thoughts and feelings) is referred to "theory of mind" or "mentalizing". Theory of mind is accomplished when the child realizes that other people might be thinking or feeling differently than he does, and when the child is able to take others' perspective (Symons, 2004). These perspective-taking skills improve with age (Symons, 2004).

Studies have shown that around 18 months of age, or even earlier, a child understands at some level that human behavior is intentional and goal-orientated (reviewed by Flavell, 2000, 2004). It has been suggested that such understanding is an antecedent to the development of a theory of mind (i.e. mental representation of other people's beliefs and desires, see Flavell, 2000; Wellman, Lopez-Duran, LaBounty, & Hamilton, 2008). Until three years of age, a child is able to understand that other people might have different desires than himself, and around four years of age, he comes to understand that other people may hold "false" beliefs (i.e. beliefs contrary to reality) (Wellman & Liu, 2004). Not until 5–6 years of age children are able to understand that a person may hide the emotion he is really experiencing and instead express another emotion (Harris, 1989). Although the

development of theory of mind usually follows this course, there are substantial individual differences in children's understanding of mental states (Cutting & Dunn, 1999).

Wellman and Liu (2004) developed a scale for assessing various aspects of theory of mind in children. One version of this scale consists of five different kinds of tasks that gradually become more difficult and target more complex aspects of theory of mind. In the easiest task, a child must understand that another person wants something different than what he wants (Diverse desires). In the second task, a child must come to understand that another person's belief may differ from his (Diverse beliefs). In the third task, the child is shown content of a box, and he must understand that another person, who has never seen inside the box, does not know what is in there (Knowledge access). In the fourth task, a child must understand that another person may hold false belief, which is a belief contrary to reality (in this version: Contents false belief). In the last and most difficult task, a child must understand that another person may hide what he is truly feeling and express another emotion, for example, in his face (Real-apparent emotion). Usually, young children's theory of mind is assessed by using only false-belief task (e.g. Liu, Meltzoff, & Wellman 2009; Wellman, Cross, & Watson, 2001).

Meta-analysis by McClure (2000) suggested that girls have better emotion recognition skills than boys already in infancy, but majority of the recent studies have not found differences between girls and boys in emotion recognition (e.g. Gao & Maurer, 2009; Herba et al., 2006; Székely et al., 2011). However, some studies suggest that preschool girls do better than boys on mentalizing tasks measuring understanding of false belief (Charman, Ruffman, & Clements, 2005; Thoermer, Sodian, Vuori, Perst, & Kristen, 2012; Walker, 2005). Scourfield, Martin, Lewis and McGuffin (1999) examined social cognitive skills in twins aged 5–17 by using questionnaires (parent-report), and found that males had poorer social cognition than females, but this was not explained by a difference in genetic effects. They did found that social cognitive skills were substantially heritable (68 % of the variance in social cognition scores was explained by genetic influence). Also, a twin study by Hughes and Cutting (1999) showed that 66 % of the variation in three-year-old children's scores on theory-of-mind tasks was explained by genetic influence. However, Hughes and her colleagues (2005) found that only 15 % of the variation in 60-month-old children's performance in theory-of-mind tasks was explained by genetic influences.

These results are partly in line with the findings that autism spectrum conditions, which are characterized by difficulties in recognizing others' emotional expressions (Herba & Phillips, 2004) and in imagining other people's minds (Baron-Cohen & Belmonte, 2005), are heritable (Baron-Cohen, 2002). These difficulties might be partly explained by the finding that when people with autism process emotional facial expressions, they do not tend to look more to eye region of human

face (Klin et al., 2003) as healthy individuals do (e.g. Haxby, Hoffman, & Gobbini, 2002; Klin et al., 2003; Gamer, & Büchel, 2009).

1.4. Summary and aims of the present study

Accumulating evidence shows that genetic variations in oxytocin function are associated with individual differences in various social cognitive and behavioral processes, including face and emotion perception, empathy, and prosocial behaviors (Poulin et al., 2012; Rodrigues et al., 2009; Tost et al., 2010). However, because the majority of the existing studies have been conducted in adults (for an exception: Beitchman et al., 2012; Chen et al., 2011; Johnson & Chen, 2011), it is not known how genetic variations in oxytocin function are associated with social cognition and behavior in infants and children, and therefore, the developmental precursors of the adult phenotype are largely unknown.

In this thesis, this question was addressed by examining the effects of OXTR *rs53576* genotype on early-emerging social behaviors in human children at different postnatal ages. Specifically, the effect of OXTR *rs53576* genotype on different forms of age-typical social behavior was investigated: face preference and attention to facial expressions of emotion in infants, prosocial behavior in toddlers, and emotion recognition and theory of mind in preschoolers. Individuals with the G/G genotype were compared to those carrying the A-allele (G/A and A/A genotypes), and also the genotype effects between boys and girl were compared because the effect of OXTR *rs53576* on social behavior might be different for females and males (Tost et al., 2010).

Although the effects of individual single nucleotide polymorphisms (SNPs) in the OXTR on some specific behavior could be small and require relatively large sample sizes in order to be statistically significant, the sample sizes used in previous studies examining SNP in the OXTR have been relatively small, varying from 45 to 348 participants (Bakermans-Kranenburg & van IJzendoorn, 2008; Beitchman et al., 2012; Chen et al, 2011; Johnson & Chen, 2011; Poulin et al., 2012; Rodrigues et al., 2009; Tost et al., 2010). Also, Leppänen et al. (2011) found a significant association of a single nucleotide polymorphism in serotonin system (TPH2 - 703, *rs4570625*) with visual attention in a small sample of 66 infants (the same sample as used in the present study). Johnson and Fearon (2011) commented on this finding by pointing out that the association between single nucleotide polymorphisms in a specific gene and specific behavior might be stronger and clearer in early development because environmental factors have not yet affected this relation as much as during later development. For this reason, it was hypothesized that the effect of OXTR

rs53576 on early-emerging social behaviors in infancy and early childhood may be evident in a relatively small sample.

Based on previous research showing consistently heightened levels of social behavior in G/G homozygotes, overall hypothesis was that the G/G genotype is associated with heightened levels of social behaviors across development, that is, individuals with the G/G genotype would exhibit stronger face preference and enhanced attention to facial expressions of emotion in infancy, more prosocial behavior as toddlers, and they would also be better at recognizing emotions and mentalizing as preschoolers compared to those carrying the A-allele. Also, explorative analyses regarding OXTR-gender interactions on social behavior were performed.

2. METHODS

2.1. Participants

The participants were children who took part in a laboratory assessment at 7 months of age. The parents of these children were later contacted for follow-up assessments at 24 and 48 months of age. Because these follow-up assessments were added after the study was commenced, only a subgroup was contacted for the 24-month assessment. All participants were contacted for the 48-month assessment. The recruitment method and the final sample size for each assessment are described in the following.

For the 7-month assessment, parents of infants were contacted through birth records and local child welfare clinics. They were sent information about the study and a contact information form by post. Interested families posted their contact information back to the researchers who again contacted them by phone and scheduled a laboratory visit when the infant was 7-month-old (± 7 days). Of the initial sample of 120 infants, 79 infants provided a blood sample. Of them, 66 infants (28 girls, *M* age = 215 days, *SD* = 3.36, range 207–222 days) had enough analyzable attention data (i.e. three or more analyzable trials in the disengagement attention test with four stimulus conditions)[1], and were included in the final sample. All participants in the final sample were born

[1] The final sample size for re-engagement analysis was 58 infants after excluding eight infants because of low number of analyzable trials (i.e. two or less trials).

full term (37–42 weeks) and had no history of visual or neurological abnormalities. The 13 infants not included in this final sample had low number of analyzable trials in some experimental conditions (i.e. two or less trials, n = 3), were born pre-term (n = 1), were excluded from the analysis due to a procedural error in testing (n = 1), or were tested with a different version of the attention paradigm (n = 8).

When participants were around two years old, their parents were contacted again by phone and asked if they could be sent information about the follow-up study (second measurement time). Only parents of the 95 participants in the initial sample were contacted because the other 25 participants were older than two years when the 24-month assessment was added to the study. Those who were interested, sent their information back to the researchers via e-mail or mail, and were contacted again by phone and scheduled for a laboratory visit. Of 95 participants, 44 (46 %) participated in the second measurement time. Of these 44 participants, 32 had given a blood sample when they were 7-month-old. Five children were excluded because they were inattentive during social referencing tasks, so the final sample size included 27 children (7 girls, M age = 742 days, SD = 14.10, range = 717–771 days). Reasons for participant attrition were, for example, that the family had moved, parent refused to participate, or researches were unable to contact parents.

Parents of children who had participated in the first laboratory assessment (n = 120) were contacted again by phone or e-mail around children's forth birthday. They were told about a follow-up study (third measurement time) and asked if they could participate. Testing was appointed for those who were interested. Of 120 children, 86 (72 %) participated in the third measurement time. Those who had given blood sample at 7-month-old (n = 61), were included in the final sample (22 girls, M age = 1513 days, SD = 63.75, range = 1402–1735 days). Reasons for participant attrition were, for example, that the family had moved, parent's refusal, or researches were unable to contact parents.

The project has been approved by the Ethical Committee of Tampere University Hospital and a written informed consent was obtained from the parents during every measurement time. Previous reports of this projects have been documented analyses of polymorphisms in serotonin genes (Leppänen et al, 2011), heart rate and attention disengagement (Leppänen et al, 2010), and two unpublished Master's theses. One of these theses examined how 7-month-old infants' attention to facial expressions of emotion was associated with socio-emotional development at 24 months of age (Haikonen, 2011), and the another one examined the effect of OXTR *rs53576* on social behavior at 24 months of age, using slightly different analytic approach as explained below (Koriseva, 2011).

2.2. DNA extraction and genotyping of the OXTR

For DNA extraction, participants had given a 3.0 ml EDTA-whole blood sample at 7 months of age. Following the procedure described in Leppänen et al. (2011), the blood samples were taken by an experienced laboratory nurse and these samples were stored in a freezer at -20 ^{o} C. Genomic DNA was extracted by using QIAampDNA Blood Minikit and automated biorobot M48 (Qiagen). Single nucleotide polymorphism (SNP) *rs53576* located in the third intron of OXTR was genotyped using Taqman SNP Genotyping Assays and ABI Prism 7900HT Sequence Detection System. No discrepancies were detected in the genotyping results of duplicate samples.

2.3. 7-month assessment: preference for faces and social signals of emotion

2.3.1. Experimental design

At 7 months of age, children participated in a laboratory assessment of attention to facial expressions of emotion (n = 66) (Leppänen et al., 2011). The children's parents were also given questionnaires about family's background information, mother's depression and child's temperament. Also, later after the laboratory visit, a mother-child interaction video (10–15 min) was recorded at the family's home. The questionnaires and mother-child interaction are not reported here.

During testing, the infant sat on a parent's lap in a dimly lit room and was presented with visual stimuli on a computer monitor 60 cm away. Screen size was 19 inches and the middle part of the screen was at the level of the infant's eyes. Above the screen was a hidden video camera that was used to monitor the infant's gaze direction during the testing. The video recordings were also used later when the infants' eye movements were analyzed. Because the room was dark and the surroundings of the screen were covered by black fabric, infants had no other stimulus to pay attention to.

The laboratory visits were scheduled for times the parent thought would be best for their infant (i.e. the infant was awake and attentive). Children were tested with a so-called overlap paradigm in which they were first presented with a central stimulus on a screen and then a peripheral target stimulus next to the central stimulus. The central stimuli used in this study were colorful pictures of

faces, in which a female model posed neutral, happy, or fearful facial expressions, or a face-shaped control stimulus on white background (Figure 1). The control stimulus was made of the fearful face picture so that the amplitude and color spectra and also the contour of the face was retained but its phase spectra was scrambled so that the stimulus was not identifiable as a face stimulus. The sizes of the central stimuli were 15.4° and 10.8° vertically and horizontally, respectively. The peripheral target stimuli were black-and-white vertically arranged circles or a checkerboard pattern, which were the size of 15.4° and 4.3° vertically and horizontally, respectively. Every trial began with an animation, in which a red circle expanded from 0.4° to 4.3° continuously, to attract infant's attention to the center of the screen. These stimuli were presented with E-Prime (Psychology Software Tools, Inc).

When the child was attending to the expanding red circle in the beginning of the trial, the experimenter pressed a button to present a face or control stimulus on the center of the screen. The four different central stimuli and peripheral target stimuli were presented in random order except that the same central stimulus was presented no more than twice in a row and the target was presented on the same side of the screen no more than three times in a row. After the face or the control stimulus had been presented alone for 1,000 milliseconds, the peripheral target stimulus appeared automatically either on the left or right side of the screen. The central stimulus and the peripheral target stimulus were kept on the screen for 3,000 milliseconds. Between every trial, an empty screen was presented for one second, and every new trial began with presenting the expanding red circle. The trials were presented until the infant had completed 25–30 trials or became fussy or inattentive.

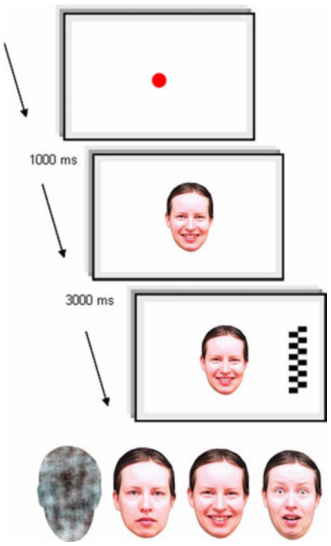

FIGURE 1. Experimental design, control stimulus, and face stimuli in the 7-month assessment (Picture from Leppänen et al., 2011).

2.3.2. Variables and analysis of the data

A hidden video camera recorded infants' eye movements that were then analyzed by an observer who was blind to the presentation order of stimuli by using VirtualDubMod (video editing software with frame-by-frame playback, www.virtualdubmod.sourceforge.net). The observer coded the times when the central stimulus appeared to the screen which was marked by a light flash on infant's face. If the infant moved her eyes from the central stimulus to the peripheral target during a time window from 160 to 1,000 milliseconds after the onset of the peripheral stimulus, observer coded the time when the saccade began. This was called "disengagement". Also, if the infant returned from the peripheral target back to the central stimulus before the screen went black, the observer coded the time when the saccade towards the central stimulus began, and this was called "re-engagement".

From these time points, reaction times for infants' disengagement from the central stimulus and re-engagement with the central stimulus were calculated. Also, probabilities of disengagement from the central stimulus and re-engagement with the central stimulus were calculated. In this thesis, only disengagement probability and re-engagement latency were used because these variables have been used in previous studies and may best reflect infants' face preference (Leppänen et al., 2011; Peltola et al., 2011). Trials in which infant was not looking towards the central stimulus when it appeared, made a saccade toward an incorrect location (i.e. not to the peripheral target), or closed his or her eyes (e.g. when rubbing her eyes) during trial were excluded from the analysis. Also, trials with anticipatory eye movements, that is, saccades beginning less than 160 milliseconds after the onset of peripheral stimulus, were excluded (M = 6.7 % of trials excluded, SD = 7.4 %).

2.4. 24-month assessment: prosocial behavior

2.4.1. Experimental design

At 24 months of age, children participated in an observational assessment where the child interacted with experimenter during playful tasks. These tasks assessed empathy, helping behavior, and social referencing. There were also tasks assessing impulse control but these are not reported here. The children's parents were also given questionnaires about background information, mother's mood, and child's temperament. Later after the assessment, a mother-child interaction video (10–15 min)

was recorded at the family's home. The questionnaires and mother-child interaction are not reported here.

The follow-up assessments were performed in an observation room at the Department of Psychology, University of Tampere. The size of the room was approximately 30 square meters. A small table (100 cm x 60 cm) was placed to the center of the room, and the child was sitting opposite to experimenter during the tasks (Figure 2). The parent was also in the room but he or she was sitting in the background approximately two meters away from the child and the experimenter. There were two video cameras in the room recording the assessment. The cameras were placed near the ceiling, and one of them was recording the child's reactions, and the other one recording both the child and the experimenter from the right side of the child (Figure 3).

FIGURE 2. The first experimenter and the child performing task assessing empathy in the 24-month assessment (Picture from Haikonen, 2011; Koriseva, 2011).

The experimenters were always the same two females. The experimenters alternated their roles so that while the first experimenter was interacting with the child during testing, the second one was in another room. The second experimenter was following the assessment from a TV-screen that showed the camera's view, and she also brought needed equipment to the assessment room. Before the assessment, there was a warm-up period during which the first experimenter played freely with the child for about 10 minutes to familiarize with him or her. The parent had been told that the child could have her own toy with him or her so that it would be easier to warm-up the child. During the warm-up period, the parent was informed about the tasks. He or she was advised to stay in the background during these tasks. Also, the parent was told that the goal was to observe the child's natural, spontaneous ways of acting and reacting, and the parent should not guide him or her in any

ways. During the assessment, the child sat by a small table interacting with the experimenter. Children did not have to perform tasks they did not want to. If the child was very shy or nervous, the parent could sit closer to the table or the child could sit on his or her parent's lap by the table. The assessment took about 15–20 minutes, and the child could have breaks whenever needed. The child was also allowed to leave the table and go to the parent.

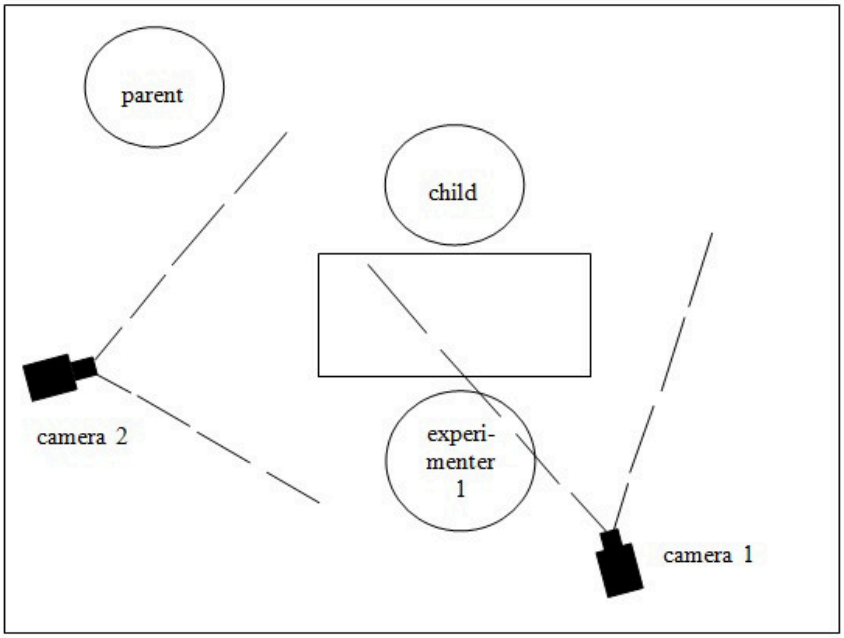

FIGURE 3. Location of the cameras, the first experimenter, the child, and the parent in the 24-month assessment (Figure modified from Haikonen, 2011; Koriseva, 2011).

The assessment began with a task assessing social referencing, that is, the child's tendency to use the experimenter's reactions to guide his or her interaction with novel objects (modified from Mumme & Fernald, 2003). The first experimenter called the second experimenter in another room and asked if she could bring some toys for them to play with. This task consisted of two trials using different toys. In the first trial, the toy was a stuffed green caterpillar. The first experimenter raised the first toy between herself and the child looking directly at the toy. Then she said in Finnish "Isn't it big… and it has these kinds of nodules" while expressing neutral facial expressions and tone of voice. After she had expressed the neutral facial and vocal signals, she placed the toy on the table and the child was given 20 seconds for interacting with the toy. If the child did not pick up the toy, the toy was left on the table for 20 seconds, and after this the experimenter and the child played with it. Then, in the second trial, the second experimenter brought another toy, which was a novel plastic ball with colorful nodules. The first experimenter acted in the same way and used the same words as during the first trial, except that now she expressed fearful facial expression and tone of

voice. After the second trial, the first experimenter called again to the second experimenter and asked her to come and tell them what the ball was. The second experimenter came and told what it was and that it is nothing to be afraid of. Then she gave a third toy (a stuffed frog) to the first experimenter who expressed positive affect (i.e. happiness) and said in Finnish "This is a fun toy. I like this toy!" This last trial was performed to end the task in a positive way and it was not included in the analysis.

After social referencing task there were two tasks assessing the child's spontaneous helping behavior when the first experimenter was in a need for instrumental help (see Warneken & Tomasello, 2006). In the first task, the second experimenter brought three wet towels to the assessment room and asked the first experimenter to hang them to dry on a line. The first experimenter agreed and told the child that she would hang them first, and then they would continue playing. She hanged the towels by using clothespins, but when she was hanging the last towel, she "accidentally" dropped a clothespin on the floor and said Finnish "Oh, I dropped my clothespin". Then the experimenter tried to signal nonverbally that she needed help. She tried to reach the clothespin three times without success and showed signs of frustration. Between every reach the experimenter had a short break and straightened herself before she reached again for the clothespin. If the child had not helped by the experimenter's third reach, the experimenter looked at the child and asked him or her to help. If the child did not help at all, the experimenter picked up the clothespin by herself.

In the second helping task, the first experimenter noted a stack of binders that was forgotten on a corner of a table. She said to the child that she would put them into a cabinet before they continued playing. The experimenter lifted the stack of binders in her arms and tried to put them into the cabinet but the doors were closed and the experimenter could not open them because her hands were full. She stopped and said in Finnish "Oh, the door is closed". Then she tried to signal nonverbally that she needed help. She walked slowly towards the door as if she was trying to open it without success and showed frustration. This was repeated three times, and the experimenter had a short break between every attempt. If the child did not help on any of the three cues, the experimenter asked the child to help her and open the door for her. If the child did not respond to the helping request, the experimenter put the binders down to the table, opened the cabinet by herself, and put the binders in.

The third task assessed empathy (modified from Kärtner et al., 2010). In this task, the first experimenter took different toys from a box and introduced them one at a time to the child. The third toy was a tractor, and it was designed so that one of its front wheels would be easily detached. The experimenter introduced the toy to the child by telling a short story, and towards the end of the

story, the front wheel of the tractor "accidentally" came off. Then the experimenter held the tractor and the detached wheel in her hands, expressed sadness, and said in Finnish: "Oh no, now the wheel detached. My tractor went broken!" Then she placed the tractor and the wheel on the table, leaned her jaw on her hand while expressing sadness on her face, moaning, and gazing towards the table for 20 seconds. The goal was to observe if the child would act prosocially when he or she saw the experimenter grieving, that is, would the child try to fix the tractor. If the child tried to fix the tractor, the experimenter stopped moaning, thanked the child, and fixed the tractor with him or her. If the child did not try to fix the tractor, the experimenter shaped up and suggested that they would fix the tractor. Regardless of how the child acted, the tractor was fixed. Then they played with the tractor for a while.

2.4.2. Variables and analysis of the data

The child's behavior was analyzed from the videotapes by using VirtualDubMod (video editing software with frame-by-frame playback, www.virtualdubmod.sourceforge.net). The video analysis was done by the two experimenters who performed the assessments. Each video was analyzed by the person who was the second experimenter in that assessment. If the child was not paying enough attention to the task, the trial was excluded from the analysis.

In the social referencing task, frequency of looks towards the experimenter and the duration of touching the toy during the free play period (i.e. when the toy was available for the child to touch it) was coded. Then "social referencing index" was calculated. For each child, an average of the difference in look frequencies and touch duration between neutral and fearful trials was calculated. The look frequencies during the neutral trial were subtracted from the look frequencies during the fearful trial (fearful-neutral). Then the time interval of the free play period (beginning when the first experimenter placed the toy on the table and ending when the experimenter began to talk) was calculated. Then the duration of touching during the above-mentioned time interval and its proportion of the time interval were calculated. This measure varied between 0.00–1.00 (no touching – touching during the whole free play period). The duration of touching in the fearful trial was subtracted from the neutral trial (neutral-fearful). More positive values of this social referencing index indicated the child's relatively greater reliance on the experimenter's emotional expressions in guiding his or her own behavior in ambiguous situation. In the helping tasks, child's behavior was coded as 2 if he or she spontaneously helped the experimenter, and as 1 if he or she helped after the experimenter asked him or her or the child did not help at all. These measures from

the two helping tasks were summed up for “helping index”. In the empathy task, child’s behavior was coded as 2 if he or she tried to fix the tractor, and as 1 if he or she did not tried to do that. The scores from the social referencing and helping indexes and empathy task were first standardized by z-transformation before summing them up for “prosocial index”.

2.5. 48-month assessment: emotion recognition and theory of mind

2.5.1. Experimental design

At 48 months of age, children participated in a laboratory assessment of emotion recognition and theory of mind. After having assessed these skills, assessments of attention and executive control were performed, but these are not reported here. This assessment was conducted in the same room as the 24-month assessment, but the two experimenters were different females than in the 24-month assessment. In this assessment, both experimenters were always the same females who alternated their roles so that while the other one was presenting the tasks for the child (experimenter 1), the other one was sitting by a table with the child’s parent and coding the child’s behavior (experimenter 2) (Figure 4). Later after the assessment, a mother-child interaction video (10–15 min) was recorded at the family’s home. Parent was also given questionnaires about background information, mother’s mood, and child’s temperament. The questionnaires and mother-child interaction are not reported here.

The setting (Figure 4), warm-up period, and instructions given to the parent were similar to those in 24-month assessment. First tasks were from an existing theory-of-mind scale (Wellman & Liu, 2004), and then emotion recognition tasks (modified from Wismer Fries & Pollak, 2004) were performed.

Children’s mentalizing abilities were assessed with the existing theory-of-mind scale (Wellman & Liu, 2004). In the present study, a version of theory-of-mind scale that includes five tasks was used, and because there is no Finnish version available, the original scale in English was translated into Finnish. The following tasks were included in the scale: Diverse desires, Diverse beliefs, Knowledge access, Contents false belief, and Real-apparent emotion. A detailed description of the tasks is given in Table 1. In the first four tasks, four different small dolls were used to present the tasks. Also, in the diverse-desires task a child was shown a colorful picture of a cookie and a carrot, and in the diverse-beliefs task a black-and-white picture of a bush and a garage was presented. In

the knowledge-access task, a small toy dog inside a wooden box was presented, and in the contents false-belief task a small toy horse inside a chewing gum bag was shown. In the real-apparent emotion task, a paper doll was used in order to help the child focus on the story, and also three black-and-white schematic face pictures (happy, sad, neutral) were used, and a child could point these pictures to give his or her answer to the questions.

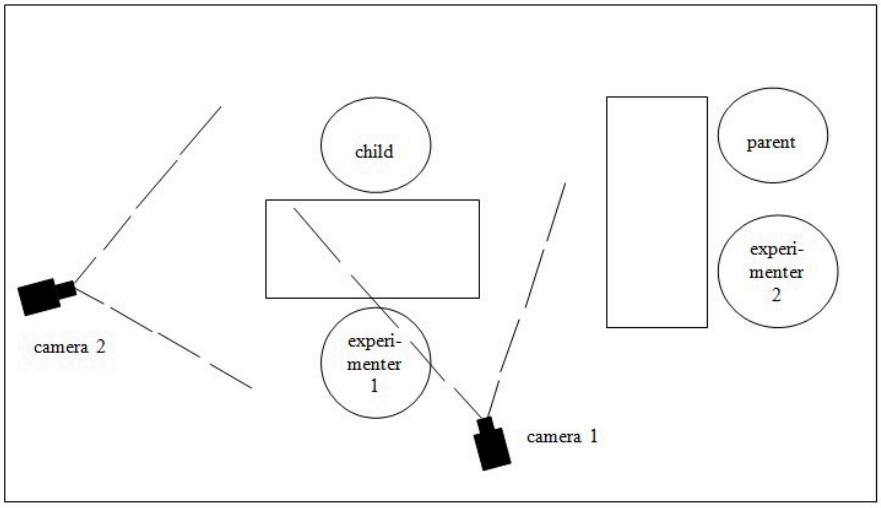

FIGURE 4. Location of the cameras, the experimenter 1, the child, the parent, and the experimenter 2 in the 48-month assessment.

In emotion recognition tasks, the child was told short stories that illustrated happy, fear, and sad emotions. The stories were the same as in study by Wismer Fries and Pollak (2004) except that they were translated into Finnish and modified slightly to make them more appropriate for 48-month-old children (Table 2). The experimenter told four stories of each emotion so in total 12 stories were told. The protagonist was a girl in the first six stories and a boy in the last six stories. While the experimenter told a story, a picture of a neutral face of an elementary school-aged girl or boy was shown, and after the story the child was shown four pictures of the same person modeling neutral, happy, sad, or fearful facial expression. The face pictures were taken from the Radboud Faces Database -site (http://www.socsci.ru.nl:8180/RaFD2/RaFD) that provides validated pictures of faces and facial expressions (Langner et al., 2010). The order of the stories illustrating happy, sad, and fear emotions were randomized so that the order of illustrated emotions would not affect emotion recognition. However, the last story of the girl and the boy were always illustrating happy emotion so that the task was ended in a positive condition. The order of the stories was same with every participant. Also, the order of different facial expressions was randomized in every story but

the order was same with every participant. The child was asked how the girl or the boy felt in the story, and the child could answer by pointing the pictures of facial expressions.

2.5.2. Variables and analysis of the data

In the theory-of-mind scale, the child got one point if he or she answered correctly to questions presented by experimenter so the maximum total score for this task was 5. In the emotion recognition task, the child got one point from every accurately recognized emotion so the maximum total score for this task was 12 and the maximum score for every emotion was 4 (happy, sad, fear). The percentage of correct answers in the emotion recognition task and in the theory-of-mind scale were calculated and analyzed.

TABLE 1. Tasks used in the theory-of-mind scale (Wellman & Liu, 2004), descriptions of the tasks, equipment used in each task, and questions presented to the child (in English).

Task of the theory- of-mind scale	**Description of the task (equipment used)**	**Questions**
1. Diverse desires	The doll wants to have a different snack than what the child wants (a doll, a colorful picture of a cookie and a carrot).	"Which snack will Jaakko (the doll) choose? A carrot or a cookie?"
2. Diverse beliefs	The doll and the child have different beliefs about where the doll's cat is hiding (a doll, a black-and-white picture of a bush and a garage).	''So where will Linda (the doll) look for her cat? In the bushes or in the garage?''
3. Knowledge access	The child knows what is in a box but the doll has never seen inside that box (a doll, a small toy dog inside a wooden box).	"Does Maija (the doll) know what is in the box?
4. Contents false belief	The child knows that inside a gum bag is a toy horse instead of chewing gum, but the doll does not know that (a doll, a small toy horse inside a chewing gum bag).	"What does Pekka (the doll) think is in the box? Chewing gum or a horse?"
5. Real-apparent emotion	The child is told a story in which a girl is trying to hide her real emotion by expressing another emotion (a paper doll, three black-and-white schematic face pictures).	"How did the girl feel? How did she try to look on her face?" (answer to the former must be more negative e.g. sad and happy)

TABLE 2. Stories used in the emotion recognition task (translated into English) and illustrated emotion in each task (modified from Wismer Fries & Pollak, 2004).

Stories about a girl (emotion)	Stories about a boy (emotion)
1. Once this girl participated in a running competition. She won the competition, and her friends were cheering for her at the finish line (happy).	1. This boy was playing outside with his friends. The boy fell down on the sidewalk and hurt his knee (sad).
2. Once this girl and her mom planned a trip to their favorite park on Saturday. But when Saturday came, it was raining so they could not go to the park (sad).	2. This boy woke up in the middle of the night and noticed a big thunder and lightning storm outside (fear).
3. Once this girl had a bad dream about a monster (fear).	3. This boy had a pet bird. One day he got home from school and saw that the bird was not in its cage. The boy thought that his bird might be gone forever (sad).
4. This girl's best friend, who she really likes to play with, moved away. Now the girl cannot play with her friend anymore (sad).	4. Once this boy drew a picture and showed it to her mom. Mom said that the boy did a good job, and that the picture was fantastic (happy).
5. This girl and her friend were walking through a forest. They heard rustle coming from the bushes and thought it might be a bear (fear).	5. This boy went shopping with his dad. There were lots of people in the store, and the boy got lost and could not find his dad anywhere (fear).
6. This girl loves dogs. On her birthday her dad gave her a dog (happy).	6. Once this boy and his mom had a picnic together at boy's favorite place (happy).

2.6. Statistical analyses

For initial, descriptive data analyses, Pearson's chi-squared (χ^2) test was used to compare the genotype distribution between boys and girls, and between those children who participated in the follow-up assessments and those who did not but had provided blood sample in the 7-month assessment. Also, the gender ratio between those children who participated in the follow-up assessments and those who did not was compared by using χ^2 test. Court's online calculator (2005–2008) was used to analyze if the allele frequencies were in the Hardy-Weinberg equilibrium, that is, if the observed genotype frequencies in the sample were similar to the expected frequencies in the population.

All statistical analyses examining the effects of OXTR *rs53576* genotype and gender on measures of social behavior were conducted cross-sectionally (i.e. by analyzing the effects of *rs53576*

genotype at each age separately) to maximize sample size for each analysis. The sample was divided to children with the G/G genotype and children carrying the A-allele (G/A and A/A genotypes). The genotype (G/G vs. A-carriers) and gender effects on the variables of interest were analyzed by using SPSS 20.0. The methods used in the analysis of the data for each assessment are described in the following.

First, one-sample Kolmogorov-Smirnov test was used to analyze if all the variables were normally distributed. All other variables were normally distributed ($p > .05$) except disengagement probability in all stimulus conditions (control stimulus, $p < .001$, neutral, $p < .001$, happy, $p < .001$, and fearful facial expressions, $p < .05$) and percentage of correct answers in the theory-of-mind scale ($p < .05$).

From the 7-month assessment's data, the genotype and gender effects on disengagement probability and re-engagement latency for each stimulus condition (control stimulus, neutral, happy, and fearful expression) were analyzed by using a repeated measures analysis of variance (ANOVA). Significant main and interaction effects in the ANOVA were further examined by using paired samples *t* test with Bonferroni-corrected significance level. Because disengagement probability in each stimulus condition was not normally distributed, additional analyses using nonparametric methods were also performed (Wilcoxon signed ranks test).

From the 24-month assessment's data, genotype and gender effects on the prosocial index were analyzed by using a two-way analysis of variance (ANOVA). Significant main and interaction effects in the ANOVA were further examined by using independent samples *t* test with Bonferroni-corrected significance level. The effect of OXTR *rs53576* on social behavior in the same 24-month-old children has been examined in an unpublished Master's Thesis (Koriseva, 2011), in which a so-called social behavior index was calculated as a sum of scores in social referencing (calculated index in which only touching duration was included), helping, and empathy tasks, and the effect of OXTR *rs53576* on children's performance on each task and social behavior index were analyzed. Also, the social behavior of all the participants was analyzed, that is, also participants who had not provided blood sample in the 7-month assessment were included. In the present thesis, social referencing index was calculated differently (both looking frequencies and touch duration were included), z-transformations for scores in each task were performed before summing them up for prosocial index, and the genotype effect on this prosocial index was compared between boys and girls.

From the 48-month assessment's data, the genotype and gender effects on emotion recognition and theory of mind were analyzed by using a two-way analysis of variance (ANOVA). Significant main and interaction effects in the ANOVA were further examined by using independent samples *t*

test with Bonferroni-corrected significance level. Because percentage of correct answers in the theory-of-mind scale was not normally distributed, control analyses using nonparametric methods were also performed (Mann-Whitney *u* test).

3. RESULTS

3.1. 7-month assessment: preference for faces and social signals of emotion

All 66 participants were included in the analyses of disengagement probability and 58 participants were included in the analyses of re-engagement latency. Eight participants were excluded from the analyses of re-engagement latency because they had two or less than two analyzable re-engagement trials. The OXTR *rs53576* genotype distribution of children participating in the 7-month assessment is presented separately for boys and girls in Table 3. The allele frequencies (n = 21 G/G, n = 36 G/A, n = 9 A/A) were in the Hardy-Weinberg equilibrium, that is, the observed genotype frequencies in the sample were similar to the expected frequencies in the population (χ^2 (1) = 1.08, $p > .10$). The genotype distribution did not differ significantly between these boys and girls (χ^2 (1) = 0.236, $p > .10$).

TABLE 3. The OXTR *rs53576* genotype distribution in 7-month assessment *(n* = 66).

	girls		**boys**		**Total**	
	n	%	*n*	%	*n*	%
G/G	8	29	13	34	21	32
G/A and A/A	20	71	25	66	45	68
n	28	100	38	100	66	100

The descriptive statistics for disengagement probability and re-engagement latency are presented in Table 4. Disengagement probability was moderately and negatively skewed in every stimulus condition (one-sample Kolmogorov-Smirnov test, $p < .05$). According to Tabachnick and Fidell (2013), when all the variables are similarly skewed to about the same moderate extent as in this case, transformations of the variables will not significantly improve the analysis. For this reason, no transformations on disengagement probability were performed. Also, because there was no nonparametric method available, parametric method of analysis was used.

TABLE 4. Descriptive statistics for disengagement probability and re-engagement latency (minimum and maximum values, mean, and standard deviation).

	n	***Min***	***Max***	***M***	***SD***
Disengagement probability					
control stimulus	66	0.60	1.00	0.94	0.12
neutral	66	0.20	1.00	0.82	0.22
happy	66	0.20	1.00	0.81	0.22
fearful	66	0.00	1.00	0.69	0.30
Re-engagement latency					
control stimulus	58	350.00	1746.67	760.02	307.67
neutral	58	296.00	1376.00	707.11	251.01
happy	58	270.00	1496.00	700.31	278.59
fearful	58	306.67	1346.67	681.09	259.18

There was a non-significant tendency for genotype and gender interaction effect on re-engagement latency (F (2.33) = 2.22, p = .11) (Figure 5). Given this tendency and the previous evidence for gender differences in the OXTR *rs53576* genotype effects (Tost et al., 2010), the genotype effects on re-engagement were analyzed separately in boys and girls. In boys, there was a statistically significant interaction between genotype and stimulus condition (F (3) = 2.97, $p < .05$). This interaction was examined further by four a priori contrasts comparing boys with the G/G and A-carrier genotype for each facial expression condition separately. Bonferroni corrected significance level for the four comparisons was .013. Boys with the G/G and A-carrier genotype did not differ in the non-face control and neutral conditions ($p > .10$), but boys with the G/G genotype had significantly faster re-engagement latency in the happy (M = 540.05, SD = 165.36) (t (27.53) = 3.24, $p < .01$) and fearful (M = 554.87, SD = 134.98) (t (26.42) = 2.879, $p < .01$) conditions than boys with the G/A and A/A genotypes (happy: M = 835.33, SD = 343.16, fearful: M = 784.84, SD = 307.58). In girls, there was no interaction between genotype and stimulus condition on re-engagement latency (F (1.84) = 1.69, $p > .10$). Aside from the interaction effects of genotype and gender on re-engagement latencies, there was a significant main effect of stimulus condition on re-engagement latency (F (3) = 2.33, $p < .05$). However, none of the six follow-up comparisons for this main effect revealed significant effects after correcting for multiple testing ($p > .0083$).

There were no main effects of genotype (F (3) = 0.48, $p > .10$) and gender (F (3) = 0.66, $p > .10$) or interaction effects of genotype and gender on disengagement probability (F (3) = 1.18, $p > .10$). Stimulus condition had a significant main effect on disengagement probability (F (3) = 17.69, $p <$ .001). This main effect of stimulus condition was explained by the fact that the disengagement probability was higher in the control stimulus condition (M = 0.94, SD = 0.12) than in the neutral (M = 0.82, SD = 0.22) (t (65) = 3.75, $p < .001$) and happy (M = 0.81, SD = 0.22) (t (65) = 4.32, $p <$

.001) conditions than in the fearful condition (M = 0.69, SD = 0.30). The disengagement probability in the fearful condition differed significantly from the control stimulus, (t (65) = 6.60, p < .001), neutral (t (65) = 3.74, p < .001), and happy (t (65) = 3.72, p < .001) conditions. Bonferroni corrected significance level for all six comparisons was .0083. Additional analysis with nonparametric Wilcoxon signed ranks test gave similar results as the parametric paired samples t test.

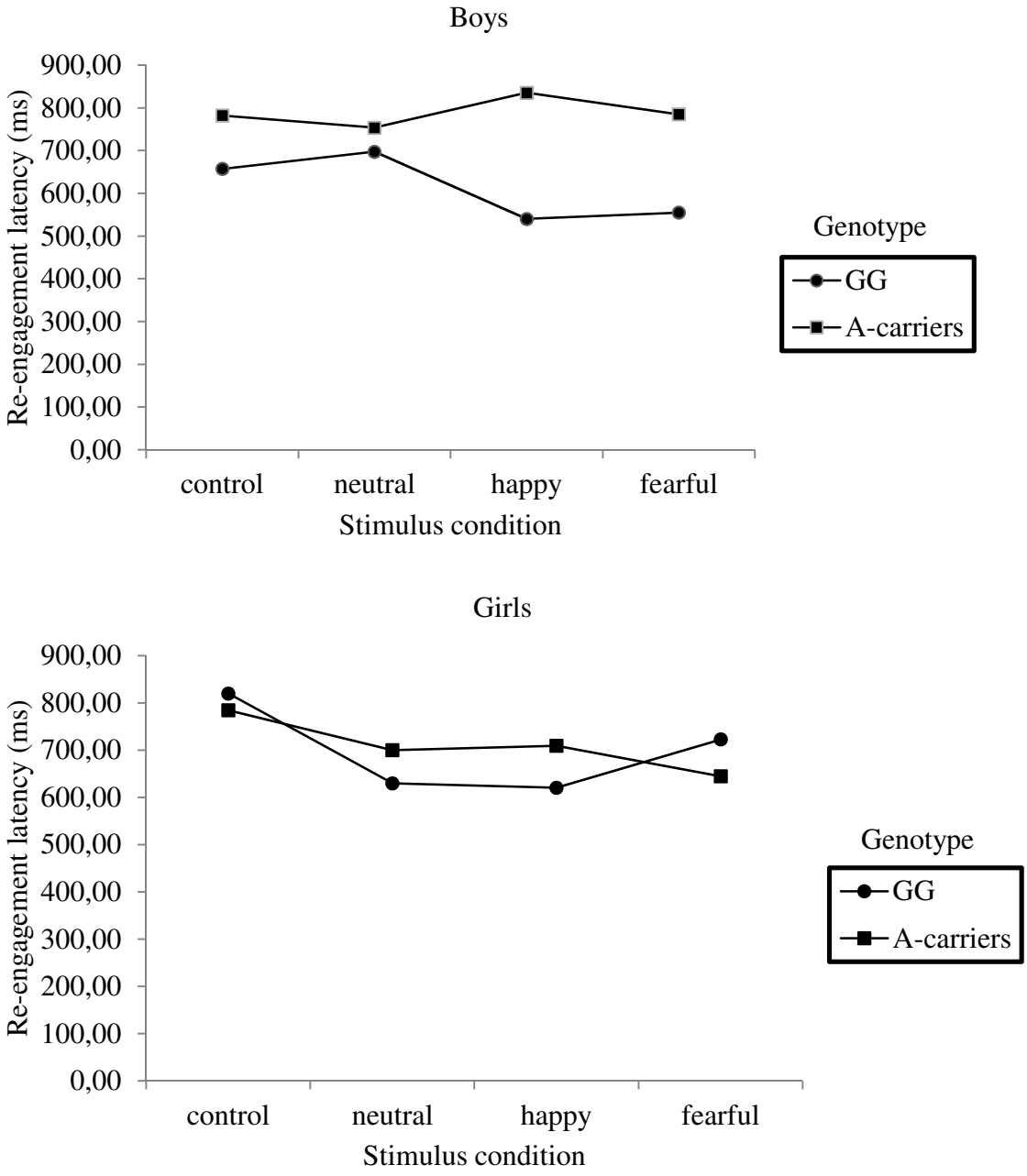

FIGURE 5. Effect of variation in the OXTR *rs53576* on re-engagement latency in 7-month-old boys (upper) and girls (lower).

3.2. 24-month assessment: prosocial behavior

All 32 participants were attentive in the helping tasks and empathy task, but five children were excluded for not paying enough attention in the social referencing task, so 27 participants were included in the final analyses. The OXTR *rs53576* genotype distribution of children participating in the 24-month assessment is presented separately for boys and girls in Table 5. The allele frequencies (n = 8 G/G, n = 15 G/A, n = 4 A/A) were in the Hardy-Weinberg equilibrium (χ^2 (1) = 0.50, $p > .10$). The genotype distribution did not differ significantly between these boys and girls (χ^2 (1) = 0.005, $p > .10$).

TABLE 5. The OXTR *rs53576* genotype distribution in 24-month assessment *(n* = 27).

	girls		**boys**		**Total**	
	n	%	*n*	%	*n*	%
G/G	2	29	6	30	8	30
A-carriers	5	71	14	70	19	70
n	7	100	20	100	27	100

TABLE 6. Descriptive statistics for variables used in the social referencing task, distance between the experimenter and the child in the helping tasks, and computed indexes (minimum and maximum values, mean, and standard deviation).

	n	***Min***	***Max***	***M***	***SD***
Social referencing					
Neutral trial					
Touch duration	27	0.00	1.00	0.59	0.40
Look frequencies	27	0.00	3.00	0.85	0.95
Fearful trial					
Touch duration	27	0.00	1.00	0.69	0.36
Look frequencies	27	0.00	6.00	1.37	1.69
Helping tasks					
Distance in the first trial	27	50.00	300.00	122.22	69.80
Distance in the second trial	27	50.00	300.00	133.33	63.55
Social Referencing Index	27	-1.50	3.50	0.21	1.02
Helping Index	27	2.00	4.00	3.44	0.80
Prosocial Index	27	-3.42	5.10	0.04	1.87

The genotype distribution of children participating in the 24-month assessment did not differ significantly from that of children who had provided blood sample and whose parents were contacted but who did not participate (χ^2 (1) = 1.38, $p > .10$). Also, children participating did not

differ significantly from those not participating in gender ratio (χ^2 (1) = 2.07, p > .10). The descriptive statistics for variables used in the social referencing task, distance between the experimenter and the child during the helping tasks, and computed indexes are presented in Table 6.

In the social referencing task, children looked more often at the experimenter in the fearful (M = 1.37, SD = 1.69) than in the neutral trial (M = 0.85, SD = 0.95), but this difference was not statistically significant (t (26) = 1.38, p > .10). Children also touched the toy for a longer period of time in the fearful (M = 0.69, SD = 0.36) than in neutral trial (M = 0.59, SD = 0.40), but this difference was not statistically significant (t (26) = 1.26, p > .10). The majority of children helped the experimenter in the helping tasks: 70 % (n = 19) of the participants helped in the first helping task and 74 % (n = 20) of them helped in the second one. In the empathy tasks, 52 % (n = 14) of the participants repaired the tractor.

There were no main effects of genotype (F (1) = 0.41, p > .10) or gender (F (1) = 0.21, p > .10) on prosocial index, but there was a suggestive interaction between genotype and gender on prosocial index (F (1) = 3.07, p = .093) (Figure 6). Given the a priori interest in gender differences in the OXTR *rs53576* genotype effects (cf. Tost et al., 2010), the genotype effects on prosocial index were analyzed separately in boys and girls with a Bonferroni corrected significance level of .025 for two comparisons. Boys with the G/G genotype showed significantly higher values on prosocial index (M = 1.59, SD = 2.01) than boys with the G/A and A/A genotypes (M = -0.45, SD = 1.57) (t (18) = 2.44, p = .025). The levels of prosocial index in girls with the G/G genotype (M = -0.99, SD = 1.24) did not differ significantly from those of girls with the G/A and A/A genotypes (M = -0.044, SD = 0.78) (t (5) = 0.59, p > .10).

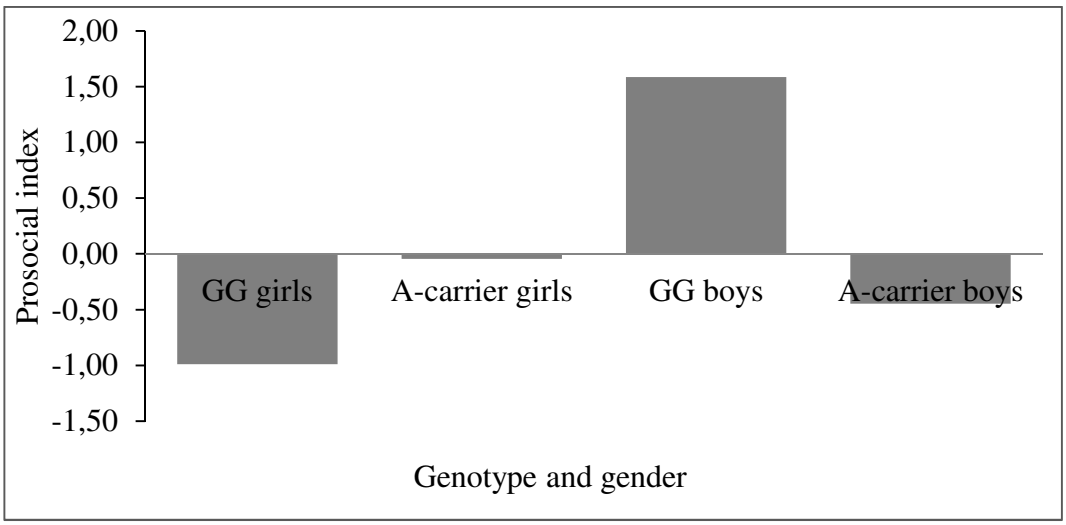

FIGURE 6. Effect of variation in the OXTR *rs53576* on prosocial index in 24-month-old boys and girls.

3.3. 48-month assessment: emotion recognition and theory of mind

The participants' age range was quite wide (1402–1735 days), but because the age during the assessment did not differ significantly in different genotype groups (G/G vs. A-carriers) (t (60) = 0.341, $p > .10$) and between different sexes (t (84) = 0.529, $p > .10$), all 61 participants were included in the analyses. The OXTR *rs53576* genotype distribution of children participating in the 48-month assessment is presented separately for boys and girls in Table 7. The allele frequencies (n = 15 G/G, n = 36 G/A, n = 10 A/A) were in the Hardy-Weinberg equilibrium (χ^2 (1) = 2.16, $p > .10$). The genotype distribution did not differ between these boys and girls (χ^2 (1) = 0.762, $p > .10$). The genotype distribution of children participating in the 48-month assessment differed significantly from that of children who had provided blood sample and whose parents were contacted but who did not participate (χ^2 (1) = 8.40, $p < .05$). Children participating did not differ significantly from those not participating in gender ratio (χ^2 (1) = 1.82, $p > .10$).

Some of the participants' answers were not scorable in the Diverse Desires task (n = 1) and False Belief task (n = 1). The percentage of correct answers in the theory-of-mind scale varied from 0.00 to 1.00 (M = 0.59, SD = 1.09). The majority of children answered correctly in the Diverse Desires task (90 %, n = 60). In the Diverse Beliefs task, 59 % of the children (n = 61) answered correctly, and 49 % of them (n = 61) succeeded in the Knowledge Access task. Only 28 % of the children (n = 60) answered correctly in the False Belief task, but in the Real-Apparent emotion task, 69 % of them (n = 61) answered correctly.

TABLE 7. The genotype OXTR *rs53576* distribution in 48-month-old assessment *(n* = 61).

	girls		**boys**		**Total**	
	n	%	*n*	%	*n*	%
G/G	4	18	11	28	15	25
A-carriers	18	82	28	72	46	75
n	22	100	39	100	61	100

The percentage of correct answers in the emotion recognition task ranged from 0.08 to 0.92 (M = 0.54, SD = 0.19), and for each emotion category (happy, sad, and fear), the percentage of correct answers ranged from 0.00 to 1.00. Recognition of happiness (M = 0.68, SD = 0.30) did not differ significantly from recognition of sadness (M = 0.63, SD =0.34) (t (60) = 1.09, $p > .10$), but compared to recognition scores for happiness and sadness, the recognition scores for fear were significantly lower (M = 0.32, SD = 0.27) (ts (60) = 6.63 and 5.83, ps < .001). Bonferroni corrected significance level was .017 for these three comparisons.

The percentage of correct answers in the theory-of-mind scale was not normally distributed according to one-sample Kolmogorov-Smirnov test ($p < .05$). The genotype effects were analyzed separately for boys and girls by using nonparametric methods (Mann-Whitney *u* test), which gave similar results as the parametric two-way ANOVA. The ANOVA results are reported here, because parametric methods were used in all other analyses, and because the ANOVA provides a method of analyzing the statistical significance of the genotype-gender interaction.

There were no main effects of genotype (F (1) = 0.071, $p > .10$) or gender (F (1) = 1.92, $p > .10$) on percentage of correct answers in the theory-of-mind scale, but there was a suggestive interaction between genotype and gender on performance in the theory-of-mind scale (F (1) = 3.42, $p = .07$) (Figure 7). Given this tendency and previous evidence for gender differences in the OXTR *rs53576* genotype effects (Tost et al., 2010), the genotype effects on performance in the theory-of-mind scale were analyzed separately in boys and girls (Bonferroni corrected significance level was .025), but no significant effects were found. Specifically, boys with the G/A and A/A genotypes (M = 0.62, SD = 0.22) performed better than boys with the G/G genotype (M = 0.47, SD = 0.24), but this difference was only suggestive (t (37) = 1.85, $p = .07$). Girls with the G/G genotype (M = 0.70, SD = 0.26) did not differ significantly from girls with the G/A and A/A genotypes (M = 0.59, SD = 0.17) in their performance (t (20) = 1.06, $p > .10$).

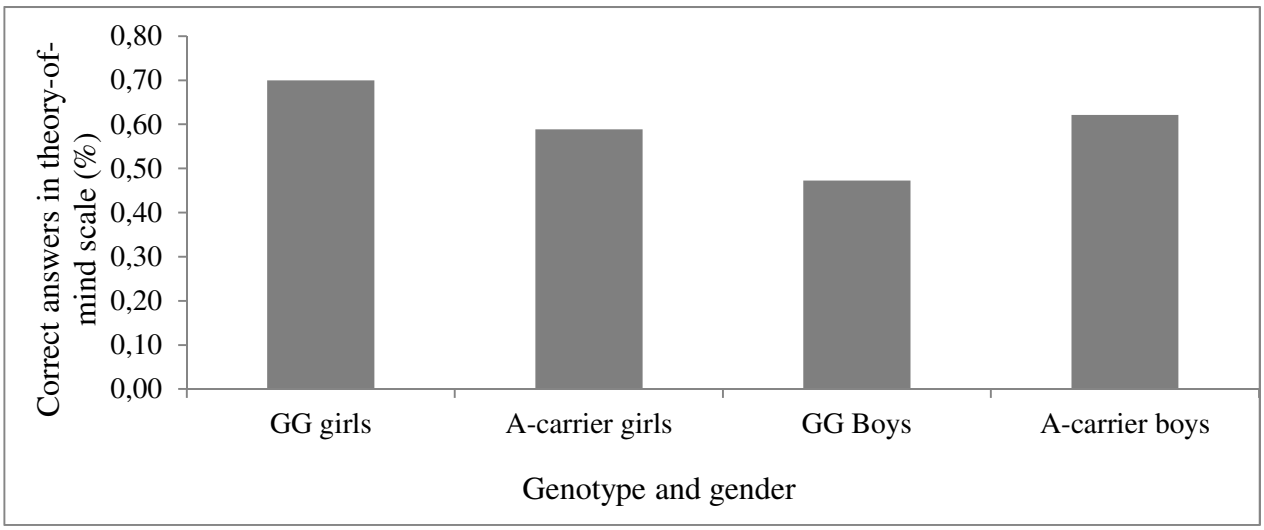

FIGURE 7. Effect of variation in the OXTR *rs53576* on percentage of correct answers in theory-of-mind scale in 48-month-old boys and girls.

There were also no main effects of genotype (F (1) = 0.24, $p > .10$) or gender (F (1) = 1.19, $p > .10$) on percentage of correct answers in the emotion recognition task, but there was a significant interaction between genotype and gender on emotion recognition (F (1) = 4.16, $p < .05$) (Figure 8).

However, when the genotype effects on emotion recognition were analyzed separately in boys and girls (Bonferroni corrected significance level was .025), no significant effects were found. Specifically, boys with the G/A and A/A genotypes (M = 0.57, SD = 0.18) did not perform significantly better than boys with the G/G genotype (M = 0.47, SD = 0.17) (t (37) = 1.52, p > .10). Also, girls with the G/G genotype (M = 0.67, SD = 0.20) did not perform significantly better than girls with the G/A and A/A genotypes (M = 0.51, SD = 0.20) in the emotion recognition task (t (20) = 1.37, p > .10).

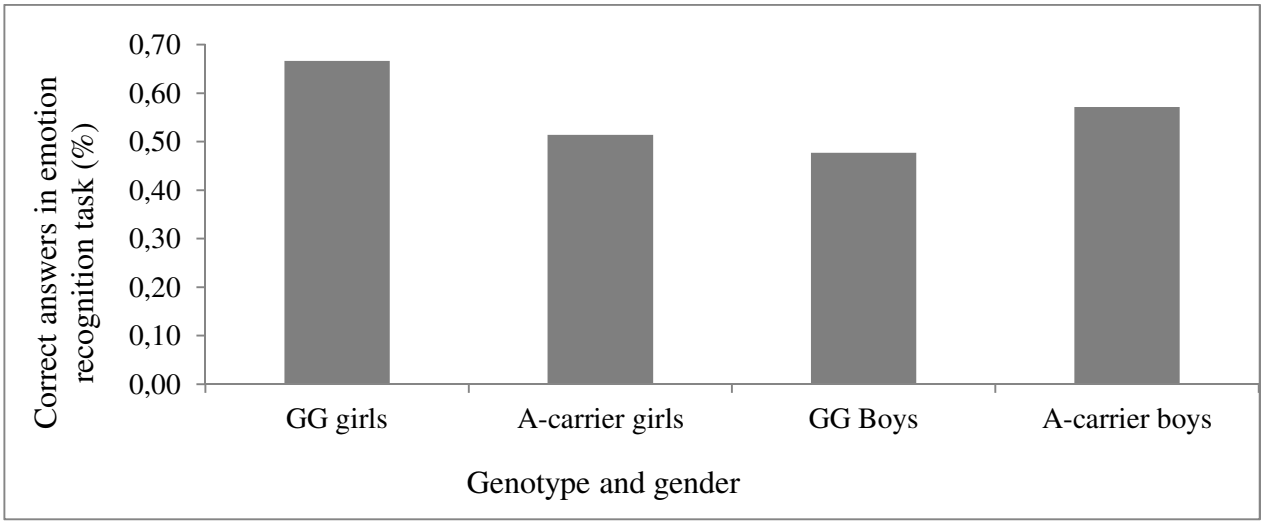

FIGURE 8. Effect of variation in the OXTR *rs53576* on percentage of correct answers in emotion recognition task in 48-month-old boys and girls.

4. DISCUSSION

In this thesis, the effects of variation in the OXTR *rs53576* genotype and gender on early-emerging social behaviors were examined. The genotype-gender interaction was analyzed on different forms of age-typical social behaviors in different postnatal ages: face preference and attention to facial expressions of emotion in 7-month-old infants, prosocial behavior in 24-month-old toddlers, and emotion recognition and theory of mind (mentalizing) in 48-month-old preschoolers. Individuals with the *rs53576* G/G genotype were compared to those carrying the A-allele (G/A and A/A genotypes), and the genotype effects were compared between boys and girls.

Because previous research done with adults has shown that the OXTR *rs53576* G/G genotype is associated with heightened levels of social behaviors when compared to the A-carrier genotype

(Bakermans-Kranenburg & van IJzendoorn, 2008; Poulin et al., 2012; Rodrigues et al., 2009; Tost et al., 2010), the overarching hypothesis of this thesis was that children with the G/G genotype would behave more socially than the A-carriers, and that this association would be seen in a small sample and across various tasks measuring spectrum of early-emerging social behaviors. More specifically, children with the G/G genotype would exhibit stronger face preference and enhanced attention to facial expressions of emotion in infancy, more prosocial behavior as toddlers, and they would also be better at mentalizing and recognizing emotions as preschoolers compared to those children with the G/A or A/A genotypes. Also, explorative analyses regarding genotype-gender interactions were performed because a recent study suggests that the effect of OXTR *rs53576* genotype on social behavior might be different for females and males (Tost et al., 2010).

4.1. Main results

The results provided partial evidence for the effects of variation in the OXTR *rs53576* on social behavior early in development in boys but not in girls. However, it is important to emphasize that these results are preliminary and need to be confirmed in further studies with larger sample sizes. The main results for each age group are discussed in the following.

4.1.1. 7-month assessment: preference for faces and social signals of emotion

The results of this thesis were consistent with previous studies that have used the overlap paradigm to assess disengagement of attention from facial expressions of emotion (e.g. Peltola et al, 2008, 2009b). Specifically, infants were less likely to disengage from faces, especially from the fearful face, as compared to the non-face control stimulus. Human face provides important social information especially for infants whose language has not yet developed (e.g. Nelson, 1987). Studies have shown that a robust preference for human face is present at the age of 4–6 months (DeNicola et al., 2013; Di Giorgio et al., 2012; Frank et al., 2009; Gliga et al., 2009). Face preference seems to be especially strong for specific emotional facial expressions at the age of 7 months when infants begin to show attentional bias towards fearful faces over happy and neutral faces (e.g. Nelson & Dolgin, 1985; Peltola et al, 2008, 2009a). In previous studies, the emotional significance of a stimulus has also affected how fast infants move their eyes from the peripheral target back to the central stimulus (i.e. re-engagement of attention). Peltola and his colleagues

(2011) used overlap paradigm and found that after disengagement of attention, 7-month-old infants moved their eyes back to the central stimulus faster when the central stimulus was a fearful face compared to a happy face (Peltola et al., 2011). However, the results of this thesis were not consistent with their study: infants moved their eyes back to the central stimulus faster when it was a fearful face compared to a control stimulus, but this difference did not survive correction for multiple testing. Also, the effect of stimulus condition on re-engagement of attention was not as strong as its effect on disengagement probability in this thesis.

The hypotheses regarding the effects of OXTR *rs53576* genotype on attention to facial expressions of emotion were partly supported. Variation in the *rs53576* genotype affected re-engagement of attention, but this genotype effect was only observed in boys when the central stimulus was a happy or fearful facial expression. Compared with the A-carrier boys, boys with the G/G genotype were faster to re-engage with the central stimulus when it was a fearful or happy facial expression. However, variation in the OXTR *rs53576* did not affect disengagement of attention.

One underlying neural mechanism for the oxytocin effects on processing emotional facial expressions might include modulations of amygdala-mediated attention to the eye region, especially when processing fearful facial expressions (Adolphs et al., 2005; Gamer & Büchel, 2009). Variation in the OXTR *rs53576* seems to affect the function of the amygdala so that it is more active in individuals with the G/G genotype during the processing of emotionally salient cues (e.g. facial expressions) than in the A-carriers (Tost et al., 2010). It has also been shown that intranasally administered oxytocin increases gaze to the eye region of human face (Andari et al., 2010; Domes et al., 2013; Guastella et al., 2008). The neural systems involved in facial-emotion processing (e.g. the amygdala, orbitofrontal cortex) develop early in life (reviewed in Leppänen & Nelson, 2009), so it is possible that the neural circuitry involving oxytocin and amygdala-mediated attention to the eye region is functional in early infancy. The results of this thesis are partially consistent with this view. Specifically, boys with the G/G genotype and possibly more reactive oxytocin-amygdala circuitry (Tost et al., 2010) exhibited enhanced attention to fearful and happy facial expressions at 7 months of age.

One reason for why OXTR-gender interaction did not affect disengagement of attention but affected re-engagement of attention could be that disengagement and re-engagement of attention are sensitive to different aspects of attention to facial expressions (i.e. reflexive and more voluntary attentional biases). In the overlap paradigm, the appearance of the peripheral target stimulus causes reflexive saccades oriented to the target stimulus, and these kinds of saccades are mediated by subcortical systems (Colombo, 2001). It is possible that when fearful expressions capture the

infant's attention, these subcortical systems are inhibited and therefore, the reflexive saccades oriented to the peripheral target are inhibited (i.e. disengagement of attention becomes less probable). The re-engagement of attention, in turn, may engage more voluntary attentional control mechanisms, which are mediated by frontal areas (Colombo, 2001). Thus, re-engagement of attention could reflect more purely infants' attention preference towards social cues provided by facial expressions.

4.1.2. 24-month assessment: prosocial behavior

To examine whether the OXTR *rs53576* genotype would affect prosocial behavior in 24-month-old children, empathy, helping behavior, and social referencing were measured by using age-appropriate playful tasks and observation.

Previous studies have clearly shown that prosocial behavior (e.g. helping, sharing and comforting) emerges during the first two years of life (Dunfield et al., 2011; Kärtner et al., 2010; Svetlova et al., 2010; Roth-Hanania et al., 2011; Vaish et al., 2009; Warneken & Tomasello, 2006; Zahn-Waxler et al., 1992). Already at 14 months of age, children are able to help others in achieving simple goals, for example, handing over objects that another person cannot reach (Warneken & Tomasello, 2007), and more complex goals (e.g. by sharing and comforting) from 18 months of age (Brownell et al., 2009, 2013; Dunfield et al., 2011; Svetlova et al. 2010; Vaish et al., 2009). Also, already at 18 months of age, children can react empathically to others' distress (Vaish et al., 2009). This thesis is in line with these previous studies: the majority of the 24-month-old children helped the experimenter and nearly half of them reacted when she was grieving over a broken toy by trying to repair it. However, there were no significant differences in children's behavior in the social referencing task between the neutral and fearful trial, so these children did not seem to use the experimenters reactions to regulate their behavior towards the new toy. It could be possible that the fearful reaction of the experimenter towards the new toy was not intensive enough. However, the purpose of this thesis was to examine individual differences in children's social behavior, and the behavior of these children varied greatly in the social referencing task.

The hypothesis regarding the genotype effects on prosocial behavior was partly supported: variation in the OXTR *rs53576* affected prosocial behavior of 24-month-old boys but not that of girls. Boys with the G/G genotype behaved more prosocially than boys carrying the A-allele. The results of this thesis are partly in line with the previous studies showing that individuals with the G/G genotype act more prosocially than the A-carriers (Poulin et al., 2012; Rodrigues et al., 2009;

Tost et al., 2010). The results also give some support for Tost and her colleagues' (2010) suggestions that males carrying the A-allele are more at risk for social dysfunction based on the effects of *rs53576* genotype on the amygdala and hypothalamus.

The results of this thesis also give some support for the suggestions that children are naturally predisposed to be prosocial (e.g. Hoffman, 1975; Warneken & Tomasello, 2009). Variation in the OXTR *rs53576* could participate in producing individual differences in prosocial behavior via its effect on oxytocin receptor function (Carter, 2007; Insel & Young, 2000), and therefore affecting oxytocin transmission in the brain (e.g. Rodrigues et al., 2009). It could be suggested that the G/G genotype might lead to more prosocial behavior because it leads to more enhanced effects of oxytocin, which has been shown to increase empathy (Bartz et al., 2010; Hurlemann et al., 2010) and prosocial behavior (reviewed in Heinrichs et al., 2009; MacDonald & MacDonald, 2010; Meyer-Lindenberg et al., 2011; Ross & Young, 2009) in adults when administered intranasally. However, according to this thesis and a study by Tost et al. (2010), the effect of *rs53576* genotype on prosocial behavior might be more pronounced in boys.

4.1.3. 48-month assessment: emotion recognition and theory of mind

To examine whether the OXTR *rs53576* genotype would affect emotion recognition and theory of mind (mentalizing) in 48-month-old children, these abilities were measured by using stories illustrating fear, happy, and sad emotions (Wismer Fries & Pollak, 2004) and pictures of facial expressions and theory-of-mind scale (Wellman & Liu, 2004).

Usually at three years of age, children are able to understand that other people might have different desires than themselves, and around four years of age they come to understand that other people may hold false beliefs (Wellman & Liu, 2004). Not until 5–6 years of age children are able to understand that a person may hide the emotion he or she is really experiencing and instead express another emotion (Harris, 1989). In this thesis, the majority of children passed the diverse-desires and diverse-beliefs tasks, nearly half of them succeeded in the knowledge-access task, and a minority of children passed the false-belief task. However, the majority passed the real-apparent emotion task, which should have been the most difficult task in the theory-of-mind scale according to Wellman and Liu (2004) (see also Harris, 1989). So the results of this thesis are not entirely consistent with the findings regarding the typical developmental course of theory of mind. In the original real-apparent emotion task (Wellman & Liu, 2004), the pictures of the schematic faces were put aside when the story began, but in the present study, the children saw these schematic

faces during the story. So it is possible that the children's performance in this task was facilitated by seeing these pictures during the story. The results of this thesis did show that there are substantial individual differences in children's mentalizing abilities, a finding that is in line with a study by Cutting and Dunn (1999).

It has been shown that four-year-old children are able to recognize and label emotions from facial expressions (Herba et al., 2006), although not as accurately as adults do (Widen & Russell, 2003, 2008). Four-year-old children are more accurate when recognizing happy expression than in discriminating between negative expressions (e.g. angry, fearful, and sad expressions) (Markham & Adams, 1992; Székely et al., 2011; Widen & Russell, 2003, 2008). This thesis is partly in line with these previous studies: fear was the most difficult emotion to recognize, but there were no differences between recognition of happy and sad emotions.

Hypotheses regarding the genotype-gender interaction were not supported: variation in the OXTR *rs53576* did not affect mentalizing abilities and emotion recognition in 48-month-old boys and girls. Boys with the G/A and A/A genotypes showed better mentalizing abilities than boys with the G/G genotype, but this difference was only suggestive. There was a significant genotype-gender interaction on emotion recognition, but when the genotype effects on emotion recognition were analyzed separately in boys and girls, no significant effects were found. So these suggestive results regarding theory of mind and emotion recognition were not in line with previous studies linking the G/G genotype to heightened levels of social behaviors (Bakermans-Kranenburg & van IJzendoorn, 2008; Poulin et al., 2012; Rodrigues et al., 2009; Tost et al., 2010).

It is possible that the hypothesized effect of variation in the OXTR *rs53576* on theory of mind was not found because the tasks used in the present study did not activate brain areas that oxytocin has been shown to modulate (e.g. the amygdala, Domes et al., 2007a, 2010; Kirsch et al., 2005; Petrovic et al., 2008; Rilling et al., 2012) as much as other brain areas involved in mentalizing (e.g. the medial prefrontal cortex, Frith & Frith, 2006). The amygdala is involved in mentalizing at least when making judgments about other people's minds by viewing their eyes (*Reading the Mind in the Eyes*, Baron-Cohen et al., 1997, 1999), perhaps because it directs attention to the eye region (Adolphs et al., 2005; Gamer & Büchel, 2009). It has been shown that intranasally administered oxytocin increases gaze to the eye region of human face (Andari et al., 2010; Domes et al., 2013; Guastella et al., 2008), possibly through its modulating effect on the amygdala, and also improves performance in the *Reading the Mind in the Eyes* test (Domes et al., 2007b), so it might be that oxytocin system is involved in this kind of mentalizing processing. However, the theory-of-mind scale used in the present study might not have involved this kind of amydgala-related mentalizing processing, so it is possible that the role of oxytocin system was not emphasized in these tasks.

Also, the emotion recognition task used in the present study might not have activated the amygdala, which could explain why the hypothesized effect of variation in the OXTR *rs53576* on emotion recognition was not found. Accumulated research considering the neural basis of emotion processing has shown the important role of amygdala in assigning emotional significance to stimuli, responding to fearful stimuli, and in recognition of fearful facial expressions (reviewed in Herba & Phillips, 2004). Oxytocin might enhance recognition of facial expressions of emotions (Van IJzendoorn & Bakermans-Kranenburg, 2012) through its effect on amygdala activity (Domes et al., 2007a, 2010; Kirsch et al., 2005; Petrovic et al., 2008; Rilling et al., 2012). However, neuroimaging studies suggest that the amygdala is more activated in implicit processing of emotional facial expressions (e.g. passive viewing or matching emotions) than in explicit processing (e.g. labeling or judgment of emotionality of facial expressions) when the prefrontal cortex is more activated (e.g. Hariri, Bookheimer, & Mazziotta, 2000; Lange et al., 2003). So it is possible that because children relied more on explicit processing of emotional facial expression in the emotion recognition task used in the present study, this lead to more activated prefrontal cortex and less activated amygdala of these children. Therefore, the role of oxytocin system might not have been emphasized in the emotion recognition task.

4.2. Genetic and environmental influences on social behavior

Overall, the present results point to an interaction of the OXTR allelic variations and gender on the early development of social behaviors in humans. While the results are of interest and partly in line with some previous studies, it is also important to view them in a wider context, acknowledging the possibility that the ontogeny of social behavior is likely to be influenced by multiple genes and environmental factors (Robinson, Fernald, & Clayton, 2008). In further studies, it would be important to examine not only the effect of other single nucleotide variations in the OXTR than the *rs53576* on social behavior, but also the effect of other genes affecting the neural systems involved in social behavior. For example, vasopressin, a peptide that act both as a hormone and neurotransmitter and is closely related to oxytocin structurally (Insel, 1997), also mediates complex social cognition and behavior in humans (reviewed in Meyer-Lindenberg et al., 2011). It is also important to study the behavioral correlates of genes coding protein for vasopressin receptors (see Ebstein et al., 2012), that are widely distributed in the brain (Insel, 1997).

Social behavior depends on interaction and communication between individuals (Robinson et al., 2008), and children's social behavior is affected by several environmental influences during

development. Early in childhood, the most important context for children's social behavior is family environment, and with increasing age it broadens to include peers and school environment. For instance, children's prosocial behavior seems to be enhanced by warm, supportive, responsive, and sensitive parenting style whereas authoritarian, strict, and punitive parenting might lead to lower prosociality (Knafo & Israel, 2010). Development of emotion recognition may also be affected by children's rearing environment, for example, lack of maternal stimulation or maltreatment (reviewed in Leppänen, 2011). The development of children's theory of mind might be affected, for example, by parents' education and occupational class (Cutting & Dunn, 1999), interaction with parents and siblings, and conversations about feelings and causality (i.e. antecedents and consequents of behavior) (Dunn, Brown, Slomkowski, Tesla, & Youngblade, 1991). Results regarding the association with number of child-aged siblings and enhanced mentalizing abilities have been mixed (Cutting & Dunn, 1999; Hughes & Ensor, 2005; McAlister & Peterson, 2006), but it seems that positive sibling relationships support development of theory of mind in young children (Hughes & Ensor, 2005).

Since genetic and environmental influences interact affecting the emergence of individual differences (Knafo & Israel, 2010; Robinson et al., 2008), further studies should examine the interaction of environmental variables and the OXTR genotype on early developing social behaviors. It is also known that environmental influences can regulate gene function and expression through so-called epigenetic modifications, which are heritable changes in activity or function of specific genes, but do not affect the underlying DNA sequence (Robinson et al., 2008; Kumsta, Hummel, Chen, & Heinrichs, 2013). There are several mechanisms for epigenetic regulation of gene function, and one of the most studied of them is DNA methylation, that is, direct chemical modification of the DNA (Kumsta et al., 2013). Preliminary studies suggest that the OXTR methylation is associated with autism, callous-unemotional traits (e.g. lack of guilt and empathy, Frick & White, 2008), and activation differences in brain regions involved in social perception, but the effects of environmental influences on the OXTR methylation have not been studied yet (reviewed in Kumsta et al., 2013).

4.3. Limitations and strengths of the present study

It should be noted that the results of this thesis are only preliminary and suggestive. The original study was designed to be longitudinal, so the attrition of the participants caused the sample sizes to be quite small. For this reason, all statistical comparisons were conducted cross-sectionally (i.e. by

analyzing the effects of OXTR *rs53576* genotype on social behaviors at each age separately) to maximize the sample size for each analysis. However, some of the groups remained small. Because individuals with the G/G genotype were compared with the A-carriers (G/G and G/A genotypes), the former group appeared to be a minority in the present study. The sizes of the genotype groups were small especially in girls who were a minority in the present study. This might have affected the results so that the genotype effects did not reach statistical significance when girls with different genotypes were compared. So the results regarding genotype-gender interaction on social behavior need to be confirmed in further studies with larger sample sizes.

The effects of other variables that might have affected the children's behavior in the assessments were not controlled. For example, it is possible that children's temperament affected their performance in the 24-month assessment so that very shy children may have inhibited their spontaneous helping behavior when they interacted with a strange adult. Although there was a warm-up period before the assessment began, that is, the experimenter played freely with the child for about 10 minutes, it is possible that it was too short for the shiest children to get familiarized with the experimenter. Also, although the participants in the follow-up assessments did not differ from those not participating in their genotype and gender distributions (except in their genotype distribution in the 48-month assessment), it is possible that they differed on some other variables (e.g. temperament, social economic status of the family, parents' education background). In further studies, it would be important to control the effect of these variables.

The strengths of this study include the use of experimental design with objective methods for assessing several aspects of children's social behavior. These methods have also been used in previous studies. The overlap paradigm has been used in several other studies examining infants' attention to emotional faces (Leppänen et al., 2011; Peltola et al., 2008, 2009b, 2011), and the tasks used in the follow-up assessments were modified from previous studies (Kärtner et al., 2010; Mumme & Fernald, 2003; Warneken & Tomasello, 2006; Wellman & Liu, 2004; Wismer Fries & Pollak, 2004). These tasks were neither too easy nor too difficult for the participants, so they seemed to be appropriate for studying individual differences. Also, one of the strengths of this study is that the genetic effects were seen in different ages and tasks: a similar genotype-gender interaction was observed at 7 months of age and later at 24 months of age with different measures of early-emerging social behaviors.

The assessments were conducted in environments that were not too stimulating for the children so they could focus on the tasks. In the first assessment, the room where the experiment took place was dark, so the infants had no other stimuli to pay attention to. However, it was quite difficult to assess how well the older children focused on the tasks, especially in the 48-month assessment when the

experimenter told them short stories in the theory-of-mind and emotion recognition tasks. In the 24-month assessment, some children were excluded because they clearly were not paying enough attention to some tasks, but in the 48-month assessment there were no clear criteria for excluding participants based on how attentive they were.

4.4. Summary and conclusions

In sum, this thesis offered preliminary and suggestive evidence about the effects of allelic variation in the OXTR *rs53576* on early-emerging social behaviors. Variation in the OXTR *rs53576* had an effect on some measures of social behaviors of boys but not that of girls. The *rs53576* genotype affected how the boys paid attention to facial expressions of emotion when they were 7-month-old, and how they used social information to guide their behavior especially for prosocial purposes when they were 24-month-old. Genotype did not affect emotion recognition or theory of mind when the children were 48-month-old. The results indicate that variation in the OXTR *rs53576* participates in producing individual differences in social behavior early in development especially in boys. However, further studies with larger sample sizes are needed to replicate these findings. Also, it would be important to take into account other genetic and environmental variables affecting social behavior in early development. The importance of studying oxytocinergic system is emphasized by the findings suggesting that oxytocin could be used as a treatment for social dysfunction (see Meyer-Lindenberg et al., 2011).

It is important to study the early development of social behavior and cognition not only to understand the typical development, but also to provide some information about how and why deficits in these behaviors and abilities emerge. This kind of information helps in developing methods of intervention and prevention for children exhibiting deficits and problems in social behavior and cognition so that these problems would not continue to adolescence and adulthood. So it is important to recognize those at high risk for social dysfunction in order to provide help and support for those in need. Also, the earlier the intervention is provided, the better the outcomes might be.

Genetic testing provides one method for recognizing those at risk for social dysfunction early in development. For example, children who have conduct problems and also display so-called callous-unemotional traits (e.g. lack of guilt and empathy, Frick & White, 2008), may be at risk for antisocial behavior later in development (reviewed in Lynam & Gudonis, 2005), and a recent study by Beitchman and his colleagues (2012) suggests that variation in the OXTR *rs237885* is associated

with these callous-unemotional traits in children aged between 6–16 years. Also, variation in the OXTR *rs53576* has been associated with autism (Wu et al., 2005; Ylisaukko-oja et al., 2006) but the results have been inconsistent (e.g. no association in Jacob et al., 2007; almost significant association in Liu et al., 2010). The results of this thesis suggest that the A-carrier boys might be at risk for developing social dysfunction (see also Tost et al., 2010). However, further research is needed before it is possible to use genetics in screening purposes.

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