The role of oxytocin in the ability of domestic dogs (*Canis familiaris*) to use human social cues and bond with humans

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Abstract

The domestic dog (*Canis familiaris*) demonstrates attachment/bonding behaviour towards humans, whilst wolves (*Canis lupus*) do not. Domestic dogs also use humans' non-verbal social cues to solve problems better than wolves do, even wolves raised in the same manner as domestic dogs. The neuropeptide oxytocin has been implicated in mammalian bonding and non-verbal intelligence and therefore the oxytocinergic system may have evolved in the dog during domestication in such a manner as to enable the formation of human-dog bonds and facilitate human-dog communication. To test this hypothesis three related studies were conducted. The first investigated the influence of intranasally-administered oxytocin on the ability of domestic dogs to perform an object choice task involving a concealed food reward. It was hypothesized that food-finding would be enhanced after the administration of oxytocin. The second study investigated whether owner-perceived level of bonding with, and intelligence of, their dog could predict the dog's performance on the object choice task. It was hypothesized that dogs highly-bonded to their owners and with owners who perceived their dog to have a high level of intelligence would perform better on the object choice task than the dogs of owners with weaker perceptions of bonding with, and the intelligence of, their dog. The third study investigated whether variation in tandem repeat length close to the oxytocin receptor gene could account for individual differences in performance on the object choice task and for the species difference in performance between dogs and wolves.

Seventy-five pet dogs and their owners were recruited for the studies, which involved two testing sessions, 5-15 days apart. An intranasal spray of oxytocin or saline was administered to the dogs in a pseudo-random, counter-balanced order at the beginning of each session. A buccal swab was also taken from the dogs for subsequent genetic analysis and the owners were required to fill out several questionnaires. Forty-five minutes after the intranasal administration, dogs commenced the object choice task which required them to find a hidden food treat using pointing and gazing cues given by the experimenter. It was found that oxytocin improved dogs' performance on the object choice task when pointing cues were available and that this enhanced performance was maintained for up to 15 days in the absence of further oxytocin administration. Oxytocin also decreased aversion to the gazing cue, whereby dogs actively avoided the gazed-at bowl after saline but performed at chance level after oxytocin. Anxious attachment to pets (measured with the Pet Attachment Questionnaire) negatively predicted performance on the object choice task with pointing cues, whilst perceived contagion of human emotions (measured with the Perceptions of Dog Intelligence and Cognition Survey) positively predicted performance using gazing cues. This suggests that human communication signals may be interpreted differently by dogs owned by anxiously-attached and

non-anxiously attached humans. No differences in tandem repeat length close to the oxytocin receptor gene could implicate this gene in affecting performance on the object choice task. However, a species difference in tandem repeat lengths was observed, suggesting that mutations of the oxytocin receptor gene played an integral role in the domestic dog's evolution from the wolf.

General Declaration

Declaration for thesis based or partially based on conjointly published or unpublished work

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer reviewed journal (refer to the appendix) and two unpublished, but submitted, publications. As a result there is some overlap among the introductory and methodological material of the chapters. The core theme of the thesis is dog-human interactions. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Biological Sciences under the supervision of Associate Professor Alan Lill.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters 2-4 my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status*	Nature and extent of candidate's contribution
2	Oxytocin enhances the appropriate use of human social cues by the domestic dog (Canis familiaris) in an object choice task	Published (refer to appendix)	95%
3	Associations between owner-perceived closeness to, and intelligence of, their dog, and the animal's actual performance level in an object-choice task	Submitted	95%
4	The oxytocin receptor gene, an integral piece of the evolution of Canis familaris from Canis lupus	Submitted	90%

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed: 

Date: 06/05/2015

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Chapter 1 - Introduction

The Human-Dog Relationship

Evidence from remains found in Siberia believed to date back to 33,000 years ago, before the last glacial maximum, suggests that this may have been the time of one of the first dog domestication events (Ovodov et al., 2011). These early dogs, however, are not believed to have survived the last glacial maximum and therefore are not thought to be the direct ancestors of modern-day domestic dogs (Ovodov, et al., 2011). However, it is fascinating that this evolutionary phenomenon may have occurred more than once and in two distinct, geographical locations. Domestication of contemporary dogs was originally believed to date back to between 5,400 and 16,300 years ago, involve wolves from China and South East Asia and probably coinciding with Chinese rice-farmer settlement (Pang et al., 2009). However, these authors have more recently found evidence of domestication occurring even earlier, approximately 32,000 years ago (Wang et al., 2013), at a similar date to the remains found in Siberia. Parker, Shearin and Ostrander (2010) have reviewed research which suggests various domestication dates and the involvement of various sub-species of wolf, although the exact place and time of domestication, whether it involved a single or multiple events, and the circumstances under which domestication occurred may never be known. Evidence from a burial site in Israel in which both human and dog remains were found together suggests that an affectionate relationship between man and dog existed approximately 10,000-12,000 years ago (Davis & Valla, 1978). Since then, dogs have become an integral part of human lives world-wide and have impacted many facets of human life, including hunting and working, but none so prominent as their role as companion animals (Clutton-Brock, 1995). As Odendaal (2000) explains, domestication of dogs occurred naturally as a mutual fulfillment of needs. The human provides the dog with food and shelter and the dog provides the human with security and utility needs. At the same time, both the human and the dog provide each other with attention, allowing for emotional bonds to form.

Today, domestic dogs are in high demand as companion animals, but nonetheless a significant percentage of pet dogs are still relinquished to shelters. A study conducted across three Melbourne shelters reported that almost 11% of relinquishments are for behavioural reasons, this percentage doubling for adopted dogs that are subsequently returned (Marston, Bennett, & Coleman, 2004). The fate of these dogs is then in the hands of the shelter staff who need to decide whether to euthanase them or make them available for (re)adoption and re-homing. So what is going wrong? As McGreevy and Bennett (2010) highlight, dogs continue to be bred as companion

animals despite a lack of: i) owner feedback on the success of the dog as a companion animal, ii) breeder accountability if the dog is unsuccessful as a companion animal, and iii) reinforcement of good companion animal genetics due to widespread de-sexing. So this begs the question, what are people looking for in a companion dog? King, Marston and Bennett (2009) surveyed 877 Australian residents about their 'ideal dog' and the most prevalent characteristics included "my ideal dog shows affection towards me", "enjoys being cuddled", "enjoys being petted", "is friendly with other dogs" and "is safe with children". Furthermore, it was considered "important" or "extremely important" for 92.7% of participants surveyed that the ideal dog was affectionate. This study suggests that people are potentially looking for dogs with a high level of central oxytocin function, as this neuropeptide is known to be involved in affiliation and bonding.

The Neurochemistry of Bonding in mammals

Two structurally similar neuropeptides have been implicated in mammalian bonding; arginine vasopressin and oxytocin (for a review see, Lim & Young, 2006). Both peptides are predominantly expressed in magnocellular neurons of the hypothalamic paraventricular and supraoptic nuclei. These neurons project to the posterior lobe of the pituitary where the peptides are released into the bloodstream and act as peripheral hormones. Smaller amounts of the peptides are also expressed in parvocelluar neurons of the paraventricular nucleus of the hypothalamus, projecting to central brain regions and to the median eminence, where peptides are released in the hypophyseal portal system. Additionally, neuropeptides are released from parts of these neurons other than the terminal bouton, including the dendrites, soma and axon, and therefore certain amounts are also released into the cerebrospinal fluid (CSF) (Ludwig & Leng, 2006).

Many mammalian studies support a role of the above-mentioned neuropeptides in bonding behavior and one of the most widely studied models is the socially monogamous prairie vole (*Microtus ochrogaster*) (Carter & Getz, 1993; Cho, DeVries, Williams, & Carter, 1999). Investigations have revealed that oxytocin and vasopressin receptor distribution differs between socially monogamous and non-monogamous vole species (Insel & Shapiro, 1992; Insel, Wang, & Ferris, 1994). For example, oxytocin receptors (OXTRs) in prairie voles are largely distributed in the striatum (both the shell and core of the nucleus accumbens and the caudate putamen) (Lim et al., 2004; Olazabal & Young, 2006b) and are sparse in the lateral septum (Olazabal & Young, 2006b), whilst vasopressin receptors are distributed in the ventral pallidum (Lim, et al., 2004). In contrast, OXTR binding is significantly lower in the nucleus accumbens of non-monogamous rodents, such as rats (*Rattus rattus*), mice (*Mus musculus*) and meadow voles (*Microtus pennsylvanicus*) (Olazabal & Young, 2006b). High densities of OXTRs in the shell of the nucleus accumbens have been associated

with spontaneous maternal behaviours observed in sexually-naïve prairie voles exposed to pups (Olazabal & Young, 2006a, 2006b) and are negatively correlated with OXTR distribution in the lateral septum (Olazabal & Young, 2006b), indicating the importance of these brain regions in maternal and bonding behaviour both within and between rodent species, behaviours which can be inhibited by the administration of an OXTR antagonist into the nucleus accumbens (Olazabal & Young, 2006a).

Studies have shown that the structures that demonstrate high oxytocin expression in prairie voles are also a part of the dopamine reward pathway (for a review see, Young & Wang, 2004). Young and Wang (2004) postulate that the interaction between dopamine and oxytocin within this neural circuitry is involved in the differences observed in social behavior among rodent species. This has been demonstrated in male prairie voles not forming social bonds with their mating partner in the presence of the nonselective dopamine antagonist, haloperidol, and in sexually-naïve males exhibiting social bonds in the presence of low doses of the dopamine agonist, apomorphine (which binds with high affinity to D2 receptors and low affinity to D1 receptors) (Aragona, Liu, Curtis, Stephan, & Wang, 2003). Studies by Gingrich, Liu, Cascio, Wang and Insel (2000) found that mating significantly increased dopamine in the nucleus accumbens of female prairie voles and that the dopamine D2 receptor antagonist, eticlopride, blocked partner preference formation, whilst the D2 agonist, quinpirole facilitated partner preference formation in females that did not mate. These findings specifically implicate the D2 dopamine receptor in the formation of social bonds. Further investigations have confirmed this conclusion. For example, Aragona et al. (2006) demonstrated that dopamine D2 receptor binding in the rostral shell of the nucleus accumbens is responsible for the formation of partner preferences in the prairie vole, as injections of the D2 agonist, quinpirole, just into this region facilitated partner preference formation. This effect was blocked by concurrent D1 receptor activation. It was found that males that had bonded with a female for two weeks demonstrated increased D1 receptor binding in both the core and shell of the nucleus accumbens, but with no differences in D2 receptor binding. This pattern of D1 receptor distribution is similar to that of the promiscuous meadow vole and the authors surmised that this prevented the formation of additional bonds and helped to maintain social monogamy in prairie voles. A further experiment showed that pair-bonded male voles were selectively aggressive to unfamiliar females and that this effect was inhibited by D1, but not D2, receptor blockade (Aragona, et al., 2006). These data suggest that whilst D2 receptor activation in the rostral shell of the nucleus accumbens is important for the formation of social bonds, D1 activation in the nucleus accumbens blocks this effect. Similar findings have been reported in rats; mothers that exhibit high levels of licking and grooming of their pups have a stronger dopamine signal in the shell of the nucleus accumbens (Champagne et al., 2004;

Shahrokh, Zhang, Diorio, Gratton, & Meaney, 2010) than those who exhibit low levels of licking and grooming. Moreover, high levels of licking and grooming behaviour could be abolished with an oxytocin antagonist, suggesting that the dopamine signal is oxytocin-dependant (Shahrokh, et al., 2010). This oxytocin-dopamine interaction suggests that a reason why some species form monogamous social bonds is because the reward pathway is activated at bond formation and as such, these species experience the maintenance of the bond as rewarding.

Although not directly related to central oxytocin, which is difficult to study in a minimally invasive way in people, human plasma oxytocin concentrations have been positively associated with maternal behaviours (Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Uvnäs-Moberg, Widström, Nissen, & Björvell, 1990), maternal gazing (Kim, Fonagy, Koos, Dorsett, & Strathearn, 2014), attachment and harm-avoidance (Tops, van Peer, Korf, Wijers, & Tucker, 2007), trust and trustworthiness (Zak, Kurzban, & Matzner, 2005), greater self-reported partner support (Grewen, Girdler, Amico, & Light, 2005), and cardio-protective physiology (Grewen, et al., 2005). This suggests that the oxytocin system can be primed by past bonding experiences. Plasma oxytocin concentrations are also significantly lower in children with autism (a disorder characterized by social deficits (Volkmar, 2011)) than in non-autistic controls (Modahl et al., 1998). However, these autistic children demonstrate high concentrations of the oxytocin precursor (Green et al., 2001), indicating that this disorder may be characterised by deficits in the metabolism of the precursor to its active form. In humans, oxytocin is also secreted after interactions with pet dogs.

Oxytocin studies in human-dog dyads

In a pioneering study, Odendaal and Meintjes (2003) investigated the effects of 5 to24 minutes of human-dog interaction on mean arterial blood pressure and plasma concentrations of β-endorphin, oxytocin, prolactin, phenylacetic acid and dopamine in both human and dogs. They found that mean arterial blood pressure decreased in parallel with significant increases in plasma levels of β-endorphin, oxytocin, prolactin, phenylacetic acid and dopamine. To differentiate the neurochemicals involved in human-dog bonding, as opposed to relaxation which these physiological indicators could also be reflecting, Odendaal and Meintjes compared the blood chemistry of owners interacting with their dogs with that when they were relaxing during quiet book reading. Dog interaction, compared to book reading, was characterized by significantly greater increases in plasma oxytocin, prolactin and β-endorphin, implicating these specific neurochemicals in bonding behaviour. This study employed both men and women and male and female dogs of differing breeds; however, gender was not controlled in analysis of the findings. To account for the potentially confounding effects of gender and breed, Miller, et al. (2009) replicated Odendaal and Meintjes'

study using ten men and ten women and analysed them separately, but measured only plasma oxytocin concentration. Blood samples were drawn from participants before arriving home from work to greet their pet dog and after 25 minutes of interacting with their dog. The same book-reading condition used by Odendaal and Meintjes was employed on a separate day as the control treatment. Interestingly, whilst human females' plasma oxytocin concentration significantly increased after interaction with their dog, males' plasma oxytocin concentration significantly decreased. The reason for this gender disparity is unknown, but it may be due to the interaction between male and female sex hormones and oxytocin (for a review see, Gabor, Phan, Clipperton-Allen, Kavaliers, & Choleris, 2012) or to differences in the type of interaction shared with dogs by men and women. For example, although not recorded in Miller, et al.'s study, calm, soft petting may be more commonly practiced by women, whilst rough play may be more common in men. Indeed, it has been demonstrated that plasma cortisol levels rose in dogs petted for 20 minutes after venipuncture by men, but not in dogs petted by women (Hennessy, Davis, Williams, Mellott, & Douglas, 1997), indicating that men did not stimulate a significant anxiolytic effect in dogs after the invasive procedure. However, when men were trained to pet in a similar fashion to most women, no difference in cortisol concentrations of the petted dogs was observed (Hennessy, Williams, Miller, Douglas, & Voith, 1998). There was, however, a large degree of individual variability in the plasma oxytocin concentrations of both men and women in Miller, et al.'s study, with two women not demonstrating any increase in oxytocin concentration and two men demonstrating one. In addition, one woman and one man appeared to be outliers, their concentrations being two standard deviations greater than the mean. More interesting still was that oxytocin concentration was not correlated with the subjects' scores on the Lexington Attachment to Pets Scale.

It is important to note, however, that these studies measured peripheral plasma oxytocin concentration, which may not reflect central oxytocin function. This was demonstrated by Engelmann, Wotjak, Ebner and Landgraf (2000), who revealed through their experiments with rats that an increase in centrally-released vasopressin and oxytocin in the supraoptic nucleus is not necessarily associated with increased levels in the peripheral circulation. This may be explained by the fact that neuropeptides, such as oxytocin and vasopressin, are released from all parts of the neuron, including the axon, soma and dendrites, which releases these peptides into the CSF, and not only the terminal bouton, which releases these peptides into the circulation (Ludwig & Leng, 2006). Importantly these two release mechanisms are not necessarily time-locked; the two "hormones" can be released independently in response to the same stimulus. There are reports of dendritic release being delayed by more than one hour and exerting its effects for much longer, as neuropeptides

released in this manner act as a kind of 'hormone' with the CSF, regulating the cells of origin and priming target cells (Ludwig & Leng, 2006). This disparity between blood and CSF concentrations of oxytocin has also been shown in lactating guinea-pigs (*Cavia porcellus*) that exhibited increased plasma concentrations of oxytocin during suckling, in the absence of any increase in CSF oxytocin concentrations (Robinson & Jones, 1982). Furthermore, it is unlikely that peripheral administration of oxytocin will influence brain function, as the conventional wisdom is that neuropeptides do not readily pass the blood-brain barrier (Robinson, 1983; Veening, de Jong, & Barendregt, 2010). However, it has been demonstrated in rats that approximately 0.0002-0.0003% of a peripheral dose of oxytocin and approximately 0.001% of a peripheral dose of vasopressin does cross the blood-brain barrier (Mens, Witter, & van Wimersma Greidanus, 1983), although this effect has not been demonstrated in dogs (Vorherr, Bradbury, Hoghoughi, & Kleeman, 1968).

Despite the lack of central and peripheral synergy, peripheral oxytocin has continued to be the most widely investigated hormone in human-dog bonding (Handlin et al., 2011; Mitsui et al., 2011; Nagasawa, Kikusui, Onaka, & Ohta, 2009). Using only female humans and male Labrador dogs in their study, Handlin, et al. (2011) found that plasma oxytocin concentrations increased in both the dog and its owner after just one minute of petting and talking to the dog. However, when only analyzing grouped data, it is difficult to conclude which dogs, if any, were more bonded, or had a greater propensity to bond after petting. To investigate this, Nagasawa, et al. (2009) separated participants into two distinct groups: group 1 comprised dog owners who expressed a significantly greater perceived degree of satisfaction and communication with their dogs compared to group 2 members. After thirty minutes of interaction with their dog, group 1 members excreted significantly higher concentrations of oxytocin in their urine than members of group 2; their dogs also gazed at them for longer durations during the interaction, but not when owners were instructed to refrain from gazing back at them. This study reveals that a dog's gaze is an important factor in bonding and attachment to a human, similar to the situation observed in human infants (Dickstein, Thompson, Estes, Malkin, & Lamb, 1984; Striano, Vaish, & Benigno, 2006).

Intranasal oxytocin administration studies in humans

Modulating the oxytocin system via intranasal administration of the neuropeptide may provide a better alternative to peripheral blood measures when investigating the influence of this molecule on behaviour. Indeed, studies have demonstrated that intranasal administration of peptides allows their access to the brain, circumventing the blood-brain barrier (Born et al., 2002; Rault, 2013). Many investigations involving humans have utilised this method and some have shown this to be an effective way of modulating social behaviour. For example, oxytocin seems to enhance

facial processing, emotion recognition and the memory and encoding of facial stimuli (for a review see, Guastella & MacLeod, 2012). Oxytocin has also been shown to increase trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), although not in situations with prior trust violation, or in out-groups or clinical populations who are rejection-sensitive (for a review see, Bartz, Zaki, Bolger, & Ochsner, 2011). In their review, Bartz, et al. found that half (50%) of the studies investigating the effect of intranasal oxytocin on social cognition show a significant effect; however, whilst the majority of effects were pro-social, a sizable minority (43%) actually involved decreased sociality. This highlights the context- and person-specific nature of this neuropeptide's action, which seems to increase the salience of social cues, be they interpreted as positive or negative. Oxytocin appears to do this by drawing the individual's attention to the social features of others. For example, it has been shown to enhance the detection of biological motion (Keri & Benedek, 2009) and increase gazing towards the eye region of other human faces (Guastella, Mitchell, & Dadds, 2008), an effect also observed in some monkeys (Dal Monte, Noble, Costa, & Averbeck, 2014).

The oxytocin receptor gene

In addition to the peptides themselves, the genes which code for the peptides also play a key role in behaviour modulation. Canine genetic analysis has aided research into canine diseases and behaviours that are homologous with human diseases and behaviours (for a review see, Sutter & Ostrander, 2004). Conversely, many studies have searched for gene variants in the dog that may be able to explain aspects of canine behavior, based on the demonstrated role of these genes in humans (for a review see, Parker, et al., 2010). The OXTR gene is a prime candidate for investigation of human-dog bonding, as variation in the human OXTR gene has numerous social implications. For example, in humans a particular single nucleotide polymorphism (SNP) of either an adenine (A) or a guanine (G) within intron 3 of the OXTR gene (rs53576) has been shown to have a great influence on human social phenotypes. For instance, the A allele has been implicated in autism (Jacob et al., 2007; Wu et al., 2005), but less extreme forms of social dysfunction have also been linked to the A allele. For example, Bakermans-Kranenburg and van IJzendoorn (2008) found that mothers with the A allele were less sensitive to their toddlers than those that were homozygous for the G allele, as subjectively rated by experimenters on a 7 point scale. This effect was later demonstrated physiologically by Riem, et al. (2011), who found that the OXTR GG genotype was associated with a greater physiological response to infants' crying than evident with the OXTR AG and AA genotypes. At the individual level, people homozygous for the G allele are more empathic and less stressed on both subjective and objective measures (Rodrigues, Saslow, Garcia, John, & Keltner, 2009). Conversely, Saphire-Bernstein et al. (2011) found that carriers of the A allele were less optimistic,

felt less personal mastery and had lower self-esteem than those not carrying this allele, whilst Lucht et al. (2009) found that the OXTR AA genotype was associated with lower values for positive affect and non-verbal intelligence. A-allele carriers of the OXTR gene also displayed lower levels of sociality, lower levels of oxytocinergic innervations of the hypothalamus and greater hypothalamic connections to both the amygdalae and the dorsal anterior cingulate cortex (Tost et al., 2010).

Evidence has only recently emerged that variants of the OXTR gene are responsible for different proximity-seeking and friendliness phenotypes in dogs, the latter being breed-dependant (Kis et al., 2014). By simultaneously screening dogs for OXTR gene variants and high central oxytocin function, associations between such genotypic variants and different phenotypes can be identified and provide breeders and shelter owners with important information about their dogs. As previously mentioned, King, Marston and Bennett (2009) found that for the vast majority of Australians, it was "important" or "extremely important" for their ideal dog to be affectionate. Therefore if more affectionate dogs could be bred, this may result in fewer pet dogs being relinquished to shelters and more dog-owners possessing positive experiences from ownership. But how can we identify dogs with high oxytocin function non-invasively?

Subjective measures of oxytocin function

Assuming that oxytocin facilitates human-dog bonding, one way to potentially identify dogs having a high level of oxytocin function is to use owner-rated questionnaires measuring the perceived relationship that they share with their dog. Several such questionnaires have been developed that aim to evaluate owner attachment to their pet (for a review see, Crawford, Worsham, & Swinehart, 2006). However, Miller, et al. (2009) have demonstrated that these scales do not necessarily reflect oxytocin function. Possible reasons for this are that (a) the authors used peripheral measures of oxytocin, which for the reasons discussed above may not reflect central oxytocin function, or (b) their choice of scale, the Lexington Attachment to Pets Scale, was inappropriate.

Firstly, as Crawford et al. (2006) highlight, many of the scales addressing owner attachment to their pet have not been based on attachment as it has been traditionally defined e.g. by Bowlby (1958). In developing the concept of attachment, Bowlby described it in the context of a human infant's interaction with its mother. He defined it as separate to 'dependency' on the fulfillment of physiological needs and as involving the expression of certain behaviours, such as following and clinging, which encourage the mother to respond by providing a 'safe haven'. Over time these behaviours become not just readily directed towards anyone who can satisfy them, but directed

preferentially towards the particular person to whom the infant is attached. We can think of attachment towards a pet in the same way, as satisfying a *psychological* need, and a satisfaction that only *our* specific pet can provide. Secondly, as Dwyer, Bennett and Coleman (2006) highlight, many scales have not been psychometrically validated and those that have are often found to be inadequate. Although the Lexington Attachment to Pets Scale *does* have good psychometric properties (Johnson, Garrity, & Stallones, 1992), it was not designed to measure attachment to dogs specifically, but is also suitable for cats. Lastly, there has been a predominant focus on the positive aspect of companion animal ownership and neglect of the measurement of negative aspects (e.g. lifestyle and necessary monetary sacrifices), in relationship scale development. To address these shortcomings, Dwyer, et al. developed an instrument based not on Attachment Theory, but on Social Exchange Theory, which has been applied to human-companion animal relationships (Netting, Wilson, & New, 1987) and assumes that they are maintained when the perceived benefits of the relationship are in balance with or outweigh the perceived costs. These authors' work generated a 28-item questionnaire, the Monash Dog Owner Relationship Scale (MDORS), which quantifies the 'perceived emotional closeness' of the human-dog bond, the degree of 'dog-owner interaction' and the 'perceived costs' (both momentary and to lifestyle) involved in dog ownership. This instrument is specific to only the domestic dog and has good content validity and internal consistency, with Cronbach alpha values (a co-efficient of internal consistency commonly used as an estimate of reliability) of 0.84 for both 'perceived emotional closeness' (subscale 2) and 'perceived costs' (subscale 3). The 'dog-owner interaction' (subscale 1) co-efficient was, however, not as strong, with alpha = 0.67 (Dwyer, et al., 2006).

An additional problem with the Lexington Attachment to Pets Scale, and similar scales, as highlighted by Zilcha-Mano, Mikulincer and Shaver (2011), is that it assesses strength of "attachment", without having been designed in the context of Bowlby's above-mentioned original definition (1958) (later expanded upon by Ainsworth (1973), and it does so without considering attachment *style* (Ainsworth, Blehar, Waters, & Wall, 1978). Ainsworth, et al. (1978) first showed that human infants demonstrate different styles of attachment to their mothers and classified these styles as either 'secure' or 'insecure'. Insecure attachment is characterised by either anxious (anger or rejection of proximity-seeking with their mother after a period of separation) or avoidant (inconsistencies in greeting their mother after a period of separation) behaviours in an unfamiliar situation. These behaviours develop as a consequence of the mother's responsiveness to her infant's needs (Ainsworth, et al., 1978). It has been postulated that internal working 'models' of attachment are developed in infancy and carried forward into adulthood (for a review see, Bretherton &

Munholland, 2008). Designed in the context of such traditional Attachment Theory, Zilcha-Mano, et al's. 26-item scale assesses the degree to which an owner forms an anxious or avoidant attachment bond with their pet. Anxious attachment to a pet involves constant thoughts that something bad might happen to it and that they will be left alone, resulting in a constant desire to be near their pet and frustration when it does not reciprocate with the same need for closeness (Zilcha-Mano, et al., 2011). In contrast, avoidant attachment involves feelings of discomfort when their pet gets too close to them and an avoidance of intimacy with it (Zilcha-Mano, et al., 2011). Low scores for both anxious and avoidant attachment would suggest a healthy, secure attachment in which the owner is comfortable with the level of intimacy their pet shows towards them and the relationship is not affected by distressing thoughts or feelings (Zilcha-Mano, et al., 2011). This questionnaire has been found to have good content and construct validity, test-retest reliability (r=.75) and internal consistency, with Cronbach alpha values for avoidant attachment of .84 - .91 and for anxious attachment of .86 - .92, and no significant correlation between the two (r = .1 to .4) (Zilcha-Mano, et al., 2011).

Several research groups have investigated attachment of the dog to its owner using a modified version of the Ainsworth Strange Situation Test (first described in, Ainsworth & Wittig, 1969). This original test involves a mother and an infant being left alone a room full of toys for three minutes, after which time a stranger enters the room. After another three minutes, the mother leaves the infant with the stranger for an additional three minutes before returning for three more minutes. The baby is then left alone in the room for another three minutes, after which time the stranger re-enters the room and remains with the baby for two or five minutes and then leaves. At this point the infant is then reunited with its mother. Throughout this procedure the infant's behaviour is monitored and used to assess attachment style (Ainsworth, et al., 1978), as a separate entity to attachment strength. Ainsworth, et al. characterised secure attachment behaviors as: using the mother as a secure base for exploration of the room, proximity-seeking towards the mother in the presence of a stranger, disrupted exploration and signs of distress in the absence of the mother, and positive proximity-seeking upon reunion. Insecure attachment was characterised in two different ways: avoidant attachment, in which infants failed to, or were inconsistent in, greeting their mother upon reunion and ambivalent/anxious attachment, in which reunion with the mother included displays of anger and/or rejection (Ainsworth, et al., 1978). Variation in the OXTR gene has been associated with infant attachment style in an ethnically-dependant manner (Chen, Barth, Johnson, Gotlib, & Johnson, 2011).

A modified version of this test was first applied to dogs by Topál, Miklósi, Csányi and Dóka (1998). The authors concluded that dogs could be separated into five distinct categories based on behaviours that paralleled those along the secure-insecure dimensions seen in human infants. These conclusions have been criticized for being too liberal, based on the modified version of the test they used and the small number of behaviours they observed (Prato-Previde, Custance, Spiezio, & Sabatini, 2003). A similar study was conducted by Prato-Previde, et al. (2003) that more closely replicated the original test by Ainsworth (1978). The authors concluded that the dog-human relationship resembled more of a strong bond than an attachment *per se*. Semantics aside, this human-canine relationship is specific to domestic dogs, and absent in wolves. Topál, et al. (2005) repeated their earlier experiment to test the effect of species differences versus rearing differences on the behaviours observed. To do this they used three groups of canids: (1) pet dog puppies that remained with their mothers for 7-9 weeks post-partum and were then adopted out to human families, (2) hand-reared dog puppies that were separated from their mother and litter mates 3-5 days after birth and hand-reared by a human, and (3) hand-reared wolf pups that were reared in the same way as the hand-reared dog pups. Differences between following an attachment figure (their owner) versus a stranger when they left the room, and greeting their owner versus a stranger when they re-entered the room, were exhibited in both groups of domestic dogs, but absent in wolves, which followed and greeted their owner and a stranger equally. Domestic dogs also spent more time playing with their owner than with a stranger than wolf pups did and spent more time standing by the door when their owner was absent (Topál, et al., 2005). Even shelter dogs demonstrate this type of relationship with their handlers, whom they have only known for 3 days and with whom they have interacted for only 10 minutes per day (Gácsi, Topál, Miklósi, Dóka, & Csányi, 2001).This indicates a really inherent tendency to bond with humans in the domestic dog that is absent in the wolf.

Working models of attachment have been well documented to operate in relationships among adult humans (Brennan, Clark, & Shaver, 1998). Secure adult attachment has been associated with (1) higher levels of peripheral oxytocin in mothers when interacting with their infants and (2) activation of oxytocinergic and dopaminergic brain regions associated with reward upon viewing their infant's faces (Strathearn, Fonagy, Amico, & Montague, 2009). Adult attachment also interacts with OXTR genotype in assessing risk and in feelings of closeness (Denes, 2015) and we now know that human adults exhibit varying attachments styles towards their pets (Zilcha-Mano, et al., 2011). However, studies are yet to show whether this variation is dependent on the pet's level of oxytocin function and/or the strength of the bond felt by the owner. If oxytocin *is* involved in this ability of

the domestic dog to bond with its owner, the fact that dogs demonstrated various levels of attachment/bonding to owners (Prato-Previde, et al., 2003; Topál, et al., 2005; Topál, et al., 1998) whilst wolves did not (Topál, et al., 2005), would suggest that the oxytocinergic system was integral in the domestic dog's evolution from the wolf. If this is true, it is reasonable to assume that, along with an ability to bond with humans, dogs would be likely to exhibit an strong ability to understand social cues that are relevant to the dogs' social environment. As the domestic dog has evolved from being a pack animal strongly bonded to other pack members, to 'man's best friend' (i.e. strongly bonded to a human owner or minder), these social cues are often likely to be *human* in derivation. Once again, we can use subjective measures of owners' perceptions of their dog's intelligence derived from questionnaires to assess the ability of dogs to use such human social cues.

The Perceptions of Dog Intelligence and Cognitive Skills survey (PoDIaCs) was developed to determine cognitive abilities in dogs, as perceived by their owners, across eight domains: 'recognition of human emotions', 'learned problem-solving abilities', 'instinctive awareness of human attention', 'learned awareness of human attention', 'deception', 'contagion of human emotions', 'instinctive problem-solving abilities' and 'general intelligence compared to humans' (Howell, Toukhsati, Conduit, & Bennett, 2013). Each cognitive domain was found to have good internal consistency, with Cronbach alpha values of 0.74-0.91. Supporting the idea that a dog's ability to understand human social cues and to bond with humans are influenced by the same neuropeptide, oxytocin, each subscale was significantly correlated with the second subscale of the MDORS, the owner's 'perceived emotional closeness' with his/her dog (Howell, et al., 2013). It is currently unknown, however, if either of these variables relate to actual cognitive ability. An alternative to relying on owner-rated questionnaires to evaluate dogs' intelligence is to test dogs on a task that requires them to use human social cues to solve. Given the role of oxytocin in social cognition in humans, by analogy maybe dogs that perform well on such a task are the individuals with a high level of oxytocin function.

The Object Choice Task

An 'object choice task' (OCT) was first used with domestic dogs by Miklósi, Polgárdi, Topál and Csányi (1998) to investigate their ability to use human social cues, and has been used in numerous studies of a variety of canids, as well as other vertebrates. The OCT involves a human gesturing to one of two (or more) objects, usually bowls, located to the right and left of them, to indicate the location of a hidden reward. It is the subject's task to correctly interpret this gesture, and then choose the 'gestured at' rather than the 'non-gestured at', object. This results in the subject receiving a reward, usually food. This task not only tests a subject's ability to interpret non-

verbal social cues to the whereabouts of an apparently uninteresting object, but also to infer that there is some purpose in so doing i.e. acquisition of food. Human social cues that have been used in OCTs include touching the target object, pointing towards the target object and orienting to and/or gazing at the target object. Cues can be given continuously until the subject makes its choice, or can be delivered momentarily for 1-2 seconds *before* the subject makes its choice. Cues can also be delivered at relatively close proximity to the object (*proximally*) or from relatively far away (*distally*). Similarly, cues can be delivered by an experimenter who is close to the subject and objects (*central*) or relatively far away from them (*peripheral*). Different species display different abilities on OCTs, as summarized in Mulcahy and Hedge (2012).

Collectively, studies demonstrate that domestic dogs have an innate ability to perform OCTs using human social cues without any training; they even outperform chimpanzees (*Pan troglodytes*) (Hare, Brown, Williamson, & Tomasello, 2002), humans' closest genetic relative, and wolves, *their* closest genetic relatives. Strikingly, this is true even if the wolves have been socialized (Hare, et al., 2002; Miklósi et al., 2003) or extensively hand-reared away from conspecifics (Virányi et al., 2008). However, caution must be exercised when interpreting these findings for several reasons. Firstly, whilst most dogs have been tested using a peripheral set-up (where the subject and objects are relatively far away, approximately 2m, from the experimenter), most great apes have been tested using a central set-up (usually separated by the length of a standard table). Mulcahy and Call (2009) were able to demonstrate that this critically affects the performance of great apes and that when tested using a peripheral set-up similar to that used in most dog studies, their performance significantly improves. As highlighted by these authors, when the containers and the cue are located close to each other, there is the possibility of divided attention i.e. 'competition' between the containers and the cue for the subject's attention. If the containers prove to be more attention-inducing stimuli, the subject may not attend to the cue delivered by the experimenter. Additionally, distance between the subject and the experimenter introduces an element of cost, i.e. if the subject makes the wrong choice, it would have expended energy moving towards a container for no reward. If it makes the correct choice, however, and obtains the reward, the cost of travelling to the correct container is offset. Therefore a subject may be motivated to pay closer attention to the cue to avoid making an energetically costly wrong choice.

Secondly, the existence of a barrier between the experimenter and wolves (a fence) and the experimenter and chimpanzees (glass) in a previous study comparing dog, chimp and wolf OCT performance may explain the poorer performance in chimps and wolves than that in dogs that were tested without a barrier (Hare, et al., 2002). The existence of a barrier between the experimenter

and the subject may well decrease the salience of the cue provided by the experimenter. Two studies have provided support for this by demonstrating that when domestic dogs are tested behind a fence, their performance decreases (Kirchhofer, Zimmermann, Kaminski, & Tomasello, 2012; Udell, Dorey, & Wynne, 2008a).

Thirdly, the environment in which an OCT is carried out may impact performance. For example, studies have shown that domesticated dogs (Miklósi, Pongrácz, Lakatos, Topál, & Csányi, 2005; Soproni, Miklósi, Topál, & Csányi, 2002; Virányi, et al., 2008) perform well on an OCT when given relatively difficult momentary distal pointing cues, whilst hand-reared wolves do not (Miklósi, et al., 2003), at least without extensive training (Virányi, et al., 2008). However, one important exception (Udell, et al., 2008a) is a study that demonstrated that when pet dogs and hand-reared wolves were both tested in an outdoor enclosure using a momentary distal pointing cue given by a familiar experimenter, wolves significantly outperformed dogs (Udell, et al., 2008a). However, pet dogs that were tested indoors, even by an unfamiliar experimenter, performed as well as the wolves tested outdoors (Udell, et al., 2008a). This was not true, however, for shelter dogs tested indoors (Udell, et al., 2008a). Ried (2009) has suggested that the olfactory, auditory and visual contact with conspecifics in this experimental set-up may have been unfairly distracting to the dogs who were likely to be unfamiliar with the other dogs in the experiment, compared with the wolves who were used to their conspecific companions. Nonetheless Udell et al.'s investigation seems to indicate that an important factor in performing accurately on an OCT is a familiar environment. However, the wolves in Udell, et al.'s study performed at above chance level when given a momentary distal pointing cue, whilst the dogs in Miklósi's (2003) study, tested in a similar environment, performed at chance level with the same cue. The fact that the wolves in Udell et al.'s study were already able to use this cue may explain the discrepancy in the findings and may be due to the fact that, as pointed out by Hare et al. (2010), "given their use in public education programmes, the wolves that Udell and colleagues tested probably had received previous training" (p. e6). Udell and Wynne (2010a) agree with this suggestion in a later publication. Indeed, hand-reared wolf puppies performed below chance level with this momentary distal pointing cue, but young wolves were able to learn this cue over time and perform at above chance level (Virányi, et al., 2008). Therefore, collectively, experimental findings do support the notion that dogs have an innate ability to use certain human cues, performing at above chance levels without training (Virányi, et al., 2008). However, results from dogs tested in an unfamiliar environment (Kirchhofer, et al., 2012; Udell, et al., 2008a; Udell, Dorey, & Wynne, 2010b) (behind a fence, outside or in a shelter) suggest that this innate ability can be compromised by environmental factors that probably impact on the animals' level of stress.

Many theories have been advanced as to why dogs are better performers than wolves on OCTs. The convergent evolution hypothesis states that domestic dogs independently evolved similar social communication skills to humans through domestication (Hare & Tomasello, 2005). However, this theory is challenged by the fact that non-domesticated species also perform above chance on OCTs (for a review see, Mulcahy & Hedge, 2012). It is difficult to draw compelling conclusions from these studies because, in many cases, sample sizes were small, the animals had been socialised and either trained on the task or trained to use human cues when performing other tasks, and because there were prominent methodological differences among studies (Miklósi & Soproni, 2006). Miklósi and Soproni highlight the importance of testing the domestication (convergent evolution) theory by comparing the performances of domesticated species and their wild counterparts that have been socialised to humans to a comparable extent. Domestic ferrets (*Mustela furo*) have provided support for this theory in that they perform as well as domestic dogs on OCTs, but tellingly outperform wild *Mustela* hybrids kept as pets in an identical way to the domestic ferrets. Similarly, silver fox (*Vulpes vulpes*) kits purpose-bred for tameness, as wolves may have been , not only started to develop similar physical features to domestic dogs, including floppy ears and fluffy tails (Trut, Oskina, & Kharlamova, 2009), but performed as well as domestic dog pups on an OCT, and outperformed control fox kits (Hare et al., 2005). Udell, Dorey and Wynne (2010c) have argued that this may be due to the shorter socialization period observed in control fox kits (i.e. not selected for tameness) compared with tame fox kits (Trut, et al., 2009), reducing the time in which they would willingly accept humans in their environment . After this critical period, a fear of humans in unsocialised individuals may override their cognitive capacity to use humans' social cues in a beneficial way. Scott (1962) reviewed this critical period in a range of different mammals and birds and defined it as the time window in which primary social bonds are formed. During this period, many mammals and birds explore their environment, enabling the formation of connections with other animals that share that environment, and show distress when alone. The end of this period is characterized by the development of a fear response to, and avoidance of contact with, strangers (Scott, 1962). In control foxes not bred for tameness this period ends at approximately 45 days old, when they start to become fearful of their surroundings and animals in them, whereas this happens at approximately 4 months old in tame foxes (Trut, et al., 2009). Hence, if a canid can be appropriately socialised to humans within *their* critical period, would we still see differences in their performance on an OCT with human social cues? Udell, et al. (2008a) have provided evidence through their experiments with hand-reared wolves that these differences may dissolve. However, as mentioned above, this may be due to the fact that these wolves were also highly trained for use in public education programmes.

Therefore, is the superior OCT performance in dogs simply explained by the effects of enculturation i.e. dogs learn social-communication skills through their experience with humans during rearing? The fact that litter-reared puppies (awaiting adoption) performed as well as same-age puppies living with a human family superficially seems to rule out human exposure influences and indicates that a correct use of human cues is an ability peculiar to this species (Hare, et al., 2002). However, this theory has been challenged on the grounds that litter-reared pups still have contact with the human breeder (Wynne, Udell, & Lord, 2008). Further to these findings in puppies, whilst one study did not demonstrate learning effects in wolves tested on the OCT (Hare, et al., 2002), others did (Miklósi, et al., 2003; Virányi, et al., 2008), with one investigation demonstrating an equivalent ability on the OCT in wolves that had learned the cue required to complete the task and naïve dogs (Virányi, et al., 2008). In contrast, most studies of domestic dogs reveal no learning (Hare, et al., 2002; Lazarowski & Dorman, 2015; Miklósi, et al., 2005; Riedel, Buttelmann, Call, & Tomasello, 2006; Wobber, Hare, Koler-Matznick, Wrangham, & Tomasello, 2009) (with the exception of the gazing cue (Miklósi, et al., 1998)), even in puppies 6-, 8-, 16- and 24 weeks old (Riedel, Schumann, Kaminski, Call, & Tomasello, 2008). Taken together, available findings seem to suggest that dogs' innate propensity to perform well on tasks that require the use of human cues transcend effects of enculturation. However, Riedel et al.'s analysis has been criticised and upon independent re-analysis of the data the 6-week old puppies were found to demonstrate learning effects (Wynne, et al., 2008). Moreover, dogs seem to possess a degree of cognitive flexibility in their use of human cues, learning *not* to follow human pointing in an OCT when it is no longer reinforcing, and can even learn to avoid a pointed at object if a non pointed at object suddenly becomes the reinforcer (Elgier, Jakovcevic, Barrera, Mustaca, & Bentosela, 2009). Furthermore, if dogs have learned to associate the hidden food reward in an OCT with a physical (non-human) cue this may then hinder their ability to successfully use a human pointing cue (Elgier, Jakovcevic, Mustaca, & Bentosela, 2012).

The inability of either the convergent evolution hypothesis or the enculturation hypothesis to univocally account for dogs' superior performance on OCTs has lead to a new hypothesis, the 'two stage hypothesis' which states that sensitivity to human cues requires i) interactions with, and acceptance of, humans during the sensitive period of the canid's social development, followed by ii) learning, through classical and operant conditioning, to pair human gestures with the acquisition of something favourable (Udell, et al., 2010c). This has been supported by a study demonstrating better OCT performance by pet dogs versus purpose-bred research dogs that have been socialised to humans at a young age, but who do not cohabit with them and have relatively less contact with them than pet dogs have with their owners (Lazarowski & Dorman, 2015). Despite the fact that the

two stage hypothesis is based on two types of ontogenetic experiences, Udell et al. suggest that selection has played a role, because the sensitive period of social development is longer in domestic dogs than in wolves (Frank & Frank, 1982), as observed in tame foxes compared to controls (Trut, et al., 2009). However, the authors believe that sensitivity to human cues can be achieved in all canids, domesticated and non-domesticated (Udell, et al. 2010c).

The two stage hypothesis cannot explain, however, the shorter latency to make eye-contact with the experimenter in both pet and hand-reared puppy dogs compared to hand-reared wolf pups when performing OCTs (Virányi, et al., 2008). Therefore this greater ability of domestic dogs versus wolves to perform an OCT may be due to the fact that they naturally gaze significantly more at the human experimenter delivering the cue than do wolves. Indeed, only with extensive hand-rearing and training do wolves demonstrate gazing and an increase in task performance efficacy comparable to that of naïve domestic dogs (Virányi, et al., 2008). More gazing has also been observed in pet dogs than in hand-reared wolves in other, more difficult behavioural tasks (Miklósi, et al., 2003). Interestingly, gazing was also less frequent in domestic cats than domestic dogs in difficult behavioural tasks (Miklósi, et al., 2005). This suggests that there is an inherent ability in dogs to communicate with humans in *humans' own way*; gazing is a common phenomenon in human communication (Dickstein, et al., 1984; Striano, et al., 2006). Although, Bentosela, Barrera, Jakovcevic, Elgier and Mustaca (2008) have shown that dogs can quickly learn not to use gazing when it is no longer reinforcing.

Further to simply gazing at a human for communication purposes, evidence suggests that dogs can interpret where a person's attention is directed by looking at their eyes and that this has implications for food acquisition. For example, one study showed that dogs retrieved a forbidden piece of food less often when a human was looking at them than when the person had their eyes closed, back turned or were facing them but distracted by a hand-held computer game (Call, Bräuer, Kaminski, & Tomasello, 2003). Dogs have also been shown to beg for food from a human who faced them rather than a human who faced away from them (Virányi, Topál, Gácsi, Miklósi, & Csányi, 2004). In contrast, chimpanzees, bonobos (*Pan paniscus*) and orangutans (*Pan pygmaeus*) also beg for food from humans facing them but not those with their backs turned, but they beg equally from humans facing them who have their eyes open or closed. Moreover, the above-mentioned apes do not beg from humans who have their back facing them, even if their head is turned towards them (Kaminski, Call, & Tomasello, 2004). In addition, significantly more dogs obeyed a command to lie down when the instructor was facing them than when the instructor was facing another human, an empty space or was visually obstructed (Virányi, et al., 2004). Interestingly, significantly more dogs

obeyed this command when the human looked towards an empty space than when they looked at another person, suggesting that dogs have some kind of concept of human attention and focus (Virányi, et al., 2004).

This 'special' ability to communicate with humans accompanies an ability to form interspecies bonds which is not seen in other canid species, even those reared in a similar way (Topál, et al., 2005). Due to its role in both social cognition and bonding, it is hypothesized here that the oxytocinergic system may have been shaped through the process of domestication in a way which allowed for dogs to both communicate and bond with humans. In the present investigation, I use three approaches to test this hypothesis: i) investigating the influence of oxytocin, administered intranasally, on a dogs' ability to perform an OCT, ii) determining whether performance on an OCT can be predicted by the owner's perceptions of the strength of bonding with his/her pet dog and of the dog's intelligence, or by factors that may influence oxytocin function in both the dog and owner iii) comparing genetic variability within the OXTR gene of good and poor dog OCT performers and of domestic dogs and wolves.

Chapter 2 – Oxytocin enhances the appropriate use of human social cues by the domestic dog (*Canis familiaris*) in an object choice task

Declaration for Thesis Chapter 2

In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I contributed the intellectual property, experimental design, conducted the experiment and all analyses and wrote the paper. Please refer to appendix A to see the published version of this paper.	95%

The following co-authors contributed to the work.

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
[name 1]	Dr. Jean-Loup Rault	
[name 2]	Dr. Belinda Appleton	
[name 3]	A/Prof Alan Lill	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 06/15/2015
Main Supervisor's Signature	[redacted]	**Date** 08/05/2015

Abstract

It has been postulated that the neuropeptide, oxytocin, is involved in human-dog bonding. This may explain why dogs, compared to wolves, are such good performers on object choice tasks which test their ability to attend to, and use, human social cues in order to find hidden food treats. The objective of this study was to investigate the effect of intranasal oxytocin administration, which is known to increase social cognition in humans, on domestic dogs' ability to perform such a task. We hypothesized that dogs would perform better on the task after an intranasal treatment of oxytocin. Sixty-two (31 male; 31 female) pet dogs completed the experiment over two different testing sessions, five to fifteen days apart. Intranasal oxytocin or a saline control was administered forty-five minutes before each session. All dogs received both treatments in a pseudo-randomised, counterbalanced order. Data were collected as scores out of ten for each of the four blocks of trials in each session. Two blocks of trials were conducted using a momentary distal pointing cue and two using a gazing cue, given by the experimenter. Oxytocin enhanced performance using momentary distal pointing cues and this enhanced level of performance was maintained over 5-15 days time in the absence of oxytocin. Oxytocin also decreased aversion to gazing cues, in that performance was below chance levels after saline administration but at chance levels after oxytocin administration.

Introduction

Domestic dogs seem to have evolved specialised abilities to communicate with humans in a way that their progenitor, the wolf, cannot. Social cognitive intelligence has been postulated to underpin human evolution (Whiten & Erdal, 2012), and in relation to using human social cues, it may also have been important in the domestic dog's evolution from the wolf. The 'Object Choice Task' (OCT) was first applied to dogs by Miklósi et al. (1998) in an attempt to investigate dogs' ability to use human social cues, and has since been utilised in numerous studies of domestic dogs and various other canids. The OCT involves a human experimenter using non-verbal, social cues to indicate the location of a hidden piece of food, located in one of two objects, usually bowls, located to the right and to the left of them. The subject's task is to correctly use these cues in order to obtain the hidden reward. The cues can involve replica cards, marker placement, pointing, tapping, orienting to and/or gazing at the object for various lengths of time and from various distances.

Of all the pointing cues used, the momentary distal point is potentially the most informative with respect to canines' ability to use human communication signals, as it is the most challenging. This is because the distance from the experimenter's finger to the bowl is relatively large and the cue relatively brief. Indeed, the cue is delivered *before* the dog is released and allowed to make its choice and is only given for 1-2 seconds. As such, the dog has to rely not only on the cue itself, but also on its *memory* of the cue. Whilst domesticated dogs (Hegedüs, Bálint, Miklósi, & Pongrácz, 2013; Miklósi, et al., 2005; Schmidjell, Range, Huber, & Virányi, 2012; Soproni, et al., 2002; Virányi, et al., 2008) and socialized dingoes (Smith & Litchfield, 2010) generally perform above chance on the OCT when given the momentary distal point cue, young, hand-reared wolves that have been highly socialised to levels comparable with pet dogs do not (Miklósi, et al., 2003; Virányi, et al., 2008), or at least not without extensive training (Virányi, et al., 2008). An additonal study where domestic dogs were tested in the same outdoor conditions as wolves (as opposed to being tested indoors as in Miklósi, et al. (2003) and Virányi, et al. (2008)) suggests that the fact the wolves in these studies were tested outdoors may have handicaped them (Udell, et al., 2008a). However, this is somewhat contradictory, as in Udell, et al.'s study mature wolves with a high level of socialisation and involvement in public education programs *were* able to demonstrate above-chance performance on this task when tested outdoors. Indeed, the authors claim that the wolves even out-performed dogs tested in the same outdoor conditions, though their methodology has been criticised (for responses see, Hare, et al., 2010; Udell & Wynne, 2010a).

Of all the cues that have been used on domestic dogs, only one has yielded OCT performance *not* above-chance level: gaze (Soproni, Miklósi, Topál, & Csányi, 2001). These authors

demonstrated that domestic dogs did respond to gazing cues, but paradoxically avoided the bowl at which the experimenter gazed, rather than approaching it. This may reflect a behaviour learned from communicating with conspecifics. However, domestic dogs do demonstrate an ability to learn to use this cue correctly to solve the task over time (Miklósi, et al., 1998). With the exception of the gaze cue, most studies on the OCT in domestic dogs reveal no learning (Hare, et al., 2002; Miklósi, et al., 2005; Riedel, et al., 2006; Schmidjell, et al., 2012; Wobber, et al., 2009), even in 6, 8, 16 and 24 week old puppies (Riedel, et al., 2008). Although Riedel, et al's analysis has been criticised and upon independent re-analysis of the data, learning was found in to be present in the very young 6 week old puppies (Wynne, et al., 2008). Nonetheless, taken together, these findings suggest that dogs may have an inherent ability to perform at above chance level on tasks that require understanding of human cues, without training.

The superior ability of domestic dogs (in comparison with wolves) to perform OCTs may be due to the fact that they gaze significantly more at the humans than do wolves. This notion has been supported by the shorter latency to make eye-contact with an experimenter in both pet dog puppies (separated from their mothers at 6-9 weeks to live with a human family) and hand-reared dog puppies (separated from their mothers at 4-10 days and hand raised by humans who either kept them as pets or re-homed them) than in hand-reared wolf pups (separated from their mothers at 4-7 days and hand raised by humans who re-homed them to a wolf farm at 2-4 months) (Virányi, et al., 2008). More gazing has also been observed in pet dogs than in hand-reared wolves performing other problem-solving tasks (Miklósi, et al., 2003). Furthermore, similar findings have been obtained in anthropomorphically-viewed and treated companion dogs that glance more at their owners and perform less well on a problem-solving task than less anthropomorphically-viewed and treated working dogs (Topál, Miklósi, & Csányi, 1997). This suggests that companion dogs not only have the ability to use human cues to solve tasks and find food, but that they have become *dependent* on them. Only with extensive hand-rearing and training do wolves demonstrate an increase in gazing and in task execution that takes their performance to the level of naïve domestic dogs (Virányi, et al., 2008). Interestingly, gazing was also less frequent in domestic cats than in domestic dogs (Miklósi, et al., 2005), supporting the idea that the human-dog bond transcends the effects of domestication. This suggests that there is an inherent ability in dogs to communicate with humans in *humans' own way,* because gazing is a common phenomenon in human communication (Dickstein, et al., 1984; Striano, et al., 2006).

A link has been found between dogs that gaze at their owners for long durations and higher urinary oxytocin concentrations in the owner (Nagasawa, et al., 2009). Given that oxytocin is

implicated in mammalian bonding (Lim & Young, 2006), this suggests that a dog's gaze, imperative for the successful completion of the OCT, may be more prominent in more strongly bonded human-dog dyads. Oxytocin increases have been observed in both humans and dogs after human-dog interactions (Handlin, et al., 2011; Miller, et al., 2009; Odendaal & Meintjes, 2003) and are thought to be associated with human-dog bonding. Indeed, a new or enhanced role of oxytocin and/or its receptors in the domestic dog brain, compared to the wolf brain, may explain why dogs gaze at their owners more than hand-reared wolves do, and, in turn, do better than wolves in tasks involving human communicative signals. In humans, intranasal oxytocin administration: (a) enhances detection of human biological motion (Keri & Benedek, 2009), (b) increases the understanding of social cues and improves social memory (see reviews by, Bartz, et al., 2011; Guastella & MacLeod, 2012), (c) increases trust (Kosfeld, et al., 2005) and (d) increases a subject's gazing towards the eye region of other human faces (Guastella, et al., 2008), a phenomenon also observed in monkeys (Dal Monte, et al., 2014).

If dogs' ability to perform well on OCTs is dependent on their ability to look at humans and use human gestures, which is dependent on their central oxytocin function (as demonstrated in humans), increasing central oxytocin availability should improve their performance on OCTs. The aim of this study was to test the effect of intranasal oxytocin administration on dogs' performance on an OCT, using two different cues, momentary distal pointing and gazing (without head-turn). It was hypothesized that: (1) dogs would perform better on the OCT after an intranasal treatment with oxytocin than after a control saline administration when momentary distal pointing cues were given, and (2) oxytocin would both increase dogs' gazing toward the experimenter's eyes and their trust of the gaze cue, which would therefore improve their performance when gaze cues were offered as well. However, as Bartz, et al. (2011) highlight in their review of the pro-social effects of oxytocin in humans, increases in trust do not occur in situations with prior trust violations, out-groups or clinical populations who are rejection-sensitive. In these groups of people, trust was actually decreased by oxytocin administration. Therefore, whilst we did not expect the dogs in our study to fall into any of the categories mentioned above, we could not rule out the possibility that they would interpret the gazing cue negatively, and that this negative interpretation would be enhanced by oxytocin, thereby decreasing performance after oxytocin administration.

Method

Subjects. Seventy-five pet dogs (33 males, 42 females) were recruited for the study. Owners with healthy dogs over 12 months old were invited to participate, but owner-reported pregnant, lactating or visually-impaired dogs were excluded. Owners were recruited through poster

advertisements at Monash University Caulfield and Clayton campuses, as well as through university e-newsletters and social media websites. Dogs were randomly allocated into two separate groups: those that received oxytocin first and saline second (oxy-sal) and those that received saline first and oxytocin second (sal-oxy). Of the 75 dogs recruited, two males and 11 females did not complete the study; two dogs failed the pre-training, five dogs failed the test of motivation, three dogs passed the test of motivation but refused to continue when the more difficult cues were introduced, one dog was too excitable, and two dogs were withdrawn from the study by their owners. Partial data could, however, be used for two of the female dogs with incomplete records, leaving a total of 31 males and 31-33 females in the analysis. This study was approved by the Monash University School of Biological Sciences Animal Ethics Committee (BSCI/2013/07).

Materials. Twenty-four international units (equivalent to 50µg) of oxytocin (Auspep, Melbourne, AU) diluted in 0.5 ml of 0.09% saline, or 0.5 ml of 0.09% saline only (acting as a control) were administered to the nostrils of each dog, with a half-dose in each nostril. Treatments were delivered using a Mucosal Atomizer Device (MAD 300, Wolfe Tory Medical Inc., Salt Lake City, UT) connected to a 1mL syringe, while the dogs were maintained in a head-up position. When it could not be determined whether a dose was successfully administered, a second administration (half-dose) was delivered in the nostril concerned.

Two identical, opaque spaniel bowls (19cm base diameter, 11cm rim diameter, 12cm high, 8cm deep) were used to conceal the food treats. Spaniel bowls were selected for their height and ability to conceal the treat from the dogs' vision. Two additional and identical spaniel bowls were placed underneath the two testing bowls and treats identical to those used in the experiment were hidden in the space between them. This method was used by Udell, Giglio and Wynne (2008b) to ensure that both bowls smelled of the treats and the dog was consequently not able to rely on olfaction when making its choice between the bowls. Treats were also hidden around the testing room so that the entire room smelled of treats. The treats used were lamb puff cubes: light, low fat cubes of lamb lung, puffed with air. Scores were recorded by the experimenter using a pen and paper and the same experimenter conducted all testing of dogs in the investigation.

Procedure. On the day of the testing session, owners were asked not to feed their dog prior to participation so that motivation to perform the task was high. In cases where testing occurred in the afternoon, some dogs were fed a small snack in the morning at the owner's discretion. Owners and their dogs came to the testing location on two separate occasions, five to fifteen days apart. When they arrived for their first testing session, the dog received one of two intranasal treatments,

oxytocin or saline. When they arrived for their second session, the dog received the other intranasal treatment. Tubes containing the treatments were labelled 'A' or 'B', so that both the experimenter and the owner were 'blind' as to which treatment the dog received on which day. Order of treatment administration was pseudo-randomised and counterbalanced. The dog was restrained by its owner while the experimenter administered the intranasal spray. The owner was then required to fill out a few questionnaires to be used in an associated study while the dog was free to roam the testing room and interact with its owner or the experimenter. The owner and dog could then leave the room to wander outside or remain inside. Forty-five minutes after the treatment was administered, the first pre-training session commenced. A forty-five minute window was selected in accordance with the majority of previous human (MacDonald et al., 2011) and a recent pig (Rault, 2013) study and can be accepted as a sufficient time period in which neuropeptides can reach the brain (Born, et al., 2002; Rault, 2013).

Pre-training. The experimental set-up was similar to that of Virányi, et al. (2008). The two spaniel bowls were placed 1.5 m apart and the experimenter kneeled 30 cm behind the mid-point between the bowls. The dog, restrained by its owner, faced the experimenter at a distance of 2.5 m. The experimenter first got the dog's attention by calling its name or an affirmatory epithet ("good girl/good boy"; no address was used if the dog was already looking and calling the dog's name proved distracting to the dog). The dog was then shown a treat before it was placed in one of the bowls. The experimenter then said the release word "ok" (in some cases a different release word, more familiar to the dog, was used, such as "okay", "free", "take" , "go on", "(go) get it". The owner then released the dog and allowed it to approach one of the food bowls. If the dog approached the bowl containing the treat, it was allowed to eat the treat before both bowls were collected by the experimenter; if the dog approached the empty bowl or the experimenter, both bowls were collected by the experimenter and the dog did not receive a treat. The dog had to select the correct bowl four times in a row to move on to the testing session proper. A 10-minute cut off time was applied to the pre-training; if the dog was unable to pass the pre-training within this time, it was excluded from the study. Most pre-training sessions required only four trials and the maximum number required was 25 trials for one dog in one of its pre-training sessions.

Testing. The experimental set-up was the same as in pre-training. Each testing session contained four blocks of fifteen trials (10 where a cue was provided and five in which no cue to the treat's whereabouts was provided). The control condition was used to verify that the dogs were not relying on scent to find the hidden food. Numerous studies have found that performance is at

chance level when a control condition is employed (Hare, et al., 2002; Riedel, et al., 2008; Soproni, et al., 2002; Udell, et al., 2008b; Wobber, et al., 2009).

The first test block (B1) comprised, in sequence: three control trials, five trials with the momentary distal point cue, two control trials and then another five trials with the momentary distal point cue. The second test block (B2) comprised, in sequence: three control trials, five trials with the gaze cue, two control trials and then another five trials with the gaze cue.
The third test block (B3) was the same as the first (B1), and the fourth block (B4) was the same as the second (B2). The ordering of the blocks was such that the easier point cue was delivered first so as not to discourage the dogs from participating by delivering a difficult gaze cue straight away. Having only 10 trials per block was also strategically designed to keep the dogs motivated. Position of the correct bowl (left or right) was predetermined according to a pseudo-randomised chart that did not allow more than two consecutive trials where food could be obtained on the same side. Each test block was preceded by a pre-training session to maintain motivation to approach the baited bowl. The dog was allowed approximately five minutes of free play with its owner between testing blocks to avoid burnout.

Momentary distal point cue. The experimenter was kneeling, propped up on her toes, with her arms by her side. She got the dog's attention and then raised her ipsilateral arm and pointed (using her index finger) towards the correct bowl for 1-2 seconds, keeping her head straight, before lowering her arm back down to her side and saying "ok" (or an alternative release word). The approximate distance between the experimenter's index finger and the rim of the baited bowl was 42cm and 50cm to the treat inside. The dog was then released and allowed to make a choice between the bowls.

Gaze cue. The experimenter was kneeling with her arms by her side, the tops of her feet flat on the floor to achieve better eye-level with the dog. She got the dog's attention and then gazed towards the correct bowl for 1-2 seconds, keeping her head straight. She then said "ok" (or an alternative release word) and the dog was then released and allowed to make a choice.

Control condition. The kneeling experimenter, propped up on her toes, got the dog's attention, then kept her head straight for 1-2 seconds, then said "ok" (or an alternative release word) before the dog was released by its owner and allowed to make a choice in the absence of any cue.

Scoring. Scores were recorded as correct responses out of 10 trials per block (20 per cue) for each testing session. If the dog did not move within five seconds of being released, the cue was

given again, as in Virányi, et al. (2008), and the dog could be prompted to move by its owner. If no choice was made, the experimenter decided subjectively whether this was due to a distraction. If it was clearly due to a distraction, the trial was repeated. In cases where the experimenter was unsure why the dog did not make a decision, the test of motivation used by Udell, et al. (2008a) (two pre-training trials, one to each side) was conducted. If the dog was found to be unmotivated, the trial was discontinued; if the dog was found to be motivated, the trial continued and the experimenter assumed that the 'no choice' outcome of the previous trial was probably due to the dog not understanding the task, so that the score for that trial was 'incorrect choice'. The vast majority of dogs were found to be motivated (i.e. did not need to be tested for motivation) throughout the entire testing session, or were excluded from the study. Choices were also considered incorrect if the dog approached the incorrect bowl or the experimenter.

Statistical analysis. The raw scores for each testing block of the OCT performed after oxytocin and saline administration were entered into IBM SPSS Statistics version 22 (SPSS IBM, New York, U.S.A, 2013). Blocks one and three were also combined to give a total score for the pointing cues and blocks two and four were combined to give a total score for the gazing cues. One sample t-tests were used to investigate whether performance on the task was different from what would be expected by chance. To test for learning within each session, we compared the mean of the first 10 point and gaze cue trials (B1 and B2, respectively) with the last 10 point and gaze cue trials (B3 and B4, respectively) using paired samples t-tests. To test the effect of treatment, an independent samples t-test was run on session 1 only. The effect sizes of all significant t-tests were measured using Cohen's *d*. The effect of treatment (oxytocin, saline), gender (male, female) and group (oxy-sal, sal-oxy) on difference scores (score after oxytocin – score after saline) was evaluated using mixed model analyses of variance (ANOVA). The effect size of all significant F-tests was measured using partial eta squared. The assumption of homogeneity of covariances was tested using Box's *M* and was not violated for any test. Likewise, the assumption of homogeneity of variances was tested using F_{max} and the Levene's test and was met for all measures. Šidák-corrected pairwise comparisons (Abdi, 2007) were employed *post-hoc* to test for the effect of treatment in the oxy-sal group and sal-oxy group dogs separately, and to test the effect of treatment in male and female dogs separately.

Results

Performance different from chance. Control trials where the dog chose the left bowl, right bowl and correct bowl were scored out of a possible 20 choices per session and the means and standard deviations are given in Table 2.1.

Table 2.1. Mean (± standard deviation) object choices out of 20 and sample size for control trials.

	M	*SD*	*N*
Left after oxytocin	9.32	4.98	62
Right after oxytocin	10.08	5.16	62
Correct after oxytocin	9.35	2.04	62
Left after saline	9.22	5.27	63
Right after saline	10.14	4.99	63
Correct after saline	8.87	1.96	63

The dogs performed significantly below chance levels (score of 10) during both testing sessions, which demonstrates that they were not relying on olfactory cues to find the hidden food treat for the session after oxytocin administration (t_{61} = -2.49, *P*=.016, *d* = -0.32), and for the session after saline administration (t_{62} = -4.58, *P*<.0001, *d* = -0.58). There were no biases for the left bowl after oxytocin administration (t_{61} = -1.07, *P*=.29), the right bowl after oxytocin administration (t_{61} = 0.12, *P*=.90), the left bowl after saline administration (t_{62} = -1.17, *P*=.25) or the right bowl after saline administration (t_{62} = 0.23, *P*=.82).

Mean scores and standard deviations for each block(s) are given in Table 2.2.

Table2. 2. Mean (± standard deviation) correct object choices out of 20 for all dogs combined, according to treatment.

	Oxytocin			Saline		
	M	*SD*	*N*	*M*	*SD*	*N*
Point B1	7.41	1.86	63	7.41	2.29	64
Point B3	8.32	1.64	63	8.05	1.74	64
Point total	15.73	3.01	63	15.45	3.63	64
Gaze B2	4.68	1.67	63	4.59	1.49	64
Gaze B4	4.82	1.49	62	4.92	1.49	63
Gaze total	9.52	2.17	62	9.51	2.09	63

Dogs performed significantly better than chance (score of 5) on point B1 after oxytocin (t_{62} = 10.28, *P*<.0001, *d* = 1.30), point B3 after oxytocin (t_{62} = 16.01, *P*<.0001, *d* = 2.02), point B1 after saline (t_{63} = 8.39, *P*<.0001, *d* = 1.05) and point B3 after saline (t_{63} = 14.00, *P*<.0001, *d* = 1.75). However, dogs performed no differently from chance on gaze B2 after oxytocin (t_{62} = -1.51, *P*=.14), gaze B4 after oxytocin (t_{61} = -0.94, *P*=.35) and gaze B4 after saline (t_{62} = -0.46, *P*=.65), and significantly worse than chance on gaze B2 after saline (t_{62} = -2.19, *P*=.033, *d* = -0.28).

The influence of cue type of the effectiveness of treatment. A repeated measures ANOVA was run using total scores for each cue (point and gaze) to test whether performance using either one was more affected by the treatment than the other. There was a main effect of cue, (F1, 61 = 232.74, P<.0001, partial η^2 = .79). There was no main effect of treatment, (F1,61 = .20, P = .66) and no interaction between cue and treatment (F1, 61 = .30, P = .59).

Learning within sessions. Paired samples t-tests revealed that learning occurred within each session using the point cue, as the subjects performed better on B3 than B1 after oxytocin, (t_{62} = -3.95, *P*<.0001, *d* = -0.52) and after saline, (t_{63} = -2.79, *P*=.007, *d* = -0.32). No learning was demonstrated within each session using the gaze cue, as dogs performed no differently on B4 than B2 after oxytocin (t_{61} = -0.44, *P*=.66) or saline (t_{62} = -1.35, *P*=.18).

As learning appeared to be taking place between the pointing trial blocks, only data relating to the second block using point cues (B3) were used in the following analysis. As no differences were observed between the gaze trial blocks, they were combined in the analysis to follow.

The effect of oxytocin on performance. Mean correct choices for the second point block (B3) of trials and the gazing blocks combined for session 1 are shown in Table 2.3.

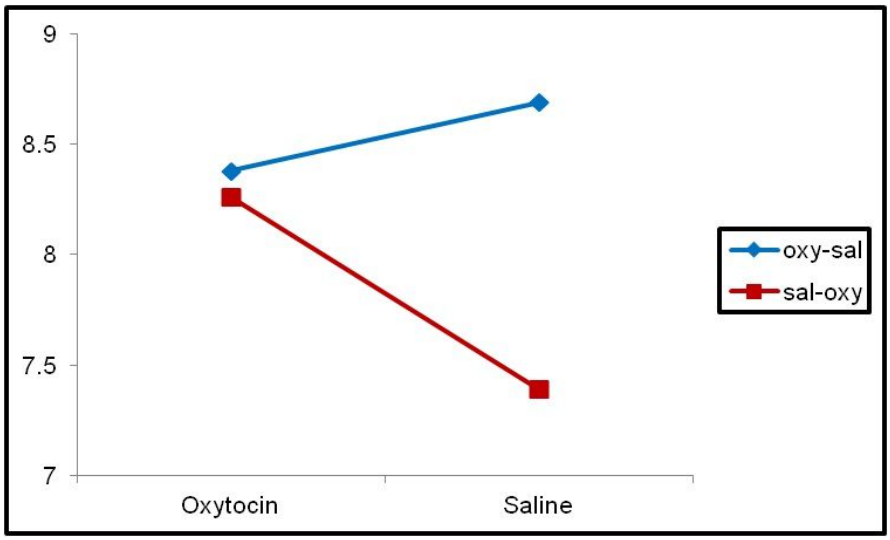
Table 2.3. Means (± standard deviation) correct choices out of 10 (point) and 20 (gaze) for dogs that received oxytocin and dogs that received saline on their first testing session.

	Oxytocin			Saline		
	M	*SD*	*N*	*M*	*SD*	*N*
Point B3	8.38	1.70	32	7.41	1.60	32
Gaze total	9.52	2.19	31	9.19	1.75	32

Independent samples t-tests were used to compare means between the dogs that received oxytocin and dogs that received saline in this session. The t-test revealed that dogs that received oxytocin on their first testing session performed significantly better than dogs that received saline on their first testing session for B3 (t_{62} = 2.35, *P*=.022, d=0.47). No significant difference was observed between the two groups of dogs for the gazing cues (t_{61} = 0.66, *P*=.51).

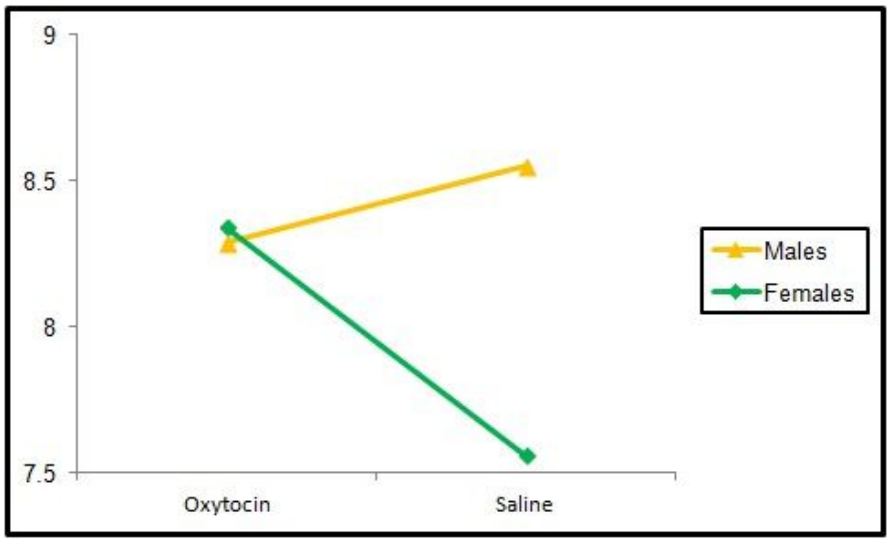
The effect of gender, group and treatment on performance. Examination of Figure 2.1 indicates that dogs in both groups performed similarly with the pointing signal in B3 after oxytocin but differently after saline, in that the oxy-sal dogs' performance improved after saline and the sal-oxy dogs' performance declined.

Figure 2.1. Mean point B3 scores (out of 10) for oxy-sal group and sal-oxy group dogs, according to treatment



There is a similar pattern in Figure 2.2 for males and females, in that males and females perform similarly after oxytocin, but male dogs' performance slightly improves after saline, whereas female dogs' performance after saline declines.

Figure 2.2. Mean point B3 scores (out of 10) for male and female dogs, according to treatment



A mixed model ANOVA revealed a significant interaction between treatment × group ($F_{1, 59}$ = 6.40, P=.014, partial η^2= .10) and treatment × gender ($F_{1, 59}$ = 5.01, P=.029, partial η^2= .08), but not among treatment × group × gender ($F_{1, 59}$ = 0.77, P=.013). There were no significant main effects of treatment ($F_{1, 59}$ = 1.36, P=.25), or gender ($F_{1, 59}$ = 1.75, P=.19), but there was a significant main effect of group ($F_{1, 59}$ = 4.35, P=.041, partial η^2= .07). Dogs that were administered oxytocin first performed more poorly after oxytocin than saline (mean difference = -0.31, SD = 1.87), whilst dogs that were administered saline first performed better after oxytocin than after saline administration (mean difference = 0.87, SD = 1.88). Male dogs performed worse after oxytocin administration (mean difference = -0.26, SD =1.81), but female dogs performed better (mean difference = 0.78, SD =1.98). Four Šidák-corrected pairwise comparisons were conducted using an adjusted alpha of .013 (1-tailed). Difference scores between treatments were significant in sal-oxy group dogs (t_{30} = 2.59, P=.0075, d = 0.54), but not oxy-sal group dogs (t_{31} = -2.40, P=.18), female dogs (t_{31} = 2.23, P=.0165) or male dogs (t_{30} = -0.80, P=.22).

For the gaze total scores, a mixed model ANOVA revealed no significant interaction effects between treatment × group ($F_{1, 58}$ = .50, P=.48), treatment × gender ($F_{1, 58}$ = .61, P=.44) and among treatment × gender × group ($F_{1, 58}$ = .57, P=.45). Nor were there any significant main effects of treatment ($F_{1, 58}$ = .01, P=.96), gender ($F_{1, 58}$ = .001, P=.98) or group ($F_{1, 58}$ = 0.73, P=.40).

Discussion

The ability of dogs to use momentary distal pointing cues, and the effect of oxytocin. Consistent with previous research, this study demonstrated an ability of domestic dogs to use

momentary distal pointing cues to find hidden food in an OCT (Hegedüs, et al., 2013; Miklósi, et al., 2005; Schmidjell, et al., 2012; Soproni, et al., 2002; Virányi, et al., 2008). In addition, consistent with our first hypothesis, a treatment effect was observed in that dogs performed significantly better after oxytocin than saline administration in session 1. This is consistent with findings for humans demonstrating that oxytocin increases perception of biologically relevant human motion (Kéri & Benedek 2009) which is imperative for social cognitive processing and communication, and supports the notion that oxytocin increases social cognition (see reviews by Bartz, et al., 2011; Guastella & MacLeod 2012). In addition, when examining difference scores between testing sessions, we observed performance improvements from session 1 to session 2 for point B3 scores in sal-oxy group dogs. Inspection of Figure 2.1 shows that their performance in session 2 was only bought up to the level of performance demonstrated by the oxy-sal group dogs in session 1, after oxytocin administration. The absence of a significant difference between sessions for the oxy-sal group dogs indicates that this group of dogs was able to maintain their performance at this level 5-15 days later, after saline administration. Thus oxytocin not only enhanced performance on the OCT, but the enhanced level of performance was maintained over time.

The effect of gender on the efficacy of oxytocin. The enhancing effect of oxytocin seems to have been driven by the female subjects in this study who performed better after oxytocin and more poorly after saline administration (see Figure 2.2). The reason why males were possibly not as influenced by oxytocin as females (whose performance was able to be brought up to the level of the males after oxytocin administration) may simply be ceiling effects, as they performed similarly after both treatments and significantly better than females after saline administration. The reason for the superior performance of male dogs compared to females after saline administration is unknown and somewhat surprising; in humans, females have shown greater social cognitive abilities than males, as demonstrated by their better perception of others' emotions (Brabec, Gfeller, & Ross, 2012; Donges, Kersting, & Suslow, 2012). However, the OCT differs in that it tests an ability to solve a task using human communicative cues, not human emotions. Estrogen is known to enhance the production of oxytocin and its receptor (Rissman, 2008) and this may explain why the female dogs in this study did not did not perform as well as human female subjects in other tests of social cognition, as the majority (88%) had been spayed, thereby reducing the volume of estrogen their bodies would be producing. However this does not explain why the male dogs (the majority of whom had also been neutered, 97%) performed so much better than the females dogs following saline.

The ability of dogs to use gazing cues, and the effect of oxytocin. Contrary to our second hypothesis, no treatment effect was observed for gazing cues. We did find some support, however,

for the negative interpretation of the gaze cue being dampened by oxytocin. For example, in gaze B2 after saline administration we obtained the same findings as Soproni, et al. (2001), who reported that dogs interpreted the gaze cue negatively, avoiding the bowl to which the experimenter gazed. Our lack of a similar finding for B2 after oxytocin administration supports our hypothesis that oxytocin increases trust in the dog, as it does in humans (Kosfeld, et al., 2005), despite the fact that the dogs were unable to use the cue, performing no better than chance after oxytocin administration. That this below-chance level performance was lacking in gaze B4 after saline administration may reflect the dogs learning that no aversive consequences would occur when they went to the bowl containing the treat, so they no longer used the gaze cue to complete the task, and just guessed.

The Clever Hans phenomenon. Whilst mean performance on the majority of the gaze cue blocks was at chance level, it is intriguing that mean control trial performance (where no cue was given) was *below* chance levels, as shown in Table 2.1. The so called 'Clever Hans' phenomenon (Pfungst, 1911), involving some form of unintentional or subconscious cueing from the owner, has been independently tested for in dogs subjected to an OCT with momentary distal pointing cues, and yielded negative findings (Hegedüs, et al., 2013; Schmidjell, et al., 2012), but we cannot completely rule this out as the reason for these unexpected results. The above-mentioned studies only tested for possible unintentional, subconscious cueing by the owner, not by the experimenter. In the current study it is conceivable that the experimenter was subconsciously 'hoping' that the dogs were not using scent to find the food in the control trials and may have been unintentionally cueing the dogs to go to the empty bowl in order to validate the experimental design. This highlights the critical importance of blind treatment testing for both the owner and experimenter, which was a strength of the current study. Nonetheless, the effect of the experimenter on the Clever Hans phenomenon warrants further study.

Learning within sessions. Another unexpected finding in the study was that of the learning observed within sessions for the pointing cues. Despite the pre-training that took place before B1, it appears that dogs were still learning to use the point cues to do the task in B1 compared to B3, where they performed better. This finding contrasts with those of previous studies, which did not report performance differences within sessions (Hare, et al., 2002; Miklósi, et al., 2005; Riedel, et al., 2006; Riedel, et al., 2008; Schmidjell, et al., 2012; Wobber, et al., 2009). As learning was observed within both treatment sessions, we do not believe this is a consequence of the oxytocin administration unique to our study. One possibility is that this 'learning' is a reflection of the dogs being less anxious about the novel environment in B3 compared to B1, and therefore less inhibited

in performing the task. However, it is still interesting that this was observed in our study but not in previous studies which employed similar testing methods and less habituation time to the testing environment. This disparity may be due to the fact that testing in the current study was carried out in a room that was not well insulated against distracting external sound disturbances, and therefore may have required more habituation time than the testing locations employed in other studies.

Limitations and future directions. The above-mentioned external sound disturbances may have varied on different testing days, which was an unavoidable limitation of our study. Other limitations of the present investigation included possible variation in the dog's hunger levels among sessions. Although efforts were made to test a particular dog at the same time of day in each session, this was not always possible. Owners were also instructed to keep the dog's day as similar as possible between sessions, but this could not be fully controlled either. Given the gender differences we observed, future studies should consider the effect that spaying and neutering has on oxytocin function, as our findings, compared to those of human studies on social cognition, may suggest this has particular influence in females. Lastly, although efforts were made to be as consistent as possible with the majority of previous studies' dosages and behavioural testing timeframes, it is currently unknown what constitutes the optimal behavioural testing time after administration of oxytocin in dogs, and how long the behavioural effects last. Extrapolating from the findings of a human study investigating the intranasal application of 40IU and 80IU of a very similar peptide, vasopressin (Born, et al., 2002), and a recent pig study investigating the intranasal application of 24IU of oxytocin (Rault 2013), we can reasonably assume that oxytocin is still active in the brain 100-120 minutes after administration, and potentially longer. Therefore the behavioural effects in the current study were likely to have been maintained for the entire testing session, which normally lasted between 90 and 120 min.

Conclusion

Administration of oxytocin was effective in aiding dogs' performance on the OCT using momentary distal pointing cues. Moreover, this enhancing effect persisted at least 5-15 days later, in the absence of further oxytocin administration. Oxytocin also appeared to decrease dogs' aversion to gazing cues, with performance being at chance level after oxytocin administration but below chance level after saline administration.

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Chapter 3 – Associations between owner-perceived closeness to, and intelligence of, their dog, and the animal's actual performance level in an object-choice task.

Declaration for Thesis Chapter 3

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I contributed the intellectual property, administered and scored the questionnaires, conducted of all analyses and wrote the paper.	95%

The following co-authors contributed to the work.

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
[name 1]	Dr. Jean-Loup Rault	
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The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 06/15/2015
Main Supervisor's Signature	[redacted]	**Date** 08/05/2015

Abstract

A positive association has been found between owner-rated dog cognition and owner-perceived closeness to their dog, using the Perceptions of Dog Intelligence and Cognitive Skills (PoDIaCS) survey and the Monash Dog Owner Relationship Scale (MDORS). Oxytocin has been positively associated with both bonding in mammals and non-verbal intelligence in humans and could therefore potentially explain this relationship in dogs. The aim of this study was to ascertain whether a pet dog's performance on an object choice task (OCT), which objectively measures dogs' ability to use human non-verbal, social gestures to find a food reward, could be predicted by their owners' scores on three different surveys: (a) the MDORS, (b) the Pet Attachment Questionnaire (PAQ), which measures levels of anxious and avoidant attachment styles, and (c) a modified version of the PoDIaCS. It was hypothesized that dogs that performed the task better would have owners with higher levels of self-reported closeness to their dog and who perceive their dog to have greater cognitive abilities. In the experiment reported by Oliva, Rault, Appleton and Lill (2015) seventy-five (33 M; 42 F) pet dogs and their owners were recruited to participate in two different OCT testing sessions, once after receiving intranasal oxytocin and once after receiving saline, 5-15 days apart. Owners completed the PoDIaCS and another survey relating to pet ownership in session 1, after the administration of the intranasal treatment and before the commencement of the task, and the MDORS and PAQ at the same time in session 2. The present study showed that the owners' responses on the anxious subscale of the PAQ were a negative predictor of their dogs' performance on the OCT using pointing cues after saline administration. Responses of owners on Subscale 6 of the PoDIaCS, 'contagion of human emotions', positively predicted performance on the OCT using gazing cues after saline administration. However, none of the questionnaire subscales significantly predicted performance on the OCT after oxytocin administration. Results suggest that a dog's ability to follow pointing cues and anxious attachment in owners are related, whilst a dog's ability to follow gazing cues is related to owner-rated empathic ability of the dog.

Introduction

Like human infants, the domestic dog displays attachment behaviors to humans that are absent in even intensively hand-reared wolves (Topál, et al., 2005), the ancestors of the domestic dog (Pang, et al., 2009). It appears as though the domestication of dogs and subsequent exploitation of them as human companion animals has facilitated the formation of human-dog bonding. One way to evaluate the strength of a human-dog bond is by using questionnaires targeting the dogs' owners. In 2006, a 28-item questionnaire, the Monash Dog Owner Relationship Scale (MDORS), was developed; it quantifies the 'perceived emotional closeness' of the human-dog bond, the degree of 'dog-owner interaction', as well as the 'perceived costs' (both monetary and to lifestyle) involved in the ownership (Dwyer, et al., 2006). This scale has good content validity and good internal consistency, with Cronbach alpha values (a co-efficient of internal consistency commonly used as an estimate of reliability) of 0.84 for both 'perceived emotional closeness' (subscale 2) and 'perceived costs' (subscale 3) (Dwyer, et al.). The 'dog-owner interaction' (subscale 1) co-efficient was not as strong, with an alpha value of 0.67 (Dwyer, et al.).

The strength of the bond, however, does not provide an indication of attachment *style*. Ainsworth, et al. (1978) first demonstrated that human infants demonstrate different styles of attachment to their mothers and classified them as either secure or insecure. Insecure attachment can be characterised by anxious (anger or rejection of proximity seeking with their mothers after a period of separation) or avoidant (inconsistent in greeting their mother after a period of separation) behaviours in a strange situation (Ainsworth, et al.). It has been postulated that internal working models of attachment are developed from infancy and are carried forward into adulthood (for a review see, Bretherton & Munholland, 2008). In adulthood, a secure style of attachment is characterised by a relative ease and comfort in close relationships with minimal distressing fears of abandonment while an anxious style of attachment is characterised by a desire to be very close in relationships, often to the point which is uncomfortable for the partner, and accompanied by fears of abandonment and inadequacy (Brennan, et al., 1998). On the other hand, an avoidant style of attachment is characterised by an uncomfortableness in being too close to, or dependent on, others (Brennan, et al., 1998). Recently, Zilcha-Mano, et al., (2011) developed a questionnaire to evaluate these adult internal working models of attachment in relation to pets, the Pet Attachment Questionnaire (PAQ), a 26-item scale that was developed to assess an owner's degree of anxious and avoidant attachment to their pet. This questionnaire has been found to have good content and construct validity, good test-retest reliability (r=.75) and good internal consistency, with Cronbach alpha values for avoidant attachment between .84 and .91 and for anxious attachment between .86

and .92, with a weak, non-significant correlation between the two (*r* = .1 to .4) (Zilcha-Mano et al.). In line with literature regarding the different styles of attachment towards other adults (Brennan, et al.), anxious attachment to a pet involves constant thoughts that something bad might happen to their pet and that they will be left alone, resulting in a constant desire to be near their pet and frustration when their pet does not reciprocate with the same need for closeness (Zilcha-Mano, et al., 2011). On the other hand, avoidant attachment involves feelings of discomfort when their pet gets too close to them and an avoidance of intimacy with their pet (Zilcha-Mano, et al.). Absence of anxious and avoidant attachment types would suggest a secure attachment in which the owner is comfortable with the level of intimacy their pet shows them and the relationship is not affected by distressing thoughts or feelings (Zilcha-Mano, et al.). Interestingly, while PAQ anxious subscale is significantly correlated with both the anxious and avoidant subscales of the Experiences in Close Relationships Scale (ECR), which measures anxious and avoidant attachment in human relationships (Brennan, et al.), the PAQ avoidant subscale is not correlated with either. This suggests that a pet may fulfil a need unable to be filled by another human for people with avoidant internal working models of attachment.

Whilst questionnaires are good tools for quantifying the human-dog bond, they obviously cannot be used on dogs and so, are inherently biased towards the owner's perspective. Currently, there is no biological marker to assess human-dog bonding; however, many studies have implicated oxytocin as having potential in this role. In a pioneering study, Odendaal and Meintjes (2003) found that during a human-dog interaction mean arterial blood pressure decreased in both species at the same time serum levels of oxytocin increased significantly and was significantly greater than if the human was simply relaxing whilst quietly reading a book. This study employed both men and women and male and female dogs of differing breeds, but these groupings were not accounted for in the analysis. To account for these potentially confounding variables, Miller, et al. (2009) replicated Odendaal and Meintjes' study using 10 men and 10 women and analysed data for the sexes separately. Interestingly, women's oxytocin secretion significantly increased, whilst men's secretion level significantly decreased after interaction with their dogs. The reason for this gender dichotomy is unknown; however, it may be due to the influence of the sex hormones, known to modulate both the oxytocin peptide and the oxytocin receptor (for a review see, Gabor, et al., 2012), or differences in the type of interaction between male and female humans, and dogs. For example, although not recorded in Miller, et al.'s study, calm, soft petting may be more common from women, whilst rough play may be more common from men. Indeed, it was demonstrated that plasma cortisol concentrations rose in dogs petted for twenty minutes by men after a venipuncture procedure, but

not in dogs petted by women (Hennessy, et al., 1997), indicating that males did not stimulate a significant anxiolytic effect in dogs petted after venipuncture. However, when men were trained to pet in a similar fashion to most women, no differences between cortisol levels in dogs petted by men and women were evident (Hennessy, et al., 1998). There was, however, considerable individual variability in serum oxytocin levels among the men and among the women in Miller, et al.'s study. In addition, one woman and one man appeared as outliers, with values two standard deviations greater than the mean. The aberrant woman had the greatest number of pet dogs of all participants in the study, whilst the man was the only participant with small children. This might suggest that their oxytocinergic systems had been primed by past bonding experiences.

It is important to note, however, that the above-mentioned studies measured peripheral oxytocin concentrations, which may not reflect central oxytocin function, as the hormone does not readily pass the blood-brain barrier (Robinson, 1983; Veening, et al., 2010). Despite this, peripheral measures of oxytocin concentration have continued to be the most widely investigated neurochemical metric in human-dog bonding studies (Handlin, et al., 2011; Mitsui, et al., 2011; Nagasawa, et al., 2009). Keeping gender and dog breed constant by only including female humans and male Labrador dogs, Handlin, et al. found that serum oxytocin levels significantly increased in both the dog and female owner after just three minutes of petting, stroking and talking to the dog. However, when analyzing grouped data, it is difficult to conclude which dogs, if any, are more bonded with their owners. To investigate this, Nagasawa, et al. separated participants into two distinct groups, one which expressed a significantly higher perceived degree of satisfaction and communication with their dogs than the other group. After 30 minutes of interaction with their dogs, members of the former group excreted significantly higher concentrations of oxytocin in their urine than members of the latter group, and owned dogs that gazed at them for longer periods within the 30 minute interaction. This was only true, however, for interactions where owners were instructed to look back at their dogs, not for interactions where they were instructed *not* to look back at their dogs. This not only supports the role of oxytocin as one of the neuropeptides involved in human-dog bonding, but also of gaze as an important factor, similar to the situation observed in human infants (Dickstein, et al., 1984; Striano, et al., 2006).

In humans, intranasal administration of neuropeptides is a popular method to study their central effects, as they are known to bypass the blood brain barrier and act directly at the brain (Born, et al. 2002). Administered intranasally, oxytocin increases the salience of social cues (see review by Bartz, et al. 2011), as well as causing an increase in gaze towards the eye region of other human faces (Guastella, et al., 2008) (also observed in monkeys (Dal Monte, et al., 2014)).

Furthermore, variations in the oxytocin receptor gene have been associated with infant attachment (Chen, et al., 2011) and interact with internal working models of attachment in adulthood in assessing risk and in feelings of closeness (Denes, 2015). Variations in this gene have also been associated with poorer non-verbal intelligence (Lucht, et al., 2009) decreased amygdala activation during the processing of emotionally-salient human faces (Tost, et al., 2010), poorer performance on the "reading the mind in the eyes" test (Rodrigues, et al., 2009) and autism (Wu, et al., 2005), a syndrome characterized by social deficits (for a review see, Volkmar, 2011). A recent study revealed that oxytocin, delivered intranasally, also influences dogs' ability to perform an 'Object Choice Task' (OCT) which tests their ability to use human social cues to find hidden food treats (Oliva, et al., 2015). Data from this study will be drawn on in the current investigation of the relationship between the dog owners' perceptions of the strength of their bond with their dog and of the dog's intelligence, and the dog's actual performance level on the OCT (see Table 3.1 for relevant means and standard deviations).

Table 3.1. Mean (± standard deviation) correct object choices out of 20 for all dogs combined, according to treatment (Oliva, et al., 2015).

	Oxytocin			Saline		
	M	*SD*	*N*	*M*	*SD*	*N*
Point total	15.73	3.01	63	15.45	3.63	64
Gaze total	9.52	2.17	62	9.51	2.09	63

Recently a survey was developed that aimed to determine owner-perceived cognitive abilities in dogs (in general), the Perceptions of Dog Intelligence and Cognitive Skills (PoDIaCs) survey (Howell, et al., 2013). The survey measures owner-perceived abilities across eight cognitive domains: 'recognition of human emotions', 'learned problem-solving abilities', 'instinctive awareness of human attention', 'learned awareness of human attention', 'deception', 'contagion of human emotions', 'instinctive problem-solving abilities' and 'general intelligence compared to humans'. Each cognitive domain was found to have good internal consistency, with Cronbach alpha values of 0.74-0.91 and each was significantly correlated with the second subscale of the MDORS, the owner's 'perceived emotional closeness' with his/her dog (Howell, et al.). It is currently unknown, however, if either of these variables relates to actual cognitive ability.

If oxytocin facilitates i) bonding of a dog to a human and ii) the ability of the dog to use human social cues, we would expect to find a correlation between owner-rated measures of bonding and social cognition, and actual ability of the dogs to perform well on a task that tests their ability to use human social cues. The aim of our study was to elucidate whether dog owners' scores on the MDORS, PAQ and PoDIaCS were related to the dogs' actual performance on an OCT. In particular, we expected to find positive associations between the MDORS subscale 2, which measures the 'perceived emotional closeness' of the bond, and the PoDIaCS subscales 3 or 4, which contain questions appropriate to the OCT, such as "my dog can instinctively understand human gestures like pointing at food or toys" (subscale 3) and "my dog can learn to understand human gestures like pointing at food or toys" (subscale 4), and OCT scores. The influence of attachment style (known to be affected by the oxytocin receptor gene (Chen, et al., 2011; Denes 2015)) and other factors relating to the dog and owner that may influence oxytocin function (such as parental and pet ownership history (Miller, et al., 2009) and order of intranasal treatments (Oliva, et al., 2015)) were also explored.

Methods

Study animals. Seventy-five pet dogs (33 males, 42 females) and their owners (14 males, 48 females) were recruited for the study, which was part of a larger investigation of the effect of oxytocin on dogs' performance on an OCT (Oliva, et al., 2015). Owners were recruited through poster advertisements at Monash University's Caulfield and Clayton campuses, as well as through University e-newsletters and social media websites. The study was approved by the Monash University School of Biological Sciences Animal Ethics Committee (BSCI/2013/07) and the Monash University Human Research Ethics Committee (CF12/0847-2012000385).

Materials. 0.5 ml saline solutions containing oxytocin (24 IU) or saline only, were administered to the nostrils of the dogs using a Mucosal Atomizer Device connected to a 1mL syringe. Four identical, opaque spaniel bowls were used to conceal the food treats and OCT scores (out of 20 per cue) were recorded by the experimenter using pen and paper. A modified version of the PoDIaCS (specifically about the owner's dog, as opposed to dogs in general), and another questionnaire about other factors relating to the dog and owner that may influence oxytocin function were administered to participants in session 1 and the MDORS and PAQ were administered in session 2, and completed by participants using pen and paper.

Procedure. Dogs were pseudo-randomly separated into two groups for the experimental study (Oliva, et al., 2015), those that received oxytocin in their first session and saline in their second

session (oxy-sal) and vice versa (sal-oxy). Upon arrival at the testing room for their first testing session, the dogs received one of two intranasal treatments, oxytocin or saline. When dogs arrived for their second session, they received the other intranasal treatment. Both the experimenter and the dog owner were 'blind' to which treatment the dog received on which day, as solutions were labelled 'A' or 'B' and only decoded after the study concluded. Regardless of which group the dogs were allocated to for the experimental study, owners of all dogs involved in the current study completed the questionnaires in the same order. In the first testing session the owner was asked to answer a few questions about dog ownership and complete the modified version of the PoDIaCS and in the second testing session, the owner completed the MDORS and the PAQ, while the dog was free to roam the testing room, interact with the owner and experimenter, or wander outside. Forty-five minutes after the treatment was administered, the OCT commenced, which involved (a) pre-training (before each 10 trial block of the OCT proper): four correct trials in a row where the dog was shown a food treat being dropped into one of two dog bowls, either side of the experimenter (delivered using the ipsilateral hand from a kneeling position, 75cm from the target) (b) 20 trials (block 1 and block 3) where the dog was given a momentary distal pointing cue (a 1-2 second point delivered using the ipsilateral index finger from the same kneeling position) to indicate the location of the hidden food reward located in one of the two dog bowls and (c) 20 trials (block 2 and block 4) using a gaze cue (a 1-2 second gaze shift delivered from the same kneeling position, keeping the head straight). Five control trials per block, where no cue was given, were also delivered to ensure the dogs were not relying on scent to solve the task. This usually lasted between 45 and 60 minutes. The test was carried out by the experimenter with the help of the owner, who called the dog back to the starting position between trials and restrained it until the experimenter said the release word "OK". The experimenter recorded the score for each trial as either correct (if the dog approached the bowl with the hidden food treat) or incorrect (if the dog approached the empty bowl or the experimenter) before starting the next trial. Trials were also considered incorrect of the dog made no choice but was deemed motivated to complete the task by participating when two of the easier pre-training cues were then given as a test of motivation.

Data analysis. The raw scores (out of 20) were calculated for each cue used in the OCT proper after oxytocin and saline administration and entered into IBM SPSS Statistics version 22 (SPSS IBM, New York, U.S.A, 2013). Totals for each subscale in the PoDIaCS, MDORS and PAQ were calculated and divided by the number of items in that subscale to obtain overall mean score values. Some items were reverse-scored according to the scoring instructions for the particular questionnaire. All MDORS subscales were scored such that higher values reflect greater relationship

quality, so higher values on MDORS subscale 3 'perceived costs' actually represent lower perceived costs.

Pearson's correlations were run between the cognition and the attachment subscales to see if previous findings of an association between owner-reported bonding/attachment and owner-perceived cognition ratings (Howell, et al., 2013) were replicated in our study, and to investigate the effect of attachment style.

Multiple regression was employed to test the predictive power of other factors relating to the dog, and ownership of the dog, in response to each subscale. To test whether dogs' performance on the OCT could be predicted by their owners' questionnaire data, other factors relating to ownership, gender of the dog and order of treatment administration. Hierarchical multiple regression was run for each of the outcome variables: point oxytocin, point saline, gaze oxytocin and gaze saline. Given the enhancing effect of oxytocin, which was maintained for at least 5-15 days (Oliva, et al., 2015), regressions were run separately for each treatment. Dummy variables were created for the 'other factors' predictors: previous dog owner, previous non-dog owner (owners with a history of owning pets other than dogs), owner parental history, young dog, old dog, gender of owner, entire dog, inside dog. Dummy variables were also created for order of treatment administration and gender of dog. Each subscale of the PoDIaCS, MDORS and PAQ was entered at step 1 of the regression; 'other factors' were entered at step 2 and gender and order of treatment administration were entered at step 3. Preliminary analyses were conducted for each of the regressions to ensure that there was no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. An alpha level of 0.05 was selected for all tests of significance.

Results

Sample means and standard deviations for each of the subscales are given in Table 3.2.

Table 3.2. Descriptive statistics for owners' scores on each subscale of the PoDIaCS, MDORS and PAQ.

	M	*SD*
PoDIaCS 1: recogition of human emotions	3.86	0.55
PoDIaCS 2: learned problem-solving abilities	3.82	0.62
PoDIaCS 3: instinctive awareness of human attention	3.75	0.68
PoDIaCS 4: learned awareness of human attention	4.34	0.50
PoDIaCS 5: deception	3.11	1.03
PoDIaCS 6: contagion of human emotions	3.06	0.66
PoDIaCS 7: instinctive problem-solving abilities	2.80	0.80
PoDIaCS 8: general intelligence compared with humans	2.36	0.97
MDORS 1: dog-owner interactions	3.83	0.60
MDORS 2: perceived emotional closeness	3.91	0.52
MDORS 3: perceived costs	4.00	0.53
PAQ avoidant	1.51	0.51
PAQ anxious	2.33	0.86

N=75

Pearson's correlations between each of the perceived intelligence subscales and each of the attachment subscales are given in Table 3.3.

Table 3.3. Pearson Correlation Matrix Describing Relationships between the 8 PoDIaCS subscales and the 3 MDORS and 2 PAQ subscales.

PoDIaCS subscales	MDORS 1: dog-owner interactions	MDORS 2: perceived emotional closeness	MDORS 3: perceived costs	PAQ: avoidant	PAQ: anxious
1: recognition of human emotions	0.22	0.15	0.02	0.07	0.04
2: learned problem solving abilities	0.16	-0.11	-0.07	0.18	-0.04
3: instinctive awareness of human attention	**0.29****	0.17	0.19	-0.08	0.13
4: learned awareness of human attention	0.20	0.03	-0.03	-0.07	0.01
5: deception	**0.25***	-0.16	-0.08	0.01	-0.18
6: contagion of human emotions	0.07	0.14	-0.03	0.20	**0.27***
7: instinctive problem-solving abilities	0.20	0.12	0.12	0.004	0.03
8: general intelligence compared with humans	**0.33****	**0.26***	**0.36****	-0.17	-0.11

*P<.05, **P<.01

As evident from Table 3.3, only subscale 8 of the PoDIaCS was positively associated with the MDORS subscale 2, subscales 3, 5, 8 were positively associated with the MDORS subscale 1 and the PoDIaCS subscale 8 was positively associated with the MDORS subscale 3. Furthermore, we also found a positive association between subscale 6 of the PoDIaCS and PAQ anxious subscale.

To test whether any 'other factors' relating to the dog and ownership of the dog contributed to questionnaire scores, multiple regressions were run for each subscale of each questionnaire with the 'other factors' as predictor variables. None of the factors were successful in predicting scores for the PoDIaCS subscales 1-5, and 8, the MDORS subscales 1-3 and the PAQ anxious subscale. However, 'previous dog owner' negatively influenced PoDIaCS 6 scores (contagion of human emotions), whilst

parental history positively influenced PODIACS 6 scores, with 20% of the variance being explained by the model ($F_{8, 66}$ = 2.11, *P*=.047). Male owners and previous non-dog owners positively influenced PoDIaCS 7 scores (instinctive problem-solving abilities), with 23% of the variance being explained by the model, ($F_{8, 66}$ = 2.49, *P*=.020). Parental history positively influenced PAQ avoidant scores, with 23% of the variance being explained by the model, ($F_{8, 66}$ = 2.52, *P*=.019).

Regression co-efficients for the above-mentioned significant models can be found in Table 3.4.

Table 3.4. Unstandardized (*B*) and Standardized (*β*) Regression Coefficients for each Predictor Variable in a Hierarchical Multiple Regression for PoDIaCS 6, PoDIaCS 7 and PAQ avoidant Scores.

	B	*SE B*	*β*
PoDIaCS 6			
Constant	2.92	0.25	
Young dog	0.17	0.17	.13
Old dog	-0.09	0.22	-.05
Entire	0.33	0.30	.13
Male owner	0.35	0.20	.21
Previous dog owner	**-0.49***	0.21	-.30
Previous non-dog owner	0.21	0.18	.15
Parental history	**0.38***	0.16	.29
Inside dog	0.09	0.19	.06
Note R^2=.20, *P<.05			
PoDIaCs7			
Constant	2.62	0.31	
Young dog	-0.03	0.20	-.02
Old dog	-0.14	0.27	-.06
Entire	-0.60	0.36	-.19
Male owner	**0.66****	0.25	.32
Previous dog owner	-0.30	0.25	-.15
Previous non-dog owner	**0.82*****	0.22	.48

Parental history	-0.11	0.20	-.07
Inside dog	-0.18	0.23	-.09
Note R^2=.23, **P<.01, ***P<.001			
PAQ avoidant			
Constant	1.52	0.19	
Young dog	0.14	0.13	.14
Old dog	-0.15	0.17	-.11
Entire	0.18	0.23	.09
Male owner	0.30	0.16	.23
Previous dog owner	-0.24	0.16	-.19
Previous non-dog owner	0.16	0.14	.15
Parental history	**0.31***	0.13	.30
Inside dog	-0.21	0.14	-.17
Note R^2=.23, *P<.05			

Of the 75 dogs involved in the study, two males and 11 females did not complete the two sessions of the experimental study successfully, leaving a total of 31 males and 31 females in the following hierarchical multiple regression analysis.

None of the models were able to predict scores for pointing or gazing after oxytocin administration. For pointing scores, the subscale scores entered at Step 1 explained an insignificant 18% of the variance, ($F_{13, 49}$ = 0.84, P=.62). The 'other factors' were entered at Step 2 but did not contribute significantly to the model (R^2 change = .18, F change $_{(21, 41)}$ = 1.39, P=.23). Additionally, 'oxytocin administered first' and 'female' were entered at Step 3, but they too did not significantly contribute to the model (R^2 change =.01, F change $_{(23, 39)}$ = 0.23, P=.79).

For gazing scores, the subscale scores entered at Step 1 explained an almost significant 35% of the variance, ($F_{13, 48}$ = 1.93, P=.050) (with only the PoDIaCS subscale 4 – learned awareness of human attention – negatively predicting performance). Once again, the 'other factors' entered at Step 2 did not contribute significantly to the model, (R^2 change = .09, F change $_{(21, 40)}$ = 0.74, P=.66), and 'oxytocin administered first' and 'female' also didn't significantly contribute to the model when entered at Step 3, (R^2 change =.02, F change $_{(23, 38)}$ = 0.76, P=.48).

In contrast, model 3 was successful in predicting pointing scores after saline administration and model 1 was successful in predicting gazing scores after saline administration. For pointing scores, the subscale scores entered at Step 1 explained an insignificant 29% of the variance, ($F_{13, 50}$ = 1.57, P=.13). The 'other factors' entered at Step 2 did not contribute significantly to the model (R^2 change = .07, F change $_{(21, 42)}$ = 0.58, P=.79). However, when 'oxytocin administered first' and 'female' were entered at Step 3, they did contribute significantly to the model (R^2 change =.22, F change $_{(23, 40)}$ = 10.44, P<.0001).

For gazing scores, the subscale scores entered at Step 1 explained a significant 35% of the variance, ($F_{13, 49}$ = 2.07, P=.034). The 'other factors' entered at Step 2 did not significantly contribute more to the model (R^2 change = .14, F change $_{(21, 41)}$ = 1.41 P=.22), nor did 'oxytocin administered first' and 'female' when entered at Step 3, (R^2 change =.02, F change $_{(23, 39)}$ = 0.77, P=.47).

Regression coefficients for the above-mentioned significant models are given in Table 3.5.

Table 3.5. Unstandardized (*B*) and Standardized (*β*) Regression Coefficients for each Predictor Variable in model 3 (MDP cues) and model 1 (gaze cues) of a Hierarchical Multiple Regression for Point and Gaze Cue Scores after Saline Administration on The Object Choice Task.

	B	*SE B*	*β*
Model 3 – Point cues			
Constant	0.21	9.00	
PoDIaCS 1	0.39	1.16	.06
PoDIaCS 2	-1.33	1.12	-.21
PoDIaCS 3	-0.30	0.85	-.05
PoDIaCS 4	2.07	1.30	.25
PoDIaCS 5	0.51	0.53	.15
PoDIaCS 6	0.30	0.98	.05
PoDIaCS 7	-0.54	0.86	-.12
PoDIaCS 8	0.71	0.67	.19
MDORS 1	1.80	1.06	.28
MDORS 2	0.67	1.34	.09

MDORS 3	-0.47	1.20	-.07
PAQ avoidant	1.14	1.40	.15
PAQ anxious	**-1.49***	0.69	-.34
Entire	-1.39	1.70	-.10
Male owner	1.58	1.44	.16
Previous dog owner	0.15	1.25	.02
Previous non-dog owner	1.61	1.37	.20
Parental history	-2.12	1.08	-.29
Inside dog	1.56	1.08	.19
Young dog	0.89	1.10	.12
Old dog	1.11	1.50	.11
Female	**-3.20****	0.96	-.44
Oxytocin First Visit	**3.12****	0.88	.43

Note R^2=.29 for Step 1; ΔR^2=.07 for step 2; ΔR^2=.22 for step 3 *P<.05, **P<.01

Model 1 – gaze cues

Constant	3.60	4.30	
PoDIaCS 1	-1.27	0.64	-.33
PoDIaCS 2	0.65	0.59	.18
PoDIaCS 3	-0.78	0.48	-.25
PoDIaCS 4	0.05	0.62	.01
PoDIaCS 5	0.28	0.31	.14
PoDIaCS 6	**1.72****	0.54	.54
PoDIaCS 7	0.31	0.41	.12
PoDIaCS 8	-0.02	0.36	-.01
MDORS 1	0.16	0.61	.04
MDORS 2	-0.67	0.74	-.16
MDORS 3	1.17	0.67	.29
PAQ avoidant	0.45	0.78	.10
PAQ anxious	0.36	0.36	.14

Note R^2=.35 for Step 1. **P<.01

As evident from Table 3.5, the PAQ anxious subscale was a significant negative predictor of performance on the OCT with pointing cues after saline administration; indeed, dogs owned by people who score one point higher on this subscale than other dog owners would be expected to score 1.49 points lower on the task than dogs owned by people who score one point lower on this subscale. Scores were also likely to be lower for female dogs and higher for dogs that received oxytocin on their first visit (thus making pointing after saline scores their pointing scores for their second visit). In contrast, the PoDIaCS 6 subscale (contagion of human emotions) was a significant positive predictor of performance on the OCT with gazing cues after saline administration whereby dogs owned by people who score one point higher on this subscale than other dog owners would be expected to score 1.72 points higher on the task than dogs owned by people who score one point lower on this subscale.

Discussion

Contrary to our hypothesis, no significant, positive correlation was obtained between the MDORS 2 (perceived emotion closeness) and the PoDIaCS 3 (instinctive awareness of human attention) or 4 (learned awareness of human attention). None of these subscales predicted OCT scores either, although, interestingly, PoDIaCS 4 was shown to negatively predict gaze scores after oxytocin, however this model was almost, but not quite, significant overall. There was, however, a significant, positive correlation between the MDORS 2 and the PoDIaCS 8 (general intelligence compared to humans). In addition, the PAQ anxious subscale negatively predicted OCT scores (as well as being female and receiving oxytocin second) using pointing cues and the PoDIaCS 6 predicted OCT scores using gazing cues. In contrast to Howell, et al.'s (2013) study, which showed a positive association between MDORS subscale 2 and all 8 subscales of the PoDIaCS, MDORS subscale 2 was correlated with only one of the eight cognitive domains assessed in the PoDIaCS subscale 8. The discrepancies between our findings and previous results are probably due to the fact that whilst our modified version of the PoDIaCS assessed perceived intelligence and cognitive skills of owned dogs, Howell et al.'s original survey assessed perceived intelligence and cognitive skills of dogs in general. Howell et al.'s study also had a much larger cohort of participants, so it is possible the discrepant findings are due to the current study having lower statistical power. The PoDIaCS subscale 8 was also associated with subscale 3 of the MDORS (perceived costs), which is consistent with Howell et al.'s findings, while the MDORS subscale 1 (dog-owner interactions), was associated with subscales 3: (instinctive awareness of human attention), 5: (deception) and 8, in the current study (and all but subscale 7 in the study by Howell, et al.). Taken together, these findings suggest that time spent with one's dog is more important than how emotionally close one feels towards their dog in perception

of intelligence and only minimally support our hypothesis that owners who are more closely bonded to their dog will perceive him/her to be more intelligent.

Additively, we found a positive association between subscale 6 of the PoDIaCS, (contagion of human emotions), and the PAQ anxious subscale. This may suggest that anxiously-attached owners believe more than non-anxiously attached owners that their dog can feel sad, afraid, angry and/or happy when its owner feels the same emotion. This may be a misperception, due to having higher levels of anxiety or the result of these owners expressing more emotions, particularly negative ones, which their dog finds distressing and to which it reacts more overtly. However, results suggest that dogs of owners who score highly on this subscale are more likely to perform more poorly on the OCT using pointing cues. O'Farrell (1995, as cited in O'Farrell, 1997) found a significant correlation between owner anxiety and over-excitement and displacement activities in the dog, hence, this poorer performance may be a direct effect of the anxious owner who was present in the room at all times.

When 'other factors' relating to ownership were considered, it was found that PoDIaCS 6 scores (contagion of human emotions) were negatively influenced by the factor 'previous dog owner', whilst 'parental history' positively influenced these scores, suggesting that first time dog owners and owners who have had children are more prone to believe that their dogs feel their emotions more than experienced dog owners and owners who have not had children. 'Male owners' and 'previous non-dog owners' positively influenced PoDIaCS 7 scores (instinctive problem-solving abilities). This may highlight differences in interactions between male and female owners and their dogs (Hennessy, et al., 1997; Hennessy, et al., 1998; Miller, et al., 2009) and also suggests that past experiences of pet ownership may affect the perception of current dog ownership, whereby experienced pet owners believe their dogs to have more instinctive problem-solving abilities. Furthermore, 'parental history' positively influenced PAQ avoidant scores, which suggests that owners with a parental history are more likely than owners who have not had children to form avoidant type attachments to their dog. According to Zilcha-Mano, et al. (2011), there is no correlation between avoidant styles of attachment towards humans and towards pets. Therefore, it is possible that due to the parent-child relationship(s) in their lives, the relationship to their pet is relatively low in priority and this is reflected as an avoidant owner-pet relationship.

Performance on the OCT after oxytocin administration could not be predicted by any of the statistical models. This may be because the dog's ability to perform the task correctly is enhanced by oxytocin administration (Oliva, et al., 2015) and therefore differs from what the owner is expecting.

Alternatively, in light of the findings relating to the PAQ anxious subscale and poorer performance on the OCT using pointing cues after saline, it could that mean that oxytocin, known also for its anxiolytic properties (de Oliveira, Zuardi, Graeff, Queiroz, & Crippa, 2012; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003), reduces the anxiety these dogs may 'absorb' from their owner, thereby disinhibiting their natural cognitive ability. The reasons mentioned above may also may explain why the PoDIaCS 4 was a negative predictor of performance, rather than a positive one as was hypothesized, in the almost significant multiple regression for gaze after oxytocin administration i.e. if the owners rated their dogs low for 'learned awareness of human attention' and the dogs performed better than expected due to oxytocin. Performance on the OCT after saline administration, however, could be predicted by the models. As previously mentioned, PAQ anxious scores negatively predicted OCT scores for pointing cues; thus, dogs owned by people who score higher on this subscale would be expected to perform more poorly on the task than dogs owned by people who score lower on this subscale. In addition, point scores after saline administration were likely to be lower for female dogs and higher for dogs performing the task for the second time (i.e. their second testing session), suggesting that male dogs are more skilled at the OCT than female dogs and that there is a learning effect associated with the task. However, findings in Oliva, et al. (2015) suggest that this is a reflection of the enhanced performance from oxy-sal group dogs being maintained in session 2, rather than a learning effect.

Performance using gazing cues was predictable from scores on the PoDIaCS 6 subscale (contagion of human emotions) after saline administration i.e. dogs owned by people who score higher on this subscale would be expected to score higher on the task than dogs owned by people who score lower on this subscale. This is interesting because this subscale was also associated with the PAQ anxious subscale, which was negatively influenced by previously owning a dog and positively influenced by parental history. This suggests that past experiences of owning a dog or parenting a child may not only affect serum oxytocin levels (Miller, et al., 2009), but perceptions about the current dog-owner relationship as well. This finding may suggest that gaze is important in human-dog communication for dogs with owners who are anxiously attached to them and hence, potentially more likely to express their emotions to their dog, who may respond accordingly.

Conclusions and Implications

Overall, this study found little evidence supporting an association between owner-perceived closeness with their dog, owner perceptions of the intelligence of their dog and the actual intelligence of their dog as manifested in the OCT. The investigation did, however, reveal that past experiences of bonding, with both pets and children, affected owners' perceptions of the cognitive

skills of the currently-owned dog and the style of attachment to it. Higher scores for anxious attachment to dogs significantly predicted lower scores on the OCT using pointing cues. An association was also found between anxious attachment and perceived 'contagion of human emotions', which significantly predicted higher scores on the OCT using gazing cues. This suggests that communicative signals may differ between, or be interpreted differently by, dogs owned by anxiously-attached humans and those owned by non-anxiously attached humans. Breeding dogs with high oxytocin function may increase the success of dogs as companion animals, as well as dogs as working animals reliant on the use of human social cues. This study has shown that owner-rated questionnaires are not a good indication of a dogs' ability to use human social cues, a possible reflection of central oxytocin function. In addition, training dogs, both for working roles and for pet obedience, may be further complicated by the attachment style of the handler/owner.

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Chapter 4 – The oxytocin receptor gene, an integral piece of the evolution of *Canis familaris* from *Canis lupus*

Declaration for Thesis Chapter 4

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
I contributed the intellectual property, designed the primers, obtained the DNA samples, conducted the analyses and wrote the paper.	90%

The following co-authors contributed to the work.

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
[name 1]	Ms. Yen Wong	
[name 2]	Dr. Jean-Loup Rault	
[name 3]	Dr. Belinda Appleton	
[name 4]	A/Prof Alan Lill	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 06/15/2015
Main Supervisor's Signature	[redacted]	**Date** 08/05/2015

Abstract

Previous research in canids has revealed both group (dog (*Canis familiaris*) versus wolf (*Canis lupus*)) and individual differences in object choice task (OCT) performance. These differences might be explained by variation in the oxytocin receptor (OXTR) gene, as intranasally administered oxytocin has recently been shown to improve performance on this task by domestic dogs (Oliva et al. 2015). This study looked at microsatellites at various distances from the OXTR gene to determine whether there was an association between this gene and: i) species (dog/wolf) and ii) good versus bad OCT performers. Ten primer sets were designed to amplify 10 microsatellites that were identified at various distances from the canine OXTR gene. We used 94 (52 males, 42 females) blood samples from shelter dogs, 75 (33 males, 42 females) saliva samples from pet dogs that took part in our experimental study (Oliva et al. 2015), and 12 (6 males, 6 females) captive wolf saliva samples to carry out our analyses. Significant species differences were found in the two markers closest to the OXTR gene, suggesting that this gene may have played an important part in the domestic dogs' evolution from the wolf, by enabling domestic dogs to use human social cues. However, no significant, meaningful differences were found in microsatellites between good versus bad OCT performers, which suggests that other factors, such as sex hormones and early life experiences, probably impacted test performance.

Introduction

Oxytocin, a neuropeptide, is commonly known for its role in mammalian bonding (Lim and Young, 2006). Several studies have found a role of peripheral oxytocin in the human-dog bond (Handlin, et al., 2011; Miller, et al., 2009; Mitsui, et al., 2011; Nagasawa, et al., 2009; Odendaal and Meintjes, 2003). However, peripheral measures of oxytocin are not necessarily a reflection of central function, as studies have demonstrated that oxytocin, as well as its structurally similar neuropeptide, arginine vasopressin, do not readily cross the blood-brain barrier (Robinson, 1983; Veening, et al., 2010; Vorherr, et al., 1968) and can be independently released in the brain and in the body (Engelmann, et al., 2000; Ludwig & Leng, 2006; Robinson & Jones, 1982). An alternative method to better evaluate the effects of central oxytocin function is to administer it intranasally. Neuropeptides administered in this way bypass the blood-brain barrier and gain direct access to the brain (Born, et al., 2002; Rault, 2013), then, rather than measuring the peptide itself, behaviors can be measured after peptide administration. This method has been exploited in many human studies and oxytocin is now receiving increasing attention for its role in human social cognition, as demonstrated in increased gaze to the eye region of human faces (Guastella, et al., 2008), improved perception of human movements (Keri and Benedek, 2009), enhanced facial processing, emotion recognition, memory and encoding of facial stimuli (see review, Guastella and MacLeod, 2012), and in increased trust (Kosfeld, et al., 2005). It is important to note, however, that the effects of oxytocin are not always pro-social; it can cause decreases in sociality under certain circumstances (see review, Bartz, et al., 2011).

Our recent study involving intranasal oxytocin administration in domestic dogs (*Canis familiaris*) found that it also increased the ability of dogs to use human communicative gestures to find food in an object choice task (OCT) (Oliva, et al., 2015). Dogs were tested on this task on two separate days, 5-15 days apart, once after oxytocin administration and once after saline. Order of treatments were pre-determined according to group allocation, *oxy-sal* or *sal-oxy*. The task involved a human experimenter using momentary distal pointing cues to indicate the location of a hidden food reward in one of two opaque containers to the right and to the left of her. Results showed that the dogs that received oxytocin in their first testing session (oxy-sal dogs) outperformed dogs that received saline, and this performance was maintained up to 15 days later, in the absence of further oxytocin administration, where they performed equally as well as dogs receiving oxytocin in their second session (sal-oxy dogs) (Oliva, et al., 2015). We have suggested that changes in the canine oxytocinergic system may have been integral to the evolution of the domestic dog from the wolf (*Canis lupus*), the ancestor of the domestic dog (Wang, et al., 2013), as wolves have exhibited poorer

performances on OCTs compared to dogs (Hare, et al., 2002; Miklósi, et al., 2003; Virányi, et al., 2008).

Geneticists have investigated the association between human social cognition and the oxytocin receptor (OXTR) gene. For example, single nucleotide polymorphisms (SNPs) have been associated with attachment style (Chen, et al., 2011; Denes, 2015), autism (Jacob, et al., 2007; Wu, et al., 2005), as well as less extreme disorders of social functioning, such as the presentation of callous-unemotional traits and conduct problems (Dadds et al., 2014). Associations have also been found between OXTR gene SNPs and mothers' sensitivity to their toddlers (Bakermans-Kranenburg & van Ijzendoorn, 2008; Riem, et al., 2011), empathy and stress (Rodrigues, et al., 2009), optimism, personal mastery and self-esteem (Saphire-Bernstein, et al., 2011), positive affect and non-verbal intelligence (Lucht, et al., 2009), and sociality (Tost, et al., 2010). Associations have also been found between OXTR gene SNPs and amounts of oxytocinergic-rich connections in the hypothalamus and connections between the hypothalamus and both the amygdala and the dorsal anterior cingulate cortex (Tost, et al., 2010). Whilst there is strong evidence to support variants of this gene functioning differently in humans, there is little evidence to date supporting differing effects of variants of this gene in dogs. However, it is reasonable to assume that this is likely, given the vast array of homologous human-dog phenotypes that have been associated with analogous genes in the two species (Parker, et al., 2010; Sutter & Ostrander, 2004).

Allelic variability of the canine OXTR gene is poorly known. One study investigated SNPs within the OXTR gene in dogs and found associations with proximity-seeking and friendliness in two different dog breeds (Kis, et al., 2014). However, the SNP associated with friendliness was associated with opposing phenotypes in the two different breeds. Similar findings have been found with OXTR gene SNPs which are associated with autism affecting people of different ethnicities differently (Jacob, et al., 2007; Wu, et al., 2005). An alternative approach to increase the probability of finding differences, especially in a relative small sample of dogs of different breeds, is to look at microsatellites close to the gene of interest. Microsatellites are short tandem repeats that can occur within both the coding and non-coding (un-translated) regions of the genome and have been used extensively to demonstrate association between a genomic location and a trait (see review, Montaldo & Meza-Herrera, 1998).

Despite the fact that dogs generally perform well on OCTs, many studies report a wide range of individual variability in performance (Agnetta, Hare, & Tomasello, 2000; Hare, et al., 2002; Miklósi, et al., 1998; Udell, et al., 2008b; Udell, et al., 2008a, 2010b; Virányi, et al., 2008; Wobber, et

al., 2009) and may reflect differences in the OXTR gene. In dogs, the OXTR gene is *2.41 million base pairs* long and located on chromosome 20 at position *9.36 million base pairs* within the genome (http://www.ncbi.nlm.nih.gov/gene/484670, annotation release 103). We used microsatellites located at different distances from the OXTR gene (refer to Table 4.1) to investigate genetic association with OCT performance phenotypes. Given the species differences demonstrated in several studies (Hare, et al., 2002; Miklósi, et al., 2003; Virányi, et al., 2008), the objectives of the current study were to search for genotypic differences near the OXTR gene between: i) good and poor OCT performers (Oliva et al. 2015) and ii) domestic dogs and wolves.

Materials and Methods

Study animals. Owners of 75 pet dogs (33 males, 42 females) volunteered their dogs for a study investigating the effect of intranasal oxytocin on dogs' performance on an OCT (Oliva, et al., 2015). Dogs were required to be more than 12 months old, healthy, and not pregnant or lactating. No restriction was put on breed, as this was too difficult to control using pet and shelter dogs whose breeds were often mixed and not confirmable. Recruitment took place through poster advertisements at university campuses and on university e-newsletters and social media websites. To increase the effective sample size of domestic dogs for genetic analysis, an additional 94 blood samples (52 from males, 42 from females) were obtained from the Animal Aid animal shelter, Coldstream, Victoria, Australia. Blood, rather than saliva, was used as a matter of convenience as 0.5-1ml of blood were routinely collected from shelter dogs to test for heart-worm, and it was easy to take an extra 2-2.5ml for the purposes of our study. Twelve samples of wolf saliva were supplied by Wolf Park, Battle Ground, Indiana, USA. All wolves were a mix of the subspecies: Arctos, Occidetalis and/or Nubilus. The study was approved by the Monash University School of Biological Sciences Animal Ethics Committee (BSCI/2013/07).

Materials. Intranasal treatments containing 24 IU of oxytocin dissolved in a 0.5 ml saline solution, and containing saline only were stored in frozen tubes labeled 'A' or 'B'. Both the experimenter and the dog owner were 'blind' as to which tubes contained which treatments. Administration to the nostrils of the dogs was conducted via a Mucosal Atomizer Device connected to a 1mL syringe. Dogs received each treatment on a separate testing day in a pre-determined, pseudo-randomized and counterbalanced order. Oragene animal swabs (Canada) were used to collect buccal deoxyribonucleic acid (DNA) samples in the pet dogs involved in the study, as well as from the wolves. Approximately 2-2.5ml blood samples were drawn from the cephalic or jugular vein of the shelter dogs. Prior to collection, the area was clipped with surgical blades and cleaned with methylated spirits. Ten tandem repeats were visually identified close to the dog OXTR gene

(http://www.ncbi.nlm.nih.gov/gene/484670) and primers were designed using the online program, Primer 3 (http://frodo.wi.mit.edu) (see Table 4.1). 'Lamb puffs' were used as dog treats in the OCT and were concealed using four identical, opaque spaniel bowls. Pen and paper were used to score performance (correct/incorrect choice of concealed food reward) on the OCT using momentary distal pointing cues (out of 20 trials).

Table 4.1. Microsatellites and their distances from OXTR gene within the canine genome.

Primer	Location (base pairs)	Period size	Copy number	A	C	G	T
1	*2.04 million*	4	25.3	77	0	22	0
2	*2.49 million*	4	21	53	0	46	0
3	*8.51 million*	3	20.3	0	32	0	67
4	*8.53 million*	2	28.5	50	49	0	0
5	*9.11 million*	2	27	0	0	50	49
6	*9.66 million*	2	22	0	0	50	50
7	*9.74 million*	4	23	0	25	0	74
8	*9.94 million*	2	26.5	50	47	0	1
10	15.98 million	2	25	50	0	0	50

The OXTR gene is located at *9.36 million base pairs* within the genome (http://www.ncbi.nlm.nih.gov/gene/484670, annotation release 103).

Procedure. When the dog arrived at the testing located he/she was administered an intranasal treatment of saline or oxytocin and then a buccal swab was rubbed against the inside of the animals' cheeks for approximately 30 seconds and stored in a fridge at the testing location (this usually occurred in session 1) before being transported to the laboratory for genetic analysis. The dog was required to wait for 45 minutes after receiving the nasal spray before the testing began. In this time the dog was free to roam the testing room, interact with its owner and/or the experimenter, or wander outside. The testing session has been described by Oliva, et al. (2015); it

involved 20 trials with a momentary distal pointing cue provided by the experimenter, normally lasting between 45 and 60 minutes.

Sample preparation and Polymerase Chain Reaction. Ninety-four genomic DNA samples were extracted from shelter dogs' blood using AxyPrep Blood Genomic DNA miniprep kit (Axygen, USA). Eighty-seven saliva samples were obtained from pet dogs and wolves using Oragene ANIMAL/saliva kits (Oragene, Canada). Polymerase chain reaction (PCR) was performed in a total of 12.5µl volume reactions in a 96 well PCR plate (Interpath Services, Australia). Each well contained a 6.25µl aliquot of genomic DNA and a 6.25µl PCR reagent mix. The PCR reagent mix contained distilled water, 2.5mM $MgCl_2$, 1mM deoxynucleotide triphosphates (dNTPs), 1x PCR buffer, 1U of taq polymerase, 0.28µM fluorescent-labelled M13(-21) primer (FAM, VIC, PET or NED) (Promega, USA), 0.072µM forward primer and 0.28µM reverse primer (Macrogen, Korea) per reaction. For all the forward primers the 5' end was modified with a M13(-21) universal sequence tag (5'-TGTAAAACGGCCAGT-3') to enable the incorporation of the universal fluorescent labelled M13(-21) primer for detection on ABI3730 capillary instrument (Macrogen, Korea) (Schuelke, 2000). All PCRs were performed with a Veriti 96 well fast thermal cycler (Applied Biosystem, Australia). The thermal cycler was programmed to 1 cycle of 5 min at 94˚C as initial hot start, then 30 sec at 94˚C, annealing step of 30 sec at 55-65˚C and extension step of 40 sec at 72˚C. This was followed by a repeat of the cycle above 30 times and then by 8 extra cycles to ensure the oligo dye attached to the maximum amount of fragments. Then followed denaturation at 94˚C for 30sec, annealing at 53˚C for 45 sec and extension at 72˚C for 45 sec. Finally, 1 cycle of 10 min at 72˚C was run for final extension and held at 14˚C.

Electrophoresis of amplified products. After amplification, a 2µl aliquot of the amplified PCR product was combined with 2µl of loading buffer (0.4% (w/v) bromo-phenol blue, 0.5M EDTA and 6ml of glycerol) and analyzed directly on 1% (w/v) agarose LE (Benchmark Scientific, Australia) gel in 1 x TAE buffer (50mM Tris acetate, 1mM EDTA). Twoµl of Hyper ladder I (Bioline, Australia) was used as a size marker to compare the molecular weight of the amplified products. Gels were run at 100 volts for 25 mins and the gel images were documented by Molecular Imager CHemi Doc XRS4 Imaging system (Syngene, UK). Then, 4µl of each of four different microsatellite amplicons were pooled for the same animal. These pooled samples were combined into a master 96 well plate and sequenced by Macrogen (Korea).

Data analysis. Deoxyribonucleic acid fragment analysis was carried out using STR and software from UCDAVIS, Veterinary Medicine

(http://www.vgl.ucdavis.edu/informatics/strand.php/). This software allowed fast analysis of the multiplexed microsatellite markers. Contingency tables were used to compare the case and control canine with the risk and wild type genotypes. This allowed assessment of the degree of association of performance and species traits. In line with recommendations by Campbell (2007), contingency tables with expected frequencies > 1 were analyzed with an N-1 χ^2, and contingency tables with expected frequencies < 1 were analyzed with a Fisher-Irwin test by Irwin's rule. These analyses were carried out using SPSS version 22.0 (SPSS IBM, New York, USA, 2013), following methods from Weaver (2013). When comparing good versus poor OCT performers, we considered good performers as dogs that scored ≥ 18/20 correct points, and poor performers as those that scored ≤ 12/20 correct points. When comparing high oxytocin responders versus poor oxytocin responders, we considered the former to be dogs that improved their performance by 3-7 points between sessions, and poor responders as those whose performance remained the same, or declined, between sessions.

Results. Primers 9-10 were not analyzed due to them not annealing correctly to the target template. As such, only results pertaining to primers 1-8 are shown below.

Given the observed effect of intranasal oxytocin on performance in the OCT which lasted across the sessions for dogs that received oxytocin in session 1 (Oliva et al. 2015), we separated good performing versus poor performing dogs in the following three ways so as not to confound the results. Firstly, we looked at only the group of dogs that received saline in their first testing session (sal-oxy dogs) and used performance data from that session only. Secondly, because there was no difference between the performance level in session 2 (with dogs that received oxytocin in their first testing session (oxy-sal dogs) having maintained their enhanced performance from session 1, and sal-oxy dogs having their performance enhanced by oxytocin in session 2), we used "oxytocin-induced performance" data from all dogs in the study. Finally, we wanted to see if some dogs were more susceptible than other dogs to the oxytocin treatment and so analyzed difference scores in performance between the two sessions for sal-oxy dogs only. Tables 2-4 show the microsatellite markers identified for the analyses.

Table 4.2. Microsatellite markers and session 1 performance association analysis by contingency N-1 χ^2 or Fisher-Irwin test by Irwin's rule.

Primer	*N*-1 χ^2	*p*-value	Fisher-Irwin *p*-value
1			1.00
2			1.00
3			0.42
4			1.00
5			1.00
6			1.00
7	1.54	0.21	
8			1.00

There was no significant association between session 1 performance and any of the primers (Table 4.2).

Table 4.3. Microsatellite markers and session 2 performance association analysis by contingency N-1 χ^2 or Fisher-Irwin test by Irwin's rule.

Primer	*N*-1 χ^2	*p*-value	Fisher-Irwin *p*-value
1			1.00
2	0.63	0.43	
3			1.00
4	0.47	0.50	
5	0.58	0.45	
6			1.00
7	0.025	0.88	
8	1.56	0.21	

There was no significant association between session 2 performance and any of the primers (Table 4.3).

Table 4.4. Microsatellite markers and difference in performance between sessions association analysis by contingency N-1 χ^2 or Fisher-Irwin test by Irwin's rule.

Primer	N-1 χ^2	p-value	Fisher-Irwin p-value
1			1.00
2	2.62	0.11	
3			1.00
4	0.47	0.50	
5			1.00
6			1.00
7	1.78	0.18	
8	0.80	0.37	

There was no significant association between oxytocin response and any of the primers (Table 4.4).

Finally, we tested whether there were any differences between the OXTR gene of the domestic dog and that of the wolf. Table 5 shows the microsatellite markers identified for the analysis. Tables 4.6-4.7 show the contingency tables for the significant analyses.

Table 4.5. Microsatellite markers and species association analysis by contingency N-1 χ^2 or Fisher-Irwin test by Irwin's rule.

Primer	N-1 χ^2	p-value	Fisher-Irwin p-value
1	1.90	0.17	
2			0.070
3	3.24	0.072	
4			1.00
5			**0.038***
6	4.93	**0.026***	
7	0.036	0.85	
8			1.00

*p <.05

There was a significant association between species and primer 5, p = .038 (Table 4.5). Odds ratio for the allele (risk/wild-type) = 12.00, indicating that a canine with the risk allele is twelve times more likely to be a dog than a wolf, than if it has a wild-type allele.

Table 4.6. Contingency table for primer 5.

	Risk	Wild-type
Case (dog)	44	11
Control (wolf)	1	3

There was also a significant association between species and primer 6, p = .026 (Table 4.5). Odds ratio for allele (risk/wild-type) = 11.61, indicating that a canine with the risk allele is almost twelve times more likely to be a dog than a wolf, than if it has a wild-type allele.

Table 4.7. Contingency table for primer 6.

	Risk	Wild-type
Case (dog)	33	37
Control (wolf)	0	6

Discussion

The study revealed significant species differences between dogs and wolves, using 2 microsatellite primers close to the OXTR gene. Considering that the domestication of the dog occurred approximately 32,000 years ago (Wang, et al., 2013), the identified allelic differences between dog and wolf are likely to reflect an old mutation. In an old mutation under strong selection, we might expect linkage disequilibrium to decay rapidly as we move away from the causative gene. Therefore we would expect to see a strong association between genetic variation and species near the OXTR gene and no association when we move along the chromosome away from the gene. This study found that two markers close to the OXTR gene, primers 5 and 6, were different in dogs and wolves. Of all the primers, these are the two closest to the OXTR gene at positions *9.11 million* and *9.66 million base pairs*, respectively, supporting the above-hypothesized strong selection. For primer 5, the wolves did not demonstrate a common allele, probably due to the very small number of wolf samples that amplified (refer to Table 4.6), all of which expressed a different microsatellite size, one of which was the risk allele. For primer 6, the risk allele was not present in any of the 6 wolf samples that amplified and could be analyzed (refer to Table 4.7). Whilst further research should be carried out with a larger sample of wolf DNA, these findings suggest the involvement of the OXTR gene in the dogs' evolution from the wolf, given that we are seeing significance in markers closest to the gene and an absence of significance in markers further away from it. Future studies could utilize other markers in this region to map the association more accurately e.g. SNPs.

The current study failed to detect genomic differences in good- versus poor -performing dogs on an OCT. This observed lack of an association could be because the differences in good-performing versus poor-performing dogs are not genetic, but rather reflect the influence of early stimulation of the oxytocinergic system, which has been shown to have implications for function in later life. Whilst it is impossible to know the early experiences of the dogs in our study, which could have been very mixed, their early experiences may have affected their performance ability on the

OCT through a process called hormonal imprinting. As Csaba (2000) explains, a critical window exists in the first few days after birth in which this hormonal imprinting can occur. The process is defined by the provocation of a hormone receptor-to-be by a circulating hormone. If this process does not occur, the receptor does not mature and is unable to bind with the hormone in a suitable quantity. For most receptor cells, this inability is life-long and passed down to daughter cells (except in the brain where most cells do not differentiate) and in some cases, even to offspring of the animal. Csaba (2008) surmises that this is a result of a change in heritable DNA methylation; however, the exact mechanism behind hormonal imprinting remains unknown. Faulty imprinting can occur as a result of a lack of the appropriate hormone which reduces receptor density (Csaba, 2008). Csaba (2000; 2008) supports the notion of imprinting as a "memory-like process" (p. 409, Csaba, 2000) in which short repeated bouts of exposure result in greater imprinting than one single, long exposure.

Faulty imprinting may explain why adult rats that demonstrate significantly less oxytocin or vasopressin receptors in the central nucleus of the amygdalae and the bed nucleus of the stria terminalis after receiving low levels of maternal licking and grooming as pups, compared to those who received high levels of maternal licking and grooming (Francis, Young, Meaney & Insel, 2002). Tanaka, Osako and Yuri (2010) also demonstrated similar findings whereby isolation-reared rat pups have fewer immunoreactive vasopressin cells (males) in the dorsal, medial parvocelluar part of the pariventricular nucleus of the hypothalamus, than socially-reared pups, and fewer oxytocin cells (females) in the ventral, medial parvocelluar part of the pariventricular nucleus of the hypothalamus. Isolation-reared males were also more cautious than socially-reared ones in an elevated plus-maze and neither male nor female rat pups displayed signs of familiarity during a social recognition test.

Faulty imprinting may also explain findings from a study of nursery-reared rhesus monkeys that displayed significantly less oxytocin in their cerebrospinal fluid and less affiliative and more aggressive and abnormal repetitive behaviors compared to mother-reared rhesus monkeys. Moreover, a retrospective study found that women with a history of childhood abuse, particularly repeated and emotional abuse, displayed lower levels of oxytocin in their cerebrospinal fluid in adulthood (Heim et al., 2009). Although all the dogs in our study were currently-owned pets, while some had come from responsible breeders, others were shelter 'rescues', and others came from pet shops renowned in Australia for selling puppies obtained from puppy farms (RSPCA, 2010). Dogs born into poor environments, such as puppy farms, may not have experienced adequate imprinting of oxytocin to its receptors due to deprivation of positive social experiences, and may have comprised the poor performers in our study for this reason. This possibility requires further research.

Alternatively, the lack of significant disparities between good and poor OCT performers may be because we were not focusing closely enough on the OXTR gene itself. Due to the novelty of the study and the unknown variation within the canine OXTR gene, rather than looking within the gene, we looked at microsatellites *close* to the gene to increase the chance of finding any association. However, it is possible that we have missed a genetic difference *within* the gene itself that differentiates good from poor OCT performers. Dog breed, which was not controlled in the current study, may also affect behavioral phenotypes as observed by Kis, et al., (2014) with respect to friendliness, and Jakovcevic, Elgier, Mustaca and Bentosela (2010) with respect to gaze towards the human face. Another limitation of our study was that the blood samples and the saliva samples were analyzed in two separate batches, with no blood and saliva samples that had come from the same dog which we could use to standardize the samples. As such, there is no way of knowing if there were biases in the samples and therefore, future studies should be mindful to collect additional samples from a subgroup of animals on which to carry out this standardization. Lastly, the transmembrane enzyme, CD38, is attracting research interest, as variations of this gene have been linked with oxytocin secretion and associated social behaviors (see review, Macdonald, 2012). Hence, future studies should also investigate this gene, in addition to the OXTR gene, when examining phenotypes believed to be governed by the oxytocin system.

Conclusion. By measuring microsatellites close to the OXTR gene in samples of domestic dogs and wolves, this study provides evidence that mutations in this gene may have played a part in the domestic dog's evolution from the wolf. This may explain why domestic dogs outperform wolves on OCTs (Hare, et al., 2002; Miklósi, et al., 2003; Virányi, et al., 2008), a performance which has been shown to improve after intranasal oxytocin administration (Oliva, et al., 2015). The study did not, however, produce evidence for the same mutations underlying dogs' varying performance on the OCT task.

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Chapter 5 – Conclusions and future research directions

Through a series of three studies this thesis explores a proposed role of the oxytocinergic system in the dog's evolution from the wolf through enabling human-dog communication and human-dog bonding. In the first study, dogs' performance on an OCT with momentary distal pointing cues, a task known to be relatively more difficult for wolves than dogs (Miklósi, et al., 2003; Miklósi, et al., 2005; Soproni, et al., 2002; Virányi, et al., 2008), was enhanced by intranasally administered oxytocin, but not saline. This suggests that the relative ease with which dogs perform this task is influenced by oxytocin. In addition, intranasal oxytocin decreased dogs' aversion to a gazing cue provided by an experimenter, possibly by increasing trust.

In the second study, a dog owner's style of attachment to their dog, known to be influenced by oxytocin (Chen, et al., 2011; Denes, 2015; Strathearn, et al., 2009), was associated with the way in which dogs interpreted human social cues. For example, anxious attachment in the owner negatively predicted the dog's ability to use an experimenter's pointing cues in the OCT, and was significantly correlated with contagion of human emotions; this in turn positively predicted the dog's ability to use experimenter-provided gazing cues. It is logical that the style in which an owner is attached to their pet would influence the way in which they interact with it and how the pet responds. This is also seen in humans when a mother's internal 'working model' of attachment, based on her infantile attachment experience, influences the way in which she parents; more positive parenting styles are associated with secure internal working models and more negative styles with insecure internal working models (Jones, Cassidy, & Shaver, 2015). There is also evidence that attachment styles of humans are transmitted across generations (Van Ijzendoorn, 1995). In addition, it has been found that children's attachment behaviour is influenced by the OXTR gene, which also affects adults' parenting behaviour (Kryski, Smith, Sheikh, Singh, & Hayden, 2014).

In human-dog relationships, avoidant (but not anxious) adult attachment styles (albeit towards other humans and not necessarily to dogs), have been associated with owning dogs with separation-related disorders, which may be due to owners' inconsistencies in responding to the dogs' needs (Konok et al., 2015). Thus, there is evidence to suggest that owner behaviours may affect dog behaviours, which in turn may influence oxytocin function in the dog; oxytocin function is likely to influence dogs' OCT performance. However, there was no support in the present study for the hypothesis that owner-rated closeness to, and perceived intelligence of, their dog were correlated. Nor was there support for the hypothesis that these variables could predict actual intelligence of the dog. This might suggest that owners' strength of attachment to, or perceived level of intelligence of, their dog is not a good predictor of their dog's oxytocin function.

In the third study, measuring microsatellites close to the OXTR gene revealed allelic differences between dogs and wolves. Given the role of this gene in human-human bonding (Bakermans-Kranenburg & van Ijzendoorn, 2008; Denes, 2015; Riem, et al., 2011) and non-verbal, pro-social communication (Kogan et al., 2011), these findings also seem to implicate its involvement in the bonding and pro-social communicative behaviours of dogs towards their owners, behaviours which are lacking in wolves (Miklósi, et al., 2003; Topál, et al., 2005; Virányi, et al., 2008). The microsatellite method did not, however, reveal any genomic differences between good and poor OCT performers.

Given that oxytocin is involved in domestic dogs' ability to use human pointing cues, and that a large amount of funding is expended on training dogs for various working roles, but with relatively low success rates (Cobb, Branson, McGreevy, Lill, & Bennett, 2015), selectively breeding dogs with high oxytocin function for working roles that require understanding of pointing cues (e.g. herding stock) is a logical application of this research. One way to do this would be to breed dogs with an innate ability to perform well on an OCT, and thus with an inherent ability to use human social cues. Alternatively, we might selectively breed dogs with gene mutations that positively influence performance on tasks requiring such cues. Although no genetic differences were found in the present study between well and poorly performed dogs on the OCT, further research should be done to search for such differences within the OXTR gene itself.

In the meantime, intranasal application of oxytocin could be used on dogs currently in training for working roles. Whilst this set of studies did not explore the effects of *chronic* oxytocin application, other investigations have shown that this can lead to detrimental effects, such as anti-sociality in prairie voles (Bales et al., 2013), anti-sociality and aggression in neonatal pigs (Rault et al., 2013), and increased anxiety and down-regulation of OXTRs in mice (Peters, Slattery, Uschold-Schmidt, Reber, & Neumann, 2014). This increased anxiety was only seen at high doses of oxytocin, however, and low doses were protective against anxiety. The authors of this study suggested that this might also be due to cross action at vasopressin receptors, a suggestion also offered by Rault et al. Oxytocin and vasopressin have been shown to act on each other's receptors with varying affinities (Mouillac et al., 1995). In contrast, however, the antisocial effects observed by Bales et al. in prairie voles were recorded only at low and medium doses of oxytocin and were not due to an increase in anxiety or to cross action at vasopressin receptors, as these effects were not seen at high doses. Bales et al. postulate that the effects were due to either up- or down-regulation of endogenous oxytocin and vasopressin, or to desensitized or down-regulated OXTRs. Research regarding the safety and effectiveness of chronic application of oxytocin is lacking. However, as Bales

et al. posit, long-term learning effects resulting from acute doses of oxytocin would be ideal, and we have provided promising evidence for this in the OCT experiment. Therefore acute administration of oxytocin may be suitable for a one-off application when working dogs-in-training are learning an important new behaviour requiring the ability to use a human social cue.

Working environments are not the only situation for potential applications of oxytocin. In a dog shelter environment, oxytocin could play a role in both reducing anxiety and establishing a bond between a newly-adopted dog and the adopter. The welfare of kennel dogs has been poorly studied, but for various reasons outlined by Taylor and Mills (2007), a shelter is likely to prove a stressful environment for a dog. In addition, establishment of a bond is critical post-adoption to prevent dogs being returned to the shelter (Patronek, Glickman, Beck, McCabe, & Ecker, 1996) and oxytocin could be applied in this situation by administering it before the dog leaves the shelter. However, Taylor, Lee and Buisman-Pijlman (2014) highlight that the effects of intranasal application of oxytocin in human children with early life trauma might be difficult to predict and consequently requires more research before being considered as a viable, safe application. Until this research has been carried out, the same cautious approach should also be applied to shelter dogs.

In summary, this project has contributed to our understanding of the likely evolutionary history of the domestic dog. The findings also have implications for contemporary human-dog bonding and communication. Whilst this study implicates the neuropeptide, oxytocin, in dogs' domestication, repeating the same OCT experiment in wolves would provide stronger evidence to support or refute this theory. If enhanced central oxytocin function was pivotal in the domestic dog's evolution from the wolf, administering oxytocin to wolves prior to performing an OCT should render their performance more 'dog-like' i.e. better. However, if this difference in central function is due to a difference in OXTR distribution in the brains of the two species (as seen in socially monogamous compared with non-monogamous vole species (Insel & Shapiro, 1992; Insel, et al., 1994)), increasing central oxytocin may have no added value. In this case, post-mortem analysis of receptor distribution in the brains of both domestic dogs and wolves would add more absolute evidence relevant to proving/disproving this hypothesis. In addition, it would be interesting to see if oxytocin has also had a role in the domestication of other animals. Future studies could look at the same enhancing effects of oxytocin on OCT performance in, for example, cats to test this theory. Given that domestic cats evolved from near-East wild cats (Driscoll et al., 2007), genomic and receptor distribution differences between domestic and wild cats would be really interesting to explore.

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ORIGINAL PAPER

Oxytocin enhances the appropriate use of human social cues by the domestic dog (*Canis familiaris*) in an object choice task

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Abstract It has been postulated that the neuropeptide, oxytocin, is involved in human–dog bonding. This may explain why dogs, compared to wolves, are such good performers on object choice tasks, which test their ability to attend to, and use, human social cues in order to find hidden food treats. The objective of this study was to investigate the effect of intranasal oxytocin administration, which is known to increase social cognition in humans, on domestic dogs' ability to perform such a task. We hypothesised that dogs would perform better on the task after an intranasal treatment of oxytocin. Sixty-two (31 males and 31 females) pet dogs completed the experiment over two different testing sessions, 5–15 days apart. Intranasal oxytocin or a saline control was administered 45 min before each session. All dogs received both treatments in a pseudo-randomised, counterbalanced order. Data were collected as scores out of ten for each of the four blocks of trials in each session. Two blocks of trials were conducted using a momentary distal pointing cue and two using a gazing cue, given by the experimenter. Oxytocin enhanced performance using momentary distal pointing cues, and this enhanced level of performance was maintained over 5–15 days time in the absence of oxytocin. Oxytocin also decreased aversion to gazing cues, in that performance was below chance levels after saline administration but at chance levels after oxytocin administration.

Keywords Cognition · Cues · Dog · Oxytocin · Social

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Introduction

Domestic dogs seem to have evolved specialised abilities to communicate with humans in a way that their progenitor, the wolf, cannot. Social cognitive intelligence has been postulated to underpin human evolution (Whiten and Erdal 2012), and in relation to using human social cues, it may also have been important in the domestic dog's evolution from the wolf. The "object choice task" (OCT) was first applied to dogs by Miklósi et al. (1998) in an attempt to investigate dogs' ability to use human social cues and has since been utilised in numerous studies of domestic dogs and various other canids. The OCT involves a human experimenter using non-verbal, social cues to indicate the location of a hidden piece of food, located in one of two objects, usually bowls, located to the right and to the left of them. The subject's task is to correctly use these cues in order to obtain the hidden reward. The cues can involve replica cards, marker placement, pointing, tapping, orienting to and/or gazing at the object for various lengths of time and from various distances.

Of all the pointing cues used, the momentary distal point is potentially the most informative with respect to canines' ability to use human communication signals, as it is the most challenging. This is because the distance from the experimenter's finger to the bowl is relatively large and the cue relatively brief. Indeed, the cue is delivered *before* the dog is released and allowed to make its choice and is only

given for 1–2 s. As such, the dog has to rely not only on the cue itself, but also on its *memory* of the cue. Whilst domesticated dogs (Hegedüs et al. 2013; Miklósi et al. 2005; Schmidjell et al. 2012; Soproni et al. 2002; Virányi et al. 2008) and socialised dingoes (Smith and Litchfield 2010) generally perform above chance on the OCT when given the momentary distal point cue, young, hand-reared wolves that have been highly socialised to levels comparable with pet dogs do not (Miklósi et al. 2003; Virányi et al. 2008), or at least not without extensive training (Virányi et al. 2008). An additional study where domestic dogs were tested in the same outdoor conditions as wolves (as opposed to being tested indoors as in Miklósi et al. 2003; Virányi et al. 2008) suggests that the fact the wolves in these studies were tested outdoors may have handicapped them (Udell et al. 2008b). However, this is somewhat contradictory, as in Udell et al.'s study, mature wolves with a high level of socialisation and involvement in public education programs *were* able to demonstrate above-chance performance on this task when tested outdoors. Indeed, the authors claim that the wolves even outperformed dogs tested in the same outdoor conditions, though their methodology has been criticised (for responses, see, Hare et al. 2010; Udell and Wynne 2010).

Of all the cues that have been used on domestic dogs, only one has yielded OCT performance *not* above chance level: gaze (Soproni et al. 2001). These authors demonstrated that domestic dogs did respond to gazing cues, but paradoxically avoided the bowl at which the experimenter gazed, rather than approaching it. This may reflect a behaviour learned from communicating with conspecifics. However, domestic dogs do demonstrate an ability to learn to use this cue correctly to solve the task over time (Miklósi et al. 1998). With the exception of the gaze cue, most studies on the OCT in domestic dogs reveal no learning (Hare et al. 2002; Miklósi et al. 2005; Riedel et al. 2006; Schmidjell et al. 2012; Wobber et al. 2009), even in 6-, 8-, 16- and 24-week-old puppies (Riedel et al. 2008). Riedel et al's analysis has been criticised, and upon independent re-analysis of the data, learning was found to be present in the very young 6-week-old puppies (Wynne et al. 2008). Nonetheless, taken together, these findings suggest that dogs may have an inherent ability to perform above chance level on tasks that require understanding of human cues, without training.

The superior ability of domestic dogs (in comparison with wolves) to perform OCTs may be due to the fact that they gaze significantly more at the humans than do wolves. This notion has been supported by the shorter latency to make eye contact with an experimenter in both pet dog puppies (separated from their mothers at 6–9 weeks to live with a human family) and hand-reared dog puppies (separated from their mothers at 4–10 days and hand raised by humans who either kept them as pets or re-homed them) than in hand-reared wolf pups (separated from their mothers at 4–7 days and hand raised by humans who re-homed them to a wolf farm at 2–4 months) (Virányi et al. 2008). More gazing has also been observed in pet dogs than in hand-reared wolves performing other problem-solving tasks (Miklósi et al. 2003). Furthermore, similar findings have been obtained in anthropomorphically viewed and treated companion dogs that glance more at their owners and perform less well on a problem-solving task than less anthropomorphically viewed and treated working dogs (Topál et al. 1997). This suggests that companion dogs not only have the ability to use human cues to solve tasks and find food, but that they have become *dependent* on them. Only with extensive hand-rearing and training do wolves demonstrate an increase in gazing and in task execution that takes their performance to the level of naïve domestic dogs (Virányi et al. 2008). Interestingly, gazing was also less frequent in domestic cats than in domestic dogs (Miklósi et al. 2005), supporting the idea that the human–dog bond transcends the effects of domestication. This suggests that there is an inherent ability in dogs to communicate with humans in *humans' own way,* because gazing is a common phenomenon in human communication (Dickstein et al. 1984; Striano et al. 2006).

A link has been found between dogs that gaze at their owners for long durations and higher urinary oxytocin concentrations in the owner (Nagasawa et al. 2009). Given that oxytocin is implicated in mammalian bonding (Lim and Young 2006), this suggests that a dog's gaze, imperative for the successful completion of the OCT, may be more prominent in more strongly bonded human–dog dyads. Oxytocin increases have been observed in both humans and dogs after human–dog interactions (Handlin et al. 2011; Miller et al. 2009; Odendaal and Meintjes 2003) and are thought to be associated with human–dog bonding. Indeed, a new or enhanced role of oxytocin and/or its receptors in the domestic dog brain, compared to the wolf brain, may explain why dogs gaze at their owners more than hand-reared wolves do, and, in turn, do better than wolves in tasks involving human communicative signals. In humans, intranasal oxytocin administration: (1) enhances detection of human biological motion (Kéri and Benedek 2009), (2) increases the understanding of social cues and improves social memory (see review by Bartz et al. 2011; Guastella and MacLeod 2012), (3) increases trust (Kosfeld et al. 2005) and (4) increases a subject's gazing towards the eye region of other human faces (Guastella et al. 2008), a phenomenon also observed in monkeys (Dal Monte et al. 2014).

If dogs' ability to perform well on OCTs is dependent on their ability to look at humans and use human gestures, which is dependent on their central oxytocin function (as

demonstrated in humans), increasing central oxytocin availability should improve their performance on OCTs. The aim of this study was to test the effect of intranasal oxytocin administration on dogs' performance on an OCT, using two different cues, momentary distal pointing and gazing (without head-turn). It was hypothesised that: (1) dogs would perform better on the OCT after an intranasal treatment with oxytocin than after a control saline administration when momentary distal pointing cues were given and (2) oxytocin would both increase dogs' gazing towards the experimenter's eyes and their trust of the gaze cue, which would therefore improve their performance when gaze cues were offered as well. However, as Bartz et al. (2011) highlight in their review of the pro-social effects of oxytocin in humans, increases in trust do not occur in situations with prior trust violations, out-groups or clinical populations who are rejection-sensitive. In these groups of people, trust was actually decreased by oxytocin administration. Therefore, whilst we did not expect the dogs in our study to fall into any of the categories mentioned above, we could not rule out the possibility that they would interpret the gazing cue negatively and that this negative interpretation would be enhanced by oxytocin, thereby decreasing performance after oxytocin administration.

Method

Subjects

Seventy-five pet dogs (33 males and 42 females) were recruited for the study. Owners with healthy dogs over 12 months old were invited to participate, but owner-reported pregnant, lactating or visually impaired dogs were excluded. Owners were recruited through poster advertisements at Monash University Caulfield and Clayton campuses, as well as through university e-newsletters and social media websites. Dogs were randomly allocated into two separate groups: those that received oxytocin first and saline second (oxy-sal) and those that received saline first and oxytocin second (sal-oxy). Of the 75 dogs recruited, two males and 11 females did not complete the study; two dogs failed the pre-training, five dogs failed the test of motivation, three dogs passed the test of motivation but refused to continue when the more difficult cues were introduced, one dog was too excitable, and two dogs were withdrawn from the study by their owners. Partial data could, however, be used for two of the female dogs with incomplete records, leaving a total of 31 males and 31–33 females in the analysis. This study was approved by the Monash University School of Biological Sciences Animal Ethics Committee (BSCI/2013/07).

Materials

Twenty-four international units (equivalent to 50 μg) of oxytocin (Auspep, Melbourne, AU) diluted in .5 ml of .09 % saline, or .5 ml of .09 % saline only (acting as a control) were administered to the nostrils of each dog, with a half-dose in each nostril. Treatments were delivered using a Mucosal Atomizer Device (MAD 300, Wolfe Tory Medical Inc., Salt Lake City, UT) connected to a 1 mL syringe, whilst the dogs were maintained in a head-up position. When it could not be determined whether a dose was successfully administered, a second administration (half-dose) was delivered in the nostril concerned.

Two identical, opaque spaniel bowls (19 cm base diameter, 11 cm rim diameter, 12 cm high, 8 cm deep) were used to conceal the food treats. Spaniel bowls were selected for their height and ability to conceal the treat from the dogs' vision. Two additional and identical spaniel bowls were placed underneath the two testing bowls, and treats identical to those used in the experiment were hidden in the space between them. This method was used by Udell et al. (2008a) to ensure that both bowls smelled of the treats, and the dog was consequently not able to rely on olfaction when making its choice between the bowls. Treats were also hidden around the testing room so that the entire room smelled of treats. The treats used were lamb puff cubes: light, low-fat cubes of lamb lung, puffed with air. Scores were recorded by the experimenter using a pen and paper, and the same experimenter conducted all testing of dogs in the investigation.

Procedure

On the day of the testing session, owners were asked not to feed their dog prior to participation so that motivation to perform the task was high. In cases where testing occurred in the afternoon, some dogs were fed a small snack in the morning at the owner's discretion. Owners and their dogs came to the testing location on two separate occasions, 5–15 days apart. When they arrived for their first testing session, the dog received one of two intranasal treatments, oxytocin or saline. When they arrived for their second session, the dog received the other intranasal treatment. Tubes containing the treatments were labelled "A" or "B", so that both the experimenter and the owner were "blind" as to which treatment the dog received on which day. Order of treatment administration was pseudo-randomised and counterbalanced. The dog was restrained by its owner whilst the experimenter administered the intranasal spray. The owner was then required to fill out a few questionnaires to be used in an associated study whilst the dog was free to roam the testing room and interacts with its owner or the experimenter. The owner and dog could then leave

the room to wander outside or remain inside. Forty-five minutes after the treatment was administered, the first pre-training session commenced. A 45-min window was selected in accordance with the majority of previous human (MacDonald et al. 2011) and a recent pig (Rault et al. 2013) study and can be accepted as a sufficient time period in which neuropeptides can reach the brain (Born et al. 2002; Rault 2013).

Pre-training

The experimental set-up was similar to that of Virányi et al. (2008). The two spaniel bowls were placed 1.5 m apart, and the experimenter kneeled 30 cm behind the mid-point between the bowls. The dog, restrained by its owner, faced the experimenter at a distance of 2.5 m. The experimenter first got the dog's attention by calling its name or an affirmatory epithet ("good girl/good boy"; no address was used if the dog was already looking and calling the dog's name proved distracting to the dog). The dog was then shown a treat before it was placed in one of the bowls. The experimenter then said the release word "ok" (in some cases, a different release word, more familiar to the dog, was used, such as "okay", "free", "take", "go on", "(go) get it". The owner then released the dog and allowed it to approach one of the food bowls. If the dog approached the bowl containing the treat, it was allowed to eat the treat before both bowls were collected by the experimenter; if the dog approached the empty bowl or the experimenter, both bowls were collected by the experimenter and the dog did not receive a treat. The dog had to select the correct bowl four times in a row to move on to the testing session proper. A 10-min cut-off time was applied to the pre-training; if the dog was unable to pass the pre-training within this time, it was excluded from the study. Most pre-training sessions required only four trials, and the maximum number required was 25 trials for one dog in one of its pre-training sessions.

Testing

The experimental set-up was the same as in pre-training. Each testing session contained four blocks of fifteen trials (ten where a cue was provided and five in which no cue to the treat's whereabouts was provided). The control condition was used to verify that the dogs were not relying on scent to find the hidden food. Numerous studies have found that performance is at chance level when a control condition is employed (Hare et al. 2002, Riedel et al. 2008, Soproni et al. 2002, Udell et al. 2008a, Wobber et al. 2009).

The first test block (B1) comprised, in sequence: three control trials, five trials with the momentary distal point cue, two control trials and then another five trials with the momentary distal point cue. The second test block (B2) comprised, in sequence: three control trials, five trials with the gaze cue, two control trials and then another five trials with the gaze cue.

The third test block (B3) was the same as the first (B1), and the fourth block (B4) was the same as the second (B2). The ordering of the blocks was such that the easier point cue was delivered first so as not to discourage the dogs from participating by delivering a difficult gaze cue straight away. Having only ten trials per block was also strategically designed to keep the dogs motivated. Position of the correct bowl (left or right) was predetermined according to a pseudo-randomised chart that did not allow more than two consecutive trials where food could be obtained on the same side. Each test block was preceded by a pre-training session to maintain motivation to approach the baited bowl. The dog was allowed approximately 5 min of free play with its owner between testing blocks to avoid burnout.

Momentary distal point cue

The experimenter was kneeling, propped up on her toes, with her arms by her side. She got the dog's attention and then raised her ipsilateral arm and pointed (using her index finger) towards the correct bowl for 1–2 s, keeping her head straight, before lowering her arm back down to her side and saying "ok" (or an alternative release word). The approximate distance between the experimenter's index finger and the rim of the baited bowl was 42 and 50 cm to the treat inside. The dog was then released and allowed to make a choice between the bowls.

Gaze cue

The experimenter was kneeling with her arms by her side, the tops of her feet flat on the floor to achieve better eye level with the dog. She got the dog's attention and then gazed towards the correct bowl for 1–2 s, keeping her head straight. She then said "ok" (or an alternative release word), and the dog was then released and allowed to make a choice.

Control condition

The kneeling experimenter, propped up on her toes, got the dog's attention, then kept her head straight for 1–2 s, then said "ok" (or an alternative release word) before the dog was released by its owner and allowed to make a choice in the absence of any cue.

Scoring

Scores were recorded as correct responses out of ten trials per block (20 per cue) for each testing session. If the dog

did not move within 5 s of being released, the cue was given again, as in Virányi et al. (2008), and the dog could be prompted to move by its owner. If no choice was made, the experimenter decided subjectively whether this was due to a distraction. If it was clearly due to a distraction, the trial was repeated. In cases where the experimenter was unsure why the dog did not make a decision, the test of motivation used by Udell et al. (2008b) (two pre-training trials, one to each side) was conducted. If the dog was found to be unmotivated, the trial was discontinued; if the dog was found to be motivated, the trial continued and the experimenter assumed that the "no choice" outcome of the previous trial was probably due to the dog not understanding the task, so that the score for that trial was "incorrect choice". The vast majority of dogs were found to be motivated (i.e. did not need to be tested for motivation) throughout the entire testing session, or were excluded from the study. Choices were also considered incorrect if the dog approached the incorrect bowl or the experimenter.

Statistical analysis

The raw scores for each testing block of the OCT performed after oxytocin and saline administration were entered into IBM SPSS Statistics version 22 (SPSS IBM, New York, USA, 2013). Blocks one and three were also combined to give a total score for the pointing cues, and blocks two and four were combined to give a total score for the gazing cues. One-sample t tests were used to investigate whether performance on the task was different from what would be expected by chance. To test for learning within each session, we compared the mean of the first ten point and gaze cue trials (B1 and B2, respectively) with the last ten points and gaze cue trials (B3 and B4, respectively) using paired-samples t tests. To test the effect of treatment, an independent-samples t test was run on session 1 only. The effect sizes of all significant t tests were measured using Cohen's d. The effect of treatment (oxytocin and saline), gender (male and female) and group (oxy-sal and sal-oxy) on difference scores (score after oxytocin—score after saline) was evaluated using mixed-model analyses of variance (ANOVA). The effect size of all significant F tests was measured using partial eta squared. The assumption of homogeneity of covariances was tested using Box's M and was not violated for any test. Likewise, the assumption of homogeneity of variances was tested using F_{max}, and the Levene's test and was met for all measures. Šidák-corrected pairwise comparisons (Abdi 2007) were employed post hoc to test for the effect of treatment in the oxy-sal group and sal-oxy group dogs separately and to test the effect of treatment in male and female dogs separately.

Results

Performance different from chance

Control trials where the dog chose the left bowl, right bowl and correct bowl were scored out of a possible 20 choices per session, and the means and standard deviations are given in Table 1. The dogs performed significantly below chance levels (score of 10) during both testing sessions, which demonstrates that they were not relying on olfactory cues to find the hidden food treat for the session after oxytocin administration ($t_{61} = -2.49$, $P = .016$, $d = -.32$) and for the session after saline administration ($t_{62} = -4.58$, $P < .0001$, $d = -.58$). There were no biases for the left bowl after oxytocin administration ($t_{61} = -1.07$, $P = .29$), the right bowl after oxytocin administration ($t_{61} = .12$, $P = .90$), the left bowl after saline administration ($t_{62} = -1.17$, $P = .25$) or the right bowl after saline administration ($t_{62} = .23$, $P = .82$).

Mean scores and standard deviations for each block(s) are given in Table 2. Dogs performed significantly better than chance (score of 5) on point B1 after oxytocin ($t_{62} = 10.28$, $P < .0001$, $d = 1.30$), point B3 after oxytocin ($t_{62} = 16.01$, $P < .0001$, $d = 2.02$), point B1 after saline ($t_{63} = 8.39$, $P < .0001$, $d = 1.05$) and point B3 after saline ($t_{63} = 14.00$, $P < .0001$, $d = 1.75$). However, dogs performed no differently from chance on gaze B2 after oxytocin ($t_{62} = -1.51$, $P = .14$), gaze B4 after oxytocin ($t_{61} = -.94$, $P = .35$) and gaze B4 after saline ($t_{62} = -.46$, $P = .65$), and significantly worse than chance on gaze B2 after saline ($t_{62} = -2.19$, $P = .033$, $d = -.28$).

Learning within sessions

Paired-samples t tests revealed that learning occurred within each session using the point cue, as the subjects performed better on B3 than B1 after oxytocin ($t_{62} = -3.95$, $P < .0001$, $d = -.52$) and after saline ($t_{63} = -2.79$, $P = .007$, $d = -.32$). No learning was demonstrated within each session using the gaze cue, as dogs performed no

Table 1 Mean (±standard deviation) object choices out of 20 and sample size for control trials

	M	*SD*	*N*
Left after oxytocin	9.32	4.98	62
Right after oxytocin	10.08	5.16	62
Correct after oxytocin	9.35	2.04	62
Left after saline	9.22	5.27	63
Right after saline	10.14	4.99	63
Correct after saline	8.87	1.96	63

Table 2 Mean (±standard deviation) correct object choices out of 20 for all dogs combined, according to treatment

	Oxytocin			Saline		
	M	*SD*	*N*	*M*	*SD*	*N*
Point B1	7.41	1.86	63	7.41	2.29	64
Point B3	8.32	1.64	63	8.05	1.74	64
Point total	15.73	3.01	63	15.45	3.63	64
Gaze B2	4.68	1.67	63	4.59	1.49	64
Gaze B4	4.82	1.49	62	4.92	1.49	63
Gaze total	9.52	2.17	62	9.51	2.09	63

Table 3 Means (±standard deviation) correct choices out of 10 (point) and 20 (gaze) for dogs that received oxytocin and dogs that received saline on their first testing session

	Oxytocin			Saline		
	M	*SD*	*N*	*M*	*SD*	*N*
Point B3	8.38	1.70	32	7.41	1.60	32
Gaze total	9.52	2.19	31	9.19	1.75	32

differently on B4 than B2 after oxytocin ($t_{61} = -.44$, $P = .66$) or saline ($t_{62} = -1.35$, $P = .18$).

As learning appeared to be taking place between the pointing trial blocks, only data relating to the second block using point cues (B3) were used in the following analysis. As no differences were observed between the gaze trial blocks, they were combined in the analysis to follow.

The effect of oxytocin on performance

Mean correct choices for the second point block (B3) of trials and the gazing blocks combined for session 1 are shown in Table 3. Independent-samples *t* tests were used to compare means between the dogs that received oxytocin and dogs that received saline in this session. The *t* test revealed that dogs that received oxytocin on their first testing session performed significantly better than dogs that received saline on their first testing session for B3 ($t_{62} = 2.35$, $P = .022$, $d = .47$). No significant difference was observed between the two groups of dogs for the gazing cues ($t_{61} = .66$, $P = .51$).

The effect of gender, group and treatment on performance

Examination of Fig. 1 indicates that dogs in both groups performed similarly with the pointing signal in B3 after oxytocin but differently after saline, in that the oxy-sal dogs' performance improved after saline and the sal-oxy dogs' performance declined. There is a similar pattern in Fig. 2 for males and females, in that males and females perform similarly after oxytocin, but male dogs' performance slightly improves after saline, whereas female dogs' performance after saline declines. A mixed-model ANOVA revealed a significant interaction between treatment × group ($F_{1,59} = 6.40$, $P = .014$, partial $\eta^2 = .10$) and treatment × gender ($F_{1,\ 59} = 5.01$, $P = .029$, partial $\eta^2 = .08$), but not amongst treatment × group × gender ($F_{1,\ 59} = .77$, $P = .013$). There were no significant main effects of treatment ($F_{1,\ 59} = 1.36$, $P = .25$) or gender ($F_{1,\ 59} = 1.75$, $P = .19$), but there was a significant main effect of group ($F_{1,\ 59} = 4.35$, $P = .041$, partial $\eta^2 = .07$). Dogs that were administered oxytocin first performed more poorly after oxytocin than saline (mean difference = −.31, $SD = 1.87$), whilst dogs that were administered saline first performed better after oxytocin than after saline administration (mean difference = .87, $SD = 1.88$). Male dogs performed worse after oxytocin administration (mean difference = −.26, $SD = 1.81$), but female dogs performed better (mean difference = .78, $SD = 1.98$). Four Šidák-corrected pairwise comparisons were conducted using an adjusted alpha of .013 (1-tailed). Difference scores between

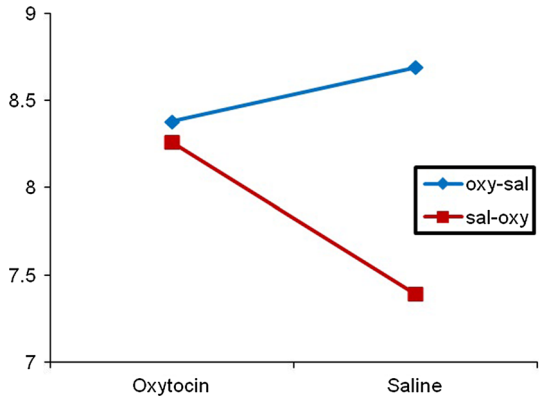

Fig. 1 Mean point B3 scores (out of 10) for oxy-sal group and sal-oxy group dogs, according to treatment

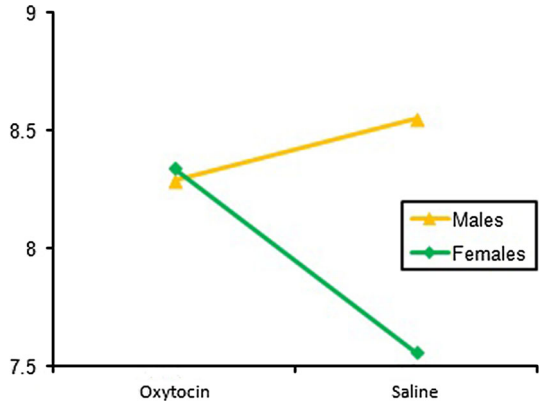

Fig. 2 Mean point B3 scores (out of 10) for male and female dogs, according to treatment

treatments were significant in sal-oxy group dogs ($t_{30} = 2.59$, $P = .0075$, $d = .54$), but not oxy-sal group dogs ($t_{31} = -2.40$, $P = .18$), female dogs ($t_{31} = 2.23$, $P = .0165$) or male dogs ($t_{30} = -.80$, $P = .22$).

For the gaze total scores, a mixed-model ANOVA revealed no significant interaction effects between treatment × group ($F_{1,58} = .50$, $P = .48$), treatment × gender ($F_{1,58} = .61$, $P = .44$) and amongst treatment × gender × group ($F_{1,58} = .57$, $P = .45$). Nor were there any significant main effects of treatment ($F_{1,58} = .01$, $P = .96$), gender ($F_{1,58} = .001$, $P = .98$) or group ($F_{1,58} = .73$, $P = .40$).

Discussion

The ability of dogs to use momentary distal pointing cues, and the effect of oxytocin

Consistent with previous research, this study demonstrated an ability of domestic dogs to use momentary distal pointing cues to find hidden food in an OCT (Hegedüs et al. 2013; Miklósi et al. 2005; Schmidjell et al. 2012; Soproni et al. 2002; Virányi et al. 2008). In addition, consistent with our first hypothesis, a treatment effect was observed in that dogs performed significantly better after oxytocin than saline administration in session 1. This is consistent with findings for humans, demonstrating that oxytocin increases perception of biologically relevant human motion (Kéri and Benedek 2009), which is imperative for social cognitive processing and communication, and supports the notion that oxytocin increases social cognition (see reviews by Bartz et al. 2011; Guastella and MacLeod 2012). In addition, when examining difference scores between testing sessions, we observed performance improvements from session 1 to session 2 for point B3 scores in sal-oxy group dogs. Inspection of Fig. 1 shows that their performance in session 2 was only bought up to the level of performance demonstrated by the oxy-sal group dogs in session 1, after oxytocin administration. The absence of a significant difference between sessions for the oxy-sal group dogs indicates that this group of dogs was able to maintain their performance at this level 5–15 days later, after saline administration. Thus, oxytocin not only enhanced performance on the OCT, but the enhanced level of performance was maintained over time.

The effect of gender on the efficacy of oxytocin

The enhancing effect of oxytocin seems to have been driven by the female subjects in this study who performed better after oxytocin and more poorly after saline administration (see Fig. 2). The reason why males were possibly not as influenced by oxytocin as females (whose performance was able to be brought up to the level of the males after oxytocin administration) may simply be ceiling effects, as they performed similarly after both treatments and significantly better than females after saline administration. The reason for the superior performance of male dogs compared to females after saline administration is unknown and somewhat surprising; in humans, females have shown greater social cognitive abilities than males, as demonstrated by their better perception of others' emotions (Brabec et al. 2012; Donges et al. 2012). However, the OCT differs in that it tests an ability to solve a task using human communicative cues, not human emotions. Oestrogen is known to enhance the production of oxytocin and its peptide (Rissman 2008), and this may explain why the female dogs in this study did not perform as well as human female subjects in other tests of social cognition, as the majority (88 %) had been spayed, thereby reducing the volume of oestrogen their bodies would be producing. However, this does not explain why the male dogs (the majority of whom had also been neutered, 97 %) performed so much better than the females dogs following saline.

The ability of dogs to use gazing cues, and the effect of oxytocin

Contrary to our second hypothesis, no treatment effect was observed for gazing cues. We did find some support, however, for the negative interpretation of the gaze cue being dampened by oxytocin. For example, in gaze B2 after saline administration, we obtained the same findings as Soproni et al. (2001), who reported that dogs interpreted the gaze cue negatively, avoiding the bowl to which the experimenter gazed. Our lack of a similar finding for B2 after oxytocin administration supports our hypothesis that oxytocin increases trust in the dog, as it does in humans (Kosfeld et al. 2005), despite the fact that the dogs were unable to use the cue, performing no better than chance after oxytocin administration. That this below-chance-level performance was lacking in gaze B4 after saline administration may reflect the dogs learning that no aversive consequences would occur when they went to the bowl containing the treat, so they no longer used the gaze cue to complete the task, and just guessed.

The Clever Hans phenomenon

Whilst mean performance on the majority of the gaze cue blocks was at chance level, it is intriguing that mean control trial performance (where no cue was given) was *below* chance levels, as shown in Table 1. The so-called Clever Hans phenomenon (Pfungst 1911), involving some form of unintentional or subconscious cueing from the

owner, has been independently tested for in dogs subjected to an OCT with momentary distal pointing cues and yielded negative findings (Hegedüs et al. 2013; Schmidjell et al. 2012), but we cannot completely rule this out as the reason for these unexpected results. The above-mentioned studies only tested for possible unintentional, subconscious cueing by the owner, not by the experimenter. In the current study, it is conceivable that the experimenter was subconsciously "hoping" that the dogs were not using scent to find the food in the control trials and may have been unintentionally cueing the dogs to go to the empty bowl in order to validate the experimental design. This highlights the critical importance of blind treatment testing for both the owner and experimenter, which was a strength of the current study. Nonetheless, the effect of the experimenter on the Clever Hans phenomenon warrants further study.

Learning within sessions

Another unexpected finding in the study was that learning was observed within sessions for the pointing cues. Despite the pre-training that took place before B1, it appears that dogs were still learning to use the point cues to do the task in B1 compared to B3, where they performed better. This finding contrasts with those of previous studies, which did not report performance differences within sessions (Hare et al. 2002; Miklósi et al. 2005; Riedel et al. 2006, 2008; Schmidjell et al. 2012; Wobber et al. 2009). As learning was observed within both treatment sessions, we do not believe this is a consequence of the oxytocin administration unique to our study. One possibility is that this "learning" is a reflection of the dogs being less anxious about the novel environment in B3 compared to B1 and therefore less inhibited in performing the task. However, it is still interesting that this was observed in our study but not in previous studies, which employed similar testing methods and less habituation time to the testing environment. This disparity may be due to the fact that testing in the current study was carried out in a room that was not well insulated against distracting external sound disturbances and therefore may have required more habituation time than the testing locations employed in other studies.

Limitations and future directions

The above-mentioned external sound disturbances may have varied on different testing days, which was an unavoidable limitation of our study. Other limitations of the present investigation included possible variation in the dog's hunger levels amongst sessions. Although efforts were made to test a particular dog at the same time of day in each session, this was not always possible. Owners were also instructed to keep the dog's day as similar as possible between sessions, but this could not be fully controlled either. Given the gender differences we observed, future studies should consider the effect that spaying and neutering have on oxytocin function, as our findings, compared to those of human studies on social cognition, may suggest this has particular influence in females. Lastly, although efforts were made to be as consistent as possible with the majority of previous studies' dosages and behavioural testing timeframes, it is currently unknown what constitutes the optimal behavioural testing time after administration of oxytocin in dogs, and how long the behavioural effects last. Extrapolating from the findings of a human study investigating the intranasal application of 40 and 80 IU of a very similar peptide, vasopressin (Born et al. 2002), and a recent pig study investigating the intranasal application of 24 IU of oxytocin (Rault 2013), we can reasonably assume that oxytocin is still active in the brain 100–120 min after administration and potentially longer. Therefore, the behavioural effects in the current study were likely to have been maintained for the entire testing session, which normally lasted between 90 and 120 min.

Conclusion

Administration of oxytocin was effective in aiding dogs' performance on the OCT using momentary distal pointing cues. Moreover, this enhancing effect persisted at least 5–15 days later, in the absence of further oxytocin administration. Oxytocin also appeared to decrease dogs' aversion to gazing cues, with performance being at chance level after oxytocin administration but below chance level after saline administration.

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Part A: Participant survey

Participant Number _____ Dog ID number _____

Section 1: Dog demographics

Age ___

Breed _____

De-sexed ☐ No
☐ Yes

Section 2: Participant demographics

1. Age group

☐ 18-25 ☐ 36-45 ☐ 56-65
☐ 26-35 ☐ 46-55 ☐ 65+

2. Gender

☐ Male
☐ Female

Section 3: Ownership history

3. Other than this dog, do you currently, or have you previously, own(ed) any other dogs as the primary owner/care-taker?

☐ No
☐ Yes How many? ___

4. Do you currently, or have you previously owned any pets other than dogs as the primary owner/care-taker?

☐ No
☐ Yes. How many? ___

If you answered yes, please specify the animals?
_____
_____

Section 4: Parental history

5. Have you had any children?

☐ No
☐ Yes. How many? ___

Section 5: Dog's living arrangements

6. Is your dog an inside or outside dog (if you dog spends time both inside and outside, please select the *most* relevant option)?

☐ Inside

☐ Outside

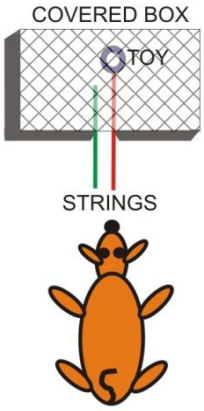
Part B.

This section will ask you how you think your dog will respond in particular situations. Please indicate the extent to which you agree or disagree with the following statements about YOUR DOG. "INSTINCTIVE" means an ability that dogs are born with.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
1. My dog **instinctively** understands human gestures like pointing at food or toys	☐	☐	☐	☐	☐
2. My dog can **learn** to understand human gestures like pointing at food or toys	☐	☐	☐	☐	☐

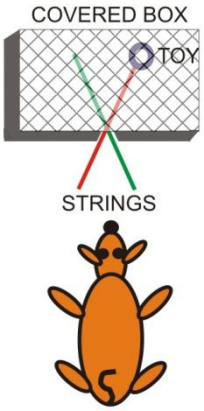
3. When faced with a problem that my dog can't solve on his/her own, such as getting a toy ball from under the sofa, my dog **instinctively** looks at me for assistance.	☐	☐	☐	☐	☐
4. When faced with a problem that my dog can't solve on his/her own, such as getting a toy ball from under the sofa, he/she can **learn** to look at me for assistance.	☐	☐	☐	☐	☐
5. When my dog looks at me, he/she **instinctively** understands when I am paying attention to him/her.	☐	☐	☐	☐	☐
6. My dog can **learn** to look at me to understand when I am paying attention to him/her.	☐	☐	☐	☐	☐
7. My dog is **instinctively** more likely to beg for food from me if I am looking at him/her rather than at something else.	☐	☐	☐	☐	☐
8. My dog can **learn** to beg for food from me when I am looking at him/her rather than at something else.	☐	☐	☐	☐	☐
9. My dog **instinctively** knows he/she can steal food more easily when I am not paying attention to him/her.	☐	☐	☐	☐	☐
10. My dog can **learn** that it is easier to steal food when I am not paying attention to him/her.	☐	☐	☐	☐	☐

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
11. My dog can **instinctively** solve problems like opening a container lid to get a treat.	☐	☐	☐	☐	☐
12. My dog can **learn** to solve problems, like opening a container lid to get a treat, by watching **me** do it first.	☐	☐	☐	☐	☐
13. My dog can **learn** to solve problems, like opening a container lid to get a treat, by watching **other dogs** do it first.	☐	☐	☐	☐	☐
14. If you put a toy or treat behind a wire barrier like a fence, my dog **instinctively** understands that he/she can go around the barrier to obtain the object.	☐	☐	☐	☐	☐
15. If you put a toy or treat behind a wire barrier like a fence, my dog can **learn** to go around the barrier to obtain the object by watching **me** do it first.	☐	☐	☐	☐	☐
16. If you put a toy or treat behind a wire barrier like a fence, my dog can **learn** to go around the barrier to obtain the object by watching **other dogs** do it first.	☐	☐	☐	☐	☐



Please answer the following 3 questions in relation to this figure.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
17. My dog **instinctively** understands that pulling the string will allow him/her to access the treat or toy at the end.	☐	☐	☐	☐	☐
18. My dog can **learn** that pulling the string will allow him/her to access the treat or toy at the end by watching **me** do it first.	☐	☐	☐	☐	☐
19. My dog can **learn** that pulling the string will allow him/her to access the treat or toy at the end by watching **other dogs** do it first.	☐	☐	☐	☐	☐



Please answer the following 3 questions in relation to this figure.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
20. My dog **instinctively** understands that pulling the string will allow him/her to access the treat or toy at the end	☐	☐	☐	☐	☐
21. My dogs can **learn** that pulling the string will allow him/her to access the treat or toy at the end by watching **me** do it first.	☐	☐	☐	☐	☐
22. My dog can **learn** that pulling the string will allow him/her to access the treat or toy at the end by watching **other dogs** do it first.	☐	☐	☐	☐	☐

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
23. My dog is capable of understanding when I am sad.	☐	☐	☐	☐	☐
24. My dog is capable of understanding when I am happy.	☐	☐	☐	☐	☐
25. My dog is capable of understanding when I am angry.	☐	☐	☐	☐	☐
26. My dog is capable of understanding when I am afraid.	☐	☐	☐	☐	☐
27. My dog is capable of understanding a stranger is sad.	☐	☐	☐	☐	☐
28. My dog is capable of understanding when a stranger is happy.	☐	☐	☐	☐	☐
29. My dog is capable of understanding when a stranger is angry.	☐	☐	☐	☐	☐
30. My dog is capable of understanding when a stranger is afraid.	☐	☐	☐	☐	☐
31. My dog is capable of trying to trick me into doing something like moving from my seat so he/she can sit there.	☐	☐	☐	☐	☐
32. My dog is capable of trying to trick other dogs into doing something like moving from their seat so he/she can sit there.	☐	☐	☐	☐	☐

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
33. My dog is capable of trying to trick strangers into doing something like moving from their seat so he/she can sit there.	☐	☐	☐	☐	☐
34. My dog is smarter than most people.	☐	☐	☐	☐	☐
35. My dog can solve **logic problems** better than most humans.	☐	☐	☐	☐	☐
36. My dog can solve **social problems** better than most humans.	☐	☐	☐	☐	☐
37. When I feel sad, my dog feels sad too.	☐	☐	☐	☐	☐
38. When I feel happy, my dog feels happy too.	☐	☐	☐	☐	☐
39. When I feel afraid, my dog feels afraid too.	☐	☐	☐	☐	☐
40. When I feel angry, my dog feels angry too.	☐	☐	☐	☐	☐

Part C: Monash Dog Owner Relationship Survey

Please read each of the following statements and mark one box that best applies to you at the present time.

1. How hard is it to look after your dog?

☐ Very hard ☐ Hard ☐ Neither hard nor easy ☐ Easy ☐ Very easy

2. My dog gives me a reason to get up in the morning.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

3. There are major aspects of owning a dog I don't like.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

4. How often do you kiss your dog?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

5. I wish my dog and I never had to be apart.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

6. My dog makes too much mess.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

7. How often do you play games with your dog?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

8. It bothers me that my dog stops me doing things I enjoyed doing before I owned it.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

9. How often do you take your dog to visit people?

☐ Once a week ☐ Once a fortnight ☐ Once a month ☐ A couple of times a year ☐ Never

10. It is annoying that I sometimes have to change my plans because of my dog.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

11. My dog costs too much money.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

12. How often do you buy your dog presents?

☐ Once a week ☐ Once a fortnight ☐ Once a month ☐ A couple of times a year ☐ Never

13. My dog is constantly attentive to me.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

14. How often do you give your dog food treats?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

15. How often do you tell your dog things you don't tell anyone else?

☐ Once a day ☐ Once a week ☐ Once a month ☐ Once a year ☐ Never

16. How often do you feel that looking after your dog is a chore?

☐ Once a day ☐ Once a week ☐ Once a month ☐ Once a year ☐ Never

17. How often do you take your dog in the car?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

18. How often does your dog stop you doing things you want to?

☐ Once a day ☐ Once a week ☐ Once a month ☐ Once a year ☐ Never

19. I would like to have my dog near me all the time.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

20. How often do you groom your dog?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

21. If everyone else left me my dog would still be there for me.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

22. How often do you feel that having a dog is more trouble than it is worth?

☐ Once a day ☐ Once a week ☐ Once a month ☐ Once a year ☐ Never

23. My dog helps me get through tough times.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

24. How often do you hug your dog?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

25. My dog provides me with constant companionship.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

26. How often do you have your dog with you while relaxing, ie watching TV?

☐ At least once a day ☐ Once every few days ☐ Once a week ☐ Once a month ☐ Never

27. My dog is there whenever I need to be comforted.

☐ Strongly agree ☐ Agree ☐ Neither agree nor disagree ☐ Disagree ☐ Strongly disagree

28. How traumatic do you think it will be for you when your dog dies?

☐ Very traumatic ☐ Traumatic ☐ Neither traumatic nor untraumatic ☐ Untraumatic ☐ Very untraumatic

Part D: Pet Attachment Questionnaire

The following statements concern how you feel in the relationship with your pet. We are interested in how you experience the relationship with your specific pet. Respond to each statement by indicating how much you agree or disagree with it, using the following scale:

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

---

1. Being close to my pet is pleasant for me

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

2. I'm often worried about what I'll do if something bad happens to my pet

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

3. I prefer not to be too close to my pet

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

4. Sometimes I feel that I force my pet to show more commitment and desire to be close to me

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

5. I prefer to keep some distance from my pet

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

6. If I can't get my pet to show interest in me, I get upset or angry

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

7. Often my pet is a nuisance to me

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐
Disagree strongly Neutral/mixed Agree strongly

8. Signs of affection from my pet bolster my self-worth

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐

Disagree strongly Neutral/mixed Agree strongly

9. I feel distant from my pet

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

10. I often feel that my pet doesn't allow me to get as close as I would like

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

11. I'm not very attached to my pet

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

12. I get angry when my pet doesn't want to be close to me as much as I would like it to

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

13. If necessary, I would be able to give away my pet without any difficulties

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

14. I get frustrated when my pet is not around as much as I would like it to be

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

15. I have no problem parting with my pet for a long time

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

16. I need shows of affection from my pet to feel there is someone who accepts me

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

17. I get uncomfortable when my pet wants to be close to me

1 2 3 4 5 6 7
☐ ☐ ☐ ☐ ☐ ☐ ☐
Disagree strongly Neutral/mixed Agree strongly

18. I feel frustrated if my pet doesn't seem to be available for me when I need it

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

19. I get nervous when my pet gets too close to me

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

20. Without acts of affection from my pet I feel worthless

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

21. I want to get close to my pet but I keep pulling away

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

22. I am worried about being left alone without my pet

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

23. I try to avoid getting too close to my pet

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

24. I need expressions of love from my pet to feel valuable

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

25. When I'm away from my pet for a long period of time, I hardly think about it

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly

26. I need a lot of reassurance from my pet that it loves me

1	2	3	4	5	6	7
☐	☐	☐	☐	☐	☐	☐
Disagree strongly			Neutral/mixed			Agree strongly