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THE EFFECTS OF OXYTOCIN ON SOCIAL BEHAVIOUR: A COMPARATIVE APPROACH

Doctoral thesis

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2017

TABLE OF CONTENTS

I. GENERAL INTRODUCTION

I/1. Social sensitivity in children and dogs: similarities and differences

1.1 Children's receptivity to communicative signals

Human communication is often accompanied by ostensive signals, which means that a human's communicative act has not only an informative but also a communicative intention. That is, in addition to expressing the signaler's *knowledge sharing intention*, human ostensive communication is specifically adapted to identify the signaler's *intention to communicate* (Sperber and Wilson, 1986) – and this makes human communication specifically human. Even young children display sensitivity to the ostensive communications of potential teachers and they are also able to learn the contents of communicative interactions through the human-specific social learning system, a basic cognitive adaptation called natural pedagogy (Csibra and Gergely, 2009). The indicated special receptivity to communicative signals enables the acquisition of generalizable, culture-specific knowledge, ultimately laying the ground for the accumulation of knowledge over generations.

One of the most commonly used ostensive communicative signals is the direct gaze, which mostly eventuates mutual eye contact. Previous studies found that newborns prefer to look at faces with direct gaze over faces with averted gaze (Farroni et al., 2002). In case of infants similar neuronal activation can be detected to adults in response to both direct gaze and eye brow raise (Grossmann et al., 2008). Infants also follow the gaze direction of interactive partners to nearby targets within their visual field as early as 3 to 6 months of age in order to identify what they look at (D'Entremont et al., 1997; Farroni et al., 2004; Gredebäck et al., 2008). However, gaze following also serves communication. Evidence showed that young infants tend to follow gaze shifts only when the gaze shift is preceded by an ostensive signal such as eye contact or infant-directed greeting (Senju and Csibra, 2008). Newborns also prefer the special intonation pattern of 'infant-directed speech' ('motherese') as an obvious signal that (s)he is the intended recipient over adult-directed speech (Cooper and Aslin, 1990) and they are able to accurately follow others' pointing gestures to distal targets by the end of their first year (Carpenter et al., 1998).

Nevertheless, Csibra and Gergely (2009) suggested that ostensive communication does not only result that children pay more attention to the action but additionally they recognize it as a special opportunity to acquire generalizable knowledge. Studies with infants have confirmed that humans already at an early age process information presented in an ostensive context in a

specific way: they expect this information to be generalizable and not restricted to the given context (e.g. Futó et al., 2010; Topál et al., 2008). For example, Topál and colleages (2008) have shown that children, after repeatedly observing that an object is hidden at one location (A), tend to erroneously search for the hidden object in its initial hiding location even after witnessing that the object has been placed in another location (B). This is the case only if the experimenter has addressed them communicatively before hiding the object. If, however the original hiding event (A) is not accompanied by ostensive communicative cues, children commit perseverative search error significantly less often. This in sum suggests that they interpret the ostensive (but not the non-ostensive) 'A' trials as a learning situation, and generalize the acquired knowledge to the 'B' trials.

1.2 Dogs' receptivity to human communication

Dogs often show infant-like communicative receptivity to human ostensive-referential signals (Miklósi and Topál, 2012). Such remarkable similarities between dogs' and children's performance have initially tempted researchers to assume that they reflect human-like social cognition in dogs (Hare et al., 2002). However, more recent rigorous analyses have shown that different cognitive mechanisms may be at play. For example, Topál et al. (2009) compared the performance of dogs and children in a novel condition where a crucial difference in their behaviour emerged: while children continued to search in location 'A' following a communicative demonstration even when there was a new social partner present, the change in social context seemed to provide a clean slate for dogs. Thus, the authors concluded that despite the similarities in superficial behaviour, the cognitive processes may be markedly different. While children's behaviour can be explained by their bias to interpret information as generalizable across contexts, what dogs may extract from such demonstrations is an instruction to produce a certain action, which retains validity as long as the person giving the instruction is present. Later results have confirmed this interpretation suggesting that dogs tend to pick up information from ostensive communication that is restricted to the 'here and now' (Sümegi et al., 2014).

1.2.1 Explanatory framework – distal and proximal causes

A few years ago, Udell et al. proposed a 'two-stage model of domestication for the emergence of dogs' social communication skills (Two Stage Hypothesis'- Udell et al., 2009). In their paper, they place great emphasis on the role of environmental effects, and suggest that

differences in social cognitive skills between wolves and dogs have environmental (developmental) origins and the specific evolutionary adaptation to human environment plays little, if any, role in the formation of dog-like behaviour (Udell et al., 2010). Others, however, argue for a more balanced approach suggesting that the evolutionary history of dogs represents a special case of changes associated with living in a human-dominated social environment (Miklósi and Topál, 2013). That is, the transition from wolf to dog was probably driven by adaptations which enabled the dog to overcome the challenges of living in close proximity to humans. Domestic dogs (*Canis familiaris*) and humans have shared essentially the same ecological niche for at least 14,000 years (Clutton-Brock, 1995), which has been highly challenging for dogs by virtue of its complex social-communicative nature. In line with these, the so-called 'Domestication Hypothesis' (Hare and Tomasello, 2005) is based on the idea, that domestication, in its early stage, affected mostly the stress tolerance and aggression/fear response of the ancestral dog populations allowing them to exploit human social environment more successfully. These individuals were then subjected to artificial selection for traits that humans considered desirable, causing genetic changes that resulted in advanced socio-cognitive skills in the modern domestic dog (Hare and Tomasello, 2005). These changes may have resulted that dogs had evolved specialized skills for recognizing and interpreting human social-communicative signals. Domestication Hypothesis has been supported by the findings that wild canids often do not perform as well on human-cued object-choice tasks as their domestic counterparts. So as a result of the domestication process, both natural and artificial selection have led to the evolution of a species whose social skills resemble that of humans to an unprecedented degree (Hare et al., 2002).

It is also worth noting, however, that dogs are highly unusual in their phenotypic variation (Parker et al., 2004) and are thus ideal to study within-species individual differences. Indeed, the more than 400 living dog breeds show extreme morphological and behavioural plasticity (Svartberg, 2006) and breed specific behavioural differences are often viewed as a consequence of the past selection during the breeds' origins (Scott and Fuller, 1965). It is increasingly assumed that different aspects of social behaviour such as enhanced cooperative ability and enduring attention have become a key requirement for the process of breed formation (Gácsi et al., 2009b) and the behavioural repertoire of modern dog breeds generally reflects the function for which the dogs were originally used (Coppinger and Coppinger, 2001). Moreover, many of the dog breeds have been selectively bred to perform specific tasks (herding, sledding etc.) that required not only different morphological and behavioural

features, but probably also various socio-cognitive capabilities (Hare and Tomasello, 2005). Concerning the potential breed differences in the domain of social cognition, there is increasing evidence that dogs' ability to utilize human signals may vary with breed. Wobber and colleages (2009), for example, found that cooperative worker dogs (e.g. shepherds) use human gaze cues more skillfully than independent workers (e.g. sledge dogs). Cooperative worker breeds were also found to be significantly more successful in utilizing human pointing gesture (Gácsi et al., 2009a). Moreover, breeds may show differences not only in their 'inborn' communicative abilities, but also in their learning skills related to these (Jakovcevic et al., 2010).

1.2.2 Potential functional analogues of social sensitivity between dogs and children

Dogs have been part of human societies for longer than any other domestic species (Clutton-Brock, 1999) and their ability to form attachment with humans is one of the most widely recognized consequences of domestication (for a review see Topál and Gácsi, 2012). They form attachment to their owners very similarly to the bond between a human infant and their caregiver (Topál et al., 1998). In case of humans attachment is an organizational construct that serves to organize the development of emotional bond between human infants and their caregivers (Bowlby, 1958). In early infancy its function is to obtain protection and care from another person by adapting one's behaviour to the characteristics of the key attachment figure (Bowlby, 1969). This early development results in different attachment styles that can be assessed in terms of two dimensions of security/insecurity: attachment-related anxiety and attachment-related avoidance (e.g. Ainsworth et al., 1978; Brennan et al., 1998; Fraley and Spieker 2003). Attachment styles have well-documented cognitive, physiological, and neurological correlates (e.g. Diamond, 2001; Gillath et al., 2005), and behavioural and psychological consequences that last into adulthood, including self-regulation of stress and emotions, influence on relationship quality with romantic partners, sexual motivation, and reactions to relationship breakups or losses (see Shaver and Clark 1994; Mikulincer et al., 2003; for reviews). Similarly to human infants, the most widely used paradigm to investigate dogs' attachment behaviour shown towards their owners is the Strange Situation Test (SST, Topál et al., 1998). The essential element of the test is that separation from the human caregiver in an unfamiliar environment evokes moderate stress, which manifests in proximity seeking while the reunion with the caregiver evokes contact-seeking behaviours. Many studies have reported that dogs show a great variability in their behaviour in the SST (Topál et al.,

1998; Gácsi et al., 2001; Naderi et al. 2002; Prato-Previde et al., 2003; Mariti et al., 2014; Scandurra et al., 2016). Until now, however, only little (and indirect) data have been available about how different genetic and environmental factors influence dogs' attachment to human.

In addition to the parallels between dogs and children in their attachment to human caregivers there is ample comparative evidence to suggest that dogs' ability to rely on different forms of human directional gestures can be equated with that of shown by 1.5–2-years old children (Lakatos et al., 2009). Dogs often use eye gaze cues in a flexible manner; they show a tendency to make eye contact with their owner in unsolvable tasks (Gaunet, 2008; Miklósi et al., 2005) and alternate their gaze between the potential human helper and the object of desire (Gaunet and Deputte, 2011; Merola et al., 2012; Miklósi et al., 2000). Gaze alternation is a three-step sequence whereby the signaler alternates its gaze directly between a target and the partner, and it seems to fulfill the requirements of active information sharing since looking at the human partner − which is subsequently followed by looking towards the target − is functionally referential signalling (Lakatos et al., 2009; Soproni et al., 2001).

It also took two decades of research to determine the underlying mechanism of dogs' outstanding success in following human pointing (Lakatos et al., 2009). In contrast to early assumptions that dogs, just as children, interpret pointing as a form of cooperative referential communication that offers them food and information where it can be found (Hare and Tomasello, 2005), recent analyses have shown that dogs take pointing as an imperative that sends them to the highlighted location (Kaminski et al., 2012; Tauzin et al., 2015). Children interpret not only pointing but also directional gaze cues as communicative signals that are supposed to provide them with generalizable knowledge (Senju et al., 2008). Also dogs have broadly been believed to use human gaze similarly to pointing, often ignoring findings that even after a communicative gaze cue they choose one of two food locations randomly (Kaminski et al., 2012). Further evidence suggests that dogs' response to human communication is mainly driven by the motivation to satisfy the human ostensive cues even when the human's action is not efficient or represents a mistaken solution to the problem (Kupán et al. 2011). In general, dogs possess a sensitivity to human communicative cues that parallels that of human children (Topál et al., 2014).

Attachment to humans and sensitivity to human ostensive-communicative signals could be considered as evolutionary novel skills in the Canis genus providing a typical case for convergent social evolution. Many assume, however, that the perception of animacy is also fundamental for guiding social interactions and thus dogs, like human (infants), may possess

such social perceptual skills. Tremoulet and Feldman (2000), for example, showed that even a single moving geometric figure can be identified by adult human observers as animate based on simple motion cues and there is also evidence that even newborns are sensitive to self-propelled motion, one of the most powerful cues for triggering perceptions of animacy in humans (Di Giorgio et al., 2017).

Perception of animacy is an ability that is fundamental to the survival of several species. This perceptual ability has also been studied in dogs. Gergely et al. (2013), for example, using a remote control car as an UMO (Unidentified Moving Object) to examine whether dogs show different behaviours toward agents on the basis of their behaviour. They found that if the UMO possessed social-like features, dogs tended to interact with it, and they looked longer at this type of UMO than at a human who showed 'mechanistic' behaviour. The interactive behaviour of the dog emerged faster and became more elaborated when the UMO was endowed with features typically linked to animacy (eyespots, self-propelled motion and contingent reactivity). These results suggest that in social interactions the behaviour of the agent is more important than its embodiment. More recently, Petró et al. (2016) investigated dogs' ability to show differential soliciting behaviour towards two physically dissimilar UMOs which assisted them in getting food by solving different problems. The authors found that dogs chose the appropriate UMO for obtaining the food, as they approached, touched and looked first at the interacting agent which was able to retrieve the reward. In a similar vein, Abdai et al. (2017a) found that dogs tend to perceive geometric shapes as animate based simply on their movement patterns, and this perceptual ability is highly similar to humans and Tauzin et al. (2017) also provide evidence that dogs are able to discriminate agents from other self-moving entities based on navigational agency. Even more recently, Abdai et al. (2017b) examined the perception of animacy in dogs with a novel approach. They studied the dogs' recognition of chasing-like movement pattern performed by inanimate agents (UMOs).They found that dogs approached and touched the agent that demonstrated chasing-like behaviour sooner than those that showed independent movement. These results suggest that dogs can discriminate between 'chasing' and 'non-chasing' behaviour patterns and tend to consider chasers (versus randomly moving agents) as interactive partners.

Biological motion perception is one of the fundamental aspects of animacy recognition(Troje and Westhoff, 2006) that can help distinguish living organisms from other objects in the environment. The perceptual cues of biological motion and the neural mechanisms mediating the perception of biological motion have been extensively investigated in humans (Giese and

Poggio, 2003). This perceptual ability appears to be functional early in life (Simion et al., 2008), even newborn infants show a spontaneous preference for biological over non-biological motion. By 3-5 months of age, infants discriminate a pattern of dots that take the form of a walking figure from similar displays (Bertenthal et al., 1987, 1984). However, biological motion perception has been shown to be impaired in individuals with social disorders (e.g. autism: Klin et al., 2009). Evidence suggests that human observers can perceive biological motion even when there are very few points of light (point-light figure or PLF, Troje and Westhoff, 2006), only limited local motion information is presented (Beintema et al., 2002), and/or the PLF is degraded by masks (Cutting et al., 1988). 3-year-old children can reliably recognize point-light displays of human and non-human forms with highly reduced and unusual structural information (Pavlova et al., 2001). This recognition ability improves rapidly with age and 5-year-olds exhibit the ceiling level of performance. The neural underpinnings of biological motion perception are overlapping with brain regions involved in perception of basic social signals such as facial expression and gaze direction (Pelphrey et al., 2005). Although biological motion perception has not yet been studied in dogs, there is evidence that a wide variety of non-human species are capable of discriminating biological from non-biological motion (e.g. chimpanzees – Tomonaga, 2001; cats – Blake, 1993) as well as to extract certain cues from biologically moving point-lights (e.g. motion direction – MacKinnon et al. 2010). There is also evidence that some species show an innate preference for biological over non-biological motion (e.g. chicks – Vallortigara et al., 2005; female marmostes – Brown et al., 2010) or are innately attracted to biological motion (medaka fish: Nakayasu et al., 2013).

In addition to the recognition of biological motion patterns the perception of contingent reactivity is also fundamental for guiding social interactions. Contingent reactivity is a consistent and predictable relation between a subject's actions and the occurrence of a partner's responses, without which social interactions could not be realized (Bigelow, 2001). Watson (1972) has shown that 2-month-old infants increased their leg kicking rate after a contingent stimulus event, which was the movement of a mobile above their cribs, but not when they experienced a noncontingent, similar event. However there was no increase when they experienced a similar, but noncontingent event. They also found that infants acted socially (with smiling and cooing) if a hanging toy moved contingently with their head movement. Findings indicate that high degrees of temporal contingency is an indicator of agency (Movellan and Watson, 2002), and it could also be crucial in the identification of

communicative interactions (Csibra and Gergely, 2006; Csibra, 2010). Gergely and Watson (1999) have proposed the existence of an innate "contingency detection module" that analyzes the conditional probability structure of the contingent relations between responses and stimulus events. During the first 2–3 months, the initial target of the contingency detection module is genetically set to seek out and explore perfectly response-contingent stimulation. Furthermore it was hypothesized that at around 3 months, the target value of the contingency analyzer in normal infants was "switched" to a preference for high-but-imperfect contingencies (Bahrick et al., 1985; Gergely and Watson, 1999; Watson, 1994). In the study of Magyar and Gergely (1998), infants between 18 and 36 months were shown two displays; one display replicated their responses perfectly and the other one was reproduced imperfectly. Results showed that infants looked significantly longer at the imperfectly contingent display than the perfectly contingent one. These results show that infants prefer turn-taking responsiveness of social animate entities, rather than contigent responses provided by non-social, inanimate entities. Johnson et al. (1998) also found that infants followed the orientation of a stuffed animal when it had previously displayed contingent behaviour irrespective of whether it had eyes or not. Taken together, these observations indicate that infants prefer highly contingent over noncontingent behaviour and contingent over perfectly contingent behaviour, because they associate highly contingent behaviour with animates and noncontingent and perfectly contingent behaviours with inanimates (Rakison and Poulin-Dubois, 2001).

To our knowledge, however, there are only two studies investigating whether dogs use contingent reactivity as a cue for attributing agency. Gergely et al. (2015) found that dogs are able to find hidden food based on the directional movement of a nonliving interactive agent (UMO) as effectively as a similar human signal, but only if they obtained previous experience with the contingently responding self-propelled object in a different context. Tauzin et al. (2016) further investigated whether dogs could recognize contingent reactivity as a marker of agents' communicative intent. After having observed an unfamiliar self-propelled agent showing different levels of contingent responding ('low-contingency', 'high-but-imperfect-contingency' and 'perfect contingency' conditions) dogs were allowed to choose one of the target objects based on the target-indicating action of the agent. They found that dogs chose the target object significantly more often in the 'perfect-contingency' condition than in the 'low-contingency' condition and showed intermediate performance in the 'high-but-imperfect-contingency' condition. These results suggest that dogs are sensitive to the

differences in the degree of contingency and this might help dogs to discriminate between social and non-social interactions.

I/2. Neurocognitive and neurohormonal aspect of social sensitivity

2.1 The effects of oxytocin in humans

Much evidence has accumulated implicating a key role of the neuropeptide oxytocin (OXT) in the regulation of a variety of human social behaviours. OXT interacts with the hypothalamo-pituitary-adrenal axis to attenuate stress response and it induces potent physiological anxiolytic effects by decreasing cortisol levels, inhibiting cardiovascular responses to stress, and attenuating amygdala responsivity to emotional stimuli (Rodrigues et al., 2009). Studies examining the effects of OXT on behaviour usually take two forms: researchers may either measure endogenous OXT levels directly (or map genotypes related to the oxytocinergic system) or administer OXT in the form of nasal spray. Previous human studies have demonstrated that oxytocin nasal spray has significant impacts on social behaviour and cognitive processes in humans in a manner that has not previously been observed from the administration of other medications (Bartz et al., 2011; Kemp and Guastella, 2011). However, OXT levels may be manipulated in another way: through intensive social stimulation. A number of studies provide evidence that social interactions may elevate the level of OXT. For example, Feldman et al. (2010a) have found that mothers and fathers who had provided high levels of tactile contact to their infants showed an increase in salivary OXT following parent–infant interactions but such an increase was not observed among parents who provided low tactile contact. Moreover, after a 15 minute long play and touch interaction, both infants' and parents' salivary OXT level increased. Other studies also demonstrated that oxytocin is released in response to stimuli such as infant suckling, somatosensory touch, or even the sight or sound of a nursing mother's infant (Johnston and Amico, 1986; Lucas et al., 1980; McNeilly et al., 1983; Uvnas-Moberg et al., 1993).

Investigations have also provided convincing evidence that oxytocin (OXT) is implicated in a wide range of human social cognitive and emotional functions (Lee et al., 2009; Meyer-Lindenberg, 2008) as it affects the activation of brain areas responsible for emotion regulation and cognitive control, including the amygdala and the prefrontal cortex (Baumgartner et al. 2008; Domes et al. 2007; Kirsch et al. 2005). Recent studies have found that OXT also has an effect on complex behaviours such as trust (Kosfeld et al., 2005), empathy (Bartz et al., 2010; Hurlemann et al., 2010) and generosity toward strangers (Zak et al., 2007; Barraza and Zak,

2009). Kosfeld and colleages (2005), for example, have found that following intranasal administration of OXT, human participants were more willing to undertake risks in a social situation when monetary gains were at stakes, showing increased trust in the social partner. Other studies have confirmed the role of OXT in trusting behaviour both when money was involved (Baumgartner et al., 2008) and in different contexts as well (Mikolajczak et al., 2010). It has also been found to be an important factor in different aspects of human social cognition, such as the amount of attention directed at the eye regions of other people's faces (Guastella et al. 2008), covert attention to positive social cues (Domes et al., 2012), recognition of complex mental states and social emotions (Domes et al. 2007), and possibly emotion detection and emotion recognition (Guastella et al., 2009; Marsh et al., 2010; Schulze et al., 2011). Other higher-order functions that may be sensitive to the level of OXT in the brain include intergroup behaviour (De Dreu et al., 2011, 2010) and memory for social stimuli (Bartz et al., 2011; Kis et al., 2013; Rimmele et al., 2009; Savaskan et al., 2008). There are also efforts to investigate the effect of OXT at the early stages of perception, on a more basic behavioural level. There has been, however, few attempts to investigate the effects of OXT on lower levels of behavioural regulation, such as unconscious visual perceptual processes (e.g. Guastella et al., 2008). In one of the few studies, for example, Kéri and Benedek (2009) have found that oxytocin enhances sensitivity to biological motion in healthy adult humans.

Much attention in the human literature has been devoted to the enhancing effect of OXT on social skills in certain psychiatric conditions such as autism (Andari et al., 2010). Oxytocin administration in patients with autism spectrum disorders led to improvement of autism-specific symptoms such as repetitive behaviours and social cognition (Hollander et al., 2007). The effect of intranasal administration of OXT on prosocial behaviours and on higher level cognitive functions has also been in the focus of many recent investigations (see Campbell, 2010 for a review).

Carter (2014) proposed that high levels of social cognition, social interactions and bonds could not have evolved without the physiological and behavioural functions of OXT. The nervous system seems to be sensitive in early life to the presence or absence of different peptides, such as oxytocin (Carter et al., 2009). Neuroendocrine events, including those that were dependent on OXT, apparently support the prolongation of infant care and slow maturation of the human brain and this provides humans with an extended period for social learning, the development of an extended network of selective relationships, and cultural intelligence. Evidence also indicates that OXT has effects on brain development: variations in

oxytocin level might redound neocortical growth by inhibiting brain cells' programmed destruction or by encouraging undifferentiated stem cells to grow into cortical cells (Gutkowska and Jankowski, 2012). The face and head muscles are also regulated partly by the autonomic nervous system in mammals, which is influenced by OXT (Grippo et al., 2009; Quintana et al., 2013). Therefore it is not surprising that functions of the face, such as facial emotions and eye gaze, can be influenced by this neurohormone (Guastella et al., 2012). The fact that the OXT system is evolutionarily conserved (both the hormone and its receptor are present in mammals and other taxa – Donaldson and Young, 2008) could allow us to use a comparative framework and test the same phenomenon in different species.

Research findings also indicate that both endogenous release and exogenous administration of oxytocin attenuate the autonomic and neuroendocrine response to stress (Altemus et al., 1995; Ditzen et al., 2009; Linnen et al., 2012). Secretion of OXT is an inherent feature of HPA axis regulation. In response to a stressor, oxytocin is released in the paraventricular nuclei of the hypothalamus, which is associated with active stress-coping behaviour (Bosch et al., 2004; Neumann and Landgraf, 2012; Nishioka et al., 1998; Smith and Wang, 2014). Other mechanisms, such as enhanced social salience via increased attention to the eyes (Domes et al., 2007a; Guastella et al., 2008a), and increased reward sensitivity via activation of reward pathways during social interaction (Shahrokh et al., 2010; Strathearn et al., 2009) may interact with the anxiolytic effects of OXT to affect social behaviour. Accordingly, the effects of OXT on social behaviour may be due to its broad physiological effects; e.g. by reducing anxiety during social interactions, other basic aspects of the social interaction (eye contact etc.) became more salient or rewarding and this could promote social responsiveness (Buttner, 2016).

2.2 The effects of oxytocin on dog-human interactions

Increasing evidence suggests that OXT also plays a key role in modulating dog-human interaction. Perhaps as a result of stress-regulating effects of OXT (reducing the amygdala activation and attenuating stress reactivity), dogs may engage in more mutual eye gaze with humans particularly during sensitive developmental periods and this opens the door for more social interaction with humans, provides more opportunities to establish attachment bonds with humans as well as to learn socially form human partners (Jakovcevic et al., 2012).

Many researchers have found that (socially positive) dog-human interaction has the potential to increase peripheral level of OXT in both humans and dogs (Handlin et al., 2011; Miller et

al., 2009; Odendaal and Meintjes, 2003). Regarding the behavioural effects of OXT in dogs, it has also recently been reported that intranasally administered OXT promotes positive social behaviours toward both humans and dogs (Romero et al., 2014), it increases their performance in an object choice task using momentary distal pointing cues (Oliva et al., 2015), induces positive expectations about ambivalent stimuli (Kis et al., 2015) and increases eye-gaze behaviour toward their owners (Hernádi et al., 2015; Nagasawa et al., 2015). Others also found a link between dogs' behaviour and changes in the owners' peripheral OXT levels: Nagasawa et al. (2009) reported that urinary OXT concentrations of owners were increased by their dog's gaze. Altogether, these results suggest that the OXT system not only modulates the dogs' social behaviours toward humans, but efficiently contributes to the development of interspecific social competence in dogs.

2.3 Genetic determinants of social sensitivity in humans and dogs: the role of oxytocin receptor gene

A number of studies have looked at the associations between different single nucleotid polymorphisms (SNP) in the oxytocin receptor (OXTR) gene and social behaviour in humans. From these studies, a few SNPs have emerged as having a prominent role in shaping socio-cognitive skills and social behaviour. The OXTR gene rs53576 polymorphism (in intron 3) is probably the most intensively investigated SNP (for a meta-analysis, see Li et al., 2015) and it has been associated with – among others – stress reactivity (Rodrigues et al., 2009), need for social support (Kim et al., 2010) and emotion processing (Tost et al., 2010). The rs2254298 polymorphisms (in intron 3) in the OXTR gene have been linked to attachment anxiety (Chen and Johnson, 2012) and depression (Thompson et al., 2011) in certain populations. This is well-demonstrated by the fact that carrying a specific genetic polymorphism can be associated with developing a particular attachment style in one kind of social environment but not in another (Gillath et al., 2008). For instance, Chen et al. (2011) found that the A allele, as compared to the G allele, of OXTR gene rs2254298 was more likely associated with secure attachment in a non-Caucasian sample but not in a Caucasian sample. Allelic variations associated with different attachment styles of infants have been shown to affect also various characteristics of the parents, allowing for an alternative, indirect, link between genotype and infant attachment. On human subjects also Haram et al. (2014) found associations between agreeableness and extraversion personality traits and oxytocin-related gene variants, like

rs2270463 and rs237878, however without correcting for multiple testing. Furthermore (Lucht et al., 2009) found that subjects with the AA genotype of rs53576 OXTR gene exhibit more social loneliness as compared with G allele carriers. Additionally, according to previous studies (Saphire-Bernstein et al., 2011; Wang et al., 2014) subjects with the A allele of rs53576 were more vulnerable when facing stress, because researchers found that the AA genotype is associated with anxiety-related personality traits and a smaller amygdala volume. Nonhuman examples can also be found, namely cats with the A allele in the SNP G738A showed significantly higher "Roughness" personality scores than cats without the A allele (Arahori et al., 2016). The rs1042778 (in exon 4 3' UTR) has also been linked to the regulation of social interactions, in particular by modulating prosocial behaviour (Israel et al., 2009).

The SNPs of the oxytocin receptor gene that may account for the variability in the social behaviour of dogs are less well known. Despite the fact that the number of studies on dog social cognition are exponentially increasing (Bensky et al., 2013; Morell, 2009), until the last few years no information on the dog OXTR gene polymorphisms was available thus the role of these polymorphisms in behaviour regulation was explored in parallel with the present thesis. A few studies have used genetic sequencing to identify loci where significant variations are exhibited between individuals (Oliva et al., 2016) and have successfully linked some SNPs (rs8679682, −212AG, 19131AG) to proximity seeking and friendliness in dogs (Kis et al., 2014a). Oliva et al. (2016) reported significant species differences between dogs and wolves, 2 microsatellite primers (primers 5 and 6, which are the two closest to the OXTR gene) and suggested that OXTR gene may have played an important role in wolf-to-dog transition during domestication. Kis et al. (2014b) found that a single OXTR gene polymorphism (-213AG) is associated with proximity seeking; carrying the G allele, was associated with lower proximity seeking in Border Collies and German Shepherds. Furthermore Persson et al. (2017) tested Golden Retriever dogs after intranasal oxytocin pre-treatment in an unsolvable problem paradigm and investigated the effect of the treatment and OXTR polymorphisms on human-directed contact seeking behaviour. They found that the oxytocin treatment decreased physical contact seeking with the experimenter and independently of treatment the 19131AG polymorphism was associated with the degree of physical contact seeking with the owner, where dogs with AA genotype sought contact earlier than dogs with AG and GG genotype. The interaction of intranasal oxytocin treatment and OXTR gene polymorphism are also associated with dogs' human-directed social skills,

namely dogs with AA genotype increased contact frequency after oxytocin treatment while GG genotypes showed the opposite reaction. In a recent study Cimarelli et al. (2017) investigated with epigenetic methods whether environmental factors possibly influenced the epigenetic variation of the OXTR gene and its behavioural effects in dogs. They found that metilated CpG sites in the OXTR gene promoter region were significantly associated with the owner-related behaviour of Border Collies, in particular with the likelihood that dogs would hide behind their owner or remain passive when approached by a threatening human. However they did not find any association between owner behaviour and methylation levels of the OXTR gene in their dogs.

II. GENERAL AIMS

The present PhD thesis aims to investigate the neuro-hormonal aspects of social sensitivity in dogs and children focusing on the modulatory role of the oxytocin system on social behaviours and the oxytocin-mediated effects of social stimulation (Figure II/1).

We have three main goals.

In the first part (PART I.) of the 'Experimental studies' section, our purpose was to investigate whether different social behaviours were in connection with single nucleotide polymorphisms of the oxytocin receptor (OXTR) gene. We aimed to investigate,

1. whether the allelic variations of OXTR gene would be associated with attachment behaviour in dogs *(Study 1).*
2. and whether gaze-following and reaction to an aversive social interaction would have corresponding associations with variation in the OXTR gene in dogs and human infants. *(Study 2);*

In the second part (PART II.)**,** we studied the effects of intranasal oxytocin administration on dogs' behaviour. In two studies we investigated

1. the effects of oxytocin pre-treatment on biological motion perception in dogs (S*tudy 3),*
2. and the effects of breed differences and the breed-specific effects of oxytocin administration on different aspects of dogs' responsiveness to social cues *(Study 4).*

Finally (PART III.), we also investigated the effects of social presensitization on social sensitivity in two studies. That is,

1. whether the receptive social attitude can be induced by presensitization in children *(Study 5)*
2. and dogs *(Study 6).*

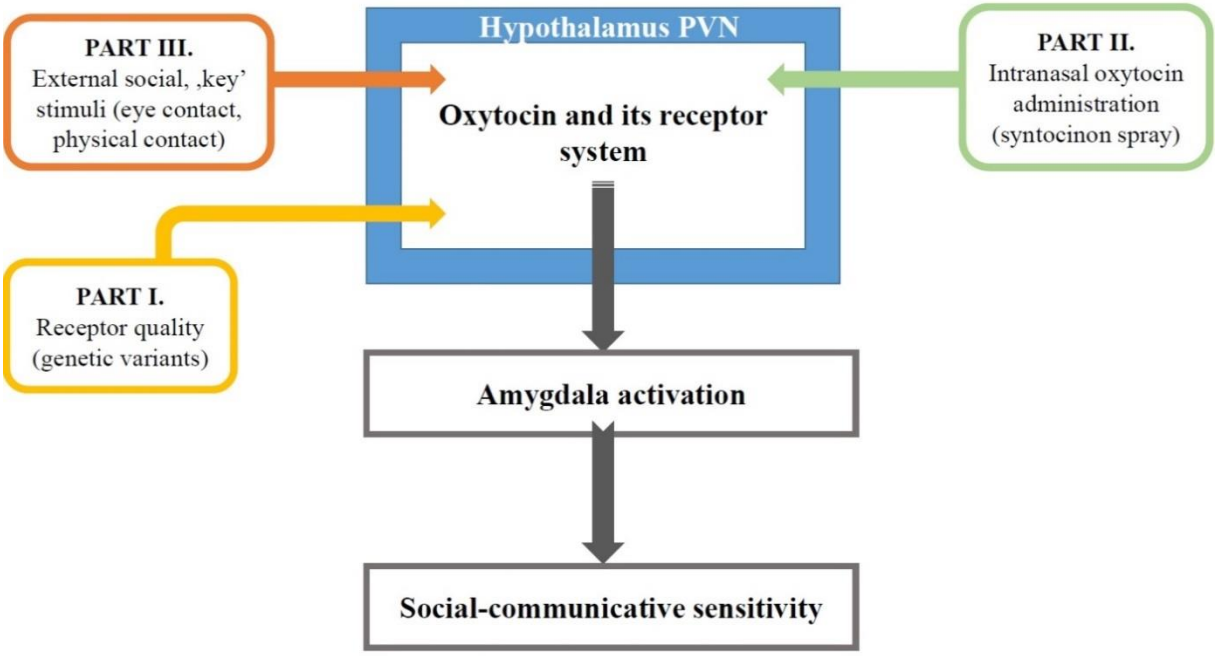

Figure II/1. Theoritical framework of the doctoral dissertation

III. EXPERIMENTAL STUDIES

PART I. Biological background of dogs's and infants' social sensitivity

III/1. Study 1: Dog-owner attachment is associated with oxytocin receptor gene polymorphisms in both parties. A comparative study on Austrian and Hungarian Border Collies[1]

III/1.1. Introduction

Until recently, most studies have focused on the environmental effects shaping attachment (such as parental behaviour, Fearon et al., 2014), even though Bowlby (1969) had already suggested that both nature and nurture play a crucial role in the development of attachment styles. Confirming his ideas, more recent candidate gene studies have reported associations between attachment styles of human infants and polymorphisms in their dopamine D4 receptor, serotonin transporter and oxytocin receptor (OXTR) genes (Barry et al., 2008; Lakatos et al., 2000; Chen et al., 2011; Spangler, 2011), suggesting that genetic polymorphisms may moderate the links between parental behaviour and other environmental effects and infant attachment. Therefore, it has become obvious that attachment styles are shaped by a combination of genetic factors and social experiences (Fonagy 2001). However such results in human infants allow for limited conclusions, because of the genetic relatedness of the infants and their parents.

As infants and their parents likely carry similar alleles in the polymorphic regions of their OXTR, in humans it is difficult to dissect whether and how infant genotypes, parent genotypes and other characteristics of parents (e.g. their personality or their own attachment style) affect infant attachment. The domestic dog, however, provides a unique opportunity to investigate this question.

Topál et al. (1998) were the first to reveal that dogs develop attachment to their owners analogous to the infant-mother attachment in humans (for a replication see Prato-Previde et

[1] This chapter is based on: Kovács K., Virányi Zs., Kis A., Turcsán B., Hudecz Á., Marmota MT., Koller D., Rónai Zs., Gácsi M., Topál J. Dog-owner attachment is associated with oxytocin receptor gene polymorphisms in both parties. A comparative study on Austrian and Hungarian Border Collies. *Front. Psychol. manuscript under review*

al., 2003). As such, pet dogs offer a good model for investigating to what extent attachment patterns are shaped by the independent genetics of the dogs and their owners and by environmental factors, such as the owners' personality, attachment style or actually the country they live in.

Similarly to the finding that polymorphism in the OXTR gene is related to security/insecurity of mother-infant attachment in humans (Chen et al., 2011), it has been suggested that oxytocin plays an important role in the relationships between dogs and their owners, higher oxytocin levels being associated with a more positive relationship from perspective of the owner (Thielke and Udell 2015).

The main purpose of the present study was to explore environmental and genetic influences on dogs' attachment behaviour. We investigated whether single nucleotide polymorphisms of pet dogs' OXTR gene (-213AG, -94TC, -74CG) and their owners' OXTR gene (rs53576, rs1042778, rs2254298) are associated with components of dog-owner attachment. In order to benefit from the genetic unrelatedness of dogs and their owners, in separate analyses we investigated *(1)* whether various OXTR polymorphisms of dogs as well as their owners are associated with the attachment behaviour of the dogs in two different countries, (2) whether such effects of the dogs' genotypes are affected by the age, sex, neutering of the dogs. Finally, to tackle potential mechanisms that may mediate affects of owner genotypes on dog attachment, we (3) analysed if owner personality, attachment style and attachment to pets have an effect on dogs' attachment behaviour. Dogs and their owners from two different countries (Austria and Hungary, N=135 in total) were tested in a modified version of the Ainsworth Strange Situation Test (SST) and questionnaires were also used to collect information about owner personality and attachment style.

III/1.2. Methods

III/1.2.1 Ethics Statement

The procedures were approved in accordance with GPS (Good Practice Statement) guidelines and national legislation by the Ethical Committees at the University of Veterinary Medicine Vienna and the Medical University of Vienna in Austria (Ref No. 04/12/97/2012 and 2073/2012, respectively) and the University Institutional Animal Care and Use Committee (UIACUC) of Eötvös Loránd University in Hungary (Ref No. XIV-I-001/531-4-2012). The owners undertook the test on a voluntary basis, they were informed that they would

participate in a scientific study and they signed an informed consent form of the study in both countries.

III/1.2.2 Subjects

Border Collies (N=135; mean age±SD: 4.17±3.01 years, range: 10 months – 14 years) kept as pet dogs were recruited in two countries, Austria and Hungary (Austria: male/female: 34/37, neutered/intact: 40/24; Hungary: male/female: 29/35, neutered/intact: 43/19); All dog−owner pairs participated in the behavioural testing (modified version of the SST test – Horn et al., 2013, see later). Buccal DNA sample was collected (see later) from N=130 dogs (Austria: 69, Hungary: 61) and N=66 owners (Austria: 33, Hungary: 33), in the remaining cases the owners refused to provide DNA samples. Owners were additionally asked to fill in three questionnaires assessing their personality (BFI_O), romantic relationships (ECR-R) and dog-owner relationship (modified ECR-R). Response rates have varied between 49.6% and 74.8% (owner personality: N=97 in total, Austria: 42, Hungary: 55; romantic relationships: N=101 in total, Austria: 40, Hungary: 61; and dog-owner relationship: N=67 in total, Austria: 34, Hungary: 33) respectively (see below for a more detailed description of questionnaires) Figure III/1.1).

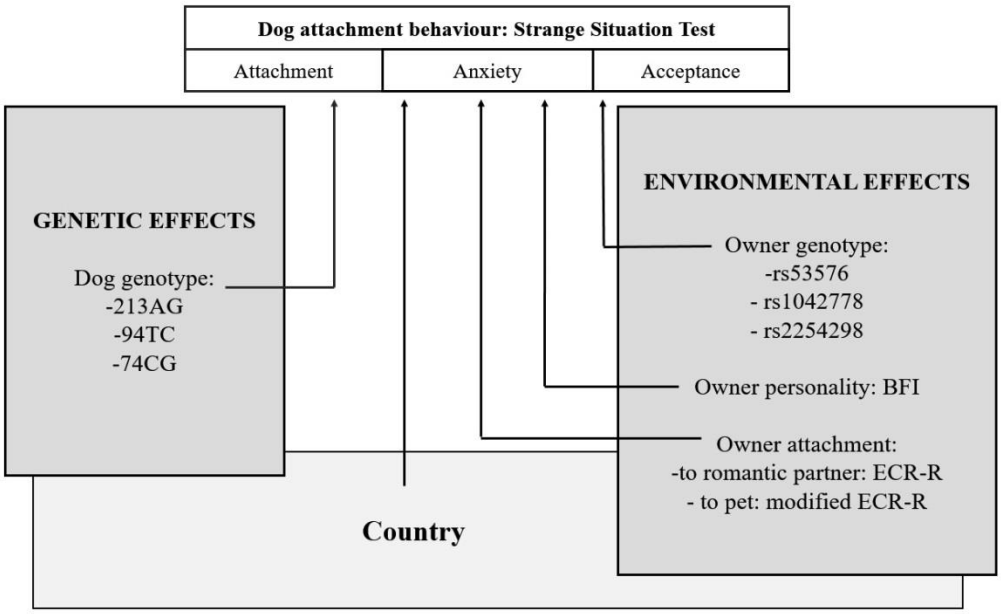

Figure III/1.1 *Examination of environmental and genetic associations of dogs' attachment behaviour to their owners.*

III/1.2.3 Experimental set-up

Dogs' attachment to their human caregivers was tested using the same protocol and experimental set-up in both countries (Horn et al., 2013). Testing took place in an experimental room that was unknown to the dog (5m × 6m; Figure III/1.2). The experimental room contained four cameras linked to monitoring and recording equipment in an adjacent room. The room contained two chairs (Chair 1, Chair 2), several toys placed on the floor, two elevated locations out of the dog's reach (i.e., windowsill, table; Location 1, Location 2), building blocks placed in Location 1, and a water bowl with fresh water. Three areas with 1 m radius were marked with tape on the floor for later video coding: "close to Chair 1", "close to Chair 2", "close to Door". Additionally, there were three lines indicating the quartiles between the table and the location with the building blocks.

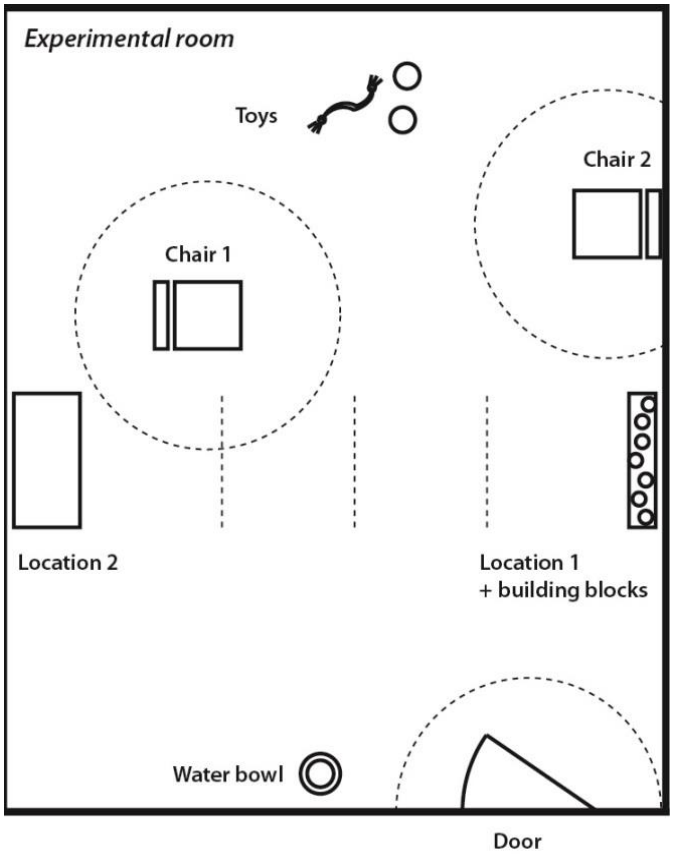

Figure III/1.2 Set-up used in the "Strange Situation Test"

III/1.2.4 Procedure

Before the start of the experiment, an experimenter explained the procedure in detail to the owner while the dog was sitting in an adjacent room. The test consisted of seven experimental

episodes of approximately 3 minutes each. In three episodes a stranger was present in the room. The stranger was of the same gender as the dog's owner and has never been seen by or interacted with the dog prior the experiment.

Episode 1 (Owner and dog): The owner entered the experimental room with the dog on leash and sat down on Chair 1. After letting the dog off the leash and placing the leash on the floor next to the chair, the owner first sat quietly and filled out a questionnaire without interacting with the dog for 2 minutes. After that the owner carried building blocks from Location 1 to Location 2 in order to build a tower without interacting with the dog for 1 minute. Then the owner sat back on Chair 1 and continued filling out the questionnaire.

Episode 2 (Owner, stranger and dog + owner leaving): A stranger entered the room quietly and sat down on Chair 2 opposite of the owner without interacting with the dog for 1 minute. Then the stranger got up and initiated play with the dog. After the first minute of the play phase the owner left the room quietly and the stranger continued to play with the dog for another minute.

Episode 3 (Stranger and dog + stranger leaving): The stranger returned to Chair 2 and did paperwork without interacting with the dog for 2 minutes. After that the stranger carried all the building blocks from Location 2 back to Location 1 without interacting with the dog for 1 minute. At the end of this phase the stranger left the room quietly.

Episode 4 (Dog alone): The dog was left alone in the room for 3 minutes. This episode was curtailed, if the dog was too distressed by the separation.

Episode 5 (Owner and dog + owner leaving): The owner entered the room, paused next to the door without interacting with the dog (approx. 5 seconds), then greeted the dog shortly (approx. 5 seconds), and finally sat back on Chair 1. The owner continued filling the questionnaire in without interacting with the dog for 3 minutes, and at the end of this phase left the room quietly again.

Episode 6 (Dog alone): The dog was left alone in the room for 3 minutes. This episode was curtailed, if the dog was too distressed by the separation.

Episode 7 (Stranger and dog): The stranger entered the room, paused next to the door without interacting with the dog (approx. 5 seconds), then greeted the dog shortly (approx. 5 seconds), and finally sat back on Chair 2. The stranger continued doing paperwork without interacting

with the dog for 3 minutes and at the end of this phase put the leash on the dog and left the room together with the dog.

III/1.2.5 Behaviour coding

Multivariate analysis of Topál et al's data (1998) (factor and cluster analyses) separated three key aspects of dogs' behavioural structure (Attachment, Anxiety and Acceptance). In our study we based our behavioural analyses on these three aspects of the dogs' behaviour in the SST. All the three key aspects of dogs' responses were built from the sum of the several independently coded scores. This method of evaluation, in contrast to the previously applied independent behaviour variables (e.g. Topál et al., 1998), allowed us to separate three scores that characterize the dogs' behaviour in the SST from three different (but somewhat interrelated) aspects. Scoring a different list of behaviours in different episodes (Appendix1) and summing these scores up for each aspect, each dog received a score of Attachment, Anxiety and Acceptance ranging from 0 to 12.

Dogs' behaviour was analyzed by using Solomon Coder (http://solomoncoder.com/). Coding was blind to subject details. Inter-rater reliability for dogs' behaviour was calculated by coding 30% of the sample by four independent coders. Intra-class correlation coefficient (ICC) was used to assess reliability ($ICC_{(2,4)}$= 0.976, p=0.002 for Attachment, $ICC_{(2,4)}$=0.923, p=0.018 for Anxiety and $ICC_{(2,4)}$=0.993, $p<0.001$ for Acceptance).

III/1.2.6 Buccal sample collection and SNP genotyping

Before the behavioural test we collected buccal cell samples from each dog with a non-invasive method, by swabbing the upper gum area of the dogs with 4 cotton tips (Wan et al., 2013; Kis et al., 2014). The cotton tips were then sealed in a tube and preserved in the freezer until genotyping (Bence et al., 2017). DNA purification was initiated by incubating the buccal samples at 56°C overnight in 0.2 mg/ml Proteinase K cell lysis buffer. It was followed by protein denaturation using saturated NaCl solution. Finally, DNA was precipitated using isopropanol and ethanol by standard procedures and DNA pellet was resuspended in 100 µl 0.5× TE (1× TE: 10 mM Tris pH=8, 1 mM EDTA) buffer. Typical DNA concentration of the dogs' genomic DNA samples isolated from buccal swabs were around 20 ng/µl. -213AG, -94TC and -74CG canine SNPs are located in the 5' flanking region (Figure III/1.3) The Qiagen Hot-StarTaq polymerase kit was used for PCR amplification. The reaction mixture contained 1 µM of each primer, approximately 5 ng of DNA template, 200 µM dNTP, 0.025

U HotStarTaq DNA polymerase, 1× buffer, and 1× Q-solution supplied together with the enzyme. The PCR cycle consisted of an initial denaturation at 95°C for 15 minutes, 40 cycles of 1-min denaturation at 95°C, 1-min annealing at various temperatures, a 1-min extension at 72°C, and a 10-min final extension at 72°C. The PCR reaction was performed in a total volume of 10 µl. -213AG and the -74CG polymorphisms were genotyped by PCR-RFLP method. PCR amplification was performed as described above using 5'-CCA TTG GAA TCC GCC CCC T-3' forward and 5'- CAC CAC CAG GTC GGC TAT G-3' reverse primers. Annealing temperature was 56˚C. PCR products were incubated for 3 h at 37˚C in a restriction enzyme mixture containing 0.5 U/µl Hpy99I restriction enzyme (NEB) for -213 SNP and 0.5 U/µl BsiEI restriction enzyme (NEB) for -74CG SNP, 1xBSA and 1x NEB4 buffer. Total reaction volume was 16 ml. -94TC SNP was genotyped by allelespecific amplification (ASA) using the primers described above. Allele specific primers were 5'-CCG ATC TGC TGG TCC CGG-3' and 5'-CCG ATC TGC TGG TCC CGA-3' and the annealing temperature was 60 ˚C. The PCR products were analysed by conventional submarine agarose gel electrophoresis (Biocenter, Szeged, Hungary), using 2.5% agarose gel and visualized by ethidium bromide staining. Genotype frequencies have been determined and Hardy-Weinberg Equilibrium analyses were carried out. The genotype frequencies were in Hardy–Weinberg equilibrium in both countries. Rare homozygote (AA) genotypes were grouped together with heterozygotes (AA+AG) (Table III/1.1).

Figure III/1.3 -213AG, -94CG and -74CG polymorphisms in the dog OXTR gene

	-213AG				-94TC				-74CG			
Austria	**AA**	**AG**	**GG**	**HWE**	**CC**	**CT**	**TT**	**HWE**	**CC**	**CG**	**GG**	**HWE**
%	**0.06**	**0.22**	**0.72**	**p=0.977**	**0.13**	**0.58**	**0.29**	**p=0.982**	**0.15**	**0.27**	**0.58**	**p=0.945**
N	**4**	**15**	**49**		**9**	**40**	**20**		**10**	**18**	**38**	

Hungary	AA	AG	GG	HWE	CC	CT	TT	HWE	CC	CG	GG	HWE
%	0.13	0.32	0.55	p=0.973	0.44	0.34	0.21	p=0.963	0.24	0.32	0.44	p=0.948
N	8	19	33		27	21	13		14	19	26	

Table III/1.1. Allele frequencies (%) and number of individuals (N) for Border collies from Austria and Hungary. Statistical tests for Hardy–Weinberg Equilibrium (HWE) are also provided

Human buccal samples were collected and DNA purification was obtained as described above. The rs53576 and the rs2254298 polymorphisms were located in intron 3 and the rs1042778 in exon 4 of the human OXTR gene (Figure III/1.4). PCR amplification was performed as described above using 5'- ACT GGG GCA ACC AAA CAT CT-3' forward and 5'- ACT CTT CAT GGC CCA GAG TG-3' reverse (rs53576), 5'- GCT CCA GCC AGA GGA G-3' forward and 5'-AGT GGG TTC AGG GTG GTA-3' reverse (rs1042778), 5'- CTG TCT TTG CAC CTT TGC TA-3' forward and 5'- ATG AAA GCA GAG GTT GTG TG-3' reverse (rs2254298) primers. Annealing temperatures were 56 ˚C (rs53576 and rs2254298) and 60 ˚C (rs1042778). OXTR rs53576 and rs2254298 SNPs were genotyped by PCR-RFLP method. PCR products were incubated for 3 h at 37˚C in a restriction enzyme mixture containing 0.5 U/µl AvaII restriction enzyme (NEB) for rs53576 SNP and 0.5 U/µl DdeI restriction enzyme (NEB) for rs2254298 SNP, 1x BSA and 1x NEB4 buffer. The rs1042778 SNP was genotyped by allele specific amplification (ASA) using 5'- AGC CAC CCC AAG GAG T-3' forward and 5'- AGC CAC CCC AAG GAG G-3' allele specific primers. The PCR products were analysed by conventional submarine agarose gel electrophoresis (Biocenter, Szeged, Hungary), using 2.5% agarose gel and visualized by ethidium bromide staining. Genotype frequencies have been determined and Hardy-Weinberg Equilibrium analyses were carried out. The genotype frequencies were in Hardy–Weinberg equilibrium in both countries. Rare homozygote (AA) genotypes were grouped together with heterozygotes (Table III/1.2).

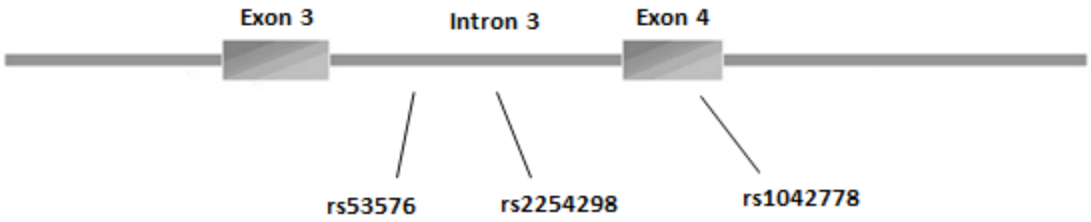

Figure III/1.4. rs53576, rs2254298 and rs1042778 SNPs in the human OXTR gene

	rs53576				**rs1042778**				**rs2254298**			
Austria	**CC**	**CT**	**TT**	**HWE**	**AA**	**AC**	**CC**	**HWE**	**CC**	**CT**	**TT**	**HWE**
Austria	**0.52**	**0.22**	**0.26**	**p=0.872**	**0.42**	**0.42**	**0.15**	**p=0.997**	**0.83**	**0.17**	**0.00**	**p=0.996**
N	**14**	**6**	**7**		**14**	**14**	**5**		**25**	**5**	**0**	
Hungary	**CC**	**CT**	**TT**	**HWE**	**AA**	**AC**	**CC**	**HWE**	**CC**	**CT**	**TT**	**HWE**
%	**0.39**	**0.36**	**0.24**	**p=0.968**	**0.13**	**0.25**	**0.63**	**p=0.946**	**0.84**	**0.16**	**0.00**	**p=0.996**
N	**13**	**12**	**8**		**4**	**8**	**20**		**26**	**5**	**0**	

Table III/1.2. Allele frequencies (%) and number of individuals (N) for owners from Austria and Hungary. Statistical tests for Hardy–Weinberg Equilibrium (HWE) are also provided

PCR amplification was performed as described above using 59-CCA TTG GAA TCC GCC CCC T-39 forward and 59- CAC CAC CAG GTC GGC TAT G-39 reverse primers. Annealing temperature was 56˚C and total reaction volume was 10 ml. PCR products were incubated for 3 h at 37˚C in a restriction enzyme mixture containing 0.5 U/ml Hpy99I restriction enzyme (NEB), 16BSA and 16NEB4 buffer. Total reaction volume was 16 ml. The digested PCR products were analysed by conventional submarine agarose gel electrophoresis (Biocenter, Szeged, Hungary), using 2.5% agarose gel and visualized by ethidium bromide staining. Genotype frequencies have been determined and Hardy-Weinberg Equilibrium analyses were carried out. The genotype frequencies were in Hardy–Weinberg equilibrium in both country (Austria: p=0.0761, Hungary: p=0.0704). Rare homozygote (AA) genotypes were grouped together with heterozygotes (AA+AG) (Table III/1.3).

			Austria	Hungary
Dogs	**-213AG**	**AA+AG**	19	26
		GG	49	34
	-94TC	**CT+TT**	60	34
		CC	9	27
	-74CG	**CC+CG**	28	33
		GG	38	26
Owners	**rs53576**	**CT+TT**	13	20
		CC	14	13
	rs1042778	**AA+AC**	28	12
		CC	5	20
	rs2254298	**CT+TT**	5	5
		CC	25	26

Table III/1.3. Number of individual dogs and owners in each OXTR genotype group in Austria and Hungary

III/1.2.7 Questionnaires

Owners both from Austria and Hungary were asked to fill out three questionnaires.

For measuring their personality the 44–item Big Five Inventory (OBFI_O; Appendix2; developed by John and Srivastava, 1999) was used. The questionnaire includes 8 questions related to extraversion (e.g. "Is full of energy"); 9 questions for agreeableness (e.g. "Can be cold and aloof"); 9 questions for conscientiousness (e.g. "Tends to be lazy"); 8 questions for neuroticism (e.g. "Is emotionally stable, not easily upset"); and 10 questions for openness (e.g. "Is curious about many different things"). All personality traits contained reverse scored items.

The relationship between the owner and his/her partner was measured by the 36-item Experiences in Close Relationship-Revised (ECR-R; Appendix3) Questionnaire (Fraley et al., 2000). Each item was rated on a Likert scale from 1 ("strongly disagree") to 7 ("strongly

agree"). The questionnaire includes 18 questions related to bond-related anxiety (e.g. "I worry a lot about my relationships") and 18 questions related for bond-related avoidance (e.g. "I tell my partner just about everything"). The trait scores were calculated by averaging the scores of the variables representing each trait.

The relationship between owner and his/her dog was measured by the modified Experiences in Close Relationship-Revised (modified ECR-R; Appendix4). This questionnaire was developed by Beck and Madresh (2008) based on the 36-item ECR-R for humans (Fraley et al., 2000). Each item was rated on a Likert scale from 1 ("strongly disagree") to 7 ("strongly agree"). The questionnaire includes 8 questions related to pet-related anxiety (e.g. "My pet makes me feel confident.") and 8 questions related for pet-related avoidance (e.g. "I prefer not to show a pet how I feel deep down"). The trait scores were calculated by averaging the scores of the variables representing each trait.

III/1.2.8 Statistical analysis

Three Generalized Estimating Equation Models using restricted maximum likelihood estimation were used. The first one (N=66) tested the effects of dog (-213AG, -94TC, -74CG) and owner (rs53576, rs1042778, rs2254298) SNPs, Country (Austria or Hungary) and two-way interactions between dog and owner SNP-s on the behavioural scales. The second model (N=130) tested the effects of dog OXTR SNPs (-213AG, -94TC, -74CG), Country (Austria or Hungary), Dogs' Sex (male or female), Neutering (Intact or Neutered), Age (covariant), as well as all two-way interactions of these on the behavioural scales measured in the SST test (Attachment, Anxiety, Acceptance), except SNP interactions with each other. The third model (N=67) tested the effects of the owners' questionnaire scales (BFI_O, ECR-R_Partner, ECR-R Dog) on the 3 behavioural scores of the dogs. Due to multiple comparisons levels of significance (p) were corrected (FDRbh adjustment, see (Benjamini and Yekutieli, 2001).

III/1.3. Results

III/1.3.1 The interactive effect of dog and owner OXTR polymorphisms (Table III/1.1)

Our analysis showed that dog and Owner OXTR SNPs had both main and interactive effects on behaviour measured in the SST (Table 5.). Attachment composite score was associated with both dog and owner OXTR SNPs (-213AG: $p<0.01$ -74CG: $p=<0.01$, rs1042778: $p<0.01$, rs2254298: $p<0.01$ respectively). Interestingly, however, dog and owner OXTR SNPs had also interactive effects, (-213AG × rs2254298: $p<0.01$, -213AG × rs53576: $p<0.05$, -74CG ×

rs53576: p<0.01). The effect of Country was further confirmed in interaction with dog OXTR SNPs (Country × -213AG: p<0.01, Country × -74CG: p<0.01).

The same holds true for the Anxiety score. Apart from the effect of dog -74CG OXTR SNP (p<0.05), interactive effects of the dog and human OXTR genotypes were also found (-213AG × rs53576: p<0.01, -74CG × rs53576: p<0.05). Interactive effect of country with dog OXTR SNPs (Country × -213AG, Country × -74CG, both p<0.01) was also significant.

Similarly, Acceptance of the stranger was also associated with dog OXTR SNPs (-74CG: p<0.05), owner OXTR SNPs (rs53576, p<0.05), and as well as the interaction of the two (-213AG × rs1042778: p<0.01, -213AG × rs53576: p<0.01, -94TC × rs1042778: p<0.01, -74CG × rs1042778: p<0.01, -74CG × rs53576: p<0.01).

Composite score	**Effect**	**WCS**	**p**	**Detail**
Attachment	**Main effects**			
	-213AG	**20.735**	**< 0.01**	**AA+AG > GG**
	-94TC	0.458	> 0.1	
	-74CG	**16.086**	**< 0.01**	**CC+CG > GG**
	rs1042778	**11.573**	**< 0.01**	**CC > AA+AC**
	rs2254298	**14.190**	**< 0.01**	**CT+TT > CC**
	rs53576	0.000	> 0.1	
	Country	1.887	> 0.1	
	Significant pairwise interactions			
	-213AG × rs2254298	**12.340**	**< 0.01**	**AA+AG (-213AG) +CC (rs2254298) > GG (-213AG) + CC (rs2254298)**
	-213AG × rs53576	**7.772**	**< 0.05**	**AA+AG (-213AG) + CT+TT (rs53576) > GG (-213AG) + CT+TT (rs53576)**
	-74CG × rs53576	**10.904**	**< 0.01**	**GG (-74CG) + CT+TT (rs53576) > CC+CG (-74CG) + CT+TT (rs53576)**

	Country × -213AG	**15.817**	**< 0.01**	**Austria AA+AG > Austria GG**
	Country × -74CG	**24.791**	**< 0.01**	**Hungary GG > Austria GG**
Anxiety	**Main effects**			
	-213AG	4.868		
	-94TC	0.000	> 0.1	
	-74CG	**7.808**	**< 0.05**	**CC+CG > GG**
	rs1042778	1.988	> 0.1	
	rs2254298	1.369	> 0.1	
	rs53576	1.222	> 0.19	
	Country	3.954		
	Significant pairwise interactions			
	-213AG × rs53576	**14.826**	**< 0.01**	**AA+AG (-213AG) + CT+TT (s53576) > AA+AG (-213AG) + CC (rs53576)**
	-74CG × rs53576	**5.955**	**< 0.05**	**GG (-74CG) + CT+TT (rs53576) > CC+CG (-74CG) + CT+TT (rs53576)**
	Country × -213AG	**20.088**	**< 0.01**	**Austria AA+AG > Austria GG**
	Country × -74TC	**10.421**	**< 0.01**	**Hungary GG > Austria GG**
Acceptance	**Main effects**			
	-213AG	4.562		
	-94TC	0.101	> 0.1	
	-74CG	**6.233**	**< 0.05**	**CC+CG > GG**
	rs1042778	0.548	> 0.1	
	rs2254298	1.480	> 0.1	
	rs53576	**6.331**	**< 0.05**	**CT+TT > CC**
	Country	1.592	> 0.1	
	Significant pairwise interactions			

	-213AG × rs1042778	**13.561**	**< 0.01**	**GG (-213AG) + CT+TT (rs53576) > GG (-213AG) + CC (rs53576)**
	-213AG × rs53576	**12.347**	**< 0.01**	**CC (-94TC) + CT+TT (rs53576) > CT+TT (-94TC) + CT+TT (rs53576)**
	-94TC × rs1042778	**12.695**	**< 0.01**	**CT+TT (-94TC) + CC (rs1042778) > CC (-94TC) + CC (rs1042778)**
	-74CG × rs1042778	**12.018**	**< 0.01**	**CC+CG (-74CG) + AA+AC (rs1042778) > GG (-74CG) + AA+AC (rs1042778)**
	-74CG × rs53576	**16.541**	**< 0.01**	**CC+CG (-74CG) + CT+TT (rs53576) > GG (-74CG) + CT+TT (rs53576)**

Table III/1.1. The effect of dog and owner OXTR polymorphisms on dog Attachment, Anxiety and Acceptance as measured in the Strange Situation Test. Significant effects are highlighted in bold.

III/1.3.2 The effect of dog OXTR polymorphisms and dog characteristics (Table III/1.2)

In our model two OXTR polymorphisms (-213AG, -74CG) and other dog characteristics (such as country, age, sex and neuter status) have been found to influence behaviour in the SST as a main effect or in interaction with each other. Attachment was most notably associated with Country ($p<0.01$) and Country × Neutered status ($p<0.05$) also had an effect. Anxiety was associated with the -213AG ($p<0.05$) SNP and country ($p<0.05$). There were interactive effects of Sex × -213AG ($p<0.05$), Sex × -74CG ($p<0.05$) and Neuter status × -213AG ($p<0.05$) . No main effect of OXTR SNP on Acceptance of the stranger was found, however it was influenced by Neuter status ($p<0.05$) and the interaction of Neuter status × Age ($p<0.05$).

Composite score	Effect	WCS	p	Details
Attachment	**Main effects**			
	-213AG	4.854		
	-94TC	0.000	> 0.1	
	74CG	1.325	> 0.1	
	Country	**16.868**	**< 0.01**	**Hungary > Austria**
	Age	4.207		
	Sex	1.762	> 0.1	
	Neuter status	0.137	> 0.1	
	Significant pairwise interactions			
	Country × Neuter status	**8.486**	**< 0.05**	**Neutered Hungary > Neutered Austria**
Anxiety	**Main effects**			
	-213AG	**12.349**	**< 0.05**	**AA+AG > GG**
	-94TC	0.099	> 0.1	
	-74CG	4.105		
	Country	**10.516**	**< 0.05**	**Hungary > Austria**
	Age	0.228	> 0.1	
	Sex	3.498	> 0.05	
	Neuter status	0.619	> 0.1	
	Significant pairwise interactions			
	Sex × -213AG	**11.027**	**< 0.05**	**Male AA+AG > Male GG**
	Sex × -74CG	**7.723**	**< 0.05**	**Female CC+CG > Female GG**
	Neuter status × -213AG	**8.975**	**< 0.05**	**Neutered AA+AG > Neutered GG**
Acceptance	**Main effects**			
	-213AG	0.179	> 0.1	
	-94TC	1.469	> 0.1	
	-74CG	1.161	> 0.1	
	Country	0.055	> 0.1	

	Age	0.167	> 0.1	
	Sex	3.098	> 0.05	
	Neuter status	**8.411**	**< 0.05**	**Neutered > Intact**
	Significant pairwise interactions			
	Neuter status × Age	**11.880**	**< 0.05**	**Intact Young > Neutered Young**

Table III/1.2. The effects of dog OXTR polymorphisms and dog characteristics on Attachment, Anxiety and Acceptance. Significant effects are highlighted in bold.

III/1.3.3 The effect of owner personality and relationship experiences (Table III/1.3)

Our analysis indicates, that dogs' behaviour in the SST is related to several aspects of owner personality and to the owner's experience with romantic partners and dogs. The dogs' attachment score was significantly associated with their owners' relationship both with their romantic partners and their dogs, in details Higher Attachment scores in dogs were in correlations associated with lower Bond-related avoidance ($p<0.01$) with their partner and higher Pet-related avoidance ($p<0.01$) with their dogs.

Higher Anxiety scores in dogs were in association with higher Extraversion ($p<0.05$) and Openness ($p<0.01$), as well as with higher Bond-related anxiety ($p<0.01$), Pet-related anxiety ($p<0.01$) and Pet related avoidancescores ($p<0.01$) and lower Bond-related avoidance ($p<0.01$) in their Owners.

As regards Acceptance scores, higher Acceptance scores in dogs were in association with lower Openness ($p<0.01$) and higher Bond-related avoidance ($p<0.01$), Pet-related anxiety ($p<0.01$), Pet-related avoidance scores ($p<0.01$) in their Owners .

Composite score	**Effect**	**WCS**	**p**	**Detail**
Attachment	Extraversion	3.545	> 0.05	
	Agreeableness	0.044	> 0.1	
	Conscientiousness	1.671	> 0.1	
	Neuroticism	0.525	> 0.1	
	Openness	1.430	> 0.1	

	Bond-related anxiety	0.545	> 0.1	
	Bond-related Avoidance	**30.691**	**< 0.01**	**lower Bond-related avoidance > higher Bond-related avoidance**
	Pet-related avoidance	**18.539**	**< 0.01**	**higher Pet-related avoidance > lower Pet-related avoidance**
	Pet-related anxiety	3.601	> 0.01	
Anxiety	**Extraversion**	**4.990**	**< 0.05**	**higher Extraversion > lower Extraversion**
	Agreeableness	0.001	> 0.1	
	Conscientiousness	4.150		
	Neuroticism	1.525	> 0.1	
	Openness	**9.577**	**< 0.01**	**higher Openness > lower Opennes**
	Bond-related anxiety	**8.944**	**< 0.01**	**higher Bond-related anxiety > lower Bond-related anxiety**
	Bond-related Avoidance	**15.345**	**< 0.01**	**lower Bond-related Avoidance > higher Bond-related Avoidance**
	Pet-related avoidance	**46.042**	**< 0.01**	**higher Pet-related avoidance > lower Pet-related avoidance**
	Pet-related anxiety	**18.790**	**< 0.01**	**higher Pet-related anxiety > lower Pet-related anxiety**
Acceptance	Extraversion	0.896	> 0.1	
	Agreeableness	0.393	> 0.1	
	Conscientiousness	2.837	> 0.05	
	Neuroticism	0.604	> 0.1	
	Openness	**11.588**	**< 0.01**	**lower Openness > higher Opennes**
	Bond-related anxiety	0.396	> 0.1	
	Bond-related Avoidance	**16.032**	**< 0.01**	**higher Pet-related avoidance > lower Pet-related avoidance**

	Pet-related avoidance	**40.075**	**< 0.01**	**higher Pet-related avoidance > lower Pet-related avoidance**
	Pet-related anxiety	**20.356**	**< 0.01**	**higher Pet-related anxiety > lower Pet-related anxiety**

Table III/1.3. The effects of owner personality and relationship experiences with both romantic partners and dogs on dogs' Attachment, Anxiety and Acceptance composite scores as measured in the Strange Situation Test. Significant effects are highlighted in bold.

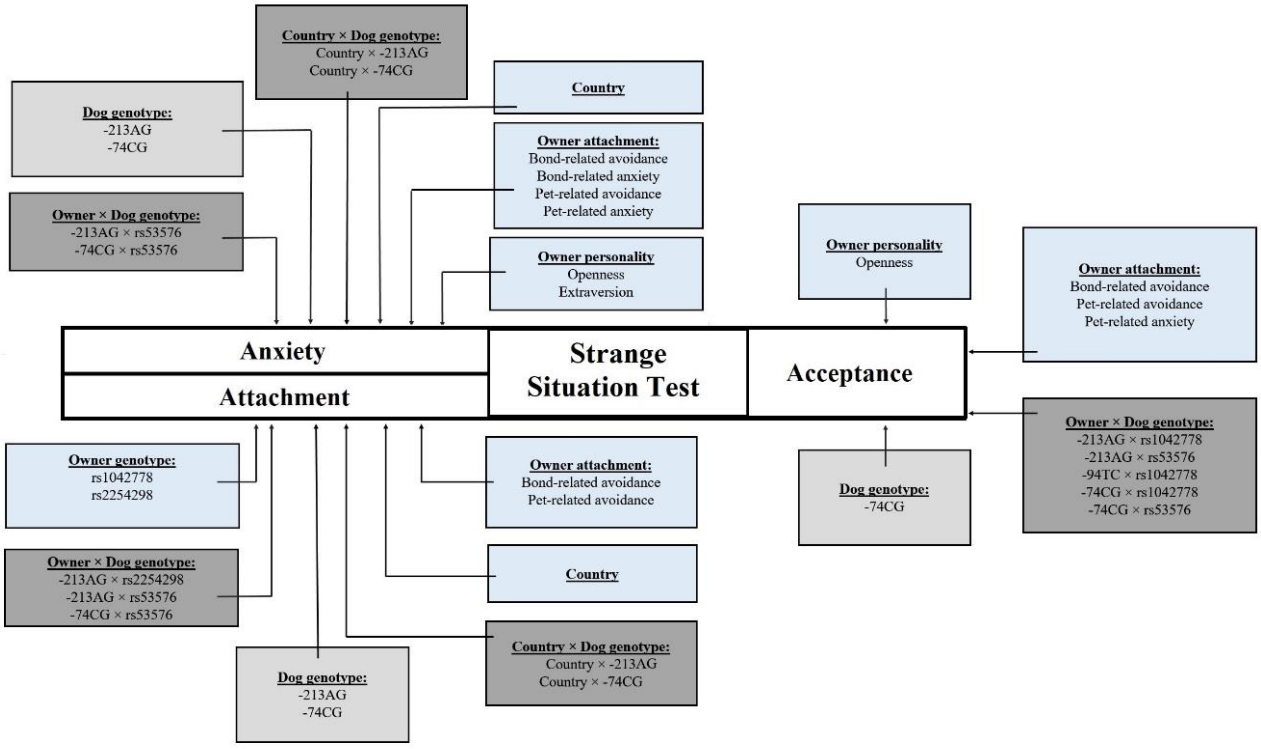

Table III/1.4. Overview of the results. Effects of environmental and genetic associations of dogs' attachment behaviour and their interactions.

III/1.4. Discussion

Chen and colleagues (2012) had suggested that one source of the variation in human infants' attachment to their mothers is the polymorphism of their OXTR gene but they remained cautious about this conclusion due to the genetic relatedness of infants and their parents. Based on the analogy between infant-mother and dog-owner attachment, our findings seem to confirm their suggestion, as the present study provides the first evidence that genetic

variations in dogs' OXTR gene are associated with their attachment behaviour to their owners.

All behavioural aspects measured in the SST (Attachment, Anxiety and Acceptance) showed significant association with all three SNPs investigated in this study (as a main or an interaction effect). While this study is the first demonstrating behavioural associations of the -74CG and -94TC SNPs of the canine OXTR, the relevant associations of the *-213AG* polymorphism has already been reported by others (Kis et al., 2014a).

We found some differences between the results of different models. For example -74CG has an effect on dogs' Attachment, Anxiety as well as Acceptance in our first model (when testing its effects in interaction with the owners' genotypes*)*, albeit it has an influence only on dogs' Anxiety and only when tested in interaction with dogs' sex instead of owner genotypes or a main effect. Furthermore however -74CG (and not -213AG) has a main effect on dogs' Anxitey in the first model, -213AG (and not -74CG) has a main effect on the Anxiety in our second model. In our study sex influenced the associations of two canine SNPs, namely the effects of -213AG and -74CG on dogs' Anxiety.

Our second important finding is that both dog and owner OXT genetic variation shapes the dog-owner attachment in an interactive manner. Earlier research has also shown a mutual effect of both dogs and their owners on the peripheral oxytocin levels of both parties (Nagasawa et al., 2009; 2015). To our knowledge, however, this is the first study to show that both dogs' and their owners'oxytocin system impact on dogs' attachment behaviour. We found significant effects of two human OXTR SNPs (rs2254298 and rs1042778) on the Attachment composite score and one SNP (rs53576) on Acceptance, as well as several significant interactive effects of the human and dog OXTR gene variants on the attachment behaviour of dogs (e.g., -213AG × rs2254298, -74CG× rs53576, -94TC × rs1042778, -74CG × rs1042778).

Confirming this assumption, we have also found that the owners' Bond-related avoidance to their partners and Pet-related avoidance influenced all the three composite scores of their dogs' attachment, Openness and Pet-related anxiety affected dogs' Anxiety and Acceptance, while Extraversion personality trait influenced the Anxiety composite score.

Finally, one of the most powerful effects we found was a difference between Austria and Hungary. There were main effects of country on two of the three behavioural components (Attachment and Anxiety composite scores) with dogs in Hungary showing higher Attachment and Anxiety, than dogs in Austria. Country has also influenced the effect of dog

genetic background on attachment; similarly to Chen et al.'s (2011) study where a certain OXTR genotype was associated with secure attachment in a non-Caucasian sample but not in a Caucasian sample.

Although the complex joint effects of genetic and environmental factors on dogs' human-directed social behaviour warrant further investigation, these findings offer a promising approach to studying causes and treatment of separation anxiety in dogs.

In conclusion, our study provides experimental evidence that both dog and owner genetic variations of the OXTR gene, as well as various aspects of dogs' environmental background are associated with their attachment to their human caregivers. Based on previous and present results we propose that polymorphism in the oxytocin receptor gene is a potentially important factor in regulating dog-human relationship.

III/2. Study 2: Gaze-following and reaction to an aversive social interaction have corresponding associations with variation in the OXTR gene in dogs but not in human infants[2]

III/2.1. Introduction

While oxytocin seems to facilitate social approach and social cognition in general both in dogs and humans (for reviews see Bartz et al., 2011; Kis et al., 2017), one of the best described mechanisms behind these facilitation effects is related to the attenuation of fear responses and anxiety. Not only oxytocin production but also the uptake of oxytocin is a key component of the oxytocinerg system. In line with this, increasing evidence suggests that genetic polymorphisms of the oxytocin receptor gene (OXTR) also play a role in modulating behaviour in social interactions, ranging from fearful behaviours through emotion processing to prosociality. A number of studies have looked at the associations between human social behaviour and different single nucleotid polymorphisms (SNP) in the oxytocin receptor gene.

The positive effect of oxytocin on following gaze cues to locate hidden objects may be exerted through at least two mechanisms: either through the reduction of social fear or through the enhancement of trust (Kirsch, 2015). That is, oxytocin may help to highlight the cooperative aspect of gazing (that is, its perception as an offer of food and information) or it

[2] This chapter based on: Oláh K., Topál J., Kovács K., Kis A., Koller D., Park SJ., Virányi Zs. 2017. Gaze-following and reaction to an aversive social interaction have corresponding associations with variation in the OXTR gene in dogs but not in human infants. *Front. Psychol.* 10.3389/fpsyg.2017.02156 in press

may facilitate approach by reducing social fear *despite* the fact that the context remains perceived as competitive. In the present study, following up on recent results described earlier, we set out to test the hypothesis that dogs perceive non-communicative gaze in an object choice task differently to children's interpretation of communicative gaze. First of all, we hypothesized that the oxytocin system would be related to the modulation of reactions shown in an aversive social context in both species. To test this hypothesis, we used well-established paradigms in both species that have already been shown to evoke distress in participants by violating the expectations of regular adult-infant or human-dog interactions (still face and threatening approach paradigms, respectively). Our second hypothesis was that in dogs oxytocin would also be related to following of non-ostensive human gaze through the same anxiolytic effect. That is, we predicted that the same OXTR genotypes will be associated with a less fearful reaction to social threat and with higher readiness to follow someone's gaze when searching for food. In contrast, in children, as they do not interpret gaze cues as a threat or competition, we predicted that following gaze will not be associated with OXTR polymorphisms or if yes different genotypes will be associated with gaze following and with reactions to a negative social situation. In order to test these hypotheses, we observed 1) the behaviour of both infants and dogs in a social context in which their human partner showed negative social behaviour unexpectedly, 2) the reaction of infants to communicative gaze, and 3) the reaction of dogs to non-communicative gaze. In addition, buccal samples were obtained from both children and dogs, in order to analyze the associations between behaviour and their OXTR polymorphisms located in the intronic as well as the UTR regions of the gene. In sum the present study investigated associations between oxytocin receptor gene polymorphisms and social behaviour in human infants and dogs with the aim to unravel potentially differential mechanisms behind their responsiveness to human gaze.

III/2.2. Methods

III/2.2.1.Ethics statement

The study with child participants was approved by the United Ethical Review Committee for Research in Psychology (Ref No. XIV-I-001/531-4-2012). For dog participants, ethical approval was obtained from in accordance with GPS (Good Practice Statement) guidelines and national legislation by the Ethical Committee for the use of animals in experiments at the

University of Veterinary Medicine Vienna (Ref No. 04/12/97/2012). Participants' owners (dogs) or caregivers (children) signed informed consent prior to participation.

III/2.2.2. Subjects

Human participants

99 toddlers of 15-16 months participated in the study (mean age: 15.73 months; SD: 0.26 months; range: 15.13-16.2 months). Children were selected from a database of families that had previously indicated interest in participating in research studies and were contacted again for this particular study. An additional 19 children were tested, but excluded from the sample due to fussiness (2), missing or insufficient DNA sample (14) or camera failure (3). 48 out of the 99 toddlers that successfully completed both tasks 76 children met the predetermined criteria only for the Gaze following task and 64 only for the Still face task (for more details see Procedure). In total, 76 child participants (36 boys / 40 girls) were included in the *Gaze following* task and 64 (32 boys / 32 girls) in the *Still face* task (Table III/2.1). Experiments with children were conducted at the Institute of Psychology, Hungarian Academy of Sciences, Budapest.

Dog participants

71 privately owned adult (older than 10 months) Border Collies (mean age: 4.27 years, SD: 2.88 years, 38 females) were recruited and tested at the Clever Dog Lab, Vienna, Austria. Out of the 71 dogs tested, 22 were castrated or spayed (12 females). An additional 5 dogs were tested, but excluded from analyses due to missing or insufficient DNA sample.

Children and dogs that could not be tested with one of the experimental tasks but provided valid data for the other were only excluded from analyses of the specific task in which they failed to participate. Similarly, if DNA could not be sequenced at a given SNP but there was valid data on the other SNPs, the participant was only excluded from the corresponding analyses (Table III/2.1 shows the number of dogs and children that were included in each analyses out of the 71 subjects and 99 participants, respectively).

Children			
	candidate SNP		
	rs1042778	**rs2254298**	**rs53576**

Gaze following task	76 (36/40)	76 (36/40)	76 (36/40)	
Still face task	64 (32/32)	64 (32/32)	64 (32/32)	
Dogs				
	rs8679682	**-213AG**	**-94CT**	**-74GC**
Gaze following task	56 (27/29)	51(24/27)	56 (27/29)	48 (24/24)
Threatening approach	56 (30/26)	50 (26/24)	56 (30/26)	48 (27/21)

Table III/2.1. Number of dogs (males/females) and children (boys/girls) included in the different analyses

III/2.2.3.Procedure

Both children and dogs took part in two tests. *Test 1* was construed to test their sensitivity to a human gaze cue. *Test 2* was construed to assess their reaction to an aversive social interaction with a human experimenter. Testing was conducted by two female experimenters for children and three female experimenters for dogs. In order to standardize their behaviour, all experimenters received a detailed experimental protocol and watched the other experimenter(s) conducting the tests. The next sections describe the species-specific testing situations separately.

Test 1: Following a human gaze cue - Children

Familiarization trials

Prior to the experiment children engaged in playful activities together with their mothers and the experimenter in order to familiarize them with the environment (10 min).

Test trials

Children were seated on their caregivers' lap on a 50 cm high chair. Parents were instructed to hold their children on their laps or were allowed to let children stand on the ground while the parent was holding them at a fixed position. The experimenter kneeled on the floor ca. 2 meters away from the child and the parent, facing them. She presented two identical opaque boxes to the participant, placing them in front of her 60 cm apart from each other. Once the child's attention was engaged, she opened the two boxes (starting always with the one on her left), revealing that one of the boxes contained a small toy. To make sure children realized the

toy in the box, the experimenter lifted the boxes, moved closer to the participant and showed them the content of the boxes close up. During this procedure, she communicated with the child in a natural manner, which included calling the child's name, using attractive facial expressions and engaging in eye-contact repeatedly. After that, she placed the boxes back at their original locations and closed the lids, starting with the one on the left. Then, she switched the location of the boxes three times in view of the child, but with a relatively fast motion in order to confuse children about the location of the baited box. This way, the baited box ended up on the opposite side of the experimenter. The experimenter then looked up at the child in order to initiate eye-contact with them. Once the child was engaged in eye-contact, the experimenter called their name and turned her head toward the baited box and kept looking at it for 5 seconds. After 5 seconds had elapsed, she turned her gaze back toward the child, smiling. At this point, parents (as a priori instructed) let go of their children, and participants were allowed to approach the boxes and look for the toy. If children touched one of the boxes or clearly pointed at one, the test was terminated and the experimenter helped open the box, revealing its content to the child. If children did not make a choice in the first 60 seconds they were coded as passive and were excluded from analyses.

Test 1: Following a human gaze cue - Dogs

Familiarization trials

This phase was included to familiarize the dogs with two small containers (10 cm diameter, 15 cm height). Before the start of the trial, the experimenter placed the two containers on the floor randomly, but ca. 1.5 m apart from each other, baiting only one of them with food (a small piece of cheese or sausage). The owner then let the dog free to enter the experimental room to search the two containers and eat the food, and waited with the experimenter outside of the room with the door open. If the dog did not start searching within 30 seconds after being released, the owner entered the room and encouraged the dog to search. A trial ended, once the dog ate the food. In total, there were 4 familiarization trials.

Test trial

Before the test trial began, the experimenter placed the two containers, in the same way as in the familiarization trials, and a chair at an equal distance of ca.2 m from the two containers. The experimenter kneeled between the two containers and waited, keeping her hands behind her back and looking straight ahead. The test began as the owner and the dog entered the

room. The owner sat on the chair, keeping the dog on a short leash so that the dog could not approach the containers closer than 1 meter. Once the owner sat down on the chair, the experimenter tried to make eye contact with the dog. If the experimenter was not able to do so within 10 seconds, she tried to get the attention of the dog by calling its name, but minimized other communication. As soon as eye contact was established, the experimenter kept looking into the dogs' eyes with a blank facial expression while staying still and silent. Once the dog broke the eye contact, the experimenter called the dog's name and made another brief eye contact and, with a clear head movement, turned her head to look down at the baited container for 5 seconds. After 5 seconds had elapsed, the owner released the dog to choose a container. The trial ended when the dog touched one of the containers with its mouth.

Test 2: Reaction to an aversive social interaction

The second test was designed to describe how the participants reacted in a socially aversive situation. As our goal was not to directly compare the behaviour of children and dogs but to compare the behavioural associations of the OXTR SNPs across tests and within species, we chose slightly different paradigms that have proved to detect individual variation both in children and dogs (still face task (Tronick et al., 1978) and threatening approach task (Vas et al., 2005), respectively). Both tasks have been described to evoke distress and frustration in participants through the violation of expectations of regular adult-infant or human-dog interactions. In the still face task, this is achieved by the withdrawal of the experimenter's communication and her lack of reactivity. In the threatening approach task, the prolongation of her approach and her intense looking evoke this mismatch. Importantly, children and dogs have also been described to show a similar range of reactions to these situations: some try to repair this mismatch by attempting to engage the partner in friendly interactions, some attempt to leave the unpleasant social situation whereas others exhibit signs of distress or frustration as a response to the violation of the expected social behaviour (Tronick et al., 1978; Vas et al., 2005).

Test 2: Reaction to an aversive social interaction - Children: Still face

Children participated in the Still face paradigm (c.f. Tronick et al., 1978) to test their reactions to the withdrawal of positive social stimulation from the experimenter. The test consisted of two one-minute-long phases. The caregiver was instructed to take a seat on one side of a 1.5 m long blanket and hold their child on their lap. The experimenter sat down at the other end of

the blanket, facing the child. In the first phase of the test, the experimenter engaged the child in a session of peek-a-boo game, where she alternated between initiating eye-contact with the child (smiling) and hiding her face behind a veil or her hands. After one minute had elapsed, a second experimenter signaled the start of the second phase. To ostensibly separate the two phases, upon hearing the signal from the second experimenter, the first experimenter turned her head away from the child and when she looked back, she began the still face phase, during which she was silently looking at the child but did not initiate any further contact and did not respond to the child's attempt to communicate. After one minute had passed, the test phase ended and the experimenter resolved the possible negative feelings caused by the still face episode by starting the peek-a-boo game again.

Test 2: Reaction to an aversive social interaction - Dogs: Threatening approach

The test procedure was similar to the procedure described in the experiment of Vas and colleagues (2005) in which the dog's response to the unexpected threatening behaviour of the experimenter was recorded. The dog was on a leash fixed on a wall in the room, while the owner was standing ca. 30 cm behind the dog. The experimenter, who had previously interacted with the dog and its owner in a friendly manner, entered the room from the side door and stood ca. 5 m away from the dog. Once the dog looked at her, the experimenter started to approach the dog slowly (one step in every 4 seconds) with her upper body slightly bent and looking steadily into the eyes of the dog without any verbal communication.

The behaviour of the experimenter was determined and standardized across subjects according to the following rules: (1) If the dog kept looking at the experimenter without any other reaction, then she continued to approach the dog until she reached it. (2) If the dog broke the eye contact with her (moving away and/or turning head away), the experimenter stopped and waited motionless for ca. 4 seconds and then tried to attract the dogs attention by making some noise (a slight cough or scratching the ground with the foot). If the dog continued to avert his gaze, the experimenter attempted to call the dog's attention two more times (with 2 seconds in between attempts). Whenever the dog looked at her again, she continued the approach. If, however, the dog did not look at her after the third attempt, the test was terminated. (3) If the dog showed active avoidance, that is, moved behind the owner, the test was immediately terminated. (4) If the dog showed signs of aggression, e.g. barked repeatedly or growled continuously (longer than 4 seconds) and/or tried to attack the experimenter, the

test was terminated. If the subject did not show any form of fear or aggression even when the experimenter reached the dog, she touched the dog's head and gently petted it.

III/2.2.4 Behaviour coding

Behavioural tests were coded offline from the recordings for pre-defined variables. For the *Gaze following task,* we coded whether the participants chose the container that had been indicated by the gaze direction of the experimenter. Participants that did not choose a container in the first 90 seconds were excluded from this part of the analyses. Both for dogs and children, we coded a correct choice if they chose the indicated container.

For the *Reaction to an aversive social interaction*, slightly different measures were used for infants and dogs due to the differences in the procedures. For children, we coded looking times, with a special interest in how much time they spent looking at the experimenter and their caregiver during the still-face period (coding categories: looking at experimenter, looking at caregiver, looking elsewhere). We also coded signs of distress (crying, negative vocalization or negative facial expressions) in the still-face phase. All of the variables were expressed in percentage of time as there could have been slight variations in the total duration times across participants. Infants who left their caregivers' laps during the still face period and spent more than 30% of the time outside of the testing context (that is, were not sitting on the caregiver's lap and were not within a 1 m radius of the experimenter) were excluded from this part of the analyses (n=21). Participants who left the caregiver's lap during the warm-up phase were excluded from all analyses (n=14).

For dogs, we also coded looking times during the threatening approach test (looking at the experimenter, the owner or elsewhere). Further on, we coded the dogs' first reaction to the threatening approach of the stranger with the following options: 1. friendly reaction to experimenter (tail wagging while moving towards the experimenter); 2, unfriendly reaction to the experimenter (looking at or approaching experimenter without wagging). Dogs that exhibited extreme stress were excluded from analyses (n=14).

III/2.2.5 Buccal sample collection and SNP genotyping

<u>Dogs</u>

Buccal cell samples were collected from each participating dog and child by swabbing the upper gum area with 4 cotton swabs. The cotton swabs were then sealed in a tube and preserved in the freezer until genotyping (Bence et al., 2017). DNA purification was initiated

by incubating the buccal samples at 56°C overnight in 0.2 mg/ml Proteinase K cell lysis buffer. It was followed by protein denaturation using saturated NaCl solution. Finally, DNA was precipitated using isopropanol and ethanol by standard procedures and DNA pellet was resuspended in 100 µl 0.5× TE (1× TE: 10 mM Tris pH=8, 1 mM EDTA) buffer.

For both species we genotyped polymorphisms that had been linked to social behaviour in former studies. For infants, these were the SNPs rs1042778; rs2254298 and rs53576 (based on Chen and Johnson, 2012; Israel et al., 2009; Rodrigues et al., 2009b, for instance). For dogs SNPs -213AG; -74CG; -94TC and rs8679682 were genotyped (Bence et al., 2017). Note that these SNPs, although all in the OXTR gene, are neither structurally nor functionally equal between dogs and humans.

Typical DNA concentration of the dogs' genomic DNA samples isolated from buccal swabs was around 20 ng/µl. The Qiagen Hot-StarTaq polymerase kit was used for PCR amplification. The reaction mixture contained 1 µM of each primer, approximately 5 ng of DNA template, 200 µM dNTP, 0.025 U HotStarTaq DNA polymerase, 1× buffer, and 1× Q-solution supplied together with the enzyme. The PCR cycle consisted of an initial denaturation at 95°C for 15 minutes, 40 cycles of 1-min denaturation at 95°C, 1-min annealing at various temperatures, a 1-min extension at 72°C, and a 10-min final extension at 72°C. The PCR reaction was performed in a total volume of 10 µl. -213AG and the -74CG polymorphisms were genotyped by PCR-RFLP method. PCR amplification was performed as described above using 5'-CCA TTG GAA TCC GCC CCC T-3' forward and 5'- CAC CAC CAG GTC GGC TAT G-3' reverse primers. Annealing temperature was 56˚C. PCR products were incubated for 3 h at 37˚C in a restriction enzyme mixture containing 0.5 U/µl Hpy99I restriction enzyme (NEB) for -213 SNP and 0.5 U/µl BsiEI restriction enzyme (NEB) for -74CG SNP, 1xBSA and 1x NEB4 buffer. Total reaction volume was 16 ml. -94TC SNP was genotyped by allelespecific amplification (ASA) using the primers described above. Allele specific primers were 5'-CCG ATC TGC TGG TCC CGG-3' and 5'-CCG ATC TGC TGG TCC CGA-3' and the annealing temperature was 60 ˚C. rs8679682 SNP was genotyped by real-time PCR using sequence specific TaqMan probes with minor groove binding (MGB) quencher. Primers were designed by Primer Express 3.0 (forward primer: 59-CTC CTT TAT TTTGGG ATC TTG TGA A-39, reverse primer: 59-CCT GCT CCTTAT TCT GAG CTT AGA A-39, probe specific for T allele: 59-FAM-AGT GGT AAG TAT AGG ATT G-MGB-39, probe specific for A allel: 59-VIC-AGT GGT AAG TAA AGG ATMGB-39.

The PCR products were analyzed by conventional submarine agarose gel electrophoresis (Biocenter, Szeged, Hungary), using 2.5% agarose gel and visualized by ethidium bromide staining. We investigated frequencies and Hardy–Weinberg Equilibrium analyses of the genotypes. Allele frequencies (Table III/2.2) did not deviate significantly from the Hardy-Weinberg equilibrium ($p>0.05$; Chi-square tests). We also tested whether there were any differences in allele frequencies across dogs tested by Experimenter 1, 2 and 3, and found no significant effects ($p>0.05$; Chi-square tests; see Table III/2.3.)

Genotype	**TT**	**CT**	**CC**	**GG**	**AG**	**AA**	**CC**	**CT**	**TT**	**GG**	**CG**	**CC**
frequency	0.257	0.60	0.143	0.657	0.20	0.143	0.114	0.571	0.314	0.528	0.257	0.1
Gaze following												
E1	9	13	3	15	6	2	3	12	10	13	5	3
E2	1	11	4	12	3	0	2	7	7	9	3	2
E3	6	8	1	10	2	1	1	11	3	9	3	1
Σ	***16***	***32***	***8***	***37***	***11***	***3***	***6***	***30***	***20***	***31***	***11***	***6***
Threatening approach												
E1	10	12	3	15	5	2	2	13	10	13	5	3
E2	1	12	4	13	3	0	3	8	6	8	5	2
E3	5	8	1	9	2	1	1	10	3	8	3	1
Σ	***16***	***32***	***8***	***37***	***10***	***3***	***6***	***31***	***19***	***29***	***13***	***6***

Table III/2.2. Allele frequencies for all dogs as well as the number of dogs by task and experimenter.

Children

SixRodrigues et al., 2009; Chen & Johnson, 2012; Israel et al., 2009 PCR amplification was performed as described above using 5'- ACT GGG GCA ACC AAA CAT CT-3' forward and 5'- ACT CTT CAT GGC CCA GAG TG-3' reverse (rs53576), 5'- GCT CCA GCC AGA GGA G-3' forward and 5'-AGT GGG TTC AGG GTG GTA-3' reverse (rs1042778), 5'- CTG TCT TTG CAC CTT TGC TA-3' forward and 5'- ATG AAA GCA GAG GTT GTG TG-3' reverse (rs2254298) primers. Annealing temperatures were 56 ˚C (rs53576 and rs2254298) and 60 ˚C (rs1042778). OXTR rs53576 and rs2254298 SNPs were genotyped by PCR-RFLP method. PCR products were incubated for 3 h at 37˚C in a restriction enzyme mixture containing 0.5 U/µl AvaII restriction enzyme (NEB) for rs53576 SNP and 0.5 U/µl DdeI

restriction enzyme (NEB) for rs2254298 SNP, 1x BSA and 1x NEB4 buffer. The rs1042778 SNP was genotyped by allele specific amplification (ASA) using 5'- AGC CAC CCC AAG GAG T-3' forward and 5'- AGC CAC CCC AAG GAG G-3' allele specific primers. The PCR products were analyzed by conventional submarine agarose gel electrophoresis (Biocenter, Szeged, Hungary), using 2.5% agarose gel and visualized by ethidium bromide staining. We investigated frequencies and Hardy–Weinberg Equilibrium analyses of the genotypes. Allele frequencies (Table III/2.3) did not deviate significantly from the Hardy-Weinberg equilibrium ($p>0.05$; Chi-square tests). We also tested whether there were any differences in allele frequencies between children tested by Experimenter 1 and Experimenter 2 and found no significant effects ($p>0.05$; Chi-square tests; see Table III/2.5.)

	rs1042778			**rs2254298**			**rs53576**		
Genotype	**TT**	**TG**	**GG**	**GG**	**AG**	**AA**	**GG**	**GA**	**AA**
frequency	**0.151**	**0.353**	**0.496**	**0.777**	**0.222**	**0**	**0.374**	**0.444**	**0.182**
Gaze following									
E1	8	20	25	42	11	0	23	22	8
E2	5	6	12	19	4	0	8	9	6
Σ	13	26	37	61	15	0	31	31	14
Still face									
E1	7	16	21	36	8	0	19	19	6
E2	2	6	12	15	5	0	8	8	4
Σ	9	22	33	51	13	0	27	27	10

Table III/2.3. Allele frequencies for all children and the number of children by task and

III/2.2.6 Statistical analysis

Statistical analyses were performed using SPSS 20.0. Based on the type of the dependent variable (behavioural measures), the associations between genotype and behaviour were analyzed using either General Linear Models (Univariate ANCOVA for durations); Binary Logistic Regression (for choice of container and first reaction in the threatening approach task for dogs). We used separate models for each SNPs, and in the ANCOVAs we included age as a covariant, sex (male vs. female), experimenter (two for children and 3 for dogs) and their two-way interactions both with each other and genotype (3 levels in all cases) in all models. For the regression analyses, we applied a backward elimination method of non-significant effects.

Finally, we also tested whether performance on one test was associated with performance on the other. Thus, we used independent samples T-tests to compare behaviours in the *Reaction to an aversive social interaction* task between participants that chose correctly vs. incorrectly in the *Gaze following* task.

III/2.3. Results

III/2.3.1 Gaze following

Children

All 76 children made a choice in this task. Out of the 76 children, 30 chose the baited container (thus, used the gaze direction of the experimenter as a cue to find the hidden object). This does not differ significantly from choosing randomly (though shows a marginal below chance effect) (binomial: p=0.085)

The SNP rs1042778 did not have a significant effect on children's choices and none of the control variables (age, sex and experimenter) did so either (all p>0.283 at removal) (Figure III/2.1). Similarly, we did not find any significant main or interaction effects in the analyses on SNP rs2254298 (all p>0.283 at removal) and SNP rs53576 (all p>0.283 at removal).

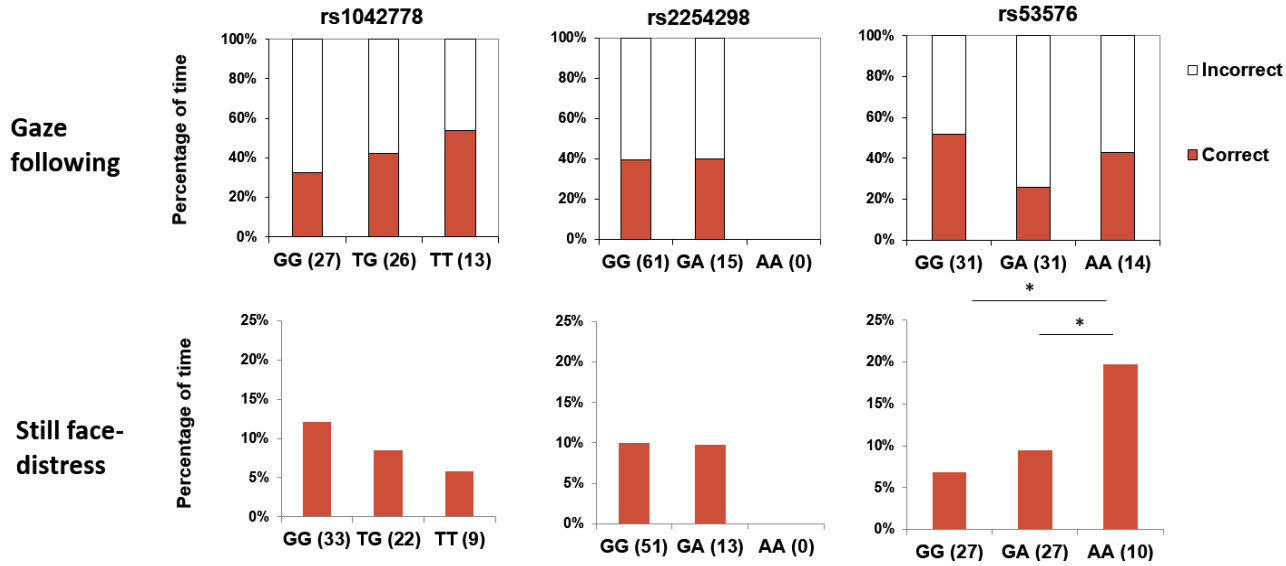

Figure III/2.1. Effects of OXTR SNPs on children's performance in the gaze following and still face tests.

Dogs

Altogether 57 dogs were included in the sample that both made a choice and had at least one identifiable SNP. Out of the 57 dogs, 38 chose the baited container (thus were successful in using gaze direction as a cue), which does not significantly differ from chance (binomial: p=0.111)

The rs8679682 polymorphism did not have a significant main effect on dogs' choices of container ($\chi^2(2)=0.754$, p=0.449), and the analyses did not yield any significant effects of the control variables or interaction effects either (all p>0.195 at removal) (Figure III/2.2).

The -94TC polymorphism, however, had a significant effect on dogs' choices of container ($\chi^2(2)=8.267$; p=0.016), showing that while dogs with the homozygous C and the heterozygous genotypes made their choices at random, dogs with the homozygous T genotype chose the baited container more often. All other effects were not significant (all p>0.222 at removal).

We also found a marginally significant effect on dogs' choices by the -213AG polymorphism ($\chi^2(2)=5.948$; p=0.051). Dogs with the homozygous G genotype were more likely to follow the correct, baited container; however, this was not true either of the homozygous A or the heterozygous genotypes. All other effects were not significant (all p>0.066 at removal)

SNP -74GC also had a significant effect on dogs' behaviour in the task ($\chi^2(2)=13.21$; p=0.001), showing that dogs with the homozygous G genotype were most likely to choose the baited container compared to the homozygous A or the heterozygous genotypes. All other effects were not significant (all p>0.109 at removal)

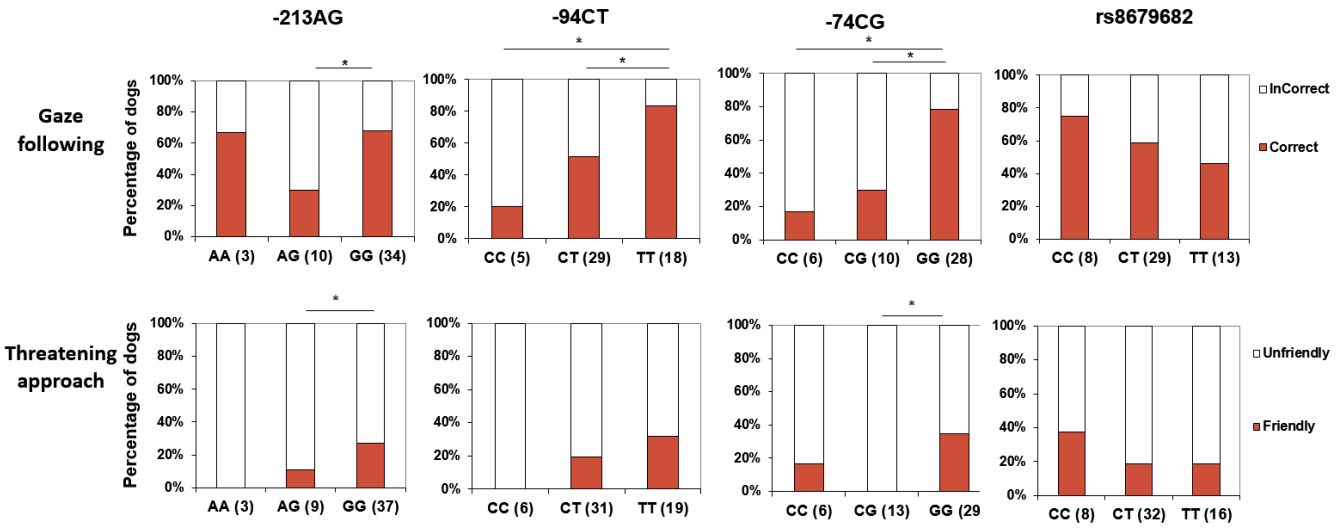

Figure III/2.2 Effects of OXTR SNPs on dogs' performance in the gaze following and threatening approach test.

III/2.3.1 Reaction to an aversive social interaction

Children – Looking at the experimenter

Allele variations at rs1042778 had no significant effect on the amount of time children spent looking at the experimenter during the still face phase ($F(2)=0.39$; $p=0.679$), nor did the analyses yield any significant interaction effects (all $p>0.319$). Similarly, no main ($F(2)=0.065$; $p=0.8$) or interaction effects were found involving SNP rs2254298 (all $p>0.15$). The model including the SNP rs53576 yielded a significant effect of experimenter ($F(1)=4.057$; $p=0.049$), but no main effect of allele variations ($F(2)=1.478$; $p=0.238$) and no interaction effects (all $p>0.231$). Children spent more time looking at Experimenter 2 than at Experimenter 1 ($M(E1)=25.4$; $M(E2)=34.24$).

Dogs – Looking at the experimenter

SNP rs8679682 had no significant main effect on the amount of time dogs spent looking at the threateningly approaching experimenter ($F(2)=0.607$; $p=0.55$). But there was a significant two-way interaction between sex and experimenter ($F(1,54)=4.578$; $p=0.017$), showing that whereas females reacted differently to the two experimenters, males did not. Allele variations at SNP -213AG had no main effect on the time dogs spent looking at the experimenter ($F(2)=1.048$; $p=0.362$), and we did not find any significant main effect of the control variables, nor any interaction effects (all $p>0.301$).

Analyzing the effects of variations at SNP -94TC, we found a marginal effect of allele variation ($F(2)=2.647$; $p=0.084$) and a marginal interaction between sex and experimenter ($F(1,54)=2.599$; $p=0.087$). Results indicate that males differentiated more between experimenters than females, and dogs with the homozygous T genotype spent less time looking at the experimenter than the other two genotypes ($M(TT)=81.06\%$; $M(CT)=93.11\%$; $M(CC)=91.39\%$).

Analyses of the SNP -74GC yielded no main effect of allele variation ($F(2)=0.783$; $p=0.466$) and no other effects (all $p>0.364$).

Children – Looking at the caregiver

The SNP rs1042778 did not have a significant effect on the amount of time children spent looking at their caregivers ($F(2)=0.2$; $p=0.146$). The interactions involving rs1042778 were not significant either (all $p>0.151$). Similarly, variations at SNP rs2254298 did not significantly modulate gazing at the caregiver ($F(2)=0.002$; $p=0.965$) and the interaction effects were not significant either (all $p>0.139$). The same was true for SNP rs53576 (main effect: $F(2)=0.165$; $p=0.849$; interaction effects: all $p>0.296$).

Dogs – Looking at the owner

rs8679682 had no main effect on the amount of time dogs spent looking back at their owners ($F(2)=0.11$; $p=0.896$) and there were no significant main effects of the control variables and no significant interactions either (all $p>0.154$). Similarly, no effects were found analyzing either SNP -213AG (main effect: $F(2)=0.034$; $p=0.967$; other effects: all $p>0.377$) and SNP -74GC (main effect: $F(2)=1.396$; $p=0.263$; other effects: all $p>0.523$).

However, allele variations at SNP -94TC had a significant effect on dogs' looking times at their owners ($F(2)=3.446$; $p=0.042$) and the analyses also yielded a main effect of experimenter ($F(2)=6.014$; $p=0.005$). These effects were qualified by significant two-way interactions between sex and experiment ($F(2,54)=4.675$; $p=0.015$); sex and allele variations ($F(2, 54)=3.673$; $p=0.035$); experimenter and allele variations ($F(4,54)=4.913$; $p=0.003$) and a three-way interaction between sex, experimenter and allele variations ($F(2,54)=6.355$; $p=0.004$). Results show greater variability in the case of males than females. Specifically, looking times increased when Experimenter 2 was administering the test for male dogs with the homozygous C genotype compared to all other cases ($M=19.55\%$, all other $Ms<7\%$.)

Children – Signs of distress

The analyses on the effects of SNP rs1042778 yielded no main effect of genotype ($F(2)=1.579$; $p=0.216$) and no interaction effects involving rs1042778 (all $p>0.102$) (Figure III/2.1). However, age and sex had marginal effects on the amount of time children exhibited signs of distress (sex: $F(1)=3.781$; $p=0.057$; age: $F(1)=3.722$; $p=0.059$) and the interaction between sex and experimenter was significant ($F(1, 63)=4.555$; $p=0.038$). The results indicate that younger children exhibited more signs of distress than did older children and boys exhibited more distress than girls ($M(girls)=8.48\%$ of the total duration of the phase; $M(boys)=11.47\%$). The interaction shows that there was not a considerable difference in the amount of distress signals in the case of girls ($M(E1)=8.069\%$; $M(E2)=7.934\%$); however

boys showed more signs of distress when the test was administered by Experimenter 2 (M(E1)=8.147%; M(E2)=21.505%).

In the analyses involving SNP rs2254298, we replicated the connection between age and distress signals (F(1)=5.208; p=0.026), but we found no main effect of genotype (F(2)=0.477; p=0.493) and no interaction effects involving rs2254298 (all p>0.352).

The rs53576 polymorphism had a significant effect on the amount of distress signals children produced in the still phase period (F(2)=5.796; p=0.005), showing that children with the homozygous AA genotype exhibited more distress (M(AA)=21.521%) than children with the other two genotypes (M(GG)=7.687%; M(GA)=7.564%). There was also a significant interaction effect between experimenter and genotype (F(1, 63)=5.601; p=0.006) showing that this difference was mainly attributable to tests administered by Experimenter 2. When Experimenter 1 administered the test, the amount of distress signals produced showed less variation across genotypes and in general, distress signals were scarcer (M(GG)=6.88%; M(GA)=10.585%; M(AA)=9.552%). The analyses also replicated the effect of age (F(1)=6.067; p=0.017).

Dogs – First reactions to the threatening experimenter

Allele variations at rs8679682 did not have a significant effect on dogs' first reactions to the experimenter ($\chi^2(2)=1.144$; p=0.564) (Figure III/2.2). However, sex ($\chi^2(2)=4.511$; p=0.034) had a significant modulatory effect, showing that while dogs were more likely to react with looking at or approaching the experimenter without tail wagging than to produce a friendly reaction, this was stronger in the case of males. All other effects were non-significant (p>0.236 at removal)

The analyses on the effects of SNP -213AG yielded a significant main effect of allele variations ($\chi^2(2)=8.383$; p=0.015), showing that while dogs with the homozygous A (n=3) or the heterozygous genotype (n=10) all reacted with looking at the experimenter without tail wagging, the behaviour of the homozygous GG genotype was more diverse with 11 out of 37 dogs reacting in a friendly way. All other effects were not significant (p>0.086 at removal).

Similarly, SNP -74GC significantly modulated dogs' behaviour ($\chi^2(2)=10.861$; p=0.004). While dogs with the heterozygous genotype all (n=13) reacted with looking at the experimenter without tail wagging, participants with the homozygous G genotype also produced friendly reactions (10 out of 29). -94TC polymorphism did not have a significant effect on dogs' first reactions ($\chi^2(2)=3.356$; p=0.187).

		Human			Dog			
		rs..576	rs..778	rs..298	rs..682	-213AG	-94TC	-74GC
Gaze following	Main effects	-	-	-	-	**G**	**G**	**G**
	Interactions	-	-	-	-	-	-	-
Still face/Threatening approach look at Caregiver/owner	Main effects	-	-	-	-	-	**G;E**	-
	Interactions	-	-	-	-	-	**S×E;S×G; E×G;**	-
Still face/Threatening approach look at Experimenter/Stranger	Main effects	**E**	-	-	-	-		-
	Interactions	-	-	-	**S×E;**	-	S×E	-
Threatening approach first reaction	Main effects	*N/A*	*N/A*	*N/A*	**A;S**	**S;G**	-	**G**
	Interactions	*N/A*	*N/A*	*N/A*	-	-	-	-
Still face signs of distress	Main effects	**G;A**	**A; S**	**A**	*N/A*	*N/A*	*N/A*	*N/A*
	Interactions	**E×G**	**S×E**	-	*N/A*	*N/A*	*N/A*	*N/A*

Table III/2.4. Summary of the results.

G= Genotype; A=Age; E=Experimenter; S=Sex Significant effects are bold, while marginally significant effects are indicated by normal font types. N/A = Not applicable

Correspondence between tasks

Children that chose correctly in the first task spent less time (mean: 24.92 sec) looking at the experimenter in the still face situation than those that could not find the reward (mean: 34.71 sec; t(46)=2.37; p=0.022). The same was true for dogs: those that chose the baited pot spent significantly less time looking at the experimenter in the threatening approach test (mean: 93.29 vs. 85.39 sec; d(49)=2.482; p=0.017). We found no other associations between performance in the gaze following task and the variables coded for reaction to an aversive social interaction task.

III/2.4. Discussion

The present study explored associations between variation in the oxytocin receptor gene and reaction to an aversive social interaction as well as use of a gaze cue to locate hidden food in

dogs and humans. Results seem to support our hypotheses that the oxytocinergic system may play a similar role in shaping dogs' and human infants' reactions to their partner's unexpected negative (distressing) behaviours but not to her gaze cue in a search task as the latter is potentially a competitive (and thus distressing) context for dogs, while it would be a cooperative context for human infants.

Our results show that single nucleotide polymorphisms in the gene coding for the uptake of oxytocin are indeed associated with both dogs' and children's reactions to a violation of normal social interactions. We found that dogs' first reactions (either friendly or neutral/fearful) were significantly modulated by two of the four polymorphisms analyzed (-213AG, -74GC). One of these polymorphisms (-213AG) had already been shown to be associated with proximity seeking, a composite measure that included latency to approach the experimenter after the threatening approach test (Kis et al., 2014a). Also, it has been shown that intranasal administration of oxytocin influences dogs' reaction in the threatening approach test (Hernádi et al., 2015). In the corresponding analyses with children, we found that the amount of distress signals produced after the withdrawal of positive social stimulation was significantly modulated by one of the three polymorphisms analyzed (rs53576). These results confirm that variation in the oxytocinerg system influences how dogs as well as humans respond to social threat or a socially ambiguous situation (Hernádi et al., 2015; Huber et al., 2005).

Analyzing participants' behaviour in the *Gaze following* test, we found that three out of the four identified polymorphisms (-213AG, -95TC and -74GC) were connected to whether dogs approached a food location the human experimenter had looked at beforehand. Importantly, two of these three polymorphisms (-213AG, -74GC) were linked to the dogs' friendliness in the threatening approach test as well. For example, dogs with the homozygous G genotype at SNP -74GC were not only more likely to search for food using the gaze direction of a human, but were also less threatened by the experimenter in the subsequent task. The same was true for dogs with the homozygous G genotype at -213AG. Although the present study does not allow us to assign specific functions to specific polymorphisms, these consistencies suggest that similar mechanisms regulate dogs' reaction to a clear social threat and to non-ostensive gaze in a food searching context.

In contrast to this, we found no such associations in the case of toddlers: none of the candidate polymorphism affected children's use of communicative human gaze to locate the hidden toy. This despite that in the same group of infants we could detect a significant association the

subjects' OXTR genotype had with the amount of distress the infants displayed in the Still face test. This may suggest different mechanisms underlying dogs' use of non-ostensive and children's use of ostensive gaze. Research in developmental psychology suggests that even younger infants are prepared to follow the gaze of an interactional partner while they ignore similar gaze cues if those are not addressed to them (e.g. Senju and Csibra, 2008). While it has been shown that infants develop an expectation that the direction of ostensive gaze is referential and it delivers generalizable knowledge (Senju et al., 2008), much less research addressed how humans interpret non-ostensive gaze. In contrast to the infants' performance, a number of studies found that without training and extended experimental pre-experiences dogs follow communicative human gaze only with their gaze but do not approach a food location indicated in this way (Duranton et al., 2017; Kaminski et al., 2012). Furthermore, dogs do not only ignore non-ostensive gaze but in fact tend to avoid a food location that another dog or a human has looked at in this way beforehand (Bálint et al., 2015; Duranton et al., 2017). Confirming these results, our findings suggest that dogs perceive such scenarios as competition over food and do not interpret non-ostensive gaze as a cooperative communicative signal that offers food to them. Dogs seem to respond to the context with markedly more social anxiety than children while at the same time it is still possible that they both interpret the non-ostensive gaze cue itself as an intentional cue that indicates the experimenter's interest in this location (Duranton et al., 2017). Further research will have to investigate this latter question. Nevertheless, in this study we did not find that dogs as a group would avoid a food location indicated with non-ostensive gaze. On the contrary, our results suggest a surprising strong effect of oxytocin on how dogs perceive such a situation. The percentage of dogs following non-ostensive gaze varied very strongly with genotype of the oxytocin receptor gene, with only 20% of the dogs carrying two C alleles on the -94CT choosing the indicated container in contrast to the 80% of the TT dogs doing so.

Interestingly, although we used different gaze cues in children and dogs and we found that only the dogs' gaze following was linked to how they reacted to a negative social situation, we found also similar associations in dogs and children between their other behaviours in the two tasks. In particular, we found that those participants – both dogs and children – that were successful in the gaze following task tended to spend less time looking at the experimenter threatening them (in dogs) or looking at them with a still face (in children). At a first sight this seems to suggest that dogs' and children's behaviour are guided by similar mechanisms. This might be even correct at the level that participants that are more skilled at utilizing gaze cues

may generally be more adept in social situations and, as such, faster to process negative social stimuli as well. Alternatively, it is also possible that gazing during social threat reflects different motivations in dogs and in children and thus, the consistency is only manifested at the behaviour level, but is not present in the underlying mechanisms. Analyses on the looking times (both in the case of children and dogs) focused on the attention participants paid to the two potential partners in the situation. The caregiver or the owner represented a secure base for participants; therefore looks directed at them can be interpreted as security or information seeking in a negative or ambivalent social situation. Gaze directed at the experimenter can either show fear or curiosity. However, looking at the experimenter in the *Still face* task may not only reflect how fearful they perceived the situation, but how much they were invested in re-engaging her in play.

Finally, an interesting puzzle in our data concerns children's generally low success in using gaze direction to locate the hidden object. As children at this age are typically good in following human communicative gaze, we suspect that the procedure we used explains their low success in this study. One could argue that the fact that the experimenter looked back at the children after her gaze cue made it more difficult for the children to remember which container they should choose. If so we would expect random choices, in contrast to which we found that children had a tendency to choose the empty container. Therefore, we suggest that children's difficulty in locating the toy stems from their immaturity of inhibitory control.

Instead of training trials that we used for the dogs, we wanted to make sure that the toddlers also understood what they would be searching by allowing them to see the toy inside the box at the non-cued location before the trial (see in procedure). As such, children's execution of action may be strongly biased by the last seen location of the object which may prevent other cognitive abilities (i.e. gaze following) from being exhibited. A similar dissociation between performance in overt behaviour and cognitive processing has been documented in other areas of cognitive development as well (e.g. Onishi, 2005). Importantly, problems with inhibition may also make genotype × behaviour associations unobservable. Thus, we cannot discard the hypothesis that similarly to dogs, children's use of communicative cues is affected by oxytocin receptor gene polymorphisms. Further studies using another experimental procedure will have to address this question. However even if OXTR genotype × gaze following association is found, our prediction is that this will not be the same association as we found between OXTR genotype and reaction to still face.

In sum, these results support the idea that similarities observed in the overt behaviour of dogs and human children may result from different mechanisms. While variations in the OXTR receptor gene affected both species behaviour in a negative social situation, we could find corresponding associations in a gaze following task only in dogs. This raises the possibility that for dogs, the two situations are more alike (potentially fear-inducing or competitive) than for human children. Although the aversive social interaction tasks differed between species, the genotype x behaviour associations we found were related to the distressing nature of these tasks both in dogs and children. However, while the same polymorphisms modulated the dogs' behaviour in the gaze following test as well, we found no such consistencies across tasks in the children. We suggest that this is because young children interpret others' object-directed behaviour as a learning opportunity (Csibra and Gergely, 2006) and as mostly cooperative, while dogs may view a social partner in a food searching task in a more antagonistic manner. If so, the oxytocin system can facilitate the success of dogs in participating in fundamentally cooperative, communicative interactions by fostering social approach through the reduction of fear responses in social interactions (Huber et al., 2005).

PART II. Effect of intranasal oxytocin pre-treatment on social sensitivity

III/3. Study 3: The effect of oxytocin on biological motion perception in dogs *(Canis familiaris)*[3]

III/3.1. Introduction

Recent studies have provided substantial insights into the neurohormonal mechanisms underlying human sociality (e.g. Skuse and Gallagher, 2009). There is, however, little research on the effect of oxytocin on basic mechanisms underlying human sociality, such as the perception of biological motion.

Dogs have a privileged status in comparative social cognition, as despite their phylogenetic distance from humans they often show a performance that is comparable to that of human infants at the behavioural level (Miklósi and Topál, 2013). Dogs also have different personalities resembling human personality types (Gosling et al., 2003) that can be characterized along the dimensions of Neuroticism, Extraversion, Agreeableness, and Openness. But despite the exponentially increasing number of studies on dog social cognition (for review see e.g. Bensky et al., 2013), relatively little is known about the effects of oxytocin on dogs' social cognition and, whether oxytocin affects their basic social cue processing such as the perception of biological motion.

In the present study our aim was to explore whether dogs show spontaneous preference for biological motion versus non-biological control stimuli, and how intranasal administration of oxytocin modulates dogs' reactions, a species adapted to the human social environment and thus widely used to model many aspects of human social behaviour. Besides we aim to study the physiological consequences of intranasal oxytocin administration (changes in heart rate and heart rate variability) and how the individuals' physiological reaction to oxytocin correlates with the looking preferences in dogs. Sex differences were also studied as based on the peripheral effects of oxytocin (i.e., to induce labour and milk ejection), a general difference in its behavioural effects on males and females can be expected. Moreover, as recent results show that the oxytocinergic system modulates the *neuroticism* personality trait

[3] This chapter is based on: Kovács, K., Kis, A., Kanizsár, O., Hernádi, A., Gácsi, M. and Topál, J., 2016. The effect of oxytocin on biological motion perception in dogs (Canis familiaris). *Animal Cognition*, 19(3): 513-522.

in humans (Chang et al., 2014), and *agreeableness* trait encompasses different prosocial attitudes such as trust, empathy and altruism that have been shown to be affected by oxytocin (see e.g. Rodrigues et al., 2009), the canine analogues of these two human personality factors were also included in the analyses.

In a within-subjects design, dogs (N=39), after having received either oxytocin (OXT) or placebo (PL) treatment, were presented with 2D projection of a moving point-light human figure and the inverted and scrambled version of the same movie. Heart rate (HR) and heart rate variability (HRV) were measured as physiological responses, behavioural response was evaluated by observing dogs' looking time, and subjects were also rated on the personality traits of neuroticism and agreeableness by their owners.

III/3.2. Methods

III/3.2.1. Ethics statement

Research was done in accordance with the Hungarian regulations on animal experimentation and the Guidelines for the use of animals in research described by the Association for the Study Animal Behaviour (ASAB). Ethical approval was obtained from the National Animal Experimentation Ethics Committee (Ref No. XIV-I-001/531-4-2012).

III/3.2.2. Subjects

N=39 task-naïve adult (older than 1 year) pet dogs (20 males and 19 females; 18 purebreds from 14 different breeds and 21 mongrels; mean age ±SD: 4.46±2.51 years; 8 of small (≤9 kg), 23 of medium (10-25 kg) and 8 of large (>25 kg) size based on average standard weight, *http://www.akc.org/* in case of pure breed dogs or based on the inspection of the videos in case of mixed breed dogs) were recruited from the Family Dog Project database that contains over a thousand owners who have volunteered to participate in behavioural experiments with their dogs. Dogs that had any type of eye or vision problem (according to the owner) were not included in the experiment. Five dogs did not return for the second test occasion, in case of five dogs no ECG recordings were conducted (due to non-compliance of the subject) and the video recording of the behavioural test could not be analysed in case of 1 dog because of technical reasons; these were all included as missing data in the analysis.

III/3.2.3. Stimuli

Stimuli consisted of a 4 s attention grabber (sound + moving rattle animation) followed by a 15 s long stimulus (point-light display) accompanied by a neutral music playback. The biological motion stimulus depicted a point-light movie of a side walking human on one side (left/right counterbalanced across subjects) ('normal point like figure – PLF'), while on the other side the inverted and scrambled version of the same point-light movie ('distractor') was shown. The point-light display was an 11-dot figure with single white dots representing the head, one shoulder, one hip, and each of the two elbows, wrists, knees, and ankles on a black background. The PLF was shown facing either left or right and walking in place, as if on a treadmill, with a stride frequency of 0.93 Hz. Both PLF and distractor displays were presented without mask dots on the first occasion, while on the second test occasion they were presented within 100 mask dots randomly plotted within the mask area (see Figure III/3.1.)

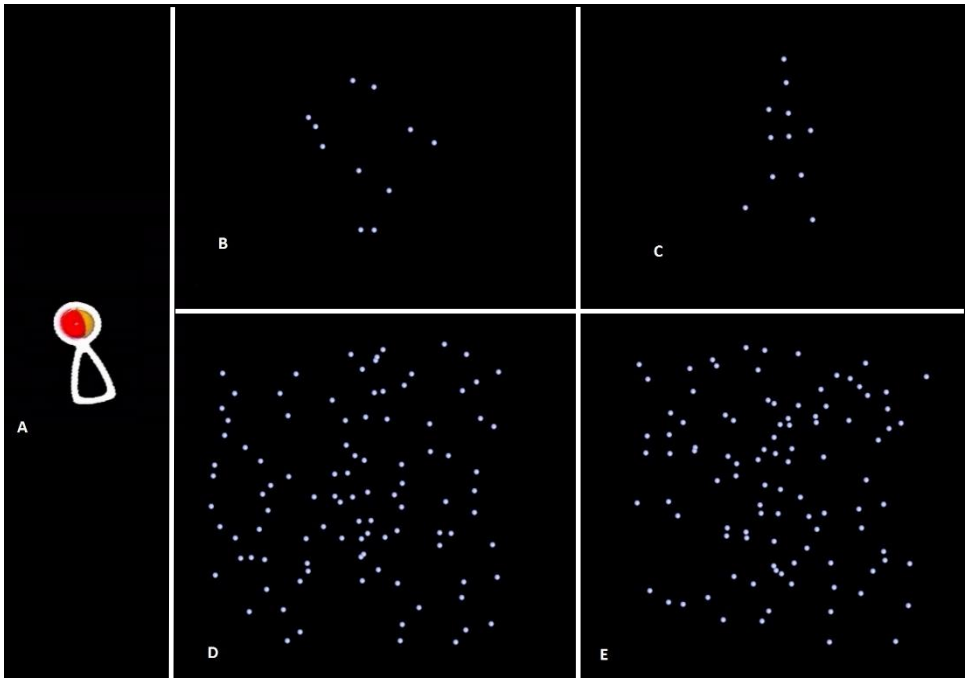

Figure III/3.1. Biological motion stimulus (A: attention grabber. B: inverted and scrambled version of a side walking human (distractor). C: normal version of a side walking human. D: inverted and scrambled version of a side walking human (distractor) with mask dots. E: normal version of a side walking human with mask dots.)

III/3.2.4. Procedure

Dogs received a single intranasal dose of 12 IU (3 puffs) oxytocin (Syntocinon, Novartis) or placebo (isotonic natriumchlorid 0.9% solution) in a double blind design.

Subjects participated in the task repeatedly, each subject received both oxytocin and placebo treatment (in a random order). The break between the two tests with different treatments was 7-14 days. The oxytocin or placebo administration was followed by a 40-minute-long waiting period (following the protocol by Kis et al., 2014b). During this waiting period, dogs spent the first 25 minutes with an on-leash walk at the University Campus (avoiding any contact with other dogs or humans) during which the experimenter ensured that the owner did not make any social contact with the dog either (e.g. did not pet or talk to it) and kept the length as well as the speed of the walk as standard as possible. Then for the remaining 15 minutes the owner and the dog were quietly sitting in an isolated room. During this time the dog was free to move and the owner was sitting and filling in questionnaires while ignoring the dog. We used the Neuroticism and Agreeableness scales of a questionnaire adapted for dogs by Gosling et al. (2003) about of dogs' personality. The 17-item questionnaire consisted of 9 statements for agreeableness (e.g. "is sensitive to the needs and feelings of others") and 8 statements for neuroticism (e.g. "gets nervous easily"). Owners were asked to score their dogs from 1 to 5 (from disagree strongly to agree strongly). Both scales contained three reverse scored items.

In order to quantify the physiological effect of oxytocin and to test its relation to the behavioural effects, ECG recordings were conducted immediately following the waiting period. The testing room was equipped with office furniture and a mattress on the floor for the dog and its owner. While we made every possible effort to keep the environmental circumstances during the waiting period before the ECG measurement as standard as possible, body posture of the dog was not controlled by the owner/experimenter in order to avoid stress inherent to external restrain. Evidently, this procedure caused slight variations in the subjects' behaviour during the waiting period, but the effect of oxytocin has been shown to be strong enough to manifest even under these semi-natural conditions (Kis et al., 2014b). When the 40 minutes waiting period elapsed a 5-10 minutes on-leash exploration and familiarization followed in the ECG measurement room, after which the owner took a seat on the mattress and assisted the experimenter throughout the process of fixing two surface attached electrodes onto the dog's chest (second rib on both sides). Gold-coated Ag|AgCl electrodes fixed with EC2 Grass Electrode Cream (Grass Technologies, USA) were used for the recordings. The electrode placement was followed by 4-minute quiet resting, and then by a 1 minute long recording (Figure III/3.2). During this last five minutes every dog was in a lying position because previous research has shown that body posture has a significant effect on dogs' heart rate (Maros et al., 2008). Signals were collected, prefiltered, amplified, and digitized at a

sampling rate of 249 Hz/channel by using the 30 channel Flat Style SLEEP La Mont Headbox with implemented second order filters at 0.5 Hz (high pass) and 70 Hz (low pass) as well as the HBX32-SLP 32 channel preamplifier (La Mont Medical Inc., USA).

Figure III/3.2. Photograph of the ECG measurement

The test setup measuring biological motion preference followed the procedure of previous experiments studying dogs' responses to projected images (e.g. Faragó et al., 2010). The experiment took place in a dark room (3 m × 5 m) with a canvas (2 m × 2.2 m) on one of the walls and a chair at a 4 m distance facing the canvas, as well as a projector on the wall opposite the canvas at a 2.2 m height. During the experiment the owners were seated on the chair and instructed to keep their dogs between their legs in a sitting position (see Figure IIII/3.3). Infrared lights and a zero lux camera focused on the dog's head were placed in front of them at 1 m distance in order to record the head and eye movements of the dogs. An additional camera, placed above the projector and synchronized with the zero lux camera, recorded the entire room in order to ensure that the looking direction of the dog was only coded during the stimuli projection phase. Dogs were allowed to look away and owners were asked not to interact with the dog.

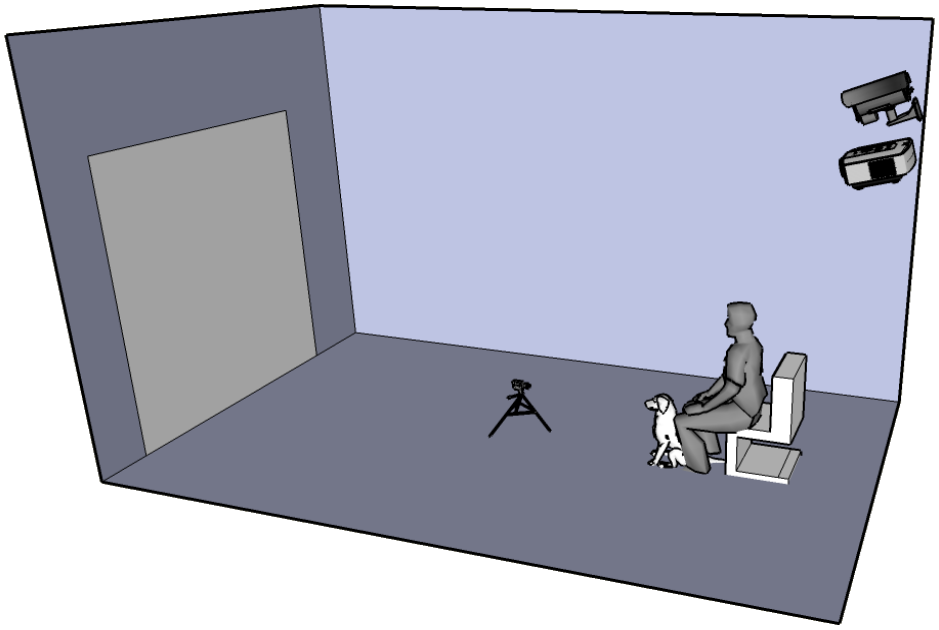

Figure III/3.3. Schematic drawing of the experimental setup measuring biological motion preference

III/3.2.2.5. Data analysis

Agreeableness and Neuroticism questionnaire scores were obtained by averaging the scores of the items representing each trait using the coding methods of Gosling et al. (2003).

Using the ECG recordings R peaks were manually detected (due to the sinus arrhythmia that characterizes dog heart rate automatic measures are hard to apply – Schöberl et al. 2014), and RR intervals were measured using the Fercio program (© Ferenc Gombos 2012). Heart rate (HR; 1/min) was derived from RR interval averages (60/meanRR), and heart rate variability (HRV; sec) was calculated as the standard deviation of RR intervals (see e.g. Gácsi et al., 2013 for similar measures).

Looking behaviour during the biological motion preference tests was analysed by frame-by-frame coding of all experimental recordings (with a 0.2 second resolution, using Solomon Coder, http://solomoncoder.com/), in order to determine the looking direction of the dogs: left side of the screen / right side of the screen / away from the screen. Coding was blind to subject details and conditions. The inclusion criterion was that dogs had to look at the screen more than 30% of the total time on a given study occasion. Therefore 2 dogs from the first occasion (non-masked) and 3 dogs from the second occasion (masked) were excluded.

In order to assess subjects' looking behaviour we measured the relative time (%) spent with looking at the point-light figure (%PLF) as well as the relative time (%) spent with looking at

the Distractor (%DISTR). Inter-rater reliability for dogs' looking behaviour was calculated by double coding of 30 random frames (on 30 different subjects) from the two stimuli by two independent coders (Cohen κ: 0.80).

Total looking was calculated as (%PLF) + (%DISTR). *Preference index* was calculated as (PLF – DISTR) / (PLF + DISTR). Linear Mixed Models using restricted maximum likelihood estimation were used to test the effects of pre-treatment (OXT or PL) and first/second test occasion (within subjects factors), gender (male or female; between subjects factor) as well as Agreeableness and Neuroticism scores (covariates) on Heart rate (HR) and Heart rate variability (HRV). Other Linear Mixed Models were used to test the effect of pre-treatment (OXT/PL; within subjects factor), stimuli type (masked or not masked; within subjects factor), gender (male or female; between subjects factor) as well as Agreeableness, Neuroticism scores, HR and HRV (covariates) on Total looking and Preference index.

III/3.3. Results

III/3.3.1. Physiological responses (HR & HRV)

The Random intercept mixed-effects model showed that HR was significantly affected by the pre-treatment, that is, OXT administration decreased HR ($F_{(1,23)}$=12.325, p=0.002, Figure III/3.4). Male dogs had higher HR, than females irrespective of OXT/PL pre-treatment ($F_{(1,24)}$= 5.012, p=0.034). Subjects with higher neuroticism ($F_{(1,25)}$= 4.422, p=0.045) and agreeableness ($F_{(1,24)}$=5.256, p=0.031) scores had higher HR. No main effect of the order (first vs. second test occasion; $F_{(1,23)}$=0.906, p=0.351) was found. All interactions were non-significant (all p>0.05).

Heart rate variability was also affected by the pre-treatment, as OXT significantly increased HRV ($F_{(1,25)}$= 5.796, p=0.024, Figure III/3.4), however, none of the other factors (gender: $F_{(1,25)}$= 1.351, p=0.256, first/second test occasion: $F_{(1,25)}$= 0.055, p=0.817, neuroticism: $F_{(1,26)}$= 0.761, p=0.391, agreeableness: $F_{(1,25)}$= 0.870, p=0.360) influenced the HRV and all interactions were non-significant (all p>0.05).

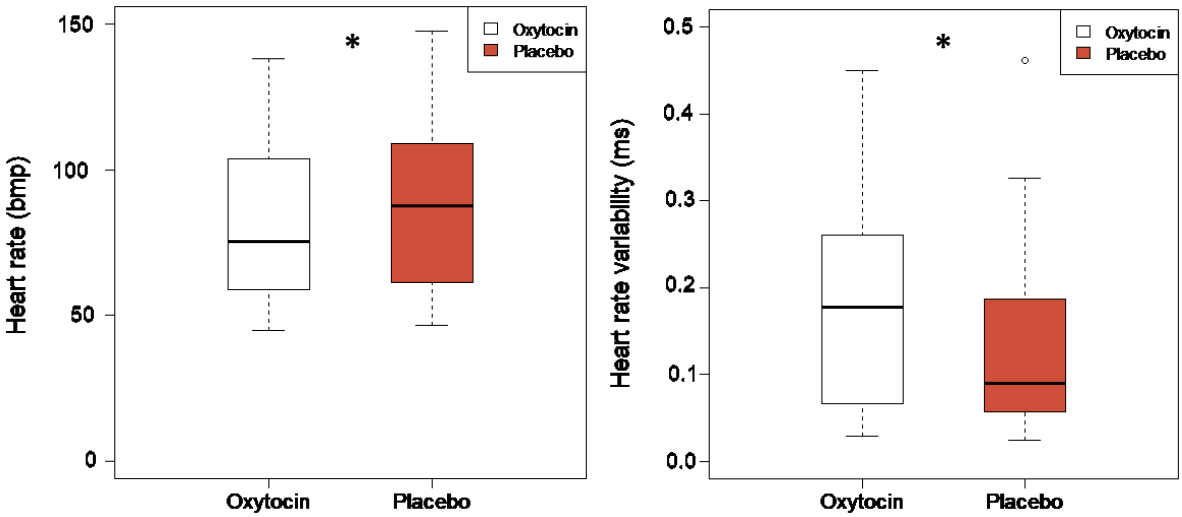

Figure III/3.4. Physiological changes after oxytocin and placebo pre-treatment. *: $p<0.05$

III/3.3.2. Total looking (%PLF + %DISTR)

Total looking was higher in case of the non-masked than the masked stimuli ($F_{(1,29)}=7.157$, $p=0.012$). Furthermore, we found that dogs who achieved higher agreeableness scores looked more at the stimuli ($F_{(1,26)}=4.589$, $p=0.042$). But no main effect of pre-treatment (OXT/PL, $F_{(1,29)}=0.218$, $p=0.644$), gender ($F_{(1,28)}=0.806$, $p=0.377$), and score for Neuroticism ($F_{(1,30)}=0.007$, $p=0.934$) was found. However, dogs with a lower HR looked more at the stimuli ($F_{(1,46)}=4.407$, $p=0.041$), and the association with HRV showed a reverse tendency; dogs with higher HRV slightly looked more at the stimuli ($F_{(1,50)}=3.451$, $p=0.069$). Furthermore, there was a significant pre-treatment × gender interaction ($F_{(2,31)}=4.385$, $p=0.021$): female dogs looked more at the stimuli after oxytocin pre-treatment (Figure III/3.5). All other interactions were non-significant ($p>0.05$).

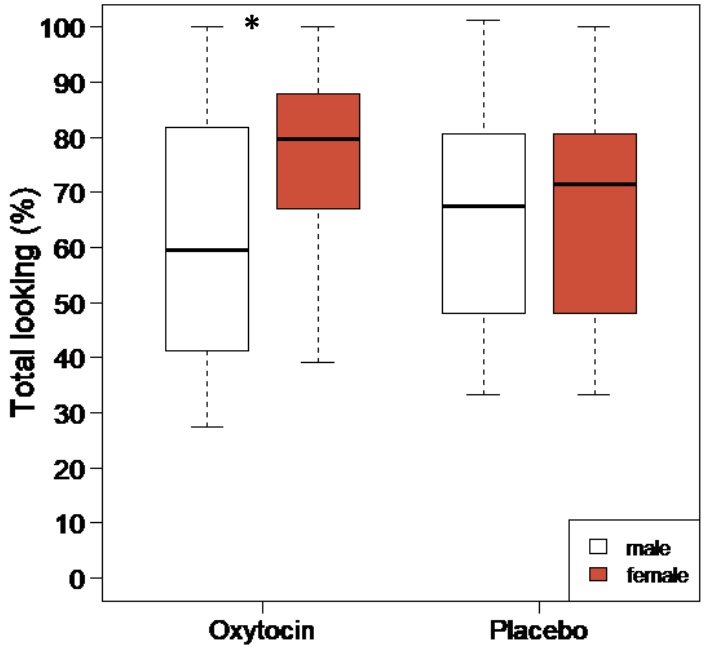

Figure III/3.5 Differential effect of OXT on the looking behaviour in males and females. Female dogs looked more at the stimuli after oxytocin pre-treatment. *: $p<0.05$

III/3.3.3. Preference index (PLF – DISTR) / (PLF + DISTR)

Pre-treatment had a significant effect on dogs' *Preference index:* subjects, after having received OXT, looked relatively less at the biological stimuli ($F_{(1,52)}=4.974$, $p=0.03$). No main effects of stimulus type (masked/non-masked, $F_{(1,52)}=2.652$, $p=0.109$), gender ($F_{(1,52)}=0.082$, $p=0.770$), and questionnaire scores (Neuroticism: $F_{(1,52)}=0.094$, $p=0.760$; Agreeableness: $F_{(1,52)}=0.351$, $p=0.556$) were found. However, there was a significant pre-treatment × stimulus type interaction ($F_{(2,47)}= 3.212$, $p=0.049$); placebo-pretreated dogs showed preference for looking at the non-masked, but not the masked biological stimuli while OXT-pretreated dogs had no preference in either of the conditions (Figure III/3.6). All other interactions were non-significant (all $p>0.05$) and we did not find significant effects of HR ($F_{(1,56)}=0.166$, $p=0.685$) and HRV ($F_{(1,56)}=0.673$, $p=0.415$).

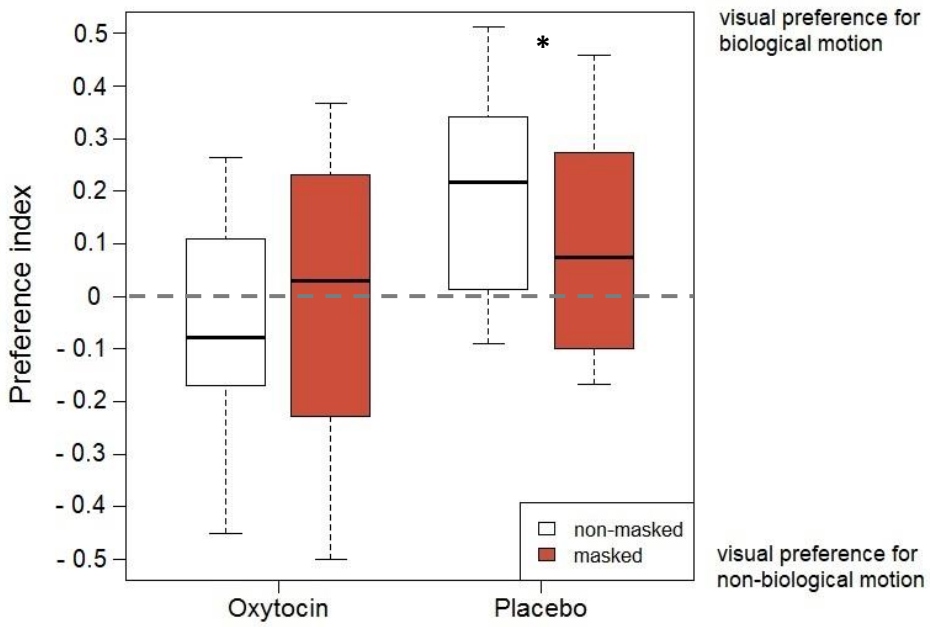

Figure III/3.6 Placebo-pretreated dogs showed a significant preference for looking at the biological motion stimuli in case of the non-masked, but not in the masked condition. *: $p<0.05$

III/3.4. Discussion

The present study provides the first evidence that dogs show spontaneous preference for biological motion. Furthermore, our results also show that intranasal administration of oxytocin affects biological motion perception in dogs, and the effects of this treatment are in interaction with physiological measures such as heart rate and heart rate variability and different aspects of the dog personality (neuroticism and agreeableness).

In humans it has been demonstrated that oxytocin enhances the perception of biological motion by increasing sensitivity for stimuli that represent living objects (a walking character) but does not change the sensitivity for nonbiological stimuli (a rotating square) (Kéri et al., 2009). Based on these results we expected that oxytocin would increase biological motion preference in dogs, but we found an opposite effect. The two findings are, however, not necessarily contradictory as the ability to more easily perceive biological motion might lead to an increased visual attention to the stimuli that are not easily recognizable due to its non-biological motion. If so, changes in dogs' attentional bias after oxytocin treatment may simply reflect changes in the visual encoding process (identification) and not changes in the relative attractiveness of PLF versus distractor stimuli (preference).

In the present study the total looking time of the dogs was only affected by oxytocin in females, but not males. Previous studies on the effect of oxytocin on dog social behaviour mostly reported no gender effects (but see Nagasawa et al. 2015 for the differential effects of intranasal oxytocin on male and female dogs' gazing behaviour).

The finding that total looking time was higher for the non-masked compared to the masked stimuli suggests that dogs were probably unable to recognize the biological motion when the point-like figures were masked. This is not surprising, as even 14-year-old children are less accurate than adults in a walker-detection task when the walking figure is embedded in moving noise dots (Pavlova et al., 2000). In our study we used a relatively high number of surrounding masking dots, thus it is possible that the supposed enhancing effect of oxytocin on biological motion perception could have been detected with a stimulus having fewer noise dots.

The present study supports previous suggestions that individual variation in the effect of oxytocin on HR and HRV makes it a good indicator of the physiological effect of oxytocin and thus can be used as a covariate in behavioural studies (Kis et al., 2014b). Our results also show (apart from confirming that OXT significantly decreases HR and increases HRV) that male dogs have higher HR, than female dogs.

In summary this study presents information about intranasal oxytocin pre-treatment affecting biological motion perception in dogs, and its connection with physiological measures and some aspects of the dog personality (neuroticism and agreeableness).

III/4. Study 4: Differential effects of oxytocin on social sensitivity in two distinct breeds of dogs (Canis familiaris)[4]

III/4.1. Introduction

Despite the above outlined variability between breeds (see: chapter *I/1.2.1*), some changes that occurred during the process of domestication are common to dogs in general. Dogs resemble humans not only in their human-analogue social behaviours, but also in that the oxytocin system is related to their social behaviour. Evidence suggests that affiliative interactions

[4] This chapter is based on: **Kovács, K.**, Kis, A., Pogány, Á., Koller, D. and Topál, J., 2016. Differential effects of oxytocin on social sensitivity in two distinct breeds of dogs (Canis familiaris). *Psychoneuroendocrinology, 74:* 212-220.

between dogs and humans have the potential to increase oxytocin in both partners (Handlin et al. 2011; Odendaal and Meintjes 2003). Making prolonged eye contact with a human partner produces intense emotional reactions and a substantial rise of circulating oxytocin in both dogs (Nagasawa et al., 2015) and humans (Gordon et al., 2010). Other studies have shown that polymorphisms in the regulatory region of the oxytocin receptor gene are related to human-directed social behaviours in dogs (see Study 1 above; Kis et al., 2014a) and that intranasal oxytocin administration influences dog's behaviour in a wide range of contexts (Kis et al., 2015; Romero et al., 2014). However there is growing evidence indicating that the effectiveness of intranasal oxytocin treatment in influencing human behaviour can vary greatly. The effects of oxytocin are indeed constrained not only by features of situations but also by those of individuals (Bartz et al., 2011).

Some research suggest that types of work for which different dog breeds have been selected may also influence the ways in which they interact with humans (Miklósi et al., 2004). For example, Gácsi et al. (2009) propose that dog breeds can be categorized in terms of the communicative role they have been bred to fill in. Namely, there are 'cooperative workers' that were originally developed for cooperative tasks, in frequent visual contact with their human partner (e.g. herding dogs), whereas others, the 'independent worker' breeds, work with no or very little human visual contact (e.g. sled dogs). The present study investigates how social behaviour is influenced by intranasal oxytocin treatment in two markedly distinct dog breeds (selected for different purposes).

Two markedly different breeds, a cooperative worker (Border Collie) and an independent worker (Siberian Husky) were selected for the study that belong to different genetic clusters (Parker et al., 2004). After having received intranasal administration of oxytocin (OXT) or placebo (PL), subjects participated in three behavioural tests measuring social responsiveness. Specifically, we investigate (1) tendency to make eye contact with a human partner and gaze alternations between the target object and the human in an unsolvable task, (2) tendency to use referential looking towards the owner, when facing an ambiguous (potentially dangerous) stimulus and (3) tolerance of prolonged eye contact with a human in an emotionally neutral situation. We predicted that dogs in these two groups differ in sociability, that is, dogs from the breed type that has been selected for cooperation in visually guided tasks would show superior performance in the use of gaze cues as compared to dogs from independent work breed. Moreover we expected that oxytocin administration would increase the use of the communicative signals in both breeds, including breed-specific changes.

III/4.2. Materials and methods

III/4.2.1. Ethics Statement

This research was done in accordance with the Hungarian regulations on animal experimentation and the Guidelines for the use of animals in research described by the Association for the Study Animal Behaviour (ASAB). Ethical approval was obtained from the National Animal Experimentation Ethics Committee (Ref No. XIV-I-001/531-4-2012). The owners volunteered to participate and gave written informed consent.

III/4.2.2. Subjects

N = 19 adult Border Collies (9 males and 10 females; 6 neutered; mean ± SD age: 3.5 ± 2.0 years) and N = 19 adult Siberian Huskies (8 males and 11 females; 15 neutered; mean ± SD age: 4.8 ± 1.9 years) kept as pet dogs were recruited and tested at the Department of Ethology, Eötvös Loránd University, Budapest, Hungary. According to owner reports there were both similarities and differences between the two breeds in their socialization background and living conditions (for details see Appendix 5). Although Huskies were older when purchased by their owners, the duration of living together with the owner was not different between the two breeds. Moreover, both breeds had similar time activity patterns (time spent indoors vs. outdoors) but more Border Collies have participated in regular training classes.

Subjects were tested in three situations to assess social responsiveness after OXT/PL treatment in a between-subject design. Based on pilot results of 8 dogs (4 Border Collies and 4 Siberian Huskies; 4 males, 4 females, mean ± SD age: 3.0 ± 1.3 years) in a within subjects design, we found a substantial habituation effect of subjects' behaviour in all tasks, therefore testing was conducted in a between subject design (see the results of the pilot study in Appendix 6).

III/4.2.3. Procedure

III/4.2.3.1. Oxytocin or placebo treatment

Dogs received a single intranasal dose of 12 IU (3 puffs) oxytocin (Syntocinon, Novartis) or placebo (isotonic sodium chloride 0.9% solution) in a double blind design that has already been proved to have both physiological (decreased heart rate and increased heart rate variability) and behavioural effects in dogs (e.g., Hernádi et al., 2015; Kis et al., 2015; see figure III/4.1). The oxytocin or placebo administration was followed by a 40-minute-long

waiting period (following the protocol by Kis et al., 2014b) that is presumed to be necessary for the central neuropeptide levels to reach plateau (Born et al., 2002). During this waiting period, dogs spent the first 25 minutes with an on-leash walk at the University Campus (avoiding any contact with other dogs or humans) during which the experimenter ensured that the owner did not make any social contact with the dog either (e.g. did not pet or talk to it) and kept the length as well as the speed of the walk as standard as possible. Then for the remaining 15 minutes the owner and the dog were quietly sitting in an isolated room. During this time the dog was free to move and the owner was sitting and filling in questionnaires about their dog keeping practices while ignoring the dog.

Figure III/4.1 Photograph of the intranasal pre-treatment

III/4.2.3.2. Behavioural tests

All test sessions were conducted in two adjacent experimental rooms (room A: 4.5 × 3.5 m; room B: 3 × 5 m) that were unfamiliar to the dogs. The test series was preceded by a 5-minute-long habituation period when subjects were allowed to explore the rooms freely, while the owner was informed about his/her tasks during the test by the experimenter. All subjects participated in the same three test situations in a fixed order, measuring different aspects of their communicative behaviour. After every test, the owner and the dog left the room and waited in the corridor until the room was prepared for the next test (approximately 2 minutes); during this time drinking water was offered to the dog. All tests were video recorded for later analysis.

Dogs first participated in the *Unreachable food* task, originally developed by Miklósi et al (2003). This procedure allows us to explore whether, and if so how, dogs change their communication towards a potential human helper when reachable food reward suddenly becomes unreachable to the dog and whether intranasal administration of oxytocin could modify this behaviour (see Figure III/4.2). Dogs were presented with four 'solvable' trials (i.e., the dog could reach food reward through an open door of a 64 × 100 × 73 cm wire mesh cage) and this was followed by a single 'blocked' (closed door) trial. Trials were recorded in room A which was empty except for the experimental cage in the middle. At the beginning of each trial, the owner (O) was standing quietly at a predetermined point (next behind the dog, while holding it by its collar) approximately 1.5 m from the cage. The experimenter (E) was standing motionless behind the dog in the opposite corner of the room. Then the E went to the cage, called the dog's attention with a piece of food in her hand (Name! + looking at the dog), and placed it in the center of the cage through a 52 × 52 cm opening. Then she fixed the door in open position and stepped back to her predetermined place (behind the dog). In this moment, the O let the dog free and it was allowed to move freely until the food reward was obtained. After the fourth solvable trial the O and his/her dog left the experimental room, while the E put ten pieces of food in the cage and closed the door so that henceforward the dog could not get the food through the opening. Then the E returned to her predetermined place (in the corner), the O and the dog re-entered the room, and took up their starting position (in the corner – see above). After this, the O released the dog to explore the cage. During this 60 seconds-long 'Blocked trial' both the E and the O were standing quietly at their predetermined place. Importantly, however, both the O and the E were watching the dog during the 'Blocked trial' thus giving the dog a chance to make eye contact.

The *Unreachable food* task was followed by the *Potentially dangerous object* task (for a similar task see Merola et al., 2012). This task was used to explore the dogs' tendency to display referential looking (a form of information seeking behaviour when facing a potentially dangerous object) towards humans, and, whether intranasal administration of oxytocin could modify this behaviour in the two distinct dog breeds (see Figure III/4.2). The test was conducted in room B which was empty except for a speaker (34 × 39 × 22 cm) covered by a blanket in the middle. The O entered the room with the dog on a leash, stepped up to a predetermined point (in the left corner), unleashed the dog and then was standing there motionless. At the moment when the O unleashed the dog, the E who was waiting in the adjacent room played back a 5-second growling sound through the speaker (stimulus from

Faragó et al., 2010). The E played back the sound four times in total with 5 s pauses between the repetitions.

Finally, dogs were presented with a *Tolerance of prolonged eye contact* trial (similar to that used by Hernádi et al., 2012) in order to assess whether, after having received intranasal oxytocin, dogs would show a higher tendency to keep eye contact with an unfamiliar human (see Figure III/4.2). Dogs' readiness to establish and maintain eye contact with the E was recorded in room B. The O was sitting on a chair opposite to the E (at a distance of 1 m) while holding the dog by its collar between his/her legs facing the E. The dog was allowed to sit, stand or lay down, but the O had to prevent it from walking away by holding its body, without talking to it. The E sat on a chair quietly facing the dog and looking at it. At the beginning of the trial the E called the dog by its name only once and when the dog made eye contact with her (it happened in less than 21 seconds for all subjects), she tried to keep continuous eye contact with it. At the moment when the dog averted its gaze from the E, the trial was terminated.

Buccal DNA samples were non-invasively collected after the test from N = 34 of the dogs participating in the study (N = 16 Border Collies and N = 18 Siberian Huskies) for a preliminary gene × behaviour analysis (see Supplementary material S3 for details).

Figure III/4. 2. The three behavioural test situations: *Unreachable food* task, *Potentially dangerous object* task and *Tolerance of prolonged eye contact* trial.

III/4.*2.4 Buccal sample collecting and SNP genotyping*

Buccal samples were collected from N = 34 of the dogs participating in the study (N = 18 Siberian Huskies and N = 16 Border Collies) in a non-invasive way, with cotton swabs from the inner surface of the cheek. Genomic DNA was extracted from buccal swabs using standard protocol. A previously described (Kis et al. 2014 PLoS One 9, e83993) single nucleotide polymorphism (SNP) in the 5' UTR regulatory region of the oxytocin receptor (OXTR) gene, -212AG, was genotyped by PCR-RFLP method. PCR amplification was

performed using 59-CCA TTG GAA TCC GCC CCC T-39 forward and 59-CAC CAC CAG GTC GGC TAT G-39 reverse primers. Annealing temperature was 56ºC and total reaction volume was 10 ml. PCR products were incubated for 3 h at 37ºC in a restriction enzyme mixture containing 0.5 U/ml Hpy99I restriction enzyme (NEB), 16BSA and 16NEB4 buffer. Total reaction volume was 16 ml. The digested PCR products were analyzed by conventional submarine agarose gel electrophoresis (Biocenter, Szeged, Hungary), using 2.5% agarose gel and visualized by ethidium bromide staining.

Fluorescent signals were detected both real-time and after the PCR amplification, and were evaluated by Sequence Detection Software 1.4. Allele frequencies were calculated for breeds separately; both breeds were in Hardy-Weinberg equilibrium for the -212AG (Table III/4.1).

	-212 AG			
	AA	**AG**	**GG**	**HWE**
Border Collie	3	6	8	p=0.34
Siberian Husky	0	7	10	p=0.28

Table III/4.1. Genotype frequencies for -212AG in Border Collies and Siberian Huskies with the respective Hardy- Weinberg equilibrium values

III/4.*2.5. Behavioural variables and statistical analyses*

Behaviours displayed by the dog during the three tasks were coded blindly to experimental conditions, frame-by-frame, using a 0.2 second resolution in Solomon Coder (version beta 16.06.26; http://solomoncoder.com/). Inter-rater reliability for dogs' behaviour was calculated by double coding 30% of the video recordings by two independent coders (Cronbach's Alpha ≥ 0.796 for all variables). Statistical analyses were carried out using R 3.2.3 (RCoreTeam, 2015).

In the *Unreachable food task* we recorded the latency to approach the food during the four training trials and the blocked trial was analyzed by measuring different aspects of social attention: (1) latency to first looking at the O; (2) latency to first looking at the E; (3) proportion of trial time spent looking at the O; (4) proportion of trial time spent looking at the E; (5) number of gaze shifts between the cage and the O; (6) number of gaze shifts between the cage and the E during the trial. In addition, a non-social looking behaviour was coded: (7) proportion of trial time spent looking at the cage. Latencies to approach the food during the training trials as well as latencies to first looking at the O and the E during the blocked trial

(response variables) were analyzed in separate Cox Models (R package 'survival'; Therneau, 2015a) with occurrence of looking as terminal event. Dogs that did not look at the human partner (O/E) within 60 seconds were treated as censored observations (N = 5 and N = 10 (of 38) censored dogs, respectively). Proportion of trial time spent looking at the O and the E (response variables) were analyzed with Tweedie Generalized Linear Models (Tweedie GLM; R packages 'stats' and 'statmod'; (RCoreTeam, 2015; Smyth et al., 2016) to take into account excess zeros in the dataset due to dogs that did not look at the O or the E. Number of gaze shifts between the cage and the O and the cage and the E were analyzed by using two separate Zero-inflated Negative Binomial GLMs (ZINB GLM, R package 'pscl'; Jackman, 2015). Proportion of trial time spent looking at the cage was analyzed in General Linear Models. Latency to approach food during the four training trials were analyzed in Cox Mixed Models (R package 'coxme'; Therneau, 2015b).

In the '*Potentially dangerous object'* situation we also measured both social and non-social aspects of dogs' behaviour: (1) latency to first looking at the O; (2) proportion of trial time spent looking at the O; (3) number of gaze shifts between the speaker and the O; (4) latency to first approaching the sound source (within 10 cm); (5) proportion of trial time spent looking at the speaker. Latency to first looking at the O and first approaching the sound source (response variables) were analyzed in two separate Cox Models with occurrence of looking or approaching as terminal event. Dogs that did not look at the O/approached the speaker within 30 seconds were treated as censored observations (N = 9 and N = 14 (of 38) censored dogs, respectively). Proportion of trial time spent looking at the O (response variable) was analyzed in Tweedie GLMs, number of gaze shifts between the speaker and the O was analyzed using Negative Binomial GLMs (NB GLM; R package 'MASS'; Venables and Ripley, 2002), proportion of trial time spent looking at the speaker was analyzed in GLMs.

In the '*Tolerance of prolonged eye contact*' trial the duration of first eye contact with the E was coded and analyzed in Linear Models (LM, R package 'stats'; RCoreTeam, 2015).

In all above Cox Models, Tweedie GLMs, NB GLMs, ZINB GLMs, GLMs, Gamma GLMs and LMs, the full models included sex (male or female), breed (Border Collie or Siberian Husky) and treatment (OXT or PL) as fixed factors with two levels, and all two-way interactions. Model selection was based on AIC values, and the effects of explanatory variables were analyzed by likelihood ratio tests: we provide χ^2 and p values of likelihood ratio tests of models with and without the explanatory variable. For Cox Models, hazard ratio

(Exp[β]) between levels of a given fixed effect with 95 percent confidence interval are given. For GLMs, we provide parameter estimates (B) of significant factors with 95% CI.

In case of the statistical analysis with the SNPs the same statistical models were run, with the only difference that subjects' genotype for -212AG was included in the models as a further explanatory variable (besides OXT/PL treatment, breed and sex). 'A' allele carriers (AA and AG genotypes) were assigned to group 1 and GG homozygotes were assigned to group 2. Latencies were analyzed in Cox Models (R package 'survival') with occurrence of looking as terminal event. Other variables were analyzed in Generalized Linear Models (Tweedie, Negative Binomial, Zero-inflated Negative Binomial) or Linear Models, according to the distribution of the data) using R statistical environment (Version 3.2.3).

III/4.3. Results

III/4.3.1. Dogs' behaviour in the Unreachable food situation

During the four solvable (training) trials 100% of the dogs ate the food. Latency to approach the food was not affected by treatment (OXT/PL), breed, sex or their interactions (Cox Mixed Models, all $p>0.173$), but trial number had a significant effect as dogs in the first trial less likely approached the food after a given time elapsed, compared to the other three trials (Cox Mixed Model, $\chi^2_{(3)}=30.002$, $p<0.001$; Exp(β) ± SE: trial 1 → trial 2 = 2.499±0.280, trial 1 → trial 3=4.146 ± 0.288, trial 1 → trial 4=3.445 ± 0.286). The analysis of the latency to first looking at the O during the blocked (unsolvable) trial showed that oxytocin-treated dogs less likely looked at the O after a given time elapsed than those receiving placebo treatment (Cox Model, treatment: $\chi^2_{(1)}=6.970$, $p=0.008$, Exp(β)=0.386 [0.190; 0.782]; Figure III/4.3a), however, probability of having looked at the O was not different between breeds ($\chi^2_{(1)}=1.785$, $p=0.182$) and sexes ($\chi^2_{(1)}=0.662$, $p=0.416$).

Moreover, experimental treatment tended to have different effects on probability of having looked at the E after a given time elapsed in the two breeds as reflected by a near-significant interaction (Cox Model, breed × treatment: $\chi^2_{(1)}=3.752$, $p=0.053$, Exp(β)=0.200 [0.039; 1.022]; Figure III/4.3b). The interaction was driven by oxytocin-treated Border Collies (but not Siberian Huskies) looking more likely to the E than those treated with placebo. In addition, females (independent of breeds) more likely looked at the E than males ($\chi^2_{(1)}=8.920$, $p=0.003$, Exp(β)=3.836 [1.495; 9.841]).

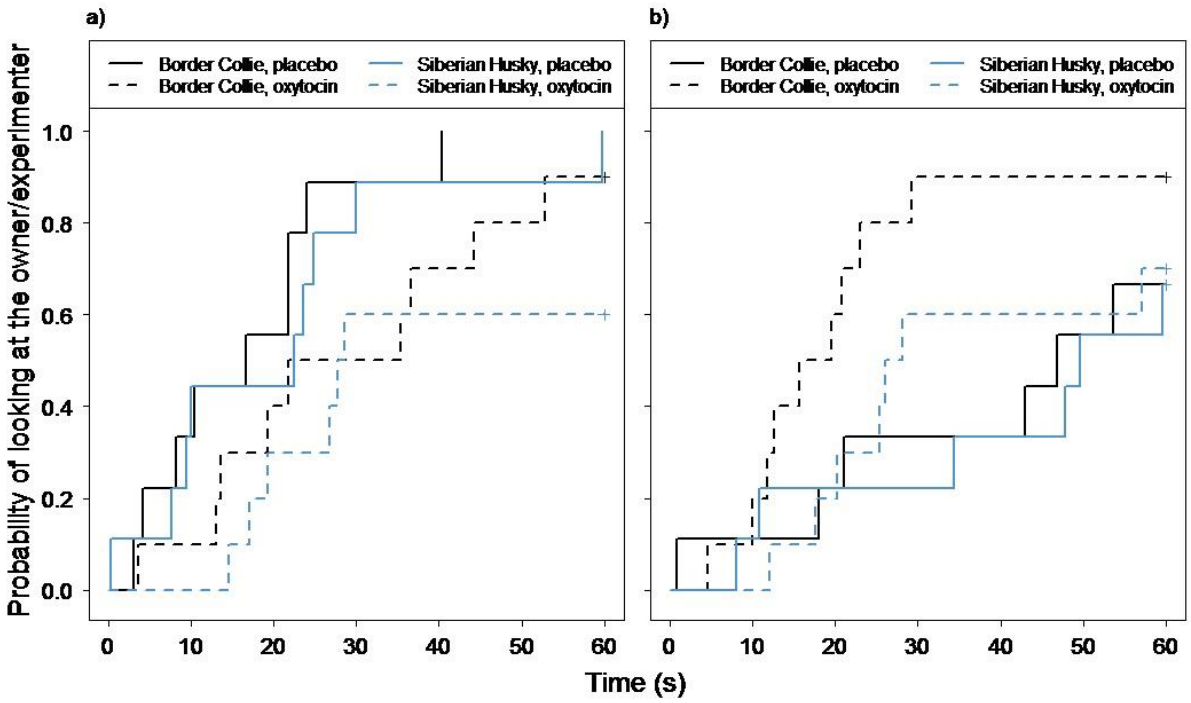

Figure III/4.3. Probability of having looked at the owner (a) and the experimenter (b) in placebo and oxytocin-treated Border Collies and Siberian Huskies after certain time elapsed in the 'Unreachable food' task

Experimental treatment had no effect on the proportion of time spent looking at the O (Tweedie GLM, treatment: $\chi^2_{(1)}$=2.240, p=0.371). Border Collies spent more time looking at the O than Siberian Huskies (breed: $\chi^2_{(1)}$=4.119, p=0.006, B=0.999 [0.281; 1.688]; Figure III/4.4a) and in both breeds, females spent more time looking at the O than males (sex: $\chi^2_{(1)}$=2.287, p=0.039, B = 0.754 [0.025; 1.446]).

Experimental treatment, however, had sex and breed-specific effects on proportion of time spent looking at the E (Tweedie GLM, sex × treatment: $\chi^2_{(1)}$=3.490, p=0.011, B=-3.106 [-5.603; -0.612]; breed × treatment: $\chi^2_{(1)}$=2.691, p=0.026, B=-2.103 [-3.974; -0.271]). The sex-specific effect was driven by male (but not female) dogs spending more time looking at the E when treated by oxytocin. The breed-specific effect was due to Border Collies (but not Siberian Huskies) spending more time looking at the E when treated with oxytocin (Figure III/4.4b).

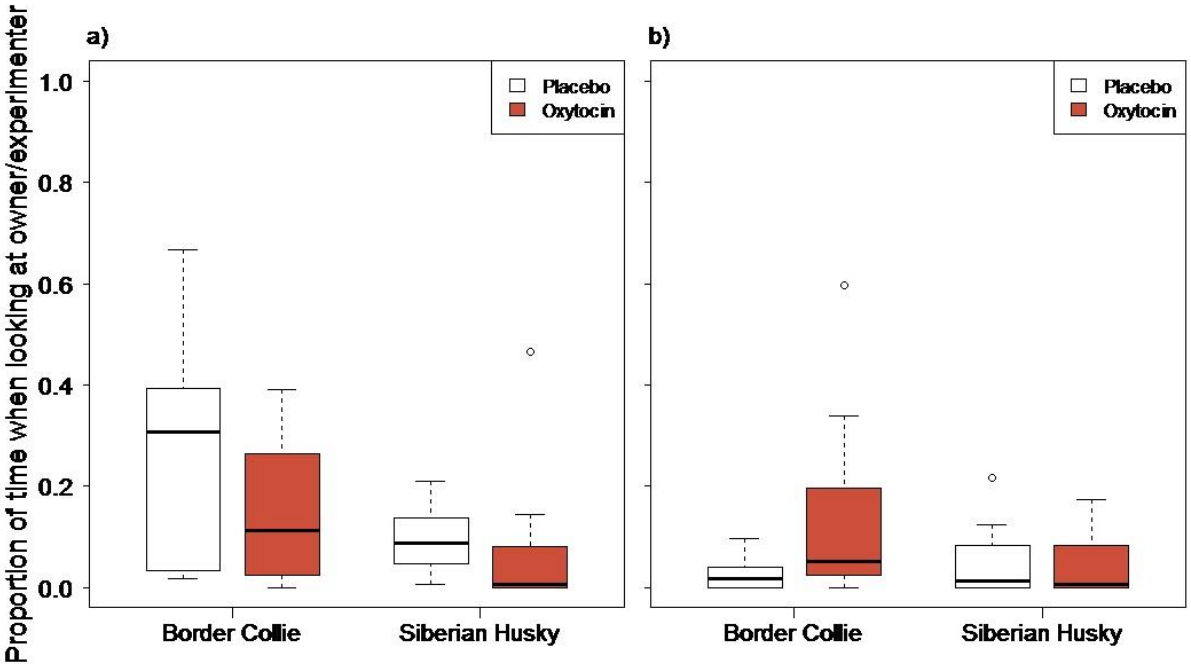

Figure III/4.4. Proportion of trial time spent looking at the owner (a) and the experimenter (b) in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Unreachable food' task.

Number of gaze shifts between the cage and the O was not different between treatments and sexes (ZINB GLM, treatment: $\chi^2_{(1)}$=1.108, p=0.293; sex: $\chi^2_{(1)}$=0.240, p=0.624). However, more gaze shifts were observed in Border Collies than in Siberian Huskies ($\chi^2_{(1)}$=9.707, p=0.002, B=0.675 [0.295; 1.055]; Figure III/4.5a). None of the investigated variables had significant effect on the number of gaze shifts between the cage and the E (NB GLM, treatment, breed, and sex: all p>0.724; Figure III/4.5b).

Duration of looking at the cage was not influenced by any of the factors investigated (treatment, breed, sex), nor by their interactions (all p>0.097).

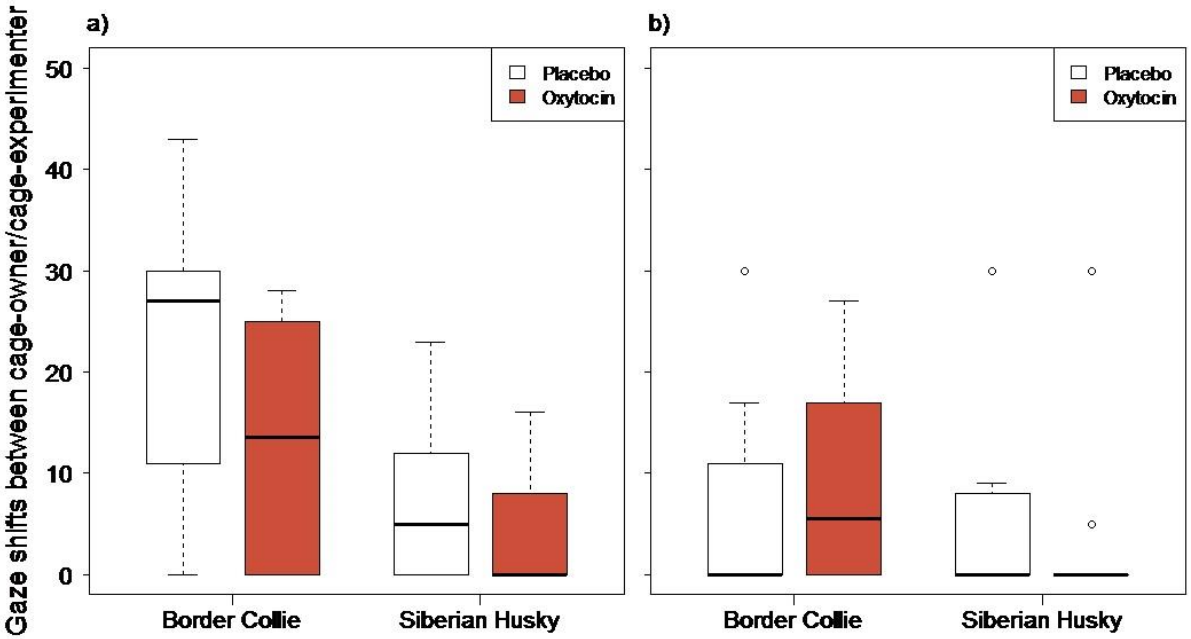

Figure III/4.5. Number of gaze shifts between the cage and the owner (a) and the cage and the experimenter (b) in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Unreachable food' task.

III/4.*3.2. Dogs' response to a 'Potentially dangerous' object*

Probability of having looked at the O in the 'Potentially dangerous object' situation was not different between experimental treatments (Cox Model, treatment: $\chi^2_{(1)}$=0.003, p=0.956). Females more likely looked at the O than males (sex: $\chi^2_{(1)}$=4.687, p=0.030, Exp(β)=2.568 [1.059; 6.227]; Figure III/4.6), whereas Siberian Huskies less likely looked at the O than Border Collies (breed: $\chi^2_{(1)}$=8.967, p=0.003, Exp(β)=0.264 [0.106; 0.659]; Figure III/4.6).

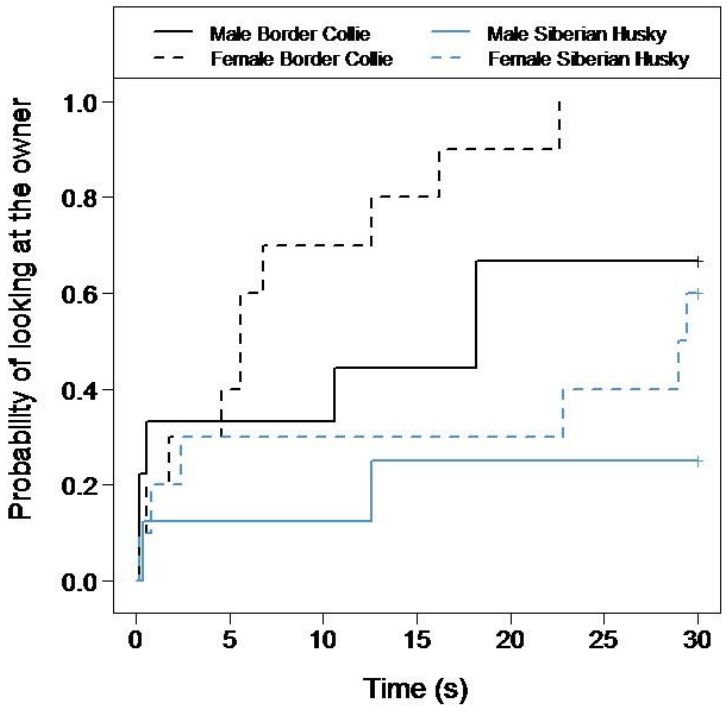

Figure III/4.6 Probability of having looked at the owner after a certain time elapsed in male and female Border Collies and Siberian Huskies in the 'Potentially dangerous object'situation.

Oxytocin had a breed-specific effect on the proportion of time spent looking at the O (Tweedie GLM, breed × treatment: $\chi^2_{(1)}$=3.171, p=0.009, B=-2.377 [-4.168; -0.581]; driven by oxytocin-treated Siberian Huskies spending less time looking at the owner than oxytocin-treated Border Collies. In addition, female dogs spent more time looking at the O (sex: $\chi^2_{(1)}$=2.370, p=0.025, B=0.999 [0.127; 1.872]).

Number of gaze shifts between the speaker and the O was also differently influenced by oxytocin treatment in the two breeds (NB GLM, breed × treatment: $\chi^2_{(1)}$=6.599, p=0.010, B=-2.154 [-3.761; -0.614]; Figure III/4.7). The interaction was driven by opposite effect of oxytocin in the breeds; in Border Collies, oxytocin increased the number of gaze shifts, whereas in Siberian Huskies, oxytocin resulted in less gaze shifts. Moreover, gaze shift was higher in females than in males (sex: $\chi^2_{(1)}$=5.506, p=0.019, B=1.035 [0.207; 1.911]).

Probability of having approached the sound source was not influenced by experimental treatment (Cox Model, treatment: $\chi^2_{(1)}$=2.338, p=0.126). We found sex and breed-specific reactions in this situation (sex × breed: $\chi^2_{(1)}$=8.213, p=0.004, Exp(β)=0.095 [0.018; 0.492]). This interaction was driven by opposite reactions of the sexes in the breeds: in Border Collies

females, while in Siberian Huskies males approached more likely the speaker than the opposite sex.

Duration of looking at the sound source was not influenced by experimental treatment (GLM: $\chi^2_{(1)}$=2.415, p=0.120), but the breeds showed sex-specific reactions in this situation (sex × breed: $\chi^2_{(1)}$=4.461, p=0.035, B=0.319 [0.003; 0.635]) resulting from female Siberian Huskies, but nor Border Collies, looking more at the speaker than males.

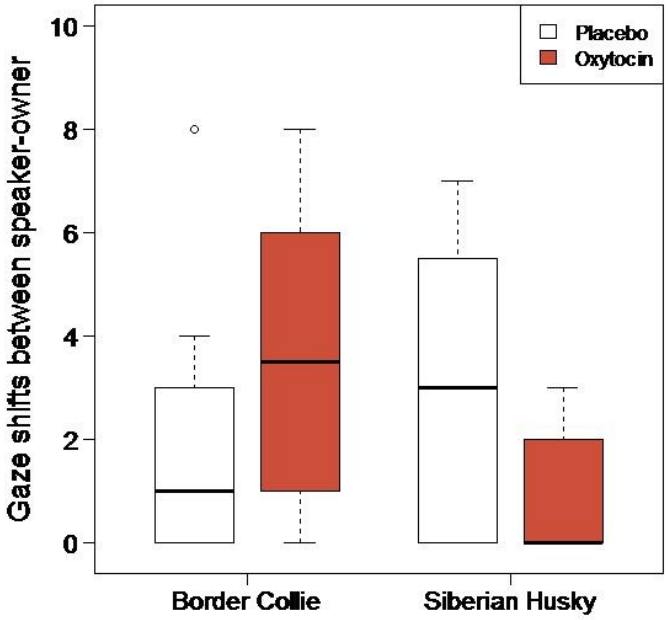

Figure III/4.7. Number of gaze shifts between the sound source and the owner in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Potentially dangerous object' situation.

III/4.*3.3. Dogs' response in the Tolerance of prolonged eye contact situation*

Oxytocin treatment had a breed-specific effect on the duration of first eye contact with the E in the 'Tolerance of prolonged eye contact' situation. In Border Collies, oxytocin increased the tendency to maintain eye contact whereas in Siberian Huskies, oxytocin resulted in a decrease (LM, breed × treatment: $\chi^2_{(1)}$=4.430, p=0.035, B=-0.073 [-0.146; 0.001]; Figure III/4.8). Besides this interaction, duration of first eye contact was longer in females than in males (sex: $\chi^2_{(1)}$=7.994, p=0.005, B=0.051 [0.014; 0.088]).

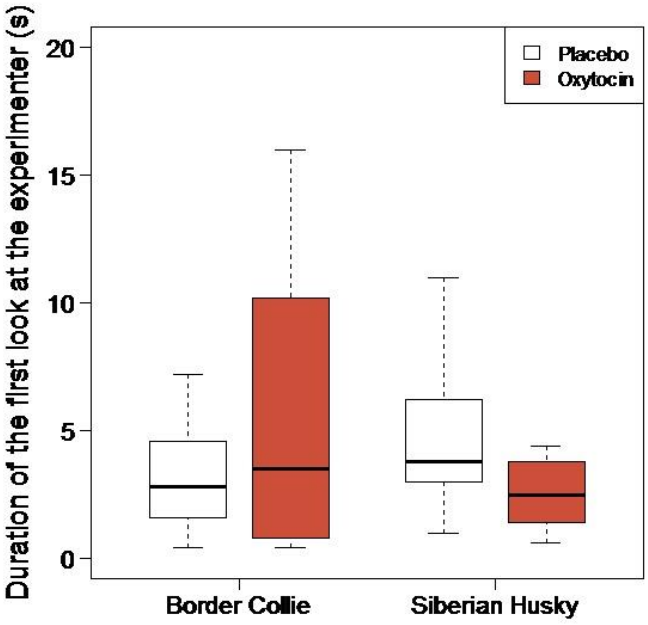

Figure III/4.8. Duration of the first eye contact with the experimenter in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Tolerance of prolonged eye contact' situation.

III/4.*3.4 Gene-behaviour associations*

Results of likelihood ratio tests of models with and without a given explanatory variable are given in Appendix 7. The results of these analyses were largely overlapping with those reported in the main text (without the inclusion of OXTR genotype), thus support our main conclusion that the effect of oxytocin treatment might differ in Border Collies and Siberian Huskies as reflected in significant breed × treatment interactions in all three behavioural tests. In some cases we found that effects that had been significant in the main models did not remain significant when genotype is included. For example in the *Unreachable food* task we did not find a main effect of sex for the proportion of trial time spent looking at the owner, instead there was a significant sex × genotype interaction. Similarly, in the *Potentially dangerous object* task sex had no effect on the proportion of trial time spent looking at the owner, but this response was influenced by a significant sex × genotype interaction effect. Moreover, the breed × treatment interaction was no longer significant in this analysis, whereas the genotype × breed as well as the genotype × treatment interactions became significant. In the *Tolerance of prolonged eye contact* task instead of the main effect of a sex a significant sex × genotype interaction was found.

A significant main effect of OXTR genotype was found in the *Unreachable food* and the *Potentially dangerous object* tasks (for the proportion of time spent looking at the owner / experimenter and the number of gaze shifts variables). The effect of OXTR genotype was also in interaction with not only OXT/PL treatment but in some cases also with breed and sex.
These results, although preliminary due to the limited sample size, suggest that (1) the results reported in the main text and the conclusions drawn are robust, and (2) the OXTR genotype of the subjects is indeed an important factor that needs to be considered in future research.

III/4.4. Discussion

Increasing evidence suggest a significant effect of intranasal oxytocin on different aspects of social behaviour in dogs (for a review see Thielke and Udell, 2015). However, previous studies all included mixed samples of dogs from various breeds. In the present study evidence was found, for the first time, that the neuro-hormonal background relating to the oxytocin system has different impact on dog breeds selected for different work purposes. In all three test situations we found a combined (interactive) influence of breed and oxytocin treatment on some aspects of human-directed social behaviour in dogs. Namely, in the *Unreachable food* task Border Collies (but not Huskies) tended to look at the potential helper (i.e. the experimenter who has previously demonstrated her ability to manipulate the apparatus) sooner and they also spent more time looking at the experimenter after receiving oxytocin. Moreover, in the *Potentially dangerous object* situation oxytocin-treated Border Collies spent more time looking at their owners and showed more 'social referencing' (i.e. shifted their gaze more frequently between the sound source and the owner) than oxytocin-treated Huskies. This finding adds further details to previous results (Hernádi et al., 2015) that in a threatening approach situation (when the object of the threat is a slowly approaching human) oxytocin-treated dogs looked more frequently at the human (owner or experimenter) standing behind them compared to placebo-treated subjects. The differential effect of oxytocin treatment on behaviour is also evidenced in Border Collies' and Siberian Huskies' tendency to maintain eye contact.
In addition to breed × treatment interactions, relatively robust main effects of treatment, breed and sex were also found. Results show that after oxytocin administration (compared to placebo treatment), dogs in general were less likely to look at their owner after a given time elapsed in the *Unreachable food* situation. Importantly, the owner was not actively involved in this task and thus looking at the owner may merely reflect task-related anxiety that can be

reduced by oxytocin treatment. The finding that oxytocin treatment does not affect dogs' looking and approach behaviour towards nonsocial targets (cage containing food in the *Unreachable food* task and speaker in the *Potentially dangerous object* situation) also supports the idea that the oxytocin system is involved in the regulation of social (but not non-social) attention in dogs.

It has been shown in both humans (e.g. Herzmann et al., 2013) and dogs (Nagasawa et al., 2015) that oxytocin can have differential effects on males and females. In line with this a combined effect of treatment and sex was found in the unsolvable task (on the duration of looking at the experimenter). A potential confound to sex × oxytocin treatment interactions in this and previous research (on both dogs and humans) is that as males and females significantly differ in body weight, the per-kilogram doses of intranasally administered oxytocin systematically differ between sexes. The effect of sex on behaviour was also found to be in interaction with breed in the social referencing test (*Potentially dangerous object* situation, latency to approach the sound source). This might be due genetic differences in the estradiol and progesterone system of the two species and/or due to sex differences in proneness to epigenetic modification of the oxytocin system during ontogeny. Sex differences were also found regardless of oxytocin administration and breed: female dogs looked longer into the eyes of the owner (*Unreachable food* and *Potentially dangerous object* situation) and they looked more likely to the experimenter (*Unreachable food* task) and longer (*Tolerance of prolonged eye contact* test) compared to male dogs. Furthermore sex × breed interactions in non-social behaviours were also found in the present study (*Potentially threatening object* task, probability of having approached the sound source and duration of looking at the sound source).

In conclusion our study provides experimental evidence that oxytocin administration can have differential effects on social responsiveness of two dog breeds selected for independent and cooperative work respectively.

PART III. Effect of social presensitization

III/5. Study 5: Priming effect of social presensitization on children' social sensitivity. [5]

III/5.1. Introduction

Previous investigations have provided convincing evidence that oxytocin (OXT) is implicated in a wide range of human social cognitive and emotional functions (Lee et al., 2009; Meyer-Lindenberg, 2008) as it affects the activation of brain areas responsible for emotion regulation and cognitive control, including the amygdala and the prefrontal cortex (Baumgartner et al. 2008; Domes et al. 2007; Kirsch et al. 2005). It has also been found to be an important factor in different aspects of human social cognition, such as the amount of attention directed at the eye regions of other people's faces (Guastella et al. 2008), covert attention to positive social cues (Domes et al., 2012), recognition of complex mental states and social emotions (Domes et al. 2007), and possibly emotion detection and emotion recognition (Guastella et al., 2009; Marsh et al., 2010; Schulze et al., 2011).

Studies examining the effects of oxytocin on behaviour usually take two forms: researchers may either measure endogenous oxytocin levels directly (or map genotypes related to the oxytocinergic system) or administer OXT in the form of nasal spray. Previous human studies have demonstrated that oxytocin nasal spray has significant impacts on social behaviour and cognitive processes in humans in a manner that has not previously been observed from the administration of other medications (Bartz et al., 2011; Kemp and Guastella, 2011).

However, OXT levels may be manipulated in another way: through intensive social stimulation. There is increasing evidence to suggest that social signals during parent-child interaction effectively stimulate the oxytocin system and elevate the level of peripheral OXT. For example, Feldman and colleagues (2010) have found that mothers and fathers who had provided high levels of tactile contact to their infants showed an increase in salivary OXT following parent–infant interactions but such an increase was not observed among parents who provided low tactile contact. Moreover, after a 15 minute long play and touch interaction, both infants' and parents' salivary OXT level increased. Other studies also demonstrated that

[5] This chapter is based on: **Kovács, K.,** Oláh, K., Lakatos, K., and Topál, J., Socially stimulating pre-treatment modulates attention to biological motion and contingent reactivity in children. *Front Psychol.* submitted manuscript.

oxytocin is released in response to stimuli such as infant suckling, somatosensory touch, or even the sight or sound of a nursing mother's infant (Johnston and Amico, 1986; Lucas et al., 1980; McNeilly et al., 1983; Uvnas-Moberg et al., 1993). Cross-species studies have also found that dog-owner affiliative interaction (gazing, petting, talking) increases owner's urinary oxytocin levels (Nagasawa et al., 2015) and results in an increase in plasma oxytocin levels in both dogs and their owners (Handlin et al., 2012, 2011). Despite these results, however, only a few studies have directly tested the effects of social stimulation on basic socio-cognitive skills that lay the ground for higher order cognitive processes.

The ability to discriminate social from non-social objects is fundamental to children's understanding of the social world and the detection of animacy and contingency from simple moving images might be a precursor to the development of such skills. Children and adults consider motion as the most important criterion for judging unfamiliar entities as animate (Poulin-Dubois et al., 1996; Richards and Siegler, 1986; Shar et al., 1985) and the perception of contingent reactivity (i.e. a consistent and predictable relation between a subject's actions and the occurrence of a partner's responses) is one of the cues triggering communicative interactions (Bigelow, 2001). There is no doubt that social interactions could not be realized without the detection of animacy and contingency, we may therefore assume that the oxytocin system modulates these basic cognitive processes.

In the present study, we set out to test the effects of intensive social stimulation on children's perception of agency and animacy as this ability is fundamental to social cognition as shown by the above presented results. Preschoolers (5-6 years of age) were presented with two different kinds of animations on an eye-tracker after a 10-min long social- or non-social pre-treatment. Our aim was to test the following two questions: (1) Does socially stimulating pre-treatment increase preference for biological motion in children; (2) Does socially stimulating pre-treatment increase the preference for high but not perfect/ or maybe for perfect contingency in children.

III/5.2. Method

III/5.2.1. Ethics statement

The study was carried out with the approval of the National Psychological Research Ethics Committee (Ref. No. 2015/23). Participants' caregivers gave written informed consent.

III/5.2.2. Partcipants

Forty-six children between the age of 5 and 6 years participated in the study. They were selected from a database of volunteer families that had previously applied for participation. One child was excluded due to a failure to calibrate properly and another one was not able to remain seated during testing. The final analysis included forty-four children (mean age ±SEM: 66.5 ±0.8 months; 23 girls, 21 boys) of whom 22 (11 girls, 11 boys) were assigned to the *Socially stimulating* (SS) pre-treatment and 22 (12 girls, 10 boys) to the *Socially ignoring* (SI) pre-treatment condition (Table III/5.1.). Parents of all children signed informed consent prior to participation. Ethical approval was obtained from the National Psychological Research Ethics Committee (Ref. No. 2011/13).

	Socially stimulating		**Socially ignoring**	
	A order	*B order*	*A order*	*B order*
Boys	6	5	5	5
Girls	6	5	7	5

Table III/5.1. Number of children participating in the *Socially stimulating* and *Socially ingoring* pre-treatment

III/5.2.3. Setup and materials

Infants were tested in the laboratories (pre-treatment room: 4 m × 4 m; eye tracking testing room: 2.0 m × 2.9 m) of the Institute of Cognitive Neuroscience and Psychology, Hungarian Academy of Sciences.

For stimuli presentation and data collection, a Tobii TX300 eye-tracker was used with the TobiiStudio 3.2 software (screen size was 52×32 cm and 1920 ×1200 pixels). We used a five point calibration throughout the experiment. Children who were included in the final sample provided at least 80% valid eye-tracking data.

III/5.2.4. Experimental stimuli

At the beginning of each video stimuli, a 4-second long attention grabber (sound + moving rattle animation) flashed on and off, to ensure that the subject's gaze was in the neutral-midline position before the presentation of the next set of stimuli. The two different kinds of animations were presented in two different orders. Children in group "A order" (N = 20) first watched *Biological motion* animation and then *Contingent motion* animation. Children in group "B order" (N = 20) watched these tasks in reverse order.

III/5.2.4.1 Biological motion animation

The biological motion stimulus depicted a point-light movie of a side walking human on one side of the screen (left/right counterbalanced across subjects; 'normal point like figure – PLF'), while on the other side, the inverted and scrambled version of the same point-light movie ('inverted scrambled display' - ISD) was shown (Figure III/5.1A). The point-light figure display was an 11-dot figure with single white dots representing the head, one shoulder, one hip, and each of the two elbows, wrists, knees, and ankles on a black background. The PLF was shown walking in place, as if on a treadmill, with a stride frequency of 0.93 Hz.
Every child watched two 25 s long video animations accompanied by a neutral music playback (e.g. right-PLF/left-ISD, then left-PLF/right-ISD); the order of the videos was counterbalanced between children. PLF was presented first on the left side and second on the right side for children in the 'A order' group, while it was presented first on the right side and then on the left side for children in the 'B order' group.

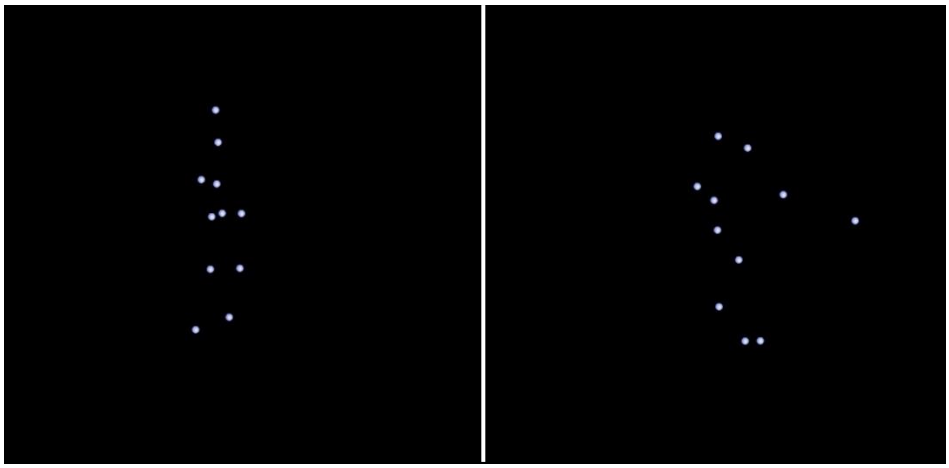

Figure III/5.1A. Photo illustration about the biological motion stimulus without masking dots (PLF on the left side, ISD on the right side)

III/5.2.4.2. Contingent motion animation

Children presented with two 25 s long video animation (*Cont-100* & *Cont-70*) accompanied by a neutral music playback (Figure III/5.1B). In *Cont-100*, a 100% contingent pair of circles displayed on the screen. One of the circles (Initiator) initiated movement back and forth in one of eight possible direction (up, down, left, right, diagonally up left, diagonally up right, diagonally down left, diagonally down right) and the other circle (Follower) always followed the movement in the same direction with a 0.5 second delay (10 turns in total; always the

same circle acted as Initiator). In *Cont-70*, however, the Follower circle took the same direction as shown by the Initiator only 7 out of 10 turns. Moreover, 7 out of 10 times the left circle initiated the movement, while 3 times the right circle initiated it. The moving circles never reached each other on their paths. Circles in *Cont-100* and *Cont-70* animations differed only in their colour shades, but not in their colour saturation and brightness. Children in the "A order" group first watched *Cont-70* and children in the "B order" group first watched *Cont-100*.

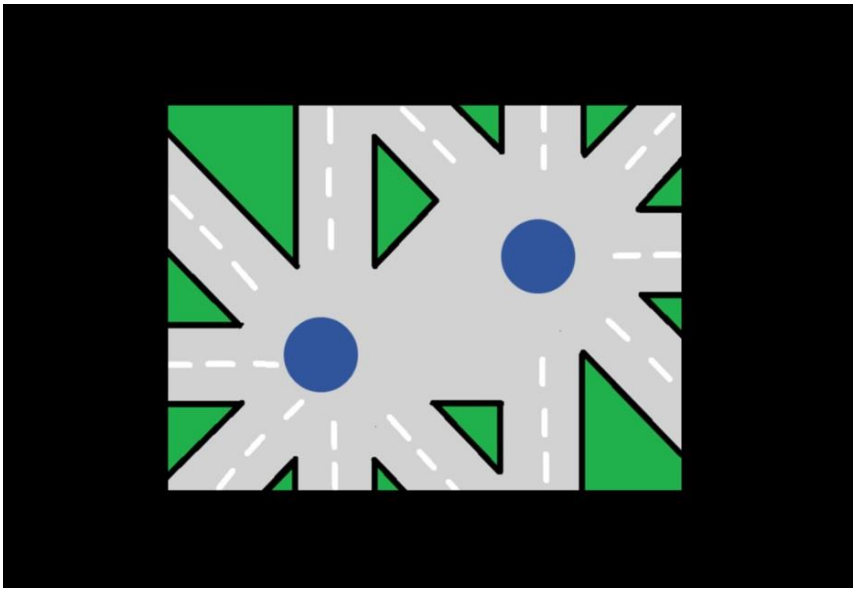

Figure III/5.1B. Photo illustration of the Contingency stimulus.

III/5.2.5. Procedure

On arriving to the laboratory, children had 5 minutes to explore the testing room and get comfortable in the company of the experimenter and the helper, while parents were briefed about the experiment and signed informed consent. After this, the helper escorted the child and his/her mother into the pre-treatment room. The pre-treatment was a 10 minute long procedure, during which only the mother and the child were in the room. When the 10-minute- long pre-treatment was over, the experimenter escorted the child and his/her mother back to the testing room, where the eye-tracker had previously been placed and set up.

III/5.2.5.1. Pre-treatment (see Figure III/5.2.)

During the 10 minutes *Socially Stimulating* pre-treatment (SS), mother and child were asked to position themselves face to face and make as much as eye contact and physical contact as they can (Figure III/5.2A).

In the *Socially Ignoring* pre-treatment (SI), however, the child was playing alone with the supplied toys, while his/her mother was filling in questionnaires. During this period, they were not allowed to make eye contact or physical contact. The mother was also asked not to talk to the child. However, if it was necessary (i.e. the child lost interest in playing with toys), mothers were allowed to ask their children to resume playing (without using eye-contact) (Figure III/5.2B).

Figure III/5.2. Photo illustration of the *Socially stimulating* (A) and *Socially ignoring* (B) pre-treatments.

III/5.2.5.2. Test

After the SS or SI pre-treatments, the experimenter escorted the child and their parent into the testing room, seated the child in the chair in front of the monitor of the eye-tracker (at a distance of approx. 60–70 cm) and the parent was seated in the other corner of the room. Testing began with a standard 5-point calibration procedure then the children were presented with the attention grabber (4s) and then the *Biological motion* and *Contingent motion* animations (see above).

III/5.2.6. Data analysis

To analyse fixations and compare them, areas of interests (AOIs) were defined for each image/video by delineating a rectangular area. In the *Biological motion* animation, 4 AOIs were created, which covered the head, body, feet regions of both PLF and ISD (Figure III/5.3). For the *Contingent motion* animation, we created moving AOIs which covered the moving circles during the whole stimulus presentation.

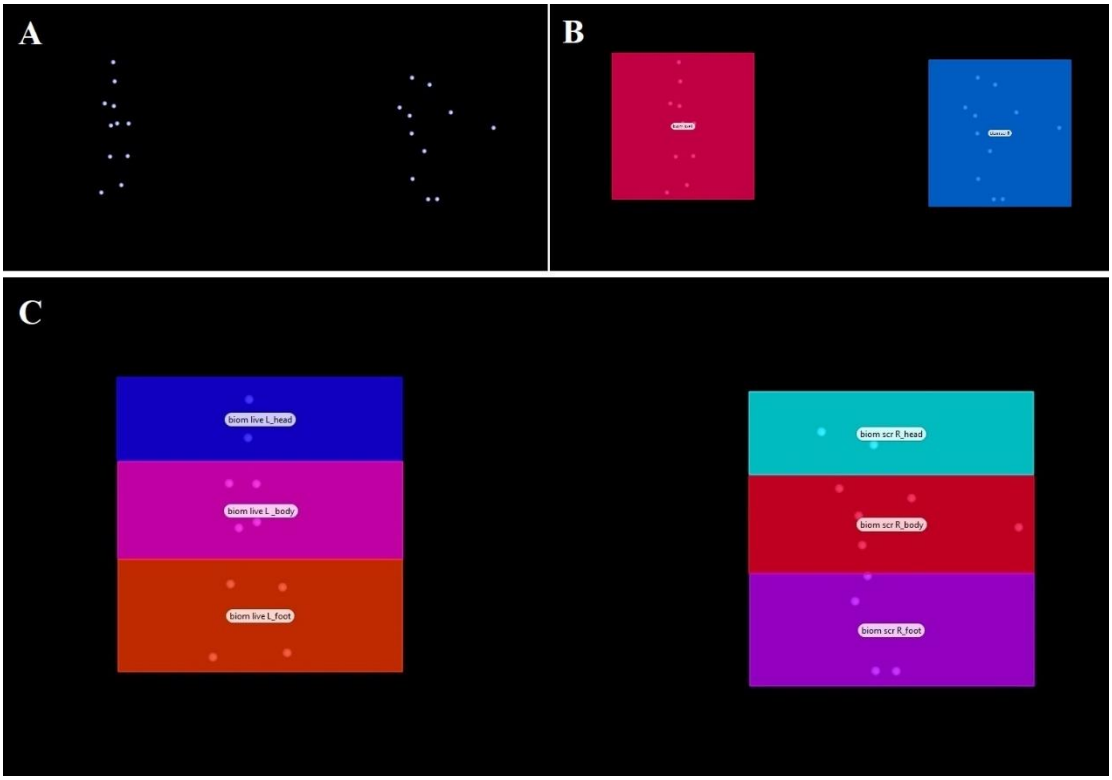

Figure III/5.3. Illustration of (A) *Biological motion* animation, (B) full AOIs of PLF and ISD, (C) AOIs of head, body and feet regions on both the PLF and ISD.

During both *'Biological motion'* and *'Contingent motion'* stimulus presentations three aspects of children's looking behaviour were recorded: (1) *Total visit duration* (duration of all visits within an AOI or an AOI group) (2) *Latency of first fixation* (how long it takes before a test participant fixates on an AOI or an AOI group for the first time) and (3) *Fixation count* (the sum of all fixations that hit on an AOI or on an AOI group during the entire stimulus presentation time).

A Generalized Estimating Equations (GEE) model using restricted maximum likelihood estimation with backward elimination method was used to test the effects of Pre-treatment (SS or SI), Order of animation (A or B) and Sex (boy or girl) as between-subjects factors as well as the effects of Stimulus type (PLF or ISD in *Biomotion*; initiator or follower in *Contingent motion*) and Contingency level (Cont-70 or Cont-100 in *Contingent motion*) as within-subjects factors. All two-way interactions were also investigated. SPSS version 21 software was used for statistical analyses.

III/5.3. Results

III/5.3.1. Biological motion animation

III/5.3.1.1 Full point-light displays

Our GEE analyses showed that the *latency of first fixation* was affected by the Stimulus type ($\chi^2_{(1)}$=3.859, p=0.049, Figure III/5.4): children tended to fixate the inverted scrambled display sooner. Other main factors (Pre-treatment: $\chi^2_{(1)}$=0.000, p=0.999; Sex: $\chi^2_{(1)}$=0.537, p=0.464; Order: $\chi^2_{(1)}$=0.348, p=0.555) and all the two-way interactions were non-significant (all p>0.05).

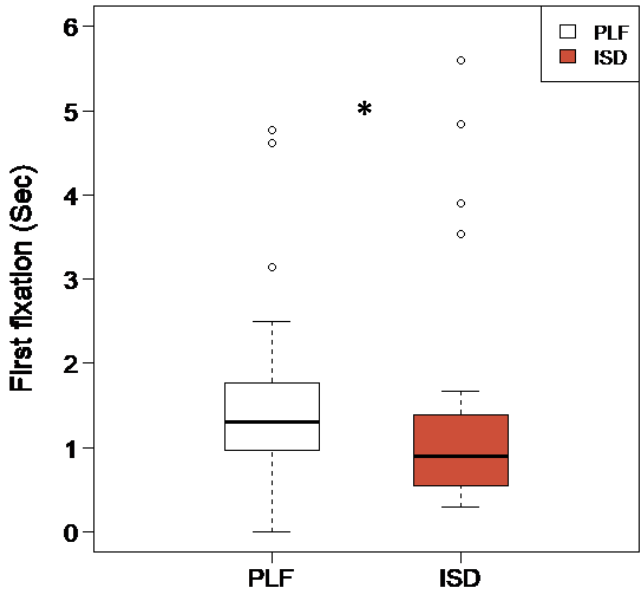

Figure III/5.4. *Latency of first fixation (mean+SD) for the presented stimuli* (PLF and ISD). * indicates difference at p<0.05 level

The *fixation count* was also affected by the Stimulus type ($\chi^2_{(1)}$=10.823, p=0.001, Figure III/5.5), children fixated on the IDS more than the PLF. Other factors (Pre-treatment: $\chi^2_{(1)}$=.360, p=0.548; Sex: $\chi^2_{(1)}$=1.346, p=0.246; Order: $\chi^2_{(1)}$=2.391, p=0.112) were non-significant. However, the Order of animation significantly interacted with the other factors. Those girls who were presented first with the *Biomotion* ('A order') fixated less the full display than girls presented with the 'B order' (i.e. who first watched Contingent motion animation; Sex × Order, $\chi^2_{(1)}$=6.916, p=0.009). Moreover, after socially stimulating pre-treatment, children in 'B order' fixated more at the stimuli than children in 'A order' (Pre-treatment × Order, $\chi^2_{(1)}$=5.009, p=0.024,) and children in 'B order' fixated more at the

inverted scramble display than children in 'A order (Stimulus type × Order, $\chi^2_{(1)}$=4.214, p=0.040). Other interactions were non-significant (all p>0.05)

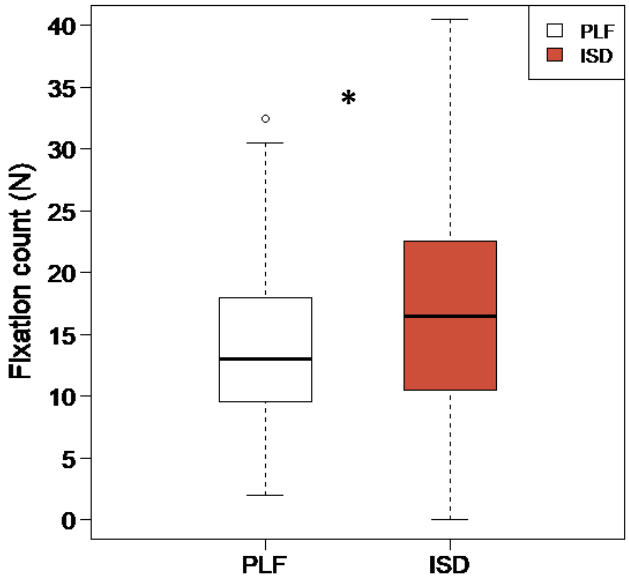

Figure III/5.5. *Latency of first fixation and fixation count (mean+SD) for the presented stimuli* (PLF and ISD). * indicates difference at p<0.05 level

The *total visit duration* was affected none of the main factors (Pre-treatment: $\chi^2_{(1)}$= 0.628, p=0.428; Order: $\chi^2_{(1)}$= 1.593, p=0.207; Sex: $\chi^2_{(1)}$= 0.656, p=0.418;Stimulus type: $\chi^2_{(1)}$= 1.513, p=0.219). However there was a significant interaction between Sex and Order ($\chi^2_{(1)}$= 3.698, p=0.045, boys in 'A order' fixated longer the stimuli than girls in 'A order') and a marginally significant Pre-treatment × Stimulus type interaction ($\chi^2_{(1)}$= 3.739, p=0.053, children looked longer at PLF after a socially stimulating than after socially ignoring pre-treatment.). All the other two-way interactions were also non-significant (all p>0.05).

III/5.3.1.2 Head region of point-light displays

Latency of first fixations were significantly associated with none of the main factors (Pre-treatment: $\chi^2_{(1)}$= 0.119, p=0.731, Sex: $\chi^2_{(1)}$= 0.197, p=0.657, Order: $\chi^2_{(1)}$= 0.846, p=0.358, Stimulus type: $\chi^2_{(1)}$= 1.535, p=0.215). However we found a significant interaction: after a socially stimulating pre-treatment, children fixated the head region of PLF sooner than at the head region of ISD (Pre-treatment × Stimulus type, $\chi^2_{(1)}$= 7.105, p=0.008, Figure III/5.6). Other interactions were non-significant (all p>0.05).

Our result showed that no main effect influenced the *fixation count* within the head regions (Pre-treatment: $\chi^2_{(1)}$= 0.070, p=0.791, Sex: $\chi^2_{(1)}$= 1.941, p=0.164, Order: $\chi^2_{(1)}$= 0.279,

p=0.597, Type of stimulus: $\chi^2_{(1)}$= 0.042, p=0.837). Moreover, girls who were presented second with the *Biomotion* ('B order') fixated more frequently within the head region of the stimuli than boys presented with the 'B order' (Sex × Order, $\chi^2_{(1)}$= 10.886, p=0.001). Pre-treatment, furthermore, significantly interacted with Sex ($\chi^2_{(1)}$= 4.251, p=0.039; after socially stimulating pre-treatment girls fixated more times at the head regions of the stimuli, than boys) and we found a marginally significant Pre-treatment x Order interaction ($\chi^2_{(1)}$= 3.699, p=0.054; after socially stimulating pre-treatment, children in 'B order' fixated less at the head regions than children in 'A order'). Other interactions were non-significant (all p>0.05).

We found that none of the main factors (Pre-treatment: $\chi^2_{(1)}$= 1.215, p=0.270; Order: $\chi^2_{(1)}$= 0.244, p=0.621; Sex: $\chi^2_{(1)}$= 0.020, p=0.886; Stimulus type: $\chi^2_{(1)}$= 0.633, p=0.426) affected the *total visit duration* in the head regions and there were only two significant interactions. Boys after socially stimulating pre-treatment looked shorter at the head region of the stimuli than after a socially ignoring pre-treatment (Pre-treatment × Sex: $\chi^2_{(1)}$= 4.243, p=0.039) and boys in 'A order' looked longer at the head region of the stimuli than boys in 'B order' (Sex × Order: $\chi^2_{(1)}$= 8.590, p=0.003).

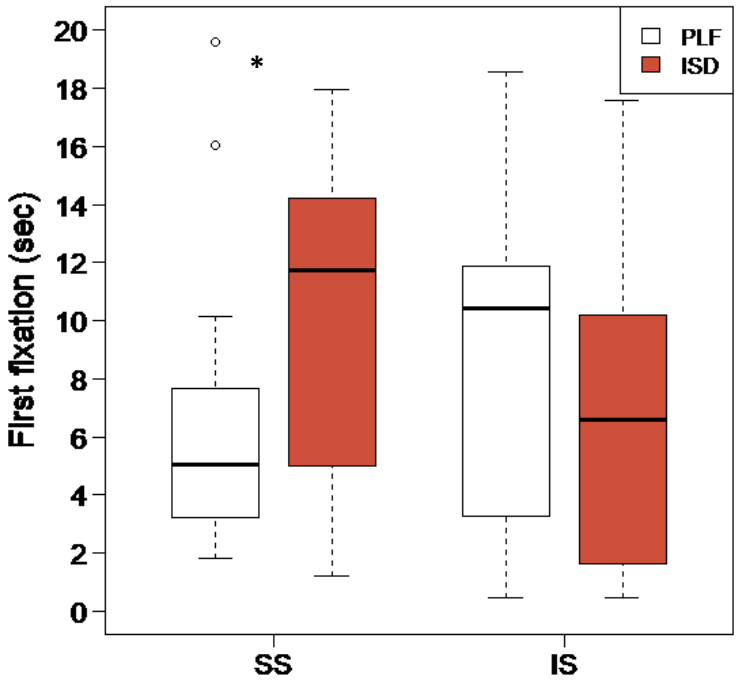

Figure III/5.6. *Latency of first fixation (mean +SD) towards the head region* of the presented stimuli (PLF and ISD). * indicates significant difference at p<0.05 level

III/5.3.1.3 Body region of point-light displays

Generalized Estimating Equation model showed that children in 'B order' fixated sooner on the body region of the stimuli, than children in 'A order' ($\chi^2_{(1)}$= 4.774, p=0.029). Stimulus type also had a significant main effect on the first fixation ($\chi^2_{(1)}$= 4.964, p=0.026; children looked sooner at the body region of ISD than the body region of PLF). There were no main effects of Pre-treatment ($\chi^2_{(1)}$= 0.016, p=0.899) and Sex ($\chi^2_{(1)}$= 0.024, p=0.876). We found a significant Stimulus type x Sex interaction ($\chi^2_{(1)}$= 7.662, p=0.006; girls looked sooner at the body region of ISD than that of the PLF). Other interactions were non-significant (all p>0.05).
The analysis of *fixation counts* showed a significant main effect of Stimulus type ($\chi^2_{(1)}$= 22.201, p<0.001; children fixated more the body region of ISD). The main effects of Pre-treatment ($\chi^2_{(1)}$= 0.409, p=0.523), Order ($\chi^2_{(1)}$= 1.986, p=0.159) and Sex ($\chi^2_{(1)}$= 0.334, p=0.563) were not significant. We also found a significant interaction between Stimulus type and Order ($\chi^2_{(1)}$= 5.951, p=0.015; children in 'B order' fixated more at the body region of ISD than on the body region of PLF). Other interactions were non-significant (all p>0.05).
Stimulus type was significantly associated with the *total visit duration* ($\chi^2_{(1)}$=9.756, p=0.002; children fixated more the body region of ISD, than the PLF). Other main factors (Pre-treatment: $\chi^2_{(1)}$= 0.588, p=0.443, Order: $\chi^2_{(1)}$= 2.358, p=0.125, Sex: $\chi^2_{(1)}$= 2.358, p=0.125) as well as the interaction between factors were non-significant (all p>0.05).

III/5.3.1.4 Feet region of point-light displays

Our results showed that *latency of first fixation* towards the feet region was not significantly associated with any of the main factors (Pre-treatment: $\chi^2_{(1)}$= 2.561, p=0.110; Order: $\chi^2_{(1)}$= 1.468, p=0.226; Sex: $\chi^2_{(1)}$= 0.029, p=0.864; Type of the stimulus: $\chi^2_{(1)}$= 0.283, p=0.595). Interactions were also non-significant (all p>0.05).
Concerning the *fixation count* toward the feet regions we found no main effects of Pre-treatment ($\chi^2_{(1)}$= 0.147, p=0.702), Order ($\chi^2_{(1)}$= 0.056, p=0.814), Sex ($\chi^2_{(1)}$= 0.265, p=0.607) and Stimulus type ($\chi^2_{(1)}$= 0.137, p=0.711). Interactions were also non-significant (all p>0.05) except Stimulus type × Order ($\chi^2_{(1)}$= 3.841, p=0.050; children in 'B order' looked more at the feet region of ISD than children in 'A order').
Similarly, the analysis of *total visit duration* showed only one significant interaction effect (Pre-treatment x Order, $\chi^2_{(1)}$=5.238 , p=0.022; children in 'A order' fixated longer the feet region of the stimuli after socially stimulating than after socially ignoring pre-treatment). Other interactions (all p>0.05) and the main effects (Pre-treatment: $\chi^2_{(1)}$= 2.408, p=0.121,

Order: $\chi^2_{(1)}$= 0.049, p=0.825; Sex: $\chi^2_{(1)}$= 0.029, p=0.865, Stimulus type: $\chi^2_{(1)}$= 0.252, p=0.616) were non-significant.

III/5.3.2. Contingent motion animation

Generalized Estimating Equation analyses showed that none of the main factors had a significant effect on the *latency of first fixations* (Stimulus type: $\chi^2_{(1)}$= 0.121, p=0.728; Pre-treatment: $\chi^2_{(1)}$= 0.032, p=0.859; Order: $\chi^2_{(1)}$= 0.008, p=0.929; Contingency level: $\chi^2_{(1)}$= 0.815, p=0.367; Sex: $\chi^2_{(1)}$= 2.278, p=0.131). However, there was a marginally significant Stimulus type × Contingency level interaction ($\chi^2_{(1)}$= 3.558, p=0.059; children looked at the initiator object sooner if they were watching the 100% contingent video stimulus, but later if they were watching the 70% contingent video stimulus). Other interactions were non-significant (all p>0.05).

Furthermore, we found that *fixation count* was not affected by the Pre-treatment ($\chi^2_{(1)}$= 1.820, p=0.177, Stimulus type ($\chi^2_{(1)}$= 0.172, p=0.678), Contingency level ($\chi^2_{(1)}$= 0.606, p=0.436), Order ($\chi^2_{(1)}$= 0.001, p=0.978) and Sex ($\chi^2_{(1)}$= 0.255, p=0.614). However we found a significant interaction between Pre-treatment and Contingency level ($\chi^2_{(1)}$= 4.654, p=0.031; after socially stimulating pre-treatment, children fixated more at the 70% contingent video stimulus than after socially ignoring pre-treatment, Figure III/5.7). All other interactions were non-significant (all p>0.05).

None of the main factors had significant effect on the *total visit duration* (Pre-treatment: $\chi^2_{(1)}$= 0.480, p=0.489; Contingency level: ($\chi^2_{(1)}$= 0.065, p=0.799;Stimulus type $\chi^2_{(1)}$= 1.246, p=0.264; Order: $\chi^2_{(1)}$= 0.236, p=0.627; Sex: $\chi^2_{(1)}$= 0.072, p=0.788), and only one marginally significant interaction was found (Pre-treatment x Contingency level, $\chi^2_{(1)}$= 3.570, p=0.059; children fixated longer the 70% contingent video stimulus after socially stimulating than after socially ignoring pre-treatment). Other interactions were non-significant (p>0.05).

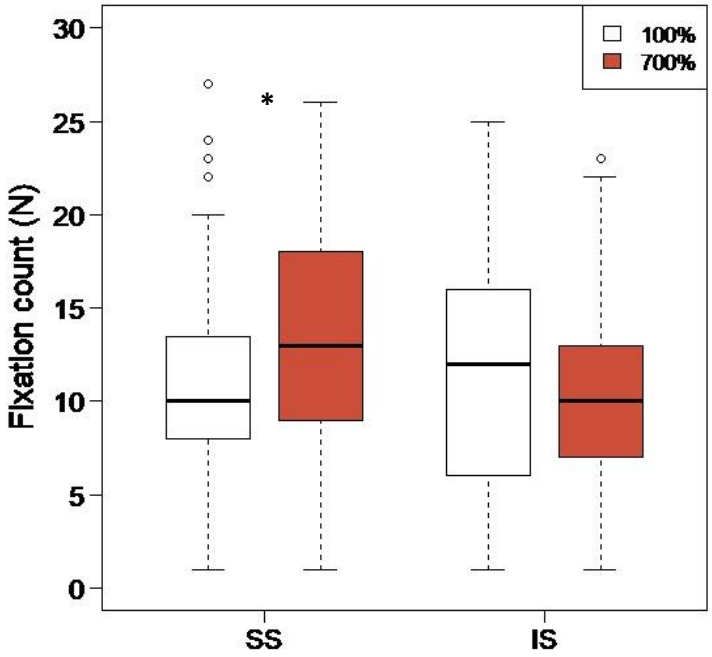

Figure III/5.7. Fixation count (mean +SD) towards the 70% and 100% contingent stimuli after socially stimulating (SS) or ignoring (IS) pre-treatments. * indicates marginally significant difference at $p<0.05$ level

III/5.4. Discussion

In the present study, we have found indication that socially stimulating pre-treatment that supposedly influences the oxytocin system (see Kis et al., 2013) can shape attention to basic aspects of social behaviour. The most importart effect we found in the *Contingent motion animation* test was that after socially stimulating pre-treatment, children fixated more at the 70% contingent video stimulus than after socially ignoring pre-treatment. It seems that priming with social signals, probably via the direct activation of the oxytocin system, strengthens childrens' preferences of high-but-imperfect contingencies. In light of previous results (Rakison and Poulin-Dubois, 2001), this suggests that pre-treatment with social signals induces a strong preference toward stimuli that can be interpreted in socially meaningful ways (in terms of agency). Furthermore, in the biological motion animation test we found significant effect of the pre-treatment on some aspects of looking behaviour in children. Specifically, when viewing point light animation of biological motion and an inverted and scrambled version of the same animation, children looked sooner and more frequently at the scrambled stimuli than at the point-light figure. However, after a socially stimulating pre-treatment, children fixated sooner the head region of the point-light figure than that of the inverted and scrambled stimulus.

Looking duration has been a widely used behavioural measure of preferences and cognitive processes. According to Sokolov's (1963) comparator model, longer looking to a novel stimulus in comparison to a familiar stimulus (i.e., a novelty preference) is indicative of recognition of a fully encoded familiar stimulus. The underlying assumption is that children will continue to look at a stimulus until it is fully encoded, at which point attention will be shifted toward novel information in the surrounding environment. Once this process is complete, the organism will no longer maintain attention toward the stimulus. Thus, we propose that our results showing an overall looking preference toward the scrambled figure reflects visual encoding difficulties. Bardi et al. (2014) suggested that increased visual attention to the inverted displays of biological motion stimuli that are not easily recognizable might arise, not because parts of the stimulus are in unfamiliar relative positions, but because the motion dynamics of dots is unnatural. With the point-light figure, children's predisposition to look for a socially meaningful interpretation of the scene is satisfied, resulting in shorter viewing times. Interestingly, almost the only body part where we found an effect of social pre-treatment was the head region. We propose that this is because through the stimulation of the oxytocin system increased attention emerged toward the most socially relevant parts of the body. When encoding the figure as socially meaningful (point-light figure), attention is probably automatically directed at the region that carries the most relevant social information. The difference in first fixations between conditions may arise because the increased level of oxytocin following the socially stimulating pre-treatment leads to a more efficient encoding of socially meaningful information (point-light figure) and thus, directs attention to the head region more efficiently.

We propose that differences between conditions can be attributed to changes in the level of oxytocin. Although in this study we did not test participants with intranasal administration of oxytocin, a previous study by Kis et al. (2013) has clearly shown that pre-treatment with nasal OXT spray and by social stimulation could result similar changes in evaluation of facial expressions and trustworthiness ratings. Furthermore, previous results showing that sensitivity to biological motion increases following oxytocin administration (Kéri and Benedek, 2009) make it a viable interpretation that the similar effects of pre-treatment in our study were exerted through the oxytocin system.

Although most of the human studies examine only males due to practical reasons, there is evidence that oxytocin has an effect on socio-cognitive behaviour in both genders, however, there might be differential effects (Herzmann et al., 2013). Pierce et al. showed that female

children pay more attention to people, whereas male children tend to pay more attention to geometric patterns (Pierce et al., 2011). Here we found that girls, after socially stimulating pre-treatment, fixated more frequently the head region of the stimuli than boys. This might be attributed to differences in oxytocin receptor affinity in men and women. Steroid hormones, such as estradiol and progesterone have been found to modulate the OXT receptor, in particular, estradiol enhances OXT receptor affinity while progesterone has been shown to decrease receptor binding (Choleris et al., 2008; Gimpl et al., 2002).

In conclusion we propose that priming with social cues relating to the oxytocin system might be a valid approach to study mechanisms underlying basic social behaviour and cognition in children.

III/6. Study 6: The effects of pre-treatment with social stimuli on dogs' tendency to conform to the human partner's behaviour: familiarity matters.

III/6.1. Introduction

Cross-species studies have found that dog-owner affiliative interaction (gazing, petting, talking) increases owner's urinary oxytocin levels (Nagasawa et al., 2015) and results in an increase in plasma OXT levels in both dogs and their owners (Handlin et al., 2012, 2011). Further evidence suggests that the release of OXT can be induced by short-term sensory interactions (touch, stroking, eye-contact, motherese) in humans (Feldman et al., 2010b; Gordon et al., 2010). It has also been shown that positive social interactions could alter the behaviour of humans in the same way as intranasal OXT administration (Kis et al., 2013; Morhenn et al., 2008). Importantly, however, a considerable inter-individual variation has been reported on the social-behavioural effects of OXT (Bartz et al., 2011; Olff et al., 2013) . Therefore it is possible that the release of OXT during social interactions depends on the relationship between partners. In line with this Crockford et al. (2013) measured urinary OXT after 10 min of grooming in wild chimpanzees. They found that OXT levels were higher after grooming with "bond partners" compared with "non-bond partners". But it is worth mentioning, that the phenomenon has not been investigated in dogs yet.

The still unpublished results of A. Hernádi (PhD thesis, in prep.) indicate, that intranasal administration of OXT as compared to placebo treatment could increase dogs' tendency to re-enact the human demonstrator's counterproductive choice in the quantity discrimination task

(for the procedure see Prato-Previde et al., 2008). The increased conformity to the human preference in the OXT pre-treated group suggests a priming effect of this neurohormone on dogs' social susceptibility. It has also been shown (Kanizsár et al. 2012) that this tendency to conform to the partner's behaviour can also be influenced by social stimuli (petting and eye contacts with the human caregiver) as primers to prosocial predispositions. Based on these findings the quantity discrimination task seems appropriate for testing whether or not the quality of social relationship is a key factor for the dogs' susceptibility to human social influence.

Thus in the present study we investigated whether positive social pre-treatment by the owner or a stranger (socially relevant and irrelevant partners) would differentially influence the dogs' tendency to follow the human's counterproductive choice (i.e. the smaller amount of food).

III/6.2. Methods

III/6.2.1. Ethics statement

This research was done in accordance with the Hungarian regulations on animal experimentation and the Guidelines for the use of animals in research described by the Association for the Study Animal Behaviour (ASAB). Ethical approval was obtained from the National Animal Experimentation Ethics Committee (Ref No. XIV-I-001/531-4-2012). The owners volunteered to participate and gave written informed consent.

III/6.2.2 Subjects

N=80 adult (older than 1 year) pet dogs (36 males and 44 females; 42 purebreds and 38 mongrels; mean age ±SD: 4.68±2.58 years) were recruited from the Family Dog Project database that contains over a thousand owners who have volunteered to participate in behavioural experiments with their dogs. 16 dogs were excluded due to side-preference (N=8) or preference of the smaller amount of food during the *Free choice test* (choose bigger amount of food less than 3 out of 6, N=8). The remaining dogs (N = 64) were assigned to one of four treatment groups (socially stimulating pre-treatment with the owner, socially stimulating pre-treatment with the experimenter, socially ignoring pre-treatment and separation from the owner).

Pre-treatment	Sep		SI		SS_s		SS_o	
Sex	male	female	male	female	male	female	male	female
N	6	10	7	9	8	8	3	13

Table III/6.1. Distribution of age and sex in the four pre-treatment group

III/6.2.3 Procedure

The experiment took place in an experimental room at the Department of Ethology, Eötvös Loránd University (3 × 5 m) that was unfamiliar to the dogs. The behaviour of the dog and its owner was videotaped and the choice behaviour of subjects was analysed later. The owner was asked to refrain from feeding his/her dog at least 4 hours prior to the test.

Familiarization

The owner entered with his/her dog into the testing area and the dog was allowed to freely explore the environment for 1 minute. Then the experimenter placed a few pellets of dry dog food on a yellow plastic plate (17 cm in diameter) and offered it to the dog by putting down the plate on the floor and encouraging the dog to take it. This phase served to familiarize the dogs with the experimental situation and to determine whether the dog can be motivated by food pellets. Different types of dry dog foods were used depending on the size of the dog (Happy Dog Adult Medium or Acana Adult Small Breed). If the dog ate the offered food pellets it was followed by a pre-training phase. No dog was excluded in this phase.

Two different quantities of food were used during testing: small, consisting of a single piece of food, and large, consisting of eight pieces of food. These two food quantities were selected based on Ward and Smuts's study (2006) which showed that dogs are capable of making subtle quantity discriminations, for example they can successfully discriminate between 1 and 5 pieces of food.

III/6.2.3.1 Pre-treatment

Every dog participated in only one of the four following pre-treatment conditions:

Socially stimulating pre-treatment with the owner (SS_o):

The owner was sitting on a chair opposite the experimenter (at a distance of 3m) while the dog was allowed to move freely in the room. In the first 5 minutes the owner tried to make eye-contact with the dog as much as she/he could, while stroking the dog and called it by its name. During the second 5 minutes the owner was asked to play with the dog at a low intensity. The

owner hid the toy (a tennis ball) behind his/her back, and as soon as the dog made eye-contact, the owner offered the toy to the dog and petted it. The experimenter did not look at the dog during this phase but verbally instructed with the owner if it was necessary.

Socially stimulating pre-treatment with the stranger (SS_s):

In this condition dogs participated in the very same procedure as above except for that the owner and experimenter switched their role (i.e. the experimenter acted as the owner in the above condition and vice versa). The owner and stranger were matched in terms of gender.

Socially ignoring pre-treatment (SI):

The owner was sitting on a chair opposite the experimenter (at a distance of 3m) while the dog was allowed to move freely in the room. Human participants were talking to each other (the experimenter asked questions about the personality of the dog) but both ignored the dog (never looked at or touched and never talked to it) throughout the 10 minutes session. The dog could play alone with the standard toy (tennis ball).

Separation pre-treatment (Sep): The dog was left alone in the room for 10 minutes (the dogs' preferred toy was left in the room). The owner and the experimenter could monitor the behaviour of the dog from outside.

III/6.2.3.2 Quantity discrimination task

The procedure consisted of three phases: Pre-training, Free choice between the large and small food quantity, and Choice between the large and small food quantity after observing the experimenter expressing a preference for the small quantity. The plates were set 1 m apart equally distant to the dog. All dogs received a total of 18 trials, i.e. six trials in each phase. To avoid the development of a side preference the same food quantity was never placed in the same location more than twice in a row.

Pre-training

The owner was asked to sit down on a chair at a predetermined point and to hold the dog there by its collar facing the middle of the room. Then the experimenter (E) approached the dog with two identical yellow plastic plates (17 cm in diameter), one baited (with 4 pellets on it) and one empty, in her hands, stopped 1.5-2 m away from the dog (depending on its body size) and placed the plates simultaneously 1 m apart equidistant from the dog. Then she stepped back half a meter along the midline in between the plates and remained motionless while avoided looking at the plates or into the dog's eyes. At the moment the dog oriented towards

the area between the two plates, the owner was allowed to release and encourage the dog (‘Go! It is yours!’), without using any other commands or gestures. Subjects were allowed to select only one plate. While the dog was eating the content of the chosen location, the non-chosen plate was removed by the experimenter. We repeated the pre-training trials with alternating the side of the food (N=6 trials), the place of food was counterbalanced in RLRLRL or LRLRLR order (half of the dogs received RLRLRL).

Free choice between large and small food quantities

Pre-treatment was immediately followed by a task in which the dogs were presented with a choice between large (8 pellets) and small (1 pellet) food quantity. During this we followed the procedure outlined in Prato-Previde et al. (2008). The owner was asked to sit down on a chair at the predetermined point (same as in pre-training) and to hold the dog there by its collar facing the middle of the room. The Experimenter entered the room and approached the dog with two identical plates (baited with 1 and 8 food pellets respectively) in her hands so that the dog’s muzzle was approximately 10 cm to the plates. This was done to ensure that the dog had the opportunity to inspect the content of both plates. The subsequent procedure was the same as in the pre-training. *Free choice* phase consisted of six trials during which the left/right position of the plates containing large amount of food was counterbalanced in RLLRRL or LRRLLR order. Only those dogs were included in the *Human influence* trials that choose the larger amount of food (8 pellets) at least three times out of the six trials.

Human influence trials

Free choice trials were immediately followed by six additional Human influence trials in which the setup and the procedure was basically the same as in the *Free choice* except that the dog was allowed to choose only after observing the experimenter expressing a preference for the small food quantity. That is, after having placed the plates containing small and large amount of food on the floor, the Experimenter approached the plate containing only one piece of pet food, picked up the pellet and, with an enthusiastic tone of voice, said: ‘Hmm! Yammi, so good!’. Then she placed it back on the plate, stepped back half a meter along the midline in between the plates and remained motionless while avoided looking at the plates or into the dog’s eyes. Similarly to the *Free choice* trials, the position of the plates containing large food quantities was counterbalanced (RLLRRL or LRRLLR order).

III/6.2.4 Behavioural coding and Statistical analysis

Based on the video recordings the number of times a dog chose the large food quantity was calculated in the *Free choice* trials and *Human influence* trials (i.e., dogs were scored 0 to 6 in both condition). A choice was noted when the dog touched one of the plates with its muzzle/nose.

To assess inter-observer agreement a second person blind to the pre-treatment condition coded a randomly selected sample of 25% of the subjects. Cohen's kappa value was 0.985 showing a high level of reliability for dogs' choice behaviour.

Change-in-bias score: In order to assess dogs' social susceptibility, that is the effect of the experimenter's preference for the smaller quantity on their behaviour, change-in-bias score was calculated by subtracting the score of *Free choice* trials from the score of *Human influence* trials. Larger values for change-in-bias indicate a greater influential effect of the human demonstrator, that is, a decrease in the dogs' preference for the larger amount of food.

We used the Mann–Whitney test for between-subjects' analysis, the Wilcoxon test for within-subjects' analysis.

A Generalized Estimating Equations (GEE) model using restricted maximum likelihood estimation with backward elimination method was also used to test the effects of Pre-treatment (SS or SI), Sex (male or female) and Age as between-subjects factors. All two-way interactions were also investigated. SPSS version 21 software was used for statistical analyses.

III/6.3. Results

Comparison of *Free choice* and *Human influence* trials showed that dogs' preference for the bigger amount of food dropped significantly after socially stimulating pre-treatment by both the owner ('SS_o': $Z=3.333$; $p<0.001$) and the stranger: ('SS_s': $Z=2.694$; $p=0.007$). That is dogs in these pre-treatment groups showed a tendency to switch towards a counterproductive behaviour (i.e. preference for the smaller quantity) in response to the experimenter's demonstration. In contrast, there were no significant differences between *Free choice* and *Human influence* trials in the other two pre-treatment groups ($Z=1.896$; $p=0.058$ for 'SI' and $Z=1.575$; $p=0.115$ for 'Sep' pre-treatment groups).

Generalized Estimating Equation model showed a significant main effect of pre-treatment: dogs in the 'SS_o' group showed higher change-in-bias scores than dogs in the 'SI' and 'Sep' group, which means that there was a greater influential effect of the human demonstrator in

case of dogs in the 'SS-o' group. ($\chi^2_{(1)}$=10.048, p=0.018, see Figure III/6.1). However this difference was not present between the groups of 'SS-o' and 'SS-s'. There were no main effects of sex ($\chi^2_{(1)}$=0.781, p=0.377) and age ($\chi^2_{(1)}$=0.384, p=0.535). However, we found two significant interactions. In case of the 'SS_s' group, younger dogs showed higher change-in bias score than older ones (pretreatment × age; $\chi^2_{(1)}$=10.284, p=0.036). Furthermore, we found a pretreatment × sex interaction ($\chi^2_{(1)}$=18.610, p<0.01), namely in the 'Sep' and 'SS_i' groups, male dogs reached higher change-in bias score than female dogs and female dogs showed higher change-in bias in the 'SS_o' group, than the 'Sep' and 'SS_I' groups. All the other interactions were non-significant (all p>0.05).

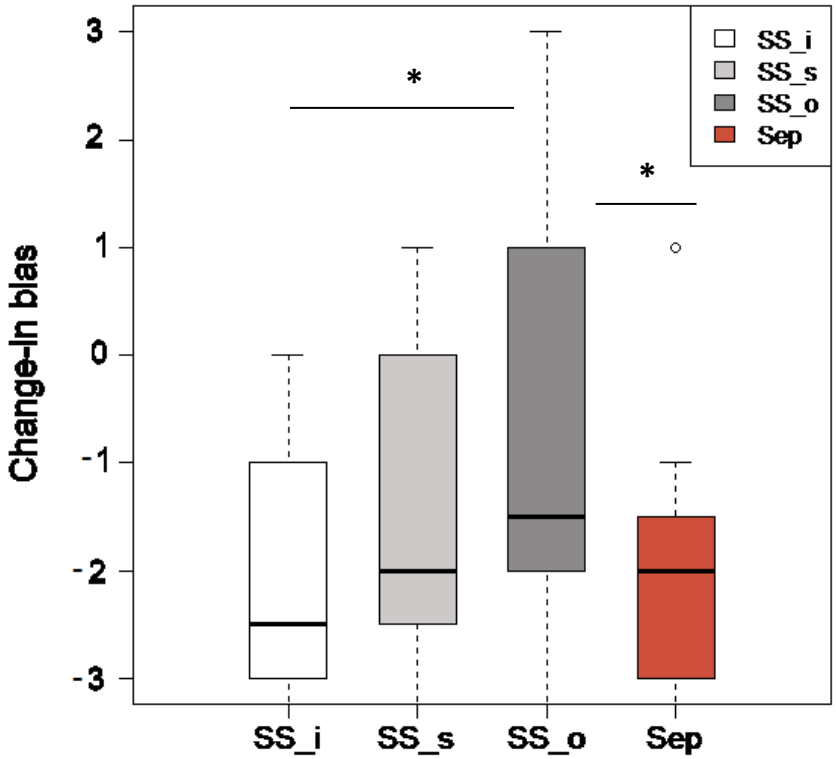

Figure III/6.1. Human influential effect (change-in-bias) in the four pre-treatment groups: Socially stimulating pre-treatment by the owner (SS_o) and by the stranger (SS_s); Socially ignoring pre-treatment (SI) and Separation (Sep).

III/6.4. Discussion

Here we investigated the effect of socially stimulating pre-treatment by the owner and a stranger on dogs' tendency to conform to the experimenter's counterproductive choice, in a food choice task. The results of the present experiment corroborate the notion that the food

choice behaviour of dogs can be strongly influenced by the expression of a human's preference even when the demonstrator is unfamiliar to the dog (see also Marshall-Pescini et al., 2011) and even if dogs are misled by a human towards a less favourable choice. In line with earlier results (Kanizsár et al., 2012) we have found that socially stimulating interaction with the owner and a stranger could similarly modify the influential effect of the experimenter's demonstration on dogs' tendency to select the larger amount of food.

However, in the present study we have also found differences between the two socially stimulating pre-treatment groups. Namely dogs, after social pre-treatment by the human caregiver, showed significantly larger values for Change-in bias scores in comparison with 'nonsocial' pre-treatments (Ignoring and Separation) and this is not true for dogs after social pre-treatment by an unfamiliar human partner. This result raises the possibility that similarly to that of found in chimpanzees (Crockford et al., 2013), positive social interaction with a socially bonded partner (the owner) has a stronger potential to increase oxytocin level in dogs. Moreover, this further supports the idea that social interaction with a human partner can increase the OXT level in dogs (Handlin et al., 2011; Odendaal and Meintjes, 2003), and that elevated oxytocin level not only makes dogs more sensitive to the difference between the owner and unfamiliar human partners (c.f. Hernádi et al., 2015) but the socially bonded partner plays a more important role in oxytocin-mediated changes in dogs' social behaviour.

This finding also highlights potentially important, yet largely neglected methodological issues in the study of dogs' social cognition. Namely, most of the research has to date paid little attention to the potential effects of social stimuli that the subjects receive during the preliminary (warm-up/familiarization) phase of an experiment. Our results clearly show that such stimulation can have a strong influence on dogs' (as well as on human infants') social-cognitive functioning in the test phase and thus social interactions with the owner (and experimenters) prior to any study procedure need to be controlled more strictly and future researches should also control for the quality of the dog-owner relationship.

In summary, our study provides the first direct experimental evidence that dogs can also be primed with social-communicative stimuli and social priming effect works also across contexts/situations (i.e. when the pre-treatment procedure is unrelated to the subsequent task situation). This is in line with human studies indicating that affiliative stimuli can act as primers for prosocial predispositions in humans (Over and Carpenter 2009a; Souza et al., 2012) and thus have important implications for further research on dog-human interactions.

IV. GENERAL DISCUSSION

Oxytocin, that can act as both hormone and neuropeptide, plays an important role in modulating social behaviour (Donaldson and Young, 2008; Goodson and Thompson, 2010; MacDonald and MacDonald, 2010). Although it has been argued (Kéri et al., 2009) that oxytocin in humans has an effect on basic social behaviours, most of the findings support the role of oxytocin in modulating higher level social cognitive functions such as emotion regulation (Rodrigues et al., 2009) or attachment (Donaldson and Young, 2008).

The aim of the present thesis was to investigate the link between the oxytocin system and different aspects of social behaviours in dogs and children. Importantly, we used different approaches to explore the oxytocin-related behavioural effects. In Studies 1 & 2 we explored the associations between genetic factors influencing the function of the oxytocin system (variations in OXTR gene) and social-communicative behaviours in dogs and children. We also studied the effects of intranasal oxytocin administration on different aspects of dogs' behaviour (Study 3 & Study 4) and whether oxytocin-like behavioural effects can be induced by social stimulation ('social priming') in children and dogs (Study 5 & Study 6). Generally speaking, our studies corroborate that oxytocin has modulatory effects on social cognition and behaviour in both dogs and children. Furthermore, the above experiments provide further support for the notion that both intranasal administration and social presesitization may be a promising methodological approach to examine the effect on oxytocin on social behaviour.

In *Study 1* results showed that single nucleotide polymorphisms (-213AG, -94TC and -74GC in case of dogs, rs53576 in children) of OXTR gene are associated with both dogs' (Border collies) and children's reactions to negative social stimuli in socially ambiguous situations. Furthermore, *Study 2* provides the first evidence that genetic variations in Border collies' OXTR gene (-213AG, -74CG and -94TC) are also associated with their attachment behaviour to their owners. It is worth noting that the -213AG polymorphism had earlier been shown to be associated with proximity seeking not only in Border Collies but also in German Shepherds (Kis et al., 2014). Romero et al. (2014) also reported that oxytocin modulates social motivation to approach and affiliate with conspecifics and human partners and this is in line with our finding that -213AG polymorphism is associated with Stranger acceptance. Oxytocin can regulate the activity of the hypothalamic–pituitary–adrenal axis, thereby modifying the stress response (Bello et al., 2008; Neumann, 2002; Windle et al., 1997) and this mechanism

may explain the associations we found in *Study 2* between the OXTR polymorphisms and the Anxiety score.

Previous investigations have found that mothers and fathers who had provided high levels of tactile contact to their infants showed an increase in salivary OXT following parent–infant interactions but such an increase was not observed among parents who provided low tactile contact (Feldman et al., 2010b). This elevation can be observed in the animal kingdom too. Among rhesus monkeys, mothers who provide more grooming and contact have higher levels of plasma OXT (Maestripieri et al., 2009), while infant monkeys reared by their mothers show greater cerebrospinal fluid OXT concentrations as compared to nursery-reared animals and display more social behaviour toward conspecifics (Winslow et al., 2003). In agreement with these observations our results indicate that priming with social cues by the caregiver presensitization can shape some aspects of social behaviour in both children and dogs (*Study 5 & Study 6)* probably via stimulation of the OXT system. A previous study by Kis et al. (2013) suggests that socially stimulating pre-treatment and the administration of oxytocin nasal spray produce similar results. They found that after having received intranasal administration of OXT or pre-treatment with social stimuli (eye contact, touch) adult human participants gave higher emotion and trustworthiness scores for faces with negative emotional expression (as compared to participants who had received placebo or non-social pre-treatment).

Dogs' social susceptibility in the 'counterproductive influence' task (see *Study 6*) seems analogous to a complex human behaviour that has been shown to be influenced by oxytocin (social conformity – Rilling and Sanfey, 2011; Stallen et al., 2012). Importantly, however we should be cautious when drawing a parallel between dogs and humans with respect to social conformity. First, it is unclear whether or not the observed functional analogy between the dogs' social suggestibility and the human tendency to conformity based on similar social-cognitive mechanisms and, second, our results in Study 6 provide no direct evidence that social priming or intranasal oxytocin administration enhanced the dogs' central oxytocin level thereby influencing the dogs' social receptivity.

Many previous studies have suggested that OXT also has a positive effect on attention for social information (Domes et al., 2007a; Donaldson and Young, 2008; Guastella et al., 2008a; Insel, 2010). For example, Fujisawa et al. (2014) investigated the relationship between visual attention for social information and salivary OXT levels in preschool children with autism compared to children with typical development using eye-tracking system. They found a positive association between salivary OXT levels and visual attention for finger pointing in

typically developing pre-schoolers but not in children with autism. This is in line with the widely held notion that patients with autism spectrum disorder have poorer visual attention for social information than typically developing individuals, especially for biological motion (e.g., Jones and Klin, 2013; Klin et al., 2009, 2002a, 2002b; Nakano et al., 2010; Pierce et al., 2011; Sasson and Touchstone, 2014). Based on these findings we may assume that children's increased visual attention toward the biological motion stimulus after socially stimulating pre-treatment (*Study 5)* was due to their elevated OXT level.

Ample evidence suggest that both humans (for a review see Nishida, 2011) and non-human animals (e.g. Vallortigara et al., 2005) show positive attentional bias toward point-light displays representing a biological motion pattern of a conspecific or a heterospecific and we also found in *Study 3* that placebo-treated dogs show spontaneous preference for biological motion pattern. Interestigly, however, we could not observe such preference in dogs' looking behaviour after intrasal oxytocin administration, maybe because OXT enhaced the encoding of biological motion stimulus and thus subjects' attention shifted toward the scrambled stimulus which is more challenging for dogs to interpret.

One way to disentangle the effect of oxytocin on encoding versus preference would be to use active choice methods (e.g. the touch screen technique, Range et al., 2008) where subjects are rewarded for selecting either the biological or the non-biological stimuli. The disadvantage of these active choice tasks, however, is that they require massive training in case of dogs prior to testing (e.g. MacKinnon et al., 2010), and they also have been criticized as learning effects could be problematic, as the stimuli become more familiar from trial to trial. Probably a combination of spontaneous preference tasks and active choice methods could produce valid data for the evaluation of the effect of oxytocin on processing biological motion. However, the combination of these two approaches could also be used in dogs. It seems that based on motion cues alone, people are capable of extracting a diverse amount of properties out of these figures, such as emotion (Dittrich et al., 1996), gender (Schouten et al., 2010) or intention (Manera et al., 2010). In order to test this, paradigms that have already been proven to be suitable for dogs could be adapted using point-light figures – e.g., gender differentiation (Takaoka and Morisaki, 2013).

In *Study 4* results show that after oxytocin administration dogs in general were less likely to look at their owner after a given time elapsed in the Unreachable food situation. The finding that oxytocin treatment does not affect dogs' looking behaviour towards nonsocial targets further supports the idea that the oxytocin system is involved in the regulation of social (but

not non-social) attention in dogs. Moreover, in *Study 4* our preliminary gene × behaviour analysis found that dog −213AG polymorphism interacts with the effect of intranasal oxytocin treatment on social behaviour in two different work breeds, and this highlights that polymorphisms in the OXTR gene might indeed be a biologically relevant underlying factor explaining some variance in IN-OT reactivity, although the analysis will need to be carried out with an increased sample size in order to confirm the findings. It has been recently argued that the field of intranasal oxytocin research would in general benefit from increasing sample sizes and/or conducting replication studies (Walum et al., 2016), and our pioneering results (although using the conventional, relatively low, sample size) suggests that taking into account the specific population in which the study was conducted is also a crucial validity issue.

Canine investigations often tested dogs from different breeds. Unfortunately, however, very little is known about the oxytocin system in the different dog breeds. We have some information about the polymorphic variations in the OXTR gene (Bence et al., 2013; Kis et al., 2014a), but the potential differences in baseline oxytocin levels across breeds – that might contribute to their differential reaction to oxytocin treatment – have not yet been investigated. In *Study 4*, our study populations (Siberian Huskies and Border Collies) have been selected for markedly different purposes (cooperative versus independent work) and they belong to different genetic clusters (Parker et al., 2004). Thus genetic differences between these breeds in the oxytocin system could be (at least partly) responsible for the differential effects of oxytocin treatment on behaviour. This is also consistent with previous reports indicating that the genetic component (polymorphisms in the dog DRD4 gene) is a key factor of looking behaviour towards humans in unsolvable task situations (Hori, 2013).

In accordance with previous studies on breed differences (e.g. Gácsi et al., 2009b) the results of *Study 4* suggest that Border Collies appeared to be more human oriented as indicated by an increased duration of looking at the owner and more gaze-shifts when faced with an unsolvable problem which may reflect the dog's tendency to interact with the owner in order to 'ask for help'. This is also supported by Border Collies' shorter latency to look at the owner in the presence of a potentially dangerous object. Although a potential confound in the investigation of breed effects is that behavioural coding cannot be blind to this factor (e.g. the coder will always see the breed of the given subject), the fact that we found high consistency across two coders is a hint that no such bias was included in the present dataset.

Another important factor that needs to be considered in oxytocin research is the effect of sex. Although most of the human studies examine only males due to practical reasons, there is evidence that oxytocin has an effect on socio-cognitive behaviours in both genders, but there might be differential effects (Herzmann et al., 2013). Sex differences may be rooted in the differences in oxytocin receptor affinity in men and women, because steroid hormones, such as estradiol and progesterone, have been found to modulate the OXT receptor, in particular, estradiol enhances OXT receptor affinity while progesterone has been shown to decrease receptor binding (Choleris et al., 2008; Gimpl et al., 2002). However, sex differences in social behaviours can also be expected because social motivation — which appears to differ between the sexes — may be a driving force behind the development of sex differences in social skills (Christov-Moore et al., 2014). Human studies that investigated the effect of OXT on amygdala reactivity also reported sex differences in oxytocin effectiveness (Domes et al., 2010). Research on other species has also shown that OXT can affect males and females differently. For example, higher binding of oxytocin receptors in the medial prefrontal cortex have been found in female prairie voles (Smeltzer et al., 2006). Furthermore it has been shown also in dogs (Nagasawa et al. 2015) that OXT can have differential effects on males and females. The findings of *Study 3 and 4* show that oxytocin can have disparate impact on the performance of male and female dogs and that these effects can be in interaction with dog breed. These results add to the growing literature that draws attention to the importance of including subjects from both genders when investigating the effects of oxytocin. This line of research might also have some indirect clinical relevance as disorders like depression, autism, and schizophrenia have been connected to oxytocin innervation and show sex differences in humans (de Vries, 2008).

Although the oxytocin system includes genetic components other than the OXTR gene that can potentially cause behavioural differences between breeds, additional (non-genetic) factors should also be considered. For example, experience during ontogeny has been shown to have an enduring effect on behaviour, and the oxytocin system (sensitivity to this neurohormone) might also be modulated through epigenetic effects (Apter-Levy et al., 2013; Feldman et al., 2010b; Kumsta et al., 2013). Indeed, Border collies and Siberian huskies in *Study 4* differed in some aspects of their socialization background (see in Appendix 5). Despite similar living conditions of Border Collies and Huskies at the time of testing, the two breeds differed in some aspects of early (social) environmental factors. Thus future research should look at the environmental background of dogs (including owner-related factors such as gender and

personality) and how these relate to oxytocin-sensitivity. This is especially important from an applied perspective as environmental factors may have the potential to modify oxytocin-related behavioural changes in different dog breeds.

The potential role of epigenetic factors contributing to breed differences is also raised by Passalacqua et al. (2011) who found that hunting and herding breeds (to which Border Collies belong) looked at a person more than dogs from Mastiff-like and ancient breeds (to which Siberian Huskies belong) at the age of 4 month and when adults, but breed group differences were not seen in 2-month-old puppies. Therefore, it is reasonable to assume that markedly different dog breeds (such as Border Collies and Siberian Huskies in *Study 4* or even different breeds in *Study 3 and 6*) develop different social behaviours as a consequence of different experiences during ontogeny as they are kept for different purposes, undergo different trainings, etc. In fact, training experience has been shown to affect dogs' performance in a wide range of tasks including independent problem solving as well as human-directed communicative abilities (Marshall-Pescini et al., 2016). Moreover, dogs' relationship with the human participants (e.g. their owner) is another factor that may have differential effects on different dog breeds (see (Horn et al., 2013) on how dog-human relationship affects problem-solving behaviour).

Oxytocin, similarly to serotonin (Yoshida et al., 2009) and dopamine function (Liu and Wang, 2003), is likely be influenced by multiple factors, including other genetic polymorphisms that vary across ethnic populations (e.g., Chang et al., 1996; Kunugi et al., 1997). These uncontrolled genetic differences (in *Study 2* dogs lived in Austria or in Hungary**)** could potentially mask the function of oxytocin and thus weakened the measurable association between variations in OXTR function and attachment behaviour of dogs. Alternatively or additionally, different components of the dogs' social environment may differ between the two countries. It has been suggested that the behavioural expression of certain genotypes is sensitive to input from the social environment (Way and Taylor, 2010). Kim et al. (2010) suggested that the social environment can alter or even reverse the phenotypic expression of different genotypes. They found that culture-specific norms as a form of social input can also affect phenotypic expression of OXTR.

For dogs' attachment behaviour one of the greatest environmental impacts can be the behaviour of their owners. It is possible that the current results of *Study 2* are an indirect reflection of the owners' genotype, particularly given Bakermans-Kranenburg's and van Ijzendoorn's (2008) finding that OXTR is related to parenting style in humans. As dogs are

unrelated to their human caregivers, the owner's genetic background may have an influence on their parenting style or other relevant behaviour that, in turn, through epigenetic processes, affects the dogs' attachment behaviour or how the effects of the dogs' own OXTR genotype on it.

Previously, Konok et al. (2015) examined in a questionnaire study whether owners' attachment style and personality traits influence the occurrence of separation-relation disorder in the dogs. They found that owners scoring higher on self-reported attachment avoidance are more likely to have dogs with separation-related disorder. The researchers suggested that owners' attachment style influences their caregiving behaviour towards their dogs, and owners with attachment avoidance may show less consistent responsiveness to their dog's needs. Albeit in that study, none of the human personality scales influenced separation-related disorder in dogs, in humans, insecure attachment, behaviour problems and separation anxiety disorders in children are often associated with mothers' neuroticism and anxiety disorder (Kochanska et al., 2004; Biederman et al., 2001; Manassis et al., 1994). Interestingly, in *Study 2* the Neuroticism personality trait affected none of the composite scores, however we have found that the owners' Bond-related avoidance to their partners and Pet-related avoidance influenced all the three composite scores of their dogs' attachment, Openness and Pet-related anxiety affected dogs' Anxiety and Acceptance composite scores, while Extraversion and Conscientiousness personality traits influenced the dogs' Anxiety behaviour. Our finding about the correlation between owners' personality and their dogs' behaviour is especially important from an applied perspective, as environmental factors may have the potential to modify oxytocin-related behavioural changes.

Taken together, our results suggest that we should be cautious in concluding that oxytocin uniformly facilitate social behaviours in dogs. Similar claims have been made in the human literature (Bartz et al., 2011), however many researchers still report their findings as generalizable to a wider population. Thereore further studies are needed to investigate how the aforementioned factors as well as other predisposing factors modulate the effects of oxytocin on dogs' and humans' social sensitivity and researchers seriously take into account when design and conclude a study also in human and non-human investigations. Furthermore, we argue that intranasal administration of OXT and social presensitization might be a valid approach to study mechanisms underlying basic and higher social behaviour and cognition in dogs.

In summary, a promising new line of research on comparative cognition has produced mounting evidence of functional similarities in the social behaviour of dogs and human children. These are usually attributed to the evolutionary convergence of the two species, and suggest that dogs can serve as a model species for the study of human social behaviour. In parallel with these advances in comparative social cognition, developments in neuro-hormonal measurement techniques and genetics have enabled scientists to begin to understand the neurobiological basis of the complexity of human social behaviours. There has been a strong interest focused on the modulatory role of oxytocin and this has led to the recognition that this neuropeptide is specifically involved in the regulation of a wide range of human social skills (e.g. attachment, trust, emotional recognition) and that variations caused by genetic polymorphisms might modulate the function of this complex system. With the combination of these two lines of research, the present dissertation aimed at a better understanding of the neuro-hormonal aspects of social behaviour and cognitive functioning in dogs. Our findings corroborate the notion that studying the relationship between different aspects of social behaviour and the oxytocin system in the dog is a promising new research area which may also have translational relevance for understanding the neuro-hormonal bases of human social cognitive abilities.

V. ACKNOWLEDGEMENTS

First of all, I would like to express my deep gratitude to my supervisor, Dr. József Topál for his endless support, bright advices, patience and professional expertise in the field. I could not have imagined having a better advisor and mentor for my Ph.D study.

I am very grateful to Ákos Pogány and Borbála Turcsán for their huge help in the statistical analysis. I would like to thank all those colleagues (especially to Anna Kis, Kata Oláh, Dóra Koller and Zsófia Virányi) who were co-authors of my publications for contributing to this thesis in a substantial way. I would also like thank all the fellow PhD students and colleagues (especially to Flóra Szánthó), for providing a joyful and inspiring environment throughout these years.

My heartfelt biggest gratitude is entitled to my husband, my parents, my brother and his wife (and also to my grandparents who are taking a big part of me loving animals) for their constant support, encouragement and belief in me in my whole life. Without you I would not write these lines. Thank you for everything.

I would like to thank all the funds and grants (Hungarian Scientific Research Fund (OTKA K100695, K112138, K109337, K100695), the Hungarian Academy of Sciences (MTA-ELTE 01 031), the Vienna Science and Technology Fund (WWTF CS11-026), the Austrian Science Fund (FWF I 1271-B24) and the BIAL Foundation (grant n^{o} 169/16)) for providing the financial support of my studies.

VI. APPENDICES

Appendix 1 ***(Study1)*** Behaviour variables observed in Strange Situation Test

		score
ATTACHMENT		
Owner PRESENT	D is mostly close to O (closest bodypart is within 1m) when does not explore or play	1
	D does not stand at the door (within 1m) for more than a few seconds	1
	during the cube-carrying the D mostly watches or follows O	1
	when O first leaves, D follows O to door (within 1m)	1
	when O leaves the second time, D follows O to door (within 1m)	1
	when O enters, D approaches (within reaching distance) at once and wags tail	1
Owner ABSENT	D plays with S (at least for 2s)	1
	any vocalisation	1
	D stands by or orients at door (at least for 2s - 1, most of the time - 2)	2
	when S enters, D does not great and tries to sneak out the door	1
	D is mostly at the chair of O (within 1m) if not at the door	1
	sum	**12**
ANXIETY		
Owner PRESENT	D stands at door (within 1m; at least 2s - 1, most of the time - 2)	2
	D does not explore or play at least for 2s	1
	D positions himself (hides) under/behind O's chair (relative to door or S) for at least 2s	1
	as soon as O stands up D approaches door within 1m (before O)	1
	D watches or approaches door while O is carrying cubes (for at least 2s)	1

	any vocalisation (if not clearly asking for the ball)	1
Owner ABSENT	any contact seeking behaviour with O before the separation	1
	at 1st separation D vocalises or runs around up and down or scretches door	1
	at 2nd separation D vocalises or runs around up and down or scretches door	1
	D follows S to the door when she leaves (within 1m)	1
	D plays or lies down comfortably (head down) but not at door for at least 2s	1
	sum	**12**
ACCEPTANCE		
Owner PRESENT	D approaches S when she 1st enters (at once, within reaching distance)	1
	D gets in physical contact and wags when S 1st enters	1
At any time	D takes toy to S (not during play)	1
	D seeks physical contact (jumps on, snuggles up to, nudges) during the episodes	1
	D avoids S during play (stands off, avoids her touch)	1
Owner ABSENT	D gets in physical contact and wags when the S enters 2nd time	1
	during cube carrying D mostly watches (1) and also follows (2) S	2
	D plays with S also during separation (at least for 2s - 1, most of the time - 2)	2
	D is close (closest bodypart is within 1m) to S during separation (at least for 2s - 1, most of the time - 2)	2
	sum	**12**

Appendix 2 (Study 2). 44–item Big Five Inventory (OBFI_O)

Disagree strongly Disagree a little Neither agree nor disagree Agree a little Agree strongly

1----------2----------3----------4----------5

I see myself *as someone who …*

___ 1. is talkative
___ 2. tends to find fault with others
___ 3. does a thorough job
___ 4. is depressed, blue
___ 5. is original, comes up with new ideas
___ 6. is reserved
___ 7. is helpful and unselfish with others
___ 8. can be somewhat careless
___ 9. is relaxed, handles stress well
___ 10. is curious about many different things
___ 11. is full of energy
___ 12. starts quarrels with others
___ 13. is a reliable worker
___ 14. can be tense
___ 23. tends to be lazy
___ 24. is emotionally stable, not easily upset
___ 25. is inventive
___ 26. has an assertive personality
___ 27. can be cold and aloof
___ 28. perseveres until the task is finished
___ 29. can be moody
___ 30. values artistic, aesthetic experiences
___ 31. is sometimes shy, inhibited
___ 32. is considerate and kind to almost everyone
___ 33. does things efficiently
___ 34. remains calm in tense situations
___ 35. prefers work that is routine
___ 36. is outgoing, sociable

___ 15. is ingenious, a deep thinker
___ 16. generates a lot of enthusiasm
___ 17. has a forgiving nature
___ 18. tends to be disorganized
___ 19. worries a lot
___ 20. has an active imagination
___ 21. tends to be quiet
___ 22. is generally trusting
___ 37. is sometimes rude to others
___ 38. makes plans and follows through with them
___ 39. gets nervous easily
___ 40. likes to reflect, play with ideas
___ 41. has few artistic interests
___ 42. likes to cooperate with others
___ 43. is easily distracted
___ 44. is sophisticated in art, music, or literature

Big Five Inventory Scoring Key

Extraversion:	1, 6R [6],11, 16, 21R, 26, 31R, 36
Neuroticism:	4, 9R, 14, 19, 24R, 29, 34R, 39
Agreeableness:	2R, 7, 12R, 17, 22, 27R, 32, 37R, 42
Openness:	5, 10, 15, 20, 25, 30, 35R, 40, 41R, 44
Conscientiousness:	3, 8R, 13, 18R, 23R, 28, 33, 38, 43R

[6] Note that "R" denotes reverse-scored items (1=5, 2=4, 3=3, 4=2, 5=1).

Appendix 3 (Study 2). 36-item Experiences in Close Relationship-Revised (ECR-R)

Bond-related anxiety

1. I'm afraid that I will lose my partner's love.
2. I often worry that my partner will not want to stay with me.
3. I often worry that my partner doesn't really love me.
4. I worry that romantic partners won't care about me as much as I care about them.
5. I often wish that my partner's feelings for me were as strong as my feelings for him or her.
6. I worry a lot about my relationships.
7. When my partner is out of sight, I worry that he or she might become interested in someone else.
8. When I show my feelings for romantic partners, I'm afraid they will not feel the same about me.
9. I rarely worry about my partner leaving me.
10. My romantic partner makes me doubt myself.
11. I do not often worry about being abandoned.
12. I find that my partner(s) don't want to get as close as I would like.
13. Sometimes romantic partners change their feelings about me for no apparent reason.
14. My desire to be very close sometimes scares people away.
15. I'm afraid that once a romantic partner gets to know me, he or she won't like who I really am.
16. It makes me mad that I don't get the affection and support I need from my partner.
17. I worry that I won't measure up to other people.
18. My partner only seems to notice me when I'm angry.

Bond-related avoidance

19. I prefer not to show a partner how I feel deep down.
20. I feel comfortable sharing my private thoughts and feelings with my partner.
21. I find it difficult to allow myself to depend on romantic partners.
22. I am very comfortable being close to romantic partners.
23. I don't feel comfortable opening up to romantic partners.
24. I prefer not to be too close to romantic partners.
25. I get uncomfortable when a romantic partner wants to be very close.

26. I find it relatively easy to get close to my partner.

27. It's not difficult for me to get close to my partner.

28. I usually discuss my problems and concerns with my partner.

29. It helps to turn to my romantic partner in times of need.

30. I tell my partner just about everything.

31. I talk things over with my partner.

32. I am nervous when partners get too close to me.

33. I feel comfortable depending on romantic partners.

34. I find it easy to depend on romantic partners.

35. It's easy for me to be affectionate with my partner.

36. My partner really understands me and my needs.

Scoring Information: The first 18 items listed below comprise the attachment-related anxiety scale. Items 19 – 36 comprise the attachment-related avoidance scale. In real research, the order in which these items are presented should be randomized. Each item is rated on a 7-point scale where 1 = strongly disagree and 7 = strongly agree. To obtain a score for attachment-related *anxiety*, please average a person's responses to items 1 – 18. However, because items 9 and 11 are "reverse keyed". To obtain a score for attachment-related *avoidance*, please average a person's responses to items 19 – 36. Items 20, 22, 26, 27, 28, 29, 30, 31, 33, 34, 35, and 36 will need to be reversed-scored before you compute this average.

Appendix 4 (Study 2). Eight-item Pet Avoidance and Anxiety Scales (modified ECR-R).

Pet Avoidance Scale

It's easy for me to be affectionate with my pet.*

I don't feel comfortable opening up to pets.

It helps to turn to my pet in times of need.*

I am nervous when pets get too close to me.

I find it relatively hard to get close to my pets.a

I prefer not to show a pet how I feel deep down.

I usually share my problems and concerns with my pet.b*

I feel comfortable sharing my private thoughts and feelings with my pet.*

Pet Anxiety Scale

I'm afraid that I will lose my pet's love.

I am confident that my pet will want to stay with me.a*

I know that pets care about me as much as I care about them.a*

I know my pet loves me.a*

My pet makes me feel confident.a*

I find that my pets don't want to get as close as I would like.

It makes me mad that I don't get the affection and support I need from my pet.

My desire to be very close sometimes scares pets away.

a Original ECR-R wording was changed to balance number of secure and insecure items.

b To make this item applicable to pets, the original "discuss" was replaced with the word "share."

* Item is reversed-scored.

Appendix 5 (Study 4). Comparison of Border Collies and Siberian Huskies in terms of body weight, demographics, socialization background and keeping conditions

	Body weight (at the time of testing; mean, 95%CI)	**Sex distribution** (number of males & females)	**Age** (in years, mean, 95%CI)	**Number of dogs who live with another dog in the household**	**Family size** (number of people living with the dog; mean, 95%CI)	**Time spent indoors** (hours/day, mean, 95%CI)
Border Collie (N=19)	18.2 (16.3-20.2)	9 & 10	3.5 (2.5-4.5)	10	2.89 (2.05-3.74)	11.8 (8.3-15.4)
Siberian Husky (N=19)	23.3 (21.3-25.2)	8 & 11	4.7 (3.8-5.7)	13	3.05 (2.19-3.91)	13.1 (9.1-17)
Breed comparison	*U=69; p=0.003*	*$\chi^2_{(1)}$=0.106; p=0.744*	*$t_{(36)}$=2.014; p=0.052*	*$\chi^2_{(1)}$=0.441; p=0.507*	*U=188.5; p=0.826*	*$t_{(36)}$=0.481; p=0.663*

	Number of dogs who participated in regular training class (obedience and/or agility and/or herding. etc.)	**Duration that dog and its owner had spent living together** (in weeks, mean, 95%CI)	**Number of dogs purchased by the owner**		**Number of dogs showing separation- or aggression- related behavioural problems**
			<6 months of age	**>1 year of age**	
Border Collie (N=19)	19	184.5 (134-234)	19	0	4 (3 & 1)
Siberian Husky (N=19)	9	153.9 (82-220)	9	10	7 (5 & 2)
Breed comparison	*$\chi^2_{(1)}$=10.99; p<0.001*	*$t_{(36)}$=0.764; p=0.445*	*$\chi^2_{(1)}$=10.99; p<0.001*		*$\chi^2_{(1)}$=0.408; p=0.683*

Appendix 6. (Study 4). GEE analyses of the behaviour of N=8 adult pet dogs (4 Border Collies and 4 Siberian Huskies; 4 males, 4 females, mean age ± SD: 3.0 ± 1.33) recorded in three situations measuring social responsiveness after oxytocin or placebo treatment in a within-subjects design. Significant effects are highlighted in bold. (O: owner, E: experimenter)

	Main factors				Interactions					
	Pre-treatment	Breed	Sex	Repetition	Pre-treatment ×Breed	Pre-treatment ×Sex	Sex ×Breed	Repetition× Pre-treatment	Repetition ×Breed	Repetition ×Sex
Unreachable food										
Latency of looking at O	**$\chi^2_{(1)}$=5.628 p=0.018**	ns.	ns.	**$\chi^2_{(1)}$=45.859 p<0.001**	**$\chi^2_{(1)}$=23.477 p<0.001**	ns.	ns.	**$\chi^2_{(1)}$=4.319 p=0.038**	ns.	**$\chi^2_{(1)}$=13.442 p<0.001**
Latency of looking at E	ns.	**$\chi^2_{(1)}$=8.022 p=0.005**	ns.	ns.	ns.	ns.	ns.	**$\chi^2_{(1)}$=6.934 p=0.008**	**$\chi^2_{(1)}$=5.799 p=0.016**	**$\chi^2_{(1)}$=11.309 p=0.001**
Total duration of looking at O	ns.	ns.	ns.	**$\chi^2_{(1)}$=6.131 p=0.013**	**$\chi^2_{(1)}$=10.840 p=0.001**	ns.	ns.	ns.	ns.	ns.
Total duration of looking at E	ns.	**$\chi^2_{(1)}$=4.265 p=0.039**	ns.	ns.	ns.	ns.	ns.	ns.	ns.	**$\chi^2_{(1)}$=5.696 p=0.017**
Number of gaze shifts between the cage and O	**$\chi^2_{(1)}$=6.643 p=0.010**	**$\chi^2_{(1)}$=4.339 p=0.037**	ns.	**$\chi^2_{(1)}$=7.919 p=0.005**	**$\chi^2_{(1)}$=12.943 p<0.001**	ns.	ns.	ns.	**$\chi^2_{(1)}$=22.394 p<0.001**	**$\chi^2_{(1)}$=7.498 p=0.006**
Number of gaze shifts between the cage and E	**$\chi^2_{(1)}$=30.029 p<0.001**	**$\chi^2_{(1)}$=31.823 p<0.001**	**$\chi^2_{(1)}$=16.192 p<0.001**	**$\chi^2_{(1)}$=4.072 p=0.044**	**$\chi^2_{(1)}$=30.029 p<0.001**	**$\chi^2_{(1)}$=5.057 p=0.025**	ns.	**$\chi^2_{(1)}$=6.362 p=0.012**	**$\chi^2_{(1)}$=4.072 p=0.044**	ns.
Response to 'Potentially dangerous' object										
Latency of looking at O	ns.	**$\chi^2_{(1)}$=36.431 p<0.001**	ns.	ns.	ns.	**$\chi^2_{(1)}$=11.861 p=0.001**	**$\chi^2_{(1)}$=21.589 p<0.001**	**$\chi^2_{(1)}$=5.719 p=0.017**	**$\chi^2_{(1)}$=8.164 p=0.004**	ns.
Latency to	ns.	**$\chi^2_{(1)}$=6.585**	ns.	ns.	ns.	ns.	ns.	ns.	**$\chi^2_{(1)}$=7.572**	**$\chi^2_{(1)}$=8.231**

approach the sound source		**p=0.010**							**p=0.006**	**p=0.004**
Total duration of looking at O	**$\chi^2_{(1)}$=12.455 p<0.001**	**$\chi^2_{(1)}$=149.413 p<0.001**	**$\chi^2_{(1)}$=22.683 p<0.001**	ns.	**$\chi^2_{(1)}$=17.445 p<0.001**	**$\chi^2_{(1)}$=9.596 p=0.002**	ns.	ns.	ns.	ns.
Number of gaze shifts between the speaker and O	ns.	**$\chi^2_{(1)}$=25.648 p<0.001**	**$\chi^2_{(1)}$=25.648 p<0.001**	ns.	**$\chi^2_{(1)}$=16.661 p<0.001**	ns.	ns.	ns.	ns.	ns.
Tolerance of prolonged eye contact										
Duration of eye contact with E	ns.	ns.	ns.	**$\chi^2_{(1)}$=7.257 p=0.007**	ns.	ns.	ns.	ns.	ns.	ns.

Appendix 7 (Study 4). Summary of results from likelihood ratio tests between models with and without the given explanatory variable. All initial statistical models included OXTR genotype and all two-way interactions with sex, experimental group and breed as explanatory variables. Final models were reached by backwards model selection based on AIC values. Non-significant effects ($p > 0.1$) are marked with ns, for significant ($p < 0.05$) and near-significant ($0.05 < p < 0.1$) effects the statistical values are given. Significance of main effects are only considered (otherwise marked by 'NA') if no two-way interaction that included that given term was significant. Effects that were found to be significant without the inclusion of OXTR genotype (see main text) but resulted non-significant or non-applicable (due to significant interactions) in the current models are marked with bold and italics ('***ns.***' or '***NA.***' respectively).

	Sex	**Breed**	**Treatment (OT/PL)**	**Sex × Breed**	**Sex × OT/PL**	**Breed × OT/PL**	**Genotype**	**Sex × Genotype**	**OT/PL × Genotype**	**Breed × Genotype**
Unreachable food										
Latency to first looking at the O	ns.	ns.	χ^2= 6.59, p = 0.01	ns.	ns.	ns.	ns.	ns.	ns.	ns.
Latency to first looking at the E	χ^2 = 7.33, p < 0.01	NA	NA	ns.	ns.	χ^2= 2.99, p = 0.08	χ^2= 3.18, p = 0.07	ns.	ns.	ns.
Proportion of time spent looking at the O	***NA***	χ^2= -2.22, p = 0.04	NA	ns.	ns.	ns.	NA	χ^2= 4.41, p < 0.01	χ^2= -1.96, p = 0.06	ns.
Proportion of time spent looking at the E	NA	ns.	NA	ns.	χ^2= -4.31, p < 0.01	***ns.***	χ^2= -4.87, p < 0.01	ns.	ns.	ns.
Gaze shifts between the cage and the O	ns.	χ^2= 9.71, p < 0.01	ns.	ns.	ns.	ns.	ns.	ns.	ns.	ns.
Gaze shifts between the cage and the E	χ^2= 8.59, p < 0.01	NA	χ^2= 6.51, p = 0.01	ns.	ns.	ns.	NA	ns.	ns.	χ^2= 14.69, p < 0.01
Potentially dangerous object										
Latency to first looking at the O	***ns.***	χ^2= 7.56, p < 0.01	NA	ns.	ns.	ns.	NA	ns.	χ^2= 5.51, p = 0.02	ns.

Latency to approach the sound source	NA	NA	ns.	χ^2= 8.21, p < 0.01	ns.	ns.	ns.	ns.	ns.	ns.
Proportion of time spent looking at the O	***NA***	NA	NA	χ^2= -2.61, p < 0.01	χ^2= -2.13, p = 0.02	***ns.***	NA	χ^2= -1.84, p = 0.03	χ^2= -2.07, p = 0.02	χ^2= -6.58, p < 0.01
Gaze shifts between the speaker and the O	NA	NA	NA	ns.	χ^2= 3.22, p = 0.07	χ^2= 6.22, p = 0.01	ns.	ns.	ns.	ns.
Tolerance of prolonged eye contact										
Duration of first eye contact with the E	***NA***	ns.	ns.	ns.	ns.	ns.	NA.	χ^2= 4.04, p = 0.04	ns.	ns.

VII. REFERENCES

Abdai, J., Baño Terencio, C., Miklósi, Á., 2017a. Novel approach to study the perception of animacy in dogs. PLoS One 12, e0177010. doi:10.1371/journal.pone.0177010

Abdai, J., Ferdinandy, B., Terencio, C.B., Pogány, Á., Miklósi, Á., 2017b. Perception of animacy in dogs and humans. Biol. Lett. 13, 20170156. doi:10.1098/rsbl.2017.0156

Ainsworth, M.D.. D.S., Blehar, M.. C., Waters, E., Wall, S., 1978. Patterns of Attachment: A Psychological Study of the Strange Situation, Journal of the American Academy of Religion. doi:10.1093/jaarel/52.2.411-a

Akiko Takaoka, Ayako Morisaki, K.F., 2013. Cross-modal concept of human gender in dogs (Canis familiaris). Japanese J. Anim. Psychol. 130, 123–130.

Altemus, M., Deuster, P.A., Galliven, E., Carter, C.S., Gold, P.W., 1995. Suppression of hypothalmic-pituitary-adrenal axis responses to stress in lactating women. J. Clin. Endocrinol. Metab. 80, 2954–2959. doi:10.1210/jcem.80.10.7559880

Andari, E., Duhamel, J.-R., Zalla, T., Herbrecht, E., Leboyer, M., Sirigu, A., 2010. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc. Natl. Acad. Sci. U. S. A. 107, 4389–4394. doi:10.1073/pnas.0910249107

Apter-Levy, Y., Feldman, M., Vakart, A., Ebstein, R.P., Feldman, R., 2013. Impact of maternal depression across the first 6 years of life on the child's mental health, social engagement, and empathy: The moderating role of oxytocin. Am. J. Psychiatry 170, 1161–1168. doi:10.1176/appi.ajp.2013.12121597

Arahori, M., Hori, Y., Saito, A., Chijiiwa, H., Takagi, S., Ito, Y., Watanabe, A., Inoue-Murayama, M., Fujita, K., 2016. The oxytocin receptor gene (OXTR) polymorphism in cats (Felis catus) is associated with "Roughness" assessed by owners. J. Vet. Behav. Clin. Appl. Res. 11, 109–112. doi:10.1016/j.jveb.2015.07.039

Bahrick, L.E., Watson, J.S., Areola Norene Huber, M., Kaufman Deborah Kennedy, B., Roscelli, N., Bahrick, E., John Watson, or S., 1985. Detection of intermodal proprioceptive-visual contingency as a potential basis of self-perception in infancy. Dev. Psychobiol. 21, 963–973. doi:10.1037/0012-1649.21.6.963

Bakermans-Kranenburg, M.J., van Ijzendoorn, M.H., 2008. Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. Soc. Cogn. Affect. Neurosci. 3, 128–134. doi:10.1093/scan/nsn004

Bálint, A., Faragó, T., Zemihn, M., Lenkei, R., Miklósi, Á., Pongrácz, P., 2015. "Do not

choose as I do!" - Dogs avoid the food that is indicated by another dog's gaze in a two-object choice task. Appl. Anim. Behav. Sci. 170, 44–53. doi:10.1016/j.applanim.2015.06.005

Bardi, L., Regolin, L., Simion, F., 2014. The First Time Ever I Saw Your Feet : Inversion Effect in Newborns ' Sensitivity to Biological Motion. Dev. Psychol. 50, 986–993. doi:10.1037/a0034678

Barraza, J.A., Zak, P.J., 2009. Empathy toward strangers triggers oxytocin release and subsequent generosity, in: Annals of the New York Academy of Sciences. pp. 182–189. doi:10.1111/j.1749-6632.2009.04504.x

Barry, R.A., Kochanska, G., Philibert, R.A., 2008. G x E interaction in the organization of attachment: mothers' responsiveness as a moderator of children's genotypes. J. Child Psychol. Psychiatry. 49, 1313–1320. doi:10.1111/j.1469-7610.2008.01935.x

Bartz, J.A., Zaki, J., Bolger, N., Hollander, E., Ludwig, N.N., Kolevzon, A., Ochsner, K.N., 2010. Oxytocin Selectively Improves Empathic Accuracy. Psychol. Sci. 21, 1426–1428. doi:10.1177/0956797610383439

Bartz, J.A., Zaki, J., Bolger, N., Ochsner, K.N., 2011. Social effects of oxytocin in humans: Context and person matter. Trends Cogn. Sci. 15, 301–309. doi:10.1016/j.tics.2011.05.002

Baumgartner, T., Heinrichs, M., Vonlanthen, A., Fischbacher, U., Fehr, E., 2008. Oxytocin Shapes the Neural Circuitry of Trust and Trust Adaptation in Humans. Neuron 58, 639–650. doi:10.1016/j.neuron.2008.04.009

Beck, L., Madresh, E.A., 2008. Romantic partners and four-legged friends: An extension of attachment theory to relationships with pets. Anthrozoos 21, 43–56. doi:10.2752/089279308X274056

Beintema, J.A., Georg, K., Lappe, M., 2006. Perception of biological motion from limited-lifetime stimuli. Percept. Psychophys. 68, 613–624. doi:10.3758/BF03208763

Beintema, J.A., Lappe, M., 2002. Perception of biological motion without local image motion. Proc. Natl. Acad. Sci. U. S. A. 99, 5661–5663. doi:10.1073/pnas.082483699

Bello, D., White-Traut, R., Schwertz, D., Pournajafi-Nazarloo, H., Carter, C.S., 2008. An exploratory study of neurohormonal responses of healthy men to massage. J. Altern. Complement. Med. 14, 387–394. doi:10.1089/acm.2007.0660

Bence, M., Elek, Z., Kis, A., Kubinyi, E., Lakatos, G., Miklósi, Á., Pergel, E., Rónai, Z., 2013. Analysis of oxytocin receptor gene as putative component of social behavior in

family dogs. Behav. Genet. 43, 509.

Bence, M., Marx, P., Szantai, E., Kubinyi, E., Ronai, Z., Banlaki, Z., 2017. Lessons from the canine Oxtr gene: Populations, variants and functional aspects. Genes, Brain Behav. 16, 427–438. doi:10.1111/gbb.12356

Benjamini, Y., Yekutieli, D., 2001. THE CONTROL OF THE FALSE DISCOVERY RATE IN MULTIPLE TESTING UNDER DEPENDENCY By Yoav Benjamini 1 and Daniel Yekutieli 2. Ann. Stat. 29, 1165–1188. doi:10.1214/aos/1013699998

Bensky, M.K., Gosling, S.D., Sinn, D.L., 2013. The world from a dog's point of view: A review and synthesis of dog cognition research. Adv. Study Behav. 45, 209–406. doi:10.1016/B978-0-12-407186-5.00005-7

Bertenthal, B.I., Proffitt, D.R., Cutting, J.E., 1984. Infant sensitivity to figural coherence in biomechanical motions. J. Exp. Child Psychol. 37, 213–230. doi:10.1016/0022-0965(84)90001-8

Bertenthal, B.I., Proffitt, D.R., Kramer, S.J., 1987. Perception of biomechanical motions by infants: implementation of various processing constraints. J. Exp. Psychol. Hum. Percept. Perform. 13, 577–585. doi:10.1037/0096-1523.13.4.577

Biederman, J., Faraone, S. V, Hirshfeld-Becker, D.R., Friedman, D., Robin, J. a, Rosenbaum, J.F., 2001. Patterns of psychopathology and dysfunction in high-risk children of parents with panic disorder and major depression. Am. J. Psychiatry 158, 49–57. doi:10.1176/appi.ajp.158.1.49

Bigelow, A.E., 2001. Discovering self through other: Infants' preference for social contingency, in: Bulletin of the Menninger Clinic. pp. 335–346. doi:10.1521/bumc.65.3.335.19852

Blake, R., 1993. Cats perceive biological motion. Psychol. Sci. doi:10.1111/j.1467-9280.1993.tb00557.x

Blake, R., Turner, L.M., Smoski, M.J., Pozdol, S.L., Stone, W.L., 2003. Visual recognition of biological motion is impaired in children with autism. Psychol. Sci. 14, 151–157. doi:10.1111/1467-9280.01434

Born, J., Lange, T., Kern, W., McGregor, G.P., Bickel, U., Fehm, H.L., 2002. Sniffing neuropeptides: a transnasal approach to the human brain. Nat. Neurosci. 5, 514–6. doi:10.1038/nn849

Bosch, O.J., Krömer, S.A., Brunton, P.J., Neumann, I.D., 2004. Release of oxytocin in the hypothalamic paraventricular nucleus, but not central amygdala or lateral septum in

lactating residents and virgin intruders during maternal defence. Neuroscience 124, 439–448. doi:10.1016/j.neuroscience.2003.11.028

Bowlby, J., 1969. Attachment and loss: Volume I: Attachment. Int. Psycho-Analytical Libr. 79, 1–401. doi:10.2307/588279

Bowlby, J., 1958. The nature of the child ' s tie to his mother 1 By. Int. J. Psychoanal. 39, 350–373.

Brennan, K.A., Clark, C.L., Shaver, P.R., 1998. Self-report measurement of adult attachment: An integrative overview, in: Attachment Theory and Close Relationships. pp. 46–76.

Brown, J., Kaplan, G., Rogers, L.J., Vallortigara, G., 2010. Perception of biological motion in common marmosets (Callithrix jacchus): By females only. Anim. Cogn. 13, 555–564. doi:10.1007/s10071-009-0306-0

Buttner, A.P., 2016. Neurobiological underpinnings of dogs' human-like social competence: How interactions between stress response systems and oxytocin mediate dogs' social skills. Neurosci. Biobehav. Rev. doi:10.1016/j.neubiorev.2016.08.029

Campbell, A., 2010. Oxytocin and human social behavior. Pers. Soc. Psychol. Rev. 14, 281–295. doi:10.1177/1088868310363594

Carpenter, M., Nagell, K., Tomasello, M., Butterworth, G., Moore, C., 1998. Social cognition, joint attention, and communicative competence from 9 to 15 months of age. Monogr. Soc. Res. Child Dev. 63, i–vi, 1–143. doi:10.2307/1166214

Carter, C.S., 2014. Oxytocin Pathways and the Evolution of Human Behavior. Annu. Rev. Psychol. 65, 17–39. doi:10.1146/annurev-psych-010213-115110

Carter, C.S., Boone, E.M., Pournajafi-Nazarloo, H., Bales, K.L., 2009. Consequences of early experiences and exposure to oxytocin and vasopressin are sexually dimorphic. Dev. Neurosci. doi:10.1159/000216544

Chang, F.M., Kidd, J.R., Livak, K.J., Pakstis, A.J., Kidd, K.K., 1996. The world-wide distribution of allele frequencies at the human dopamine D4 receptor locus. Hum. Genet. 98, 91–101. doi:10.1007/s004390050166

Chang, W.H., Lee, I.H., Chen, K.C., Chi, M.H., Chiu, N.T., Yao, W.J., Lu, R.B., Yang, Y.K., Chen, P.S., 2014. Oxytocin receptor gene rs53576 polymorphism modulates oxytocin-dopamine interaction and neuroticism traits-A SPECT study. Psychoneuroendocrinology 47, 212–220. doi:10.1016/j.psyneuen.2014.05.020

Chen, F.S., Barth, M.E., Johnson, S.L., Gotlib, I.H., Johnson, S.C., 2011. Oxytocin receptor (OXTR) polymorphisms and attachment in human infants. Front. Psychol. 2.

doi:10.3389/fpsyg.2011.00200

Chen, F.S., Johnson, S.C., 2012. An Oxytocin Receptor Gene Variant Predicts Attachment Anxiety in Females and Autism-Spectrum Traits in Males. Soc. Psychol. Personal. Sci. 3, 93–99. doi:10.1177/1948550611410325

Choleris, E., Devidze, N., Kavaliers, M., Pfaff, D.W., 2008. Steroidal/neuropeptide interactions in hypothalamus and amygdala related to social anxiety. Prog. Brain Res. doi:10.1016/S0079-6123(08)00424-X

Christov-Moore, L., Simpson, E. a., Coudé, G., Grigaityte, K., Iacoboni, M., Ferrari, P.F., 2014. Empathy: Gender effects in brain and behavior. Neurosci. Biobehav. Rev. 46, 604–627. doi:10.1016/j.neubiorev.2014.09.001

Cimarelli, G., Virányi, Z., Turcsán, B., Rónai, Z., Sasvári-Székely, M., Bánlaki, Z., 2017. Social Behavior of Pet Dogs Is Associated with Peripheral OXTR Methylation. Front. Psychol. 8, 549. doi:10.3389/fpsyg.2017.00549

Clutton-Brock, J., 1999. A natural history of domesticated mammals. Cambridge University Press.

Clutton-Brock, J., 1995. Domestication and Evolution. Origins of the dog: Domestication and early history, in: The Domestic Dog: Its Evolution, Behaviour and Interactions with People. pp. 7–50. doi:10.1016/S0140-6736(00)02054-7

Cooper, R.P., Aslin, R.N., 1990. Preference for Infant-directed Speech in the First Month after Birth. Child Dev. 61, 1584–1595. doi:10.1111/j.1467-8624.1990.tb02885.x

Coppinger, R., Coppinger, L., 2001. Dogs: A Startling New Understanding of Canine Origin, Behavior and Evolution. Chicago University Press, Chicago.

Crockford, C., Wittig, R.M., Langergraber, K., Ziegler, T.E., Zuberbühler, K., Deschner, T., 2013. Urinary oxytocin and social bonding in related and unrelated wild chimpanzees Urinary oxytocin and social bonding in related and unrelated wild chimpanzees. Proc. R. Soc. London. Ser. B, Biol. Sci. 280.

Cutting, J.E., Moore, C., Morrison, R., 1988. Masking the motions of human gait. Percept. Psychophys. 44, 339–347. doi:10.3758/BF03210415

Csibra, G., 2010. Recognizing Communicative Intentions in Infancy. Mind Lang. 25, 141–168. doi:10.1111/j.1468-0017.2009.01384.x

Csibra, G., Gergely, G., 2009. Natural pedagogy. Trends Cogn. Sci. 13, 148–153. doi:10.1016/j.tics.2009.01.005

Csibra, G., Gergely, G., 2006. Social learning and social cognition: The case for pedagogy.

Process. Chang. Brain Cogn. Dev. 249–274. doi:10.1.1.103.4994

D'Entremont, B., Hains, S.M.J., Muir, D.W., 1997. A demonstration of gaze following in 3- to 6-month-olds. Infant Behav. Dev. 20, 569–572. doi:10.1016/S0163-6383(97)90048-5

De Dreu, C.K.W., Greer, L.L., Handgraaf, M.J.J., Shalvi, S., Van Kleef, G.A., Baas, M., Ten Velden, F.S., Van Dijk, E., Feith, S.W.W., 2010. The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. Science 328, 1408–1411. doi:10.1126/science.1189047

De Dreu, C.K.W., Greer, L.L., Van Kleef, G.A., Shalvi, S., Handgraaf, M.J.J., 2011. Oxytocin promotes human ethnocentrism. Proc. Natl. Acad. Sci. 108, 1262–1266. doi:10.1073/pnas.1015316108

de Vries, G.J., 2008. Sex differences in vasopressin and oxytocin innervation of the brain. Prog. Brain Res. doi:10.1016/S0079-6123(08)00402-0

Di Giorgio, E., Lunghi, M., Simion, F., Vallortigara, G., 2017. Visual cues of motion that trigger animacy perception at birth: the case of self-propulsion. Dev. Sci. 20. doi:10.1111/desc.12394

Diamond, L.M., 2001. Contributions of Psychophysiology to Research on Adult Attachment: Review and Recommendations. Personal. Soc. Psychol. Rev. doi:10.1207/S15327957PSPR0504_1

Dittrich, W.H., Troscianko, T., Lea, S.E.G., Morgan, D., 1996. Perception of emotion from dynamic point-light displays represented in dance. Perception 25, 727–738. doi:10.1068/p250727

Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., Heinrichs, M., 2009. Intranasal Oxytocin Increases Positive Communication and Reduces Cortisol Levels During Couple Conflict. Biol. Psychiatry 65, 728–731. doi:10.1016/j.biopsych.2008.10.011

Domes, G., Heinrichs, M., Gläscher, J., Büchel, C., Braus, D.F., Herpertz, S.C., 2007a. Oxytocin Attenuates Amygdala Responses to Emotional Faces Regardless of Valence. Biol. Psychiatry 62, 1187–1190. doi:10.1016/j.biopsych.2007.03.025

Domes, G., Heinrichs, M., Michel, A., Berger, C., Herpertz, S.C., 2007b. Oxytocin Improves "Mind-Reading" in Humans. Biol. Psychiatry 61, 731–733. doi:10.1016/j.biopsych.2006.07.015

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., Herpertz, S.C., 2010. Effects of intranasal oxytocin on emotional face processing in women.

Psychoneuroendocrinology 35, 83–93. doi:10.1016/j.psyneuen.2009.06.016

Domes, G., Sibold, M., Schulze, L., Lischke, A., Herpertz, S.C., Heinrichs, M., 2012. Intranasal oxytocin increases covert attention to positive social cues. Psychol. Med. 1–7. doi:S0033291712002565 [pii]\n10.1017/S0033291712002565

Donaldson, Z.R., Young, L.J., 2008. Oxytocin, vasopressin, and the neurogenetics of sociality. Science 322, 900–904. doi:10.1126/science.1158668

Duranton, C., Range, F., Virányi, Z., 2017. Do pet dogs (Canis familiaris) follow ostensive and non-ostensive human gaze to distant space and to objects? R. Soc. Open Sci. 4, 170349. doi:10.1098/rsos.170349

Faragó, T., Pongrácz, P., Miklósi, À., Huber, L., Virányi, Z., Range, F., 2010. Dogs' expectation about signalers' body size by virtue of their growls. PLoS One 5. doi:10.1371/journal.pone.0015175

Farroni, T., Csibra, G., Simion, F., Johnson, M.H., 2002. Eye contact detection in humans from birth. Proc. Natl. Acad. Sci. 99, 9602–9605. doi:10.1073/pnas.152159999

Farroni, T., Massaccesi, S., Pividori, D., Johnson, M.H., 2004. Gaze following in newborns. Infancy 5, 39–60. doi:10.1207/s15327078in0501_2

Fearon, P., Shmueli-Goetz, Y., Viding, E., Fonagy, P., Plomin, R., 2014. Genetic and environmental influences on adolescent attachment. J. Child Psychol. Psychiatry Allied Discip. 55, 1033–1041. doi:10.1111/jcpp.12171

Feldman, R., Gordon, I., Schneiderman, I., Weisman, O., Zagoory-Sharon, O., 2010a. Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent-infant contact. Psychoneuroendocrinology 35, 1133–1141. doi:10.1016/j.psyneuen.2010.01.013

Feldman, R., Gordon, I., Zagoory-Sharon, O., 2010b. The cross-generation transmission of oxytocin in humans. Horm. Behav. 58, 669–676. doi:10.1016/j.yhbeh.2010.06.005

Fonagy, P., 2001. The human genome and the representational world: The role of early mother-infant interaction in creating an interpersonal interpretive mechanism, in: Bulletin of the Menninger Clinic. pp. 427–448. doi:10.1521/bumc.65.3.427.19844

Fox, R., McDaniel, C., 1982. The perception of biological motion by human infants. Science (80-.). doi:10.1126/science.7123249

Fraley, R.C., Spieker, S.J., 2003. Are infant attachment patterns continuously or categorically distributed? A taxometric analysis of strange situation behavior. Dev. Psychol. 39, 387–404. doi:10.1037/0012-1649.39.3.387

Fraley, R.C., Waller, N.G., Brennan, K.A., 2000. An item response theory analysis of self-report measures of adult attachment. J. Pers. Soc. Psychol. 78, 350–365. doi:10.1037/0022-3514.78.2.350

Fujisawa, T.X., Tanaka, S., Saito, D.N., Kosaka, H., Tomoda, A., 2014. Visual attention for social information and salivary oxytocin levels in preschool children with autism spectrum disorders: An eye-tracking study. Front. Neurosci. 8. doi:10.3389/fnins.2014.00295

Futó, J., Téglás, E., Csibra, G., Gergely, G., 2010. Communicative function demonstration induces kind-based artifact representation in preverbal infants. Cognition 117, 1–8. doi:10.1016/j.cognition.2010.06.003

Gácsi, M., Kara, E., Belényi, B., Topál, J., Miklósi, Á., 2009a. The effect of development and individual differences in pointing comprehension of dogs. Anim. Cogn. 12, 471–479. doi:10.1007/s10071-008-0208-6

Gácsi, M., McGreevy, P., Kara, E., Miklósi, Á., 2009b. Effects of selection for cooperation and attention in dogs. Behav. Brain Funct. 5, 31. doi:10.1186/1744-9081-5-31

Gácsi, M., Topál, J., Miklósi, a, Dóka, a, Csányi, V., 2001. Attachment behavior of adult dogs (Canis familiaris) living at rescue centers: forming new bonds. J. Comp. Psychol. 115, 423–431. doi:10.1037/0735-7036.115.4.423

Gaunet, F., 2008. How do guide dogs of blind owners and pet dogs of sighted owners (Canis familiaris) ask their owners for food? Anim. Cogn. 11, 475–483. doi:10.1007/s10071-008-0138-3

Gaunet, F., Deputte, B.L., 2011. Functionally referential and intentional communication in the domestic dog: Effects of spatial and social contexts. Anim. Cogn. 14, 849–860. doi:10.1007/s10071-011-0418-1

Gergely, A., Abdai, J., Petró, E., Kosztolányi, A., Topál, J., Miklósi, Ádám, 2015. Dogs rapidly develop socially competent behaviour while interacting with a contingently responding self-propelled object. Anim. Behav. 108, 137–144. doi:10.1016/j.anbehav.2015.07.024

Gergely, A., Petró, E., Topál, J., Miklósi, Á., 2013. What Are You or Who Are You? The Emergence of Social Interaction between Dog and an Unidentified Moving Object (UMO). PLoS One 8. doi:10.1371/journal.pone.0072727

Gergely, G., Watson, J.S., 1999. Early Socio-Emotional Development: Contingency Perception and the Social-Biofeedback Model, in: Early Social Cognition:

Understanding Others in the First Months of Life. pp. 101–136. doi:10.1111/j.1365-2214.1992.tb00355.x

Giese, M.A., Poggio, T., 2003. Neural mechanisms for the recognition of biological movements. Nat. Rev. Neurosci. 4, 179–192. doi:10.1038/nrn1057

Gillath, O., Bunge, S.A., Shaver, P.R., Wendelken, C., Mikulincer, M., 2005. Attachment-style differences in the ability to suppress negative thoughts: Exploring the neural correlates. Neuroimage 28, 835–847. doi:10.1016/j.neuroimage.2005.06.048

Gillath, O., Shaver, P.R., Baek, J.-M., Chun, D.S., 2008. Genetic correlates of adult attachment style. Personal. Soc. Psychol. Bull. 34, 1396–1405. doi:10.1177/0146167208321484

Gimpl, G., Fahrenholz, F., 2001. The oxytocin receptor system: structure, function, and regulation. Physiol. Rev. 81, 629–683.

Gimpl, G., Wiegand, V., Burger, K., Fahrenholz, F., 2002. Cholesterol and steroid hormones: Modulators of oxytocin receptor function, in: Progress in Brain Research. pp. 43–55. doi:10.1016/S0079-6123(02)39006-X

Goodson, J.L., Thompson, R.R., 2010. Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. Curr. Opin. Neurobiol. doi:10.1016/j.conb.2010.08.020

Gordon, I., Zagoory-Sharon, O., Leckman, J.F., Feldman, R., 2010. Oxytocin, cortisol, and triadic family interactions. Physiol. Behav. 101, 679–684. doi:10.1016/j.physbeh.2010.08.008

Gosling, S.D., Kwan, V.S.Y., John, O.P., 2003. A dog's got personality: a cross-species comparative approach to personality judgments in dogs and humans. J. Pers. Soc. Psychol. 85, 1161–1169. doi:10.1037/0022-3514.85.6.1161

Gredebäck, G., Theuring, C., Hauf, P., Kenward, B., 2008. The Microstructure of Infants' Gaze as They View Adult Shifts in Overt Attention. Infancy 13, 533–543. doi:10.1080/15250000802329529

Grippo, A.J., Trahanas, D.M., Zimmerman, R.R., Porges, S.W., Carter, C.S., 2009. Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. Psychoneuroendocrinology 34, 1542–1553. doi:10.1016/j.psyneuen.2009.05.017

Grossmann, T., Johnson, M.H., Lloyd-Fox, S., Blasi, A., Deligianni, F., Elwell, C., Csibra, G., 2008. Early cortical specialization for face-to-face communication in human infants.

Proc. Biol. Sci. 275, 2803–2811. doi:10.1098/rspb.2008.0986

Guastella, A.J., Mitchell, P.B., Dadds, M.R., 2008a. Oxytocin Increases Gaze to the Eye Region of Human Faces. Biol. Psychiatry 63, 3–5. doi:10.1016/j.biopsych.2007.06.026

Guastella, A.J., Mitchell, P.B., Dadds, M.R., 2008b. Oxytocin increases gaze to the eye region of human faces. Biol. Psychiatry 63, 3–5.

Guastella, A.J., Carson, D.S., Dadds, M.R., Mitchell, P.B., Cox, R.E., 2009. Does oxytocin influence the early detection of angry and happy faces? Psychoneuroendocrinology 34, 220–225. doi:10.1016/j.psyneuen.2008.09.001

Guastella, A.J., Graustella, A.J., MacLeod, C., 2012. A critical review of the influence of oxytocin nasal spray on social cognition in humans: evidence and future directions. Horm. Behav. 61, 410–8. doi:10.1016/j.yhbeh.2012.01.002

Gutkowska, J., Jankowski, M., 2012. Oxytocin Revisited: Its Role in Cardiovascular Regulation. J. Neuroendocrinol. doi:10.1111/j.1365-2826.2011.02235.x

Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdebäck, M., Jansson, A., Uvnäs-Moberg, K., 2011. Short-term interaction between dogs and their owners: Effects on oxytocin, cortisol, insulin and heart rate-an exploratory study. Anthrozoos 24, 301–315. doi:10.2752/175303711X13045914865385

Handlin, L., Nilsson, A., Ejdebäck, M., Hydbring-Sandberg, E., Uvnäs-Moberg, K., 2012. Associations between the psychological characteristics of the human-dog relationship and oxytocin and cortisol levels. Anthrozoos 25, 215–228. doi:10.2752/175303712X13316289505468

Haram, M., Tesli, M., Dieset, I., Steen, N.E., Røssberg, J.I., Djurovic, S., Andreassen, O.A., Melle, I., 2014. An attempt to identify single nucleotide polymorphisms contributing to possible relationships between personality traits and oxytocin-related genes. Neuropsychobiology 69, 25–30. doi:10.1159/000356965

Hare, B., Brown, M., Williamson, C., Tomasello, M., 2002. The domestication of social cognition in dogs. Science 298, 1634–1636. doi:10.1126/science.1072702

Hare, B., Tomasello, M., 2005a. Human-like social skills in dogs? Trends Cogn. Sci. doi:10.1016/j.tics.2005.07.003

Hare, B., Tomasello, M., 2005b. Human-like social skills in dogs? Trends Cogn. Sci. 9, 439–444. doi:10.1016/j.tics.2005.07.003

Hernádi, A., Kis, A., Kanizsár, O., Tóth, K., Miklósi, B., Topál, J., 2015. Intranasally administered oxytocin affects how dogs (Canis familiaris) react to the threatening

approach of their owner and an unfamiliar experimenter. Behav. Processes 119, 1–5. doi:10.1016/j.beproc.2015.07.001

Hernádi, A., Kis, A., Turcsán, B., Topál, J., 2012. Man's underground best friend: Domestic ferrets, unlike the wild forms, show evidence of dog-like social-cognitive skills. PLoS One 7. doi:10.1371/journal.pone.0043267

Herzmann, G., Bird, C.W., Freeman, M., Curran, T., 2013. Effects of oxytocin on behavioral and ERP measures of recognition memory for own-race and other-race faces in women and men. Psychoneuroendocrinology 38, 2140–2151. doi:10.1016/j.psyneuen.2013.04.002

Hollander, E., Bartz, J.A., Chaplin, W., Phillips, A., Sumner, J., Soorya, L., Anagnostou, E., Wasserman, S., 2007. Oxytocin increases retention of social cognition in autism. Biol. Psychiatry 61, 498–503.

Hori, Y., 2013. Dopamine receptor D4 gene (DRD4) is associated with gazing toward humans in domestic dogs (Canis familiaris). Open J. Anim. Sci. 03, 54–58. doi:10.4236/ojas.2013.31008

Horn, L., Huber, L., Range, F., 2013. The Importance of the Secure Base Effect for Domestic Dogs - Evidence from a Manipulative Problem-Solving Task. PLoS One 8, e65296. doi:10.1371/journal.pone.0065296

Huber, D., Veinante, P., Stoop, R., 2005. Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. Science 308, 245–8. doi:10.1126/science.1105636

Hurlemann, R., Patin, A., Onur, O.A., Cohen, M.X., Baumgartner, T., Metzler, S., Dziobek, I., Gallinat, J., Wagner, M., Maier, W., Kendrick, K.M., 2010. Oxytocin Enhances Amygdala-Dependent, Socially Reinforced Learning and Emotional Empathy in Humans. J. Neurosci. 30, 4999–5007. doi:10.1523/JNEUROSCI.5538-09.2010

Insel, T.R., 2010. The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior. Neuron. doi:10.1016/j.neuron.2010.03.005

Israel, S., Lerer, E., Shalev, I., Uzefovsky, F., Riebold, M., Laiba, E., Bachner-Melman, R., Maril, A., Bornstein, G., Knafo, A., Ebstein, R.P., 2009. The oxytocin receptor (OXTR) contributes to prosocial fund allocations in the Dictator Game and the social value orientations task. PLoS One 4. doi:10.1371/journal.pone.0005535

Jakovcevic, A., Elgier, A.M., Mustaca, A.E., Bentosela, M., 2010. Breed differences in dogs' (Canis familiaris) gaze to the human face. Behav. Processes 84, 602–607.

doi:10.1016/j.beproc.2010.04.003

Jakovcevic, A., Mustaca, A., Bentosela, M., 2012. Do more sociable dogs gaze longer to the human face than less sociable ones? Behav. Processes 90, 217–222. doi:10.1016/j.beproc.2012.01.010

Johansson, G., 1976. Spatio-temporal differentiation and integration in visual motion perception. Psychol. Res. doi:10.1007/BF00309043

Johansson, G., 1973. Visual perception of biological motion and a model for its analysis. Percept. Psychophys. doi:10.3758/BF03212378

John, O.P., Srivastava, S., 1999. The Big Five trait taxonomy: History, measurement, and theoretical perspectives. Handb. Personal. Theory Res. 2, 102–138. doi:citeulike-article-id:3488537

Johnson, S.C., Slaughter, V., Carey, S., 1998. Whose gaze will infants follow? The elicitation of gaze-following in 12-month-olds. Dev. Sci. 1, 233–238. doi:10.1111/1467-7687.00036

Johnston, J.M., Amico, J.A., 1986. A prospective longitudinal study of the release of oxytocin and prolactin in response to infant suckling in long term lactation. J. Clin. Endocrinol. Metab. 62, 653–657. doi:10.1210/jcem-62-4-653

Jones, W., Klin, A., 2013. Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism. Nature 504, 427–431. doi:10.1038/nature12715

Kaminski, J., Schulz, L., Tomasello, M., 2012. How dogs know when communication is intended for them. Dev. Sci. 15, 222–232. doi:10.1111/j.1467-7687.2011.01120.x

Kemp, A.H., Guastella, A.J., 2011. The Role of Oxytocin in Human Affect: A Novel Hypothesis. Curr. Dir. Psychol. Sci. 20, 222–231. doi:10.1177/0963721411417547

Kéri, S., Benedek, G., 2009. Oxytocin enhances the perception of biological motion in humans. Cogn. Affect. Behav. Neurosci. 9, 237–241. doi:10.3758/CABN.9.3.237

Kim, H.S., Sherman, D.K., Sasaki, J.Y., Xu, J., Chu, T.Q., Ryu, C., Suh, E.M., Graham, K., Taylor, S.E., 2010. Culture, distress, and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. Proc. Natl. Acad. Sci. U. S. A. 107, 15717–15721. doi:10.1073/pnas.1010830107

Kirsch, P., 2015. Oxytocin in the socioemotional brain: Implications for psychiatric disorders. Dialogues Clin. Neurosci. 17, 463–476. doi:10.1093/acprof:oso/9780195130157.003.0006

Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., Gruppe, H., Mattay, V.S.,

Gallhofer, B., Meyer-Lindenberg, A., 2005. Oxytocin modulates neural circuitry for social cognition and fear in humans. J. Neurosci. 25, 11489–11493. doi:10.1523/JNEUROSCI.3984-05.2005

Kis, A., Bence, M., Lakatos, G., Pergel, E., Turcsán, B., Pluijmakers, J., Vas, J., Elek, Z., Brúder, I., Földi, L., Sasvári-Székely, M., Miklósi, A., Rónai, Z., Kubinyi, E., 2014a. Oxytocin receptor gene polymorphisms are associated with human directed social behavior in dogs (Canis familiaris). PLoS One 9, e83993. doi:10.1371/journal.pone.0083993

Kis, A., Ciobica, A., Topál, J., 2017. The effect of oxytocin on human-directed social behaviour in dogs (Canis familiaris). Horm. Behav. doi:10.1016/j.yhbeh.2017.06.001

Kis, A., Hernádi, A., Kanizsár, O., Gácsi, M., Topál, J., 2015. Oxytocin induces positive expectations about ambivalent stimuli (cognitive bias) in dogs. Horm. Behav. 69, 1–7. doi:10.1016/j.yhbeh.2014.12.004

Kis, A., Kanizsár, O., Gácsi, M., Topál, J., 2014b. Intranasally administered oxytocin decreases heart rate and increases heart rate variability in dogs. J. Vet. Behav. Clin. Appl. Res. 9, e15. doi:10.1016/j.jveb.2014.09.050

Kis, A., Kemerle, K., Hernádi, A., Topál, J., 2013. Oxytocin and social pretreatment have similar effects on processing of negative emotional faces in healthy adult males. Front. Psychol. 4, 1–9. doi:10.3389/fpsyg.2013.00532

Klin, A., Jones, W., Schultz, R., Volkmar, F., Cohen, D., 2002a. Defining and quantifying the social phenotype in autism. Am. J. Psychiatry. doi:10.1176/appi.ajp.159.6.895

Klin, A., Jones, W., Schultz, R., Volkmar, F., Cohen, D., 2002b. Visual Fixation Patterns During Viewing of Naturalistic Social Situations as Predictors of Social Competence in Individuals With Autism. Arch. Gen. Psychiatry 59, 809. doi:10.1001/archpsyc.59.9.809

Klin, A., Lin, D.J., Gorrindo, P., Ramsay, G., Jones, W., 2009. Two-year-olds with autism orient to non-social contingencies rather than biological motion. Nature 459, 257–261. doi:10.1038/nature07868

Kochanska, G., Friesenborg, A.E., Lange, L. a, Martel, M.M., Kochanska, G., 2004. Parents' personality and infants' temperament as contributors to their emerging relationship. J. Pers. Soc. Psychol. 86, 744–759. doi:10.1037/0022-3514.86.5.744

Konok, V., Kosztolányi, A., Rainer, W., Mutschler, B., Halsband, U., Miklósi, Á., 2015. Influence of owners' attachment style and personality on their dogs' (Canis familiaris) separation-related disorder. PLoS One 10. doi:10.1371/journal.pone.0118375

Kosfeld, M., Heinrichs, M., Zak, P.J., Fischbacher, U., Fehr, E., 2005. Oxytocin increases trust in humans. Nature 435, 673–676. doi:10.1038/nature03701

Kovács, K., Kis, A., Kanizsár, O., Hernádi, A., Gácsi, M., Topál, J., 2016a. The effect of oxytocin on biological motion perception in dogs (Canis familiaris). Anim. Cogn. doi:10.1007/s10071-015-0951-4

Kovács, K., Kis, A., Pogány, Á., Koller, D., Topál, J., 2016b. Differential effects of oxytocin on social sensitivity in two distinct breeds of dogs (Canis familiaris). Psychoneuroendocrinology 74, 212–220.

Kumsta, R., Hummel, E., Chen, F.S., Heinrichs, M., 2013. Epigenetic regulation of the oxytocin receptor gene: Implications for behavioral neuroscience. Front. Neurosci. 7, 1–6. doi:10.3389/fnins.2013.00083

Kunugi, H., Hattori, M., Kato, T., Tatsumi, M., Sakai, T., Sasaki, T., Hirose, T., Nanko, S., 1997. Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. Mol. Psychiatry 2, 457–462. doi:10.1038/sj.mp.4000334

Lakatos, G., Soproni, K., Dóka, A., Miklósi, Á., 2009. A comparative approach to dogs' (Canis familiaris) and human infants' comprehension of various forms of pointing gestures. Anim. Cogn. 12, 621–631. doi:10.1007/s10071-009-0221-4

Lakatos, K., Toth, I., Nemoda, Z., Ney, K., Sasvari-Szekely, M., Gervai, J., 2000. Dopamine D4 receptor (DRD4) gene polymorphism is associated with attachment disorganization in infants. Mol. Psychiatry 5, 633–637. doi:10.1038/sj.mp.4000773

Lee, H.J., Macbeth, A.H., Pagani, J.H., Scott Young, W., 2009. Oxytocin: The great facilitator of life. Prog. Neurobiol. doi:10.1016/j.pneurobio.2009.04.001

Li, J., Zhao, Y., Li, R., Broster, L.S., Zhou, C., Yang, S., 2015. Association of oxytocin receptor gene (OXTR) rs53576 polymorphism with sociality: A meta-analysis. PLoS One 10. doi:10.1371/journal.pone.0131820

Linnen, A.-M., Ellenbogen, M.A., Cardoso, C., Joober, R., 2012. Intranasal oxytocin and salivary cortisol concentrations during social rejection in university students. Stress 15, 393–402. doi:10.3109/10253890.2011.631154

Liu, Y., Wang, Z.., 2003. Nucleus accumbens oxytocin and dopamine interact to regulate pair bond formation in female prairie voles. Neuroscience 121, 537–544.

Lucas, a, Drewett, R.B., Mitchell, M.D., 1980. Breast-feeding and plasma oxytocin concentrations. Br. Med. J. 281, 834–5. doi:10.1136/bmj.281.6244.834

Lucht, M.J., Barnow, S., Sonnenfeld, C., Rosenberger, A., Grabe, H.J., Schroeder, W., Völzke, H., Freyberger, H.J., Herrmann, F.H., Kroemer, H., Rosskopf, D., 2009. Associations between the oxytocin receptor gene (OXTR) and affect, loneliness and intelligence in normal subjects. Prog. Neuro-Psychopharmacology Biol. Psychiatry 33, 860–866. doi:10.1016/j.pnpbp.2009.04.004

MacDonald, K., MacDonald, T.M., 2010. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv. Rev. Psychiatry 18, 1–21. doi:10.3109/10673220903523615

MacKinnon, L.M., Troje, N.F., Dringenberg, H.C., 2010. Do rats (Rattus norvegicus) perceive biological motion? Exp. Brain Res. 205, 571–576. doi:10.1007/s00221-010-2378-0

Maestripieri, D., Hoffman, C.L., Anderson, G.M., Carter, C.S., Higley, J.D., 2009. Mother-infant interactions in free-ranging rhesus macaques: Relationships between physiological and behavioral variables. Physiol. Behav. 96, 613–619. doi:10.1016/j.physbeh.2008.12.016

Magyar, J. and Gergely, G., 1998. Perception of self-generated contingencies in normal and autistic children., in: Infant Behavior and Development. p. 547.

MANASSIS, K., BRADLEY, S., GOLDBERG, S., HOOD, J., SWINSON, R.P., 1994. Attachment in Mothers with Anxiety Disorders and Their Children. J. Am. Acad. Child Adolesc. Psychiatry 33, 1106–1113. doi:10.1097/00004583-199410000-00006

Manera, V., Schouten, B., Becchio, C., Bara, B.G., Verfaillie, K., 2010. Inferring intentions from biological motion: a stimulus set of point-light communicative interactions. Behav. Res. Methods 42, 168–178. doi:10.3758/BRM.42.1.168

Mariti, C., Carlone, B., Ricci, E., Sighieri, C., Gazzano, A., 2014. Intraspecific attachment in adult domestic dogs (Canis familiaris): Preliminary results. Appl. Anim. Behav. Sci. 152, 64–72. doi:10.1016/j.applanim.2013.12.002

Maros, K., Doka, A., Miklósi, Á., 2008. Behavioural correlation of heart rate changes in family dogs. Appl. Anim. Behav. Sci. 109, 329–341. doi:10.1016/j.applanim.2007.03.005

Marsh, A.A., Yu, H.H., Pine, D.S., Blair, R.J.R., 2010. Oxytocin improves specific recognition of positive facial expressions. Psychopharmacology (Berl). 209, 225–232. doi:10.1007/s00213-010-1780-4

Marshall-Pescini, S., Frazzi, C., Valsecchi, P., 2016. The effect of training and breed group on

problem-solving behaviours in dogs. Anim. Cogn. doi:10.1007/s10071-016-0960-y

Marshall-Pescini, S., Prato-Previde, E., Valsecchi, P., 2011. Are dogs (Canis familiaris) misled more by their owners than by strangers in a food choice task? Anim. Cogn. 14, 137–142. doi:10.1007/s10071-010-0340-y

McNeilly, A.S., Robinson, I.C., Houston, M.J., Howie, P.W., 1983. Release of oxytocin and prolactin in response to suckling. Br. Med. J. (Clin. Res. Ed). 286, 257–9. doi:10.1136/bmj.286.6361.257

Merola, I., Prato-Previde, E., Marshall-Pescini, S., 2012. Dogs' Social Referencing towards Owners and Strangers. PLoS One 7. doi:10.1371/journal.pone.0047653

Meyer-Lindenberg, A., 2008. Impact of prosocial neuropeptides on human brain function. Prog. Brain Res. doi:10.1016/S0079-6123(08)00436-6

Miklósi, Á., Polgárdi, R.-, Topál, J., Csányi, V., 2000. Intentional behaviour in dog-human communication: An experimental analysis of "showing" behaviour in the dog. Anim. Cogn. 3, 159–166. doi:10.1007/s100710000072

Miklósi, A., Pongrácz, P., Lakatos, G., Topál, J., Csányi, V., 2005. A comparative study of the use of visual communicative signals in interactions between dogs (Canis familiaris) and humans and cats (Felis catus) and humans. J. Comp. Psychol. 119, 179–86. doi:10.1037/0735-7036.119.2.179

Miklósi, Á., Topál, J., 2013. What does it take to become "best friends"? Evolutionary changes in canine social competence. Trends Cogn. Sci. 17, 287–294. doi:10.1016/j.tics.2013.04.005

Miklósi, Á., Topál, J., 2012. The Evolution of Canine Cognition, in: The Oxford Handbook of Comparative Evolutionary Psychology. doi:10.1093/oxfordhb/9780199738182.013.0011

Mikolajczak, M., Gross, J.J., Lane, A., Corneille, O., de Timary, P., Luminet, O., 2010. Oxytocin makes people trusting, not gullible. Psychol. Sci. a J. Am. Psychol. Soc. / APS 21, 1072–1074. doi:10.1177/0956797610377343

Mikulincer Phillip R.Pereg, Dana, M., 2003. Attachment Theory and Affect Regulation: The Dynamics, Development, and Cognitive Consequences of Attachment-Related Strategies. Motiv. Emot. 27, 77–102.

Miller, S.C., Kennedy, C., DeVoe, D., Hickey, M., Nelson, T., Kogan, L., 2009. An examination of changes in oxytocin levels in men and women before and after interaction with a bonded dog. Anthrozoos. doi:10.2752/175303708X390455

Morell, V., 2009. Going to the dogs. Science (80-.). 325, 1062–1065.

Morhenn, V.B., Park, J.W., Piper, E., Zak, P.J., 2008. Monetary sacrifice among strangers is mediated by endogenous oxytocin release after physical contact. Evol. Hum. Behav. 29, 375–383. doi:10.1016/j.evolhumbehav.2008.04.004

Movellan, J.R.J.R., Watson, J.S.J.S., 2002. The development of gaze following as a Bayesian systems identification problem. Proc. 2nd Int. Conf. Dev. Learn. ICDL 2002 34–40. doi:10.1109/DEVLRN.2002.1011728

Naderi, S., Miklósi, a, Dóka, a, Csányi, V., 2002. Does dog-human attachment affect their inter-specific cooperation? Acta Biol. Hung. 53, 537–550. doi:10.1556/ABiol.53.2002.4.13

Nagasawa, M., Kikusui, T., Onaka, T., Ohta, M., 2009. Dog's gaze at its owner increases owner's urinary oxytocin during social interaction. Horm. Behav. 55, 434–441. doi:10.1016/j.yhbeh.2008.12.002

Nagasawa, M., Mitsui, S., En, S., Ohatani, N., Ohta, M., Sakuma, Y., Onaka, T., Mogi, K. & Kikusui, T., 2015. Oxytocin-gaze positive loop and the coevolution of human-dog bonds. Science (80-.). 348, 333–336.

Nakano, T., Tanaka, K., Endo, Y., Yamane, Y., Yamamoto, T., Nakano, Y., Ohta, H., Kato, N., Kitazawa, S., 2010. Atypical gaze patterns in children and adults with autism spectrum disorders dissociated from developmental changes in gaze behaviour. Proc. R. Soc. B Biol. Sci. 277, 2935–2943. doi:10.1098/rspb.2010.0587

Nakayasu, T., Watanabe, E., 2013. Biological motion stimuli are attractive to medaka fish. Anim. Cogn. 1–17. doi:10.1007/s10071-013-0687-y

Neumann, I.D., 2002. Involvement of the brain oxytocin system in stress coping: Interactions with the hypothalamo-pituitary-adrenal axis, in: Progress in Brain Research. pp. 147–162. doi:10.1016/S0079-6123(02)39014-9

Neumann, I.D., Landgraf, R., 2012. Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. Trends Neurosci. doi:10.1016/j.tins.2012.08.004

Nishida, S., 2011. Advancement of motion psychophysics: Review 2001-2010. J. Vis. doi:10.1167/11.5.11

Nishioka, T., Anselmo-Franci, J.A., Li, P., Callahan, M.F., Morris, M., 1998. Stress increases oxytocin release within the hypothalamic paraventricular nucleus. Brain Res. 781, 57–61. doi:10.1016/S0006-8993(97)01159-1

Odendaal, J.S.J., Meintjes, R. a., 2003. Neurophysiological correlates of affiliative behaviour

between humans and dogs. Vet. J. 165, 296–301. doi:10.1016/S1090-0233(02)00237-X

Olff, M., Frijling, J.L., Kubzansky, L.D., Bradley, B., Ellenbogen, M.A., Cardoso, C., Bartz, J.A., Yee, J.R., van Zuiden, M., 2013. The role of oxytocin in social bonding, stress regulation and mental health: An update on the moderating effects of context and interindividual differences. Psychoneuroendocrinology 38, 1883–1894. doi:10.1016/j.psyneuen.2013.06.019

Oliva, J.L., Rault, J.-L., Appleton, B., Lill, a., 2015. Oxytocin enhances the appropriate use of human social cues by the domestic dog (Canis familiaris) in an object choice task. Anim. Cogn. doi:10.1007/s10071-015-0843-7

Oliva, J.L., Wong, Y.T., Rault, J.-L., Appleton, B., Lill, A., 2016. The oxytocin receptor gene, an integral piece of the evolution of Canis familaris from Canis lupus. Pet Behav. Sci. 2, 1–15. doi:10.21071/pbs.v0i2.4000

Onishi, K.H., 2005. Do 15-Month-Old Infants Understand False Beliefs? Science (80-.). 308, 255–258. doi:10.1126/science.1107621

Parker, H.G., Kim, L. V, Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E. a, Kruglyak, L., 2004. Genetic structure of the purebred domestic dog. Science 304, 1160–1164. doi:10.1126/science.1097406

Parron, C., Deruelle, C., Fagot, J., 2007. Processing of biological motion point-light displays by baboons (Papio papio). J. Exp. Psychol. Anim. Behav. Process. 33, 381–391. doi:10.1037/0097-7403.33.4.381

Passalacqua, C., Marshall-Pescini, S., Barnard, S., Valsecchi, P., Prato-Previde, E., 2011. Breed and age group differences in human-directed gazing behaviour. Anim. Behav. 1043–1050.

Pavlova M, Krägeloh-Mann I, Sokolov A, Birbaumer N, 2000. Simultaneous masking of a point-light walker in children. Fechner Day 2000 Ed. C Bonnet (strasbg. ISP) pp 279–284.

Pavlova, M., Krägeloh-Mann, I., Sokolov, A., Birbaumer, N., 2001. Recognition of point-light biological motion displays by young children. Perception 30, 925–933. doi:10.1068/p3157

Pelphrey, K.A., Morris, J.P., Michelich, C.R., Allison, T., McCarthy, G., 2005. Functional anatomy of biological motion perception in posterior temporal cortex: An fMRI study of eye, mouth and hand movements. Cereb. Cortex 15, 1866–1876. doi:10.1093/cercor/bhi064

Persson, M.E., Trottier, A.J., Bélteky, J., Roth, L.S. V, Jensen, P., 2017. Intranasal oxytocin and a polymorphism in the oxytocin receptor gene are associated with human-directed social behavior in golden retriever dogs. Horm. Behav. 95, 85–93. doi:10.1016/j.yhbeh.2017.07.016

Petró, E., Abdai, J., Gergely, A., Topál, J., Miklósi, Á., 2016. Dogs (Canis familiaris) adjust their social behaviour to the differential role of inanimate interactive agents. Anim. Cogn. 19, 367–374. doi:10.1007/s10071-015-0939-0

Pierce, K., Conant, D., Hazin, R., Stoner, R., Desmond, J., 2011. Preference for geometric patterns early in life as a risk factor for autism. Arch. Gen. Psychiatry 68, 101–109. doi:10.1001/archgenpsychiatry.2010.113

Poulin-Dubois, D., Lepage, A., Ferland, D., 1996. Infants' concept of animacy. Cogn. Dev. 11, 19–36. doi:10.1016/S0885-2014(96)90026-X

Prato-Previde, E., Custance, D., Spiezio, C., Sabatini, F., 2003. Is the dog-human relationship an attachment bond? An observational study using Ainsworth's Strange Situation. Behaviour 140, 225–254. doi:10.1163/156853903321671514

Prato-Previde, E., Custance, D., Spiezio, C., Sabatini, F., 2003. Is the dog–human relationship an attachment bond? An observational study using Ainsworth's strange situation. Behaviour 140, 225–254.

Prato-Previde, E., Marshall-Pescini, S., Valsecchi, P., 2008. Is your choice my choice? the owners' effect on pet dogs' (Canis lupus familiaris) performance in a food choice task. Anim. Cogn. 11, 167–174. doi:10.1007/s10071-007-0102-7

Quintana, D.S., Kemp, A.H., Alvares, G.A., Guastella, A.J., 2013. A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness. Front. Neurosci. doi:10.3389/fnins.2013.00048

Rakison, D.H., Poulin-Dubois, D., 2001. Developmental origin of the animate-inanimate distinction. Psychol. Bull. 127, 209–228. doi:10.1037/0033-2909.127.2.209

Range, F., Aust, U., Steurer, M., Huber, L., 2008. Visual categorization of natural stimuli by domestic dogs. Anim. Cogn. 11, 339–347. doi:10.1007/s10071-007-0123-2

Richards, D.D., Siegler, R.S., 1986. Children's understandings of the attributes of life. J. Exp. Child Psychol. 42, 1–22. doi:10.1016/0022-0965(86)90013-5

Rilling, J.K., Sanfey, A.G., 2011. The Neuroscience of Social Decision Making. Annu. Rev. Psychol. 62, 23–48. doi:10.1146/annurev.psych.121208.131647

Rimmele, U., Hediger, K., Heinrichs, M., Klaver, P., 2009. Oxytocin makes a face in memory

familiar. J. Neurosci. 29, 38–42. doi:10.1523/JNEUROSCI.4260-08.2009

Rodrigues, S.M., Saslow, L.R., Garcia, N., John, O.P., Keltner, D., 2009. Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. Proc. Natl. Acad. Sci. U. S. A. 106, 21437–21441. doi:10.1073/pnas.0909579106

Romero, T., Nagasawa, M., Mogi, K., Hasegawa, T., Kikusui, T., 2014. Oxytocin promotes social bonding in dogs. Proc. Natl. Acad. Sci. U. S. A. 111, 9085–90. doi:10.1073/pnas.1322868111

Saphire-Bernstein, S., Way, B.M., Kim, H.S., Sherman, D.K., Taylor, S.E., 2011. Oxytocin receptor gene (OXTR) is related to psychological resources. Proc. Natl. Acad. Sci. U. S. A. 108, 15118–22. doi:10.1073/pnas.1113137108

Sasson, N.J., Touchstone, E.W., 2014. Visual attention to competing social and object images by preschool children with autism spectrum disorder. J. Autism Dev. Disord. 44, 584–592. doi:10.1007/s10803-013-1910-z

Savaskan, E., Ehrhardt, R., Schulz, A., Walter, M., Schächinger, H., 2008. Post-learning intranasal oxytocin modulates human memory for facial identity. Psychoneuroendocrinology 33, 368–374. doi:10.1016/j.psyneuen.2007.12.004

Scandurra, A., Alterisio, A., D'Aniello, B., 2016. Behavioural effects of training on water rescue dogs in the Strange Situation Test. Appl. Anim. Behav. Sci. 174, 121–127. doi:10.1016/j.applanim.2015.10.007

Schouten, B., Troje, N.F., Brooks, A., van der Zwan, R., Verfaillie, K., 2010. The facing bias in biological motion perception: Effects of stimulus gender and observer sex. Atten. Percept. Psychophys. 72, 1256–1260. doi:10.3758/APP.72.5.1256

Schöberl, I., Kortekaas, K., Schöberl, F.F., Kotrschal, K., 2014. Algorithm-supported visual error correction (AVEC) of heart rate measurements in dogs, Canis lupus familiaris. Behav. Res. Methods. doi:10.3758/s13428-014-0546-z

Schulze, L., Lischke, A., Greif, J., Herpertz, S.C., Heinrichs, M., Domes, G., 2011. Oxytocin increases recognition of masked emotional faces. Psychoneuroendocrinology 36, 1378–1382. doi:10.1016/j.psyneuen.2011.03.011

Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. The University of Chicago Press, Chicago.

Senju, A., Csibra, G., 2008. Gaze Following in Human Infants Depends on Communicative Signals. Curr. Biol. 18, 668–671. doi:10.1016/j.cub.2008.03.059

Senju, A., Csibra, G., Johnson, M.H., 2008. Understanding the referential nature of looking:

Infants' preference for object-directed gaze. Cognition 108, 303–319. doi:10.1016/j.cognition.2008.02.009

Shahrokh, D.K., Zhang, T.Y., Diorio, J., Gratton, A., Meaney, M.J., 2010. Oxytocin-dopamine interactions mediate variations in maternal behavior in the rat. Endocrinology 151, 2276–2286. doi:10.1210/en.2009-1271

Sharp, K.C., Candy-Gibbs, S., Barlow-Elliott, L. and Petrun, C.J., 1985. No TitleChildren's judgment and reasoning about aliveness: Effects of object, age, and cultural/social background., in: Merrill-Palmer Quarterly (1982-). pp. pp.47–65.

Shaver, P.R., Clark, C.L., 1994. The psychodynamics of adult romantic attachment, in: Empirical Perspectives on Object Relations Theory. pp. 105–156. doi:10.1037/11100-004

Simion, F., Regolin, L., Bulf, H., 2008. A predisposition for biological motion in the newborn baby. Proc. Natl. Acad. Sci. U. S. A. 105, 809–813. doi:10.1073/pnas.0707021105

Skuse, D.H., Gallagher, L., 2009. Dopaminergic-neuropeptide interactions in the social brain. Trends Cogn. Sci. doi:10.1016/j.tics.2008.09.007

Smeltzer, M.D., Curtis, J.T., Aragona, B.J., Wang, Z., 2006. Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. Neurosci. Lett. 394, 146–151. doi:10.1016/j.neulet.2005.10.019

Smith, A.S., Wang, Z., 2014. Hypothalamic oxytocin mediates social buffering of the stress response. Biol. Psychiatry 76, 281–288. doi:10.1016/j.biopsych.2013.09.017

Sokolov, E.N., 1963. Perception and the Conditioned Reflex. Oxford: Pergamon Press.

Soproni, K., Miklósi, a, Topál, J., Csányi, V., 2001. Comprehension of human communicative signs in pet dogs (Canis familiaris). J. Comp. Psychol. 115, 122–126. doi:10.1037/0735-7036.115.2.122

Spangler, G., 2011. Genetic and environmental determinants of attachment disorganization., in: Disorganized Attachment and Caregiving. pp. 110–130.

Sperber, D., Wilson, D., 1986. Relevance: Communication and Cognition. Blackwell.

Stallen, M., De Dreu, C.K.W., Shalvi, S., Smidts, a., Sanfey, a. G., 2012. The Herding Hormone: Oxytocin Stimulates In-Group Conformity. Psychol. Sci. 23, 1288–1292. doi:10.1177/0956797612446026

Strathearn, L., Fonagy, P., Amico, J., Montague, P.R., 2009. Adult Attachment Predicts Maternal Brain and Oxytocin Response to Infant Cues. Neuropsychopharmacology 34, 2655–2666. doi:10.1038/npp.2009.103

Sümegi, Z., Kis, A., Miklósi, Á., Topál, J., 2014. Why do adult dogs (Canis familiaris) commit the A-not-B search error? J. Comp. Psychol. 128, 21–30. doi:10.1037/a0033084

Svartberg, K., 2006. Breed-typical behaviour in dogs - Historical remnants or recent constructs? Appl. Anim. Behav. Sci. 96, 293–313. doi:10.1016/j.applanim.2005.06.014

Tauzin, T., Csík, A., Kis, A., Topál, J., 2015. What or where? The meaning of referential human pointing for dogs (Canis familiaris). J. Comp. Psychol. 129, 334–338. doi:10.1037/a0039462

Tauzin, T., Csík, A., Lovas, K., Gergely, G., Topál, J., 2017. The attribution of navigational- and goal-directed agency in dogs (Canis familiaris) and human toddlers (Homo sapiens). J. Comp. Psychol. 131, 1–9. doi:10.1037/com0000053

Tauzin, T., Kovacs, K., Topál, J., 2016. Dogs Identify Agents in Third-Party Interactions on the Basis of the Observed Degree of Contingency. Psychol. Sci. 27, 0956797616647518. doi:10.1177/0956797616647518

Thielke, L.E., Udell, M.A.R., 2015. The role of oxytocin in relationships between dogs and humans and potential applications for the treatment of separation anxiety in dogs. Biol. Rev. 92, 378–388. doi:10.1111/brv.12235

Thompson, R.J., Parker, K.J., Hallmayer, J.F., Waugh, C.E., Gotlib, I.H., 2011. Oxytocin receptor gene polymorphism (rs2254298) interacts with familial risk for psychopathology to predict symptoms of depression and anxiety in adolescent girls. Psychoneuroendocrinology 36, 144–147. doi:10.1016/j.psyneuen.2010.07.003

Tomonaga, M., 2001. Visual search for biological motion patterns in chimpanzees (Pan troglodytes). Psychol. An Int. J. Psychol. Orient 44, 46–59.

Topál, J., Gácsi, M., 2012. Lessons we should learn from our relationship with dogs: an ethological approach. Crossing boundaries Investig. human-animal relationships 161–188.

Topál, J., Gergely, G., Erdohegyi, A., Csibra, G., Miklósi, A., 2009. Differential sensitivity to human communication in dogs, wolves, and human infants. Science 325, 1269–1272. doi:10.1126/science.1176960

Topal, J., Gergely, G., Miklosi, A., Erdohegyi, A., Csibra, G., 2008. Infants' Perseverative Search Errors Are Induced by Pragmatic Misinterpretation. Science (80-.). 321, 1831–1834. doi:10.1126/science.1161437

Topál, J., Kis, A., Oláh, K., 2014. Dogs' Sensitivity to Human Ostensive Cues: A Unique Adaptation? A Unique Adaptation?, in: The Social Dog: Behavior and Cognition. pp.

319–346. doi:10.1016/B978-0-12-407818-5.00011-5

Topál, J., Miklósi, Á., Csányi, V., Dóka, A., Miklósi, a, Csányi, V., Dóka, A., 1998. Attachment behavior in dogs (Canis familiaris): a new application of Ainsworth's (1969) Strange Situation Test. J. Comp. Psychol. 112, 219–229. doi:10.1037/0735-7036.112.3.219

Tost, H., Kolachana, B., Hakimi, S., Lemaitre, H., Verchinski, B.A., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2010. A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. Proc. Natl. Acad. Sci. U. S. A. 107, 13936–13941. doi:1003296107 [pii]\r10.1073/pnas.1003296107

Tremoulet, P.D., Feldman, J., 2000. Perception of animacy from the motion of a single object. Perception 29, 943–951. doi:10.1068/p3101

Troje, N.F., 2008. Retrieving information from human movement patterns, in: Understanding Events: How Humans See, Represent, and Act on Events. pp. 308–334. doi:10.1093/acprof:oso/9780195188370.003.0014

Troje, N.F., Westhoff, C., 2006. The Inversion Effect in Biological Motion Perception: Evidence for a "Life Detector"? Curr. Biol. 16, 821–824. doi:10.1016/j.cub.2006.03.022

Tronick, E., Als, H., Adamson, L., Wise, S., Brazelton, T.B., 1978. The Infant's Response to Entrapment between Contradictory Messages in Face-to-Face Interaction. J. Am. Acad. Child Psychiatry 17, 1–13. doi:10.1016/S0002-7138(09)62273-1

Turcsán, B., Range, F., Virányi, Z., Miklósi, Á., Kubinyi, E., 2012. Birds of a feather flock together? Perceived personality matching in owner–dog dyads. Appl. Anim. Behav. Sci. 140, 154–160. doi:10.1016/j.applanim.2012.06.004

Udell, M.A.R., Dorey, N.R., Wynne, C.D.L., 2010. What did domestication do to dogs? A new account of dogs' sensitivity to human actions. Biol. Rev. 85, 327–345. doi:10.1111/j.1469-185X.2009.00104.x

Udell, M.A.R., Dorey, N.R., Wynne, C.D.L., 2008. Wolves outperform dogs in following human social cues. Anim. Behav. 76, 1767–1773. doi:10.1016/j.anbehav.2008.07.028

Uvnas-Moberg K, Bruzelius G, Alster P, Lundeberg T, 1993. The antinociceptive effect of non-noxious sensory stimulation is mediated partly through oxytocinergic mechanisms. Acta Physiol. Scand. 149, 199–204. doi:10.1111/j.1748-1716.1993.tb09612.x

Vallortigara, G., Regolin, L., Marconato, F., 2005. Visually inexperienced chicks exhibit spontaneous preference for biological motion patterns. PLoS Biol. 3, 1312–1316.

doi:10.1371/journal.pbio.0030208

Vas, J., Topál, J., Gácsi, M., Miklósi, Á., Csányi, V., 2005. A friend or an enemy? Dogs' reaction to an unfamiliar person showing behavioural cues of threat and friendliness at different times. Appl. Anim. Behav. Sci. 94, 99–115. doi:10.1016/j.applanim.2005.02.001

Walum, H., Waldman, I.D., Young, L.J., 2016. Statistical and Methodological Considerations for the Interpretation of Intranasal Oxytocin Studies. Biol. Psychiatry. doi:10.1016/j.biopsych.2015.06.016

Wan, M., Hejjas, K., Ronai, Z., Elek, Z., Sasvari-Szekely, M., Champagne, F.A., Miklõsi, Á., Kubinyi, E., 2013. DRD4 and TH gene polymorphisms are associated with activity, impulsivity and inattention in Siberian Husky dogs. Anim. Genet. 44, 717–727. doi:10.1111/age.12058

Wang, J., Qin, W., Liu, B., Zhou, Y., Wang, D., Zhang, Y., Jiang, T., Yu, C., 2014. Neural mechanisms of oxytocin receptor gene mediating anxiety-related temperament. Brain Struct. Funct. 219, 1543–1554. doi:10.1007/s00429-013-0584-9

Watanabe, S., Troje, N.F., 2006. Towards a "virtual pigeon": A new technique for investigating avian social perception. Anim. Cogn. 9, 271–279. doi:10.1007/s10071-006-0048-1

Watson, J., 1994. Detection of self: The perfect algorithm., in: Self-Awareness in Animals and Humans: Developmental Perspectives. pp. 131–148.

Watson, J., 1972. Smiling, cooing and "the game." Merrill. Palmer. Q. Behav. Dev. 18, 323–339. doi:10.2307/23084026

Way, B.M., Taylor, S.E., 2010. Social influences on health: is serotonin a critical mediator? Psychosom. Med. 72, 107–12. doi:10.1097/PSY.0b013e3181ce6a7d

Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology 138, 2829–2834. doi:10.1210/en.138.7.2829

Winslow, J.T., Noble, P.L., Lyons, C.K., Sterk, S.M., Insel, T.R., 2003. Rearing effects on cerebrospinal fluid oxytocin concentration and social buffering in rhesus monkeys. Neuropsychopharmacology 28, 910–918. doi:10.1038/sj.npp.1300128

Wobber, V., Hare, B., Koler-Matznick, J., Wrangham, R., Tomasello, M., 2009. Breed differences in domestic dogs' (<I>Canis familiaris</I>) comprehension of human communicative signals. Interact. Stud. 10, 206–224. doi:10.1075/is.10.2.06wob

Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., Nishimori, K., 2009. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. J. Neurosci. 29, 2259–71. doi:10.1523/JNEUROSCI.5593-08.2009

Zink, C.F., Meyer-Lindenberg, A., 2012. Human neuroimaging of oxytocin and vasopressin in social cognition. Horm. Behav. doi:10.1016/j.yhbeh.2012.01.016

VIII. SUMMARY

The present dissertation aimed at better understanding of the neuro-hormonal aspects of social sensitivity and cognitive functioning in dogs and children in focus with the oxytocin system.

In Part I we investigated whether allelic variations of pet dogs' and children's OXTR gene modulate their social behaviour. *Study 1* provides evidence, for the first time, that both genetic variation in the OXTR gene and various aspects of dogs' environmental background (like country of origin, owner OXTR gene, owner personality and attachment) are associated with their attachment to their human caregivers. Furthermore *In Study 2* we focused the potential differential mechanisms behind dogs' and children's responsiveness to human gaze. We found that OXTR genotype was associated with reactions to an aversive social interaction both in dogs and children. However in dogs, the genotypes linked to a higher willingness to follow gaze whereas in children OXTR gene polymorphisms did not affect gaze following behaviour.

In Part II we investigated the effect of oxytocin administration on dogs' social behaviour. *In Study 3* we examined whether dogs show spontaneous preference for biological motion, and whether the intranasal oxytocin administration affects dogs' looking behaviour towarsa animated stimuli. Dogs' physiological response to oxytocin administration was also measured. We found that dogs show spontaneous preference for biological motion, and this preference was not observable after intranasal administration of oxytocin. Furthermore heart-rate and heart-rate variabilty were significantly affected by oxytocin treatment. In S*tudy 4* we aimed to explore the breed differences and breed-specific effects of oxytocin administration on different aspects of social responsiveness. Our study provided experimental evidence that there are several behavioural differences between the independent and cooperative worker breed groups and also that there are differential effects of the oxytocin treatment.

In **Part III** we examined whether social stimulation (probably via stimulation of the OXT system) can shape some aspects of social behaviour in both children and dogs. In *Study 5* we have found some indication that socially stimulating pre-treatment can shape perception to basic aspects of social behaviour in pre-schoolers, however other factors (e.g. gender) can have an influential effect. In *Study 6* we further investigated whether socially relevant and irrelevant partners would differentially influence dogs' social susceptibility after socially stimulating pre-treatment. We found that social interaction with a socially bonded partner (the owner) strongly influence dogs' tendency to conform to the partner's behaviour whereas

social priming with an unfamiliar human partner produces weaker effects. This supports the idea that the socially bonded partner plays a more important role in oxytocin-mediated changes in dogs' social behaviour.

In summary, these studies corroborate the notion that studying the relationship between different aspects of social behaviour and the oxytocin system in the dog is a promising new research area which may also have translational relevance for understanding the neuro-hormonal bases of human social cognitive abilities.

IX. ÖSSZEFOGLALÓ

Disszertációm fő célja a kutyák és gyerekek szociális érzékenységének, társas-kognitív kézségeinke jobb megértése, a viselkedés-szabályozáss neuro-kognitív aspektusainak és az oxytocin szerepének feltárásával.

Kutatásunk **Első részében** azt vizsgáltuk, hogy a házi kedvencként tartott kutyák és gyerekek oxytocin receptor gén allélváltozatai hogyan befolyásolják szociális viselkedésüket. Az *Első vizsgálatban* bizonyítékot találtunk arra, hogy az oxytocin receptort kódoló gén egyes változatai, valamint a kutyák környezetét alakító tényezők (mint például a származási országuk, gazdáik genetikai háttere, személyisége és kötődése) egyaránt befolyásolják a kutyák kötődését gazdáik irányába. A *Második vizsgálatban* a kutyák és gyerekek emberi tekintetre adott válaszkészségének potenciálisan eltérő mechanizmusaira fókuszáltunk. Eredményeink arra utalnak, hogy mind a kutyáknál, mind pedig a gyerekeknél az oxytocin receptor gén polimorfizmusai összefüggésbe hozhatók az averzív szociális interakcióra adott válaszreakciókkal. Azonban amíg az oxytocin receptor gén polimorfizmai nem befolyásolták a gyerekek tekintetkövetési képességét, addig a kutyáknál bizonyos genotípusok erősebb „tekintetkövetési hajlandóságot" eredményeztek.

A kutatás **Második részében** az intranazális úton (orrspray formájában) szervezetbe juttatott oxytocin kezelés hatásait vizsgáltuk kutyák esetében. A *Harmadik vizsgálatban* azt tanulmányoztuk, hogy a kutyák mutatnak-e spontán preferenciát a biológiai mozgást mutató egyszerű animációk nézésekor, valamint megnéztük, hogy hatással van-e az oxytocin előkezelés a kutyák nézési viselkedésére. Mindemellett megvizsgáltuk az oxytocinnal való előkezelés élattani következményeit (szívritmus és szívfrekvencia-variabilitás). Azt találtuk, hogy a kutyák spontán preferálják a biológiai mozgást, viszont oxytocin kezelés hatására ez a preferencia eltűnik. Az oxytocin kezelés ugyanakkor kimutatható volt a kutyák szívritmus és szívfrekvencia-variabilitás értékei alapján. A *Negyedik vizsgálat* során arra kerestünk válaszokat, hogy a kutyák szociális érzékenységének különböző aspektusait hogyan befolyásolják a fajtakülönbségek valamint hogy van-e az oxytocin kezelésnek fajta-specifikus hatása. A kísérlet során bizonyítékot találtunk arra, hogy számos eltérés tapasztalható az önálló- illetve kooperatív munkára szelektált fajták viselkedése között, továbbá hogy az oxytocin előkezelés eltérő módon hathat a különböző munkákra szelektált fajtákra.

A disszertációban szereplő kísérletek **Harmadik részében** arra voltunk kíváncsiak, hogy a szociális stimuláció (feltehetően az oxytocin rendszeren keresztül) hogyan képes befolyásolni a kutyák és gyerekek társas viselkedését. Az *Ötödik vizsgálatban* bizonyítékot találtunk arra, hogy a társas „kulcsingerekkel való előkezelés (szemkontaktus, érintés) képes befolyásolni a

szociális viselkedés alapvető aspektusainak észlelését óvodás gyerekek esetében. Ugynakkor azonban egyéb tényezők – mint pl. a nemi különbségek – is modulálják a társas ingerek „előfeszítő” hatását. Végül a *Hatodik vizsgálatban* azt tanulmányoztuk, hogy ismerős vagy ismeretlen emberrel történő társas előkezelés eltérő módon hat-e a kutyák azon hajlandóságára, hogy viselkedésüket másokhoz igazítsák. Eredményeink azt mutatják, hogy a kutyák saját gazdáikkal történő előzetes interakciója erősen befolyásolja azt, hogy egy rákövetkező feladatban a viselkedésüket a kísérletvezetőhöz igazítsák, míg az efféle alkalmazkodásra való hajlandóságukat kevésbé képes stimulálni az idegennel emberrel történt előkezelés. Mindez alátámasztja azt az elképzelést miszerint a társas partner fontosabb szerepet játszik a szociális viselkedés oxytocin által közvetített változásaiban.

Ezek a kísérletes vizsgálatok összességében megerősítik azt az elképzelést, hogy a kutya bevonása az oxytocin rendszer és a társas vislekedés különböző megnyilvánulásai közötti kapcsolat vizsgálatába egy olyan új és ígéretes kutatási terület, amely hozzásegíthet ahhoz hogy jobban megértsük az ember társas-kognitív képességeinek neurohormonális szabályozását.

ADATLAP

a doktori értekezés nyilvánosságra hozatalához*

I. A doktori értekezés adatai

A szerző neve: Hegedűs-Kovács Krisztina

MTMT-azonosító: 10052723

A doktori értekezés címe és alcíme: The effects of oxytocin on social behaviour: a comparative approach

DOI-azonosító[46]: 10.15476/ELTE.2017.202

A doktori iskola neve: Biológia Doktori Iskola

A doktori iskolán belüli doktori program neve: Etológia Doktori Program

A témavezető neve és tudományos fokozata: Topál József, PhD, DSc

A témavezető munkahelye: Magyar Tudományos Akadémia, Természettudományi Kutatóközpont

II. Nyilatkozatok

1. A doktori értekezés szerzőjeként
a) hozzájárulok, hogy a doktori fokozat megszerzését követően a doktori értekezésem és a tézisek nyilvánosságra kerüljenek az ELTE Digitális Intézményi Tudástárban. Felhatalmazom a Természettudományi kar Dékáni Hivatal Doktori, Habilitációs és Nemzetközi Ügyek Csoportjának ügyintézőjét, hogy az értekezést és a téziseket feltöltse az ELTE Digitális Intézményi Tudástárba, és ennek során kitöltse a feltöltéshez szükséges nyilatkozatokat.
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d) kérem, hogy a mű kiadására vonatkozó mellékelt kiadó szerződésre tekintettel a doktori értekezést a könyv megjelenéséig ne bocsássák nyilvánosságra az Egyetemi Könyvtárban, és az ELTE Digitális Intézményi Tudástárban csak a könyv bibliográfiai adatait tegyék közzé. Ha a könyv a fokozatszerzést követőn egy évig nem jelenik meg, hozzájárulok, hogy a doktori értekezésem és a tézisek nyilvánosságra kerüljenek az Egyetemi Könyvtárban és az ELTE Digitális Intézményi Tudástárban.
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b) a doktori értekezés és a tézisek nyomtatott változatai és az elektronikus adathordozón benyújtott tartalmak (szöveg és ábrák) mindenben megegyeznek.
3. A doktori értekezés szerzőjeként hozzájárulok a doktori értekezés és a tézisek szövegének plágiumkereső adatbázisba helyezéséhez és plágiumellenőrző vizsgálatok lefuttatásához.

Kelt: Budapest, 2017.12.12

Hegedűs-Kovács Krisztina
.....
a doktori értekezés szerzőjének aláírása

**ELTE SZMSZ SZMR 12. sz. melléklet*