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Intranasal oxytocin and a polymorphism in the oxytocin receptor gene are associated with human-directed social behavior in golden retriever dogs

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ABSTRACT

8The oxytocin system may play an important role in dog domestication from the wolf. Dogs have evolved unique human analogue social skills enabling them to communicate and cooperate efficiently with people. Genomic differences in the region surrounding the oxytocin receptor (*OXTR*) gene have previously been associated with variation in dogs' communicative skills. Here we have utilized the unsolvable problem paradigm to investigate the effects of oxytocin and *OXTR* polymorphisms on human-directed contact seeking behavior in 60 golden retriever dogs. Human-oriented behavior was quantified employing a previously defined unsolvable problem paradigm. Behaviors were tested twice in a repeated, counterbalanced design, where dogs received a nasal dose of either oxytocin or saline 45 min before each test occasion. Buccal DNA was analysed for genotype on three previously identified SNP-markers associated with *OXTR*. The same polymorphisms were also genotyped in 21 wolf blood samples to explore potential genomic differences between the species. Results showed that oxytocin treatment decreased physical contact seeking with the experimenter and one of the three polymorphisms was associated with degree of physical contact seeking with the owner. Dogs with the AA-genotype at this locus increased owner physical contact seeking in response to oxytocin while the opposite effect was found in GG-genotype individuals. Hence, intranasal oxytocin treatment, an *OXTR* polymorphism and their interaction are associated with dogs' human-directed social skills, which can explain previously described breed differences in oxytocin response. Genotypic variation at the studied locus was also found in wolves indicating that it was present even at the start of dog domestication.

1. Introduction

Domestic dogs have apparently evolved unique human analogue communicative skills during the course of domestication and through sharing our ecological niche (Topal et al., 2009). This social competence involves comprehension of referential gestures as well as ostensive cues such as pointing and gazing (Lakatos et al., 2012; Soproni et al., 2001). Dogs are e.g. more skillful than both their wolf ancestors and chimpanzees in understanding human communicative behavioral cues during an object choice paradigm (Hare and Tomasello, 1999, 2005). Although primate species have previously been the main focus organisms in comparative social cognition research trying to understand the origin of humans' social skills (Hare et al., 2012), dogs may therefore be equally interesting models.

The differences between dogs and wolves are evident also when studying fully socialised individuals. Wolves do not seek as much human contact as dogs (Gacsi et al., 2009; Topal et al., 2005) and do not use mutual gazing as a mean of communication (Nagasawa et al., 2015). Dogs are able to display intentional referential communicative gestures towards humans, involving both an attention-seeking component as well directional “showing” behaviors (Marshall-Pescini et al., 2013; Miklosi et al., 2000; Passalacqua et al., 2011). When faced with an unsolvable problem, dogs usually turn to a nearby human for help while wolves do not show this behavior to the same extent (Miklosi et al., 2003). Hence, the evidence suggests that this human-directed social behavior has largely been shaped during domestication.

In spite of the much larger inter-species social competence of dogs, there is still considerable within-breed variation in this trait (Persson et al., 2015). Recent evidence shows that there is a significant genetic basis for this variation: In one population of beagles, the narrow-sense heritability of human-directed social behavior was estimated to 0.23 and candidate genes were identified through genome-wide association analysis (Persson et al., 2015, 2016). Based on its well-established function in social bonding in humans and other mammals (Lim and

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Young, 2006), it has been suggested that the neuropeptide oxytocin may also play a central role for the unique dog-human bond (Nagasawa et al., 2015). Hence, genetic variations in the oxytocin system may play a role in within- and between-breed differences in human-oriented social behavior and in the bond with the owner (Beetz et al., 2012).

Oxytocin is produced in the hypothalamus as well as in the periphery and can both act as a neurotransmitter and a neurohormone (Gimpl and Fahrenholz, 2001; Neumann, 2008). Peripheral oxytocin concentrations can increase in both humans and dogs as a result of tactile interaction (Handlin et al., 2011; Mitsui et al., 2011; Odendaal and Meintjes, 2003; Rehn et al., 2014) and mutual gazing (Nagasawa et al., 2009, 2015). However, it has been suggested that it is mainly central and not peripheral oxytocin playing a role in influencing behavior (Leng and Ludwig, 2016). Although the mechanisms are yet poorly understood, peripheral and central (Neumann et al., 2013; Rault, 2016) oxytocin concentrations can be experimentally manipulated though intranasal administration (Leng and Ludwig, 2016), although the increase in central oxytocin upon intranasal administration seems to be small (Rault, 2016).

In spite of this, intranasal administration of this hormone in dogs has been shown to increase positive expectations (Kis et al., 2015), mutual gazing with the owner (Nagasawa et al., 2015), affiliative behavior towards both their owners and conspecifics (Romero et al., 2014), play behavior (Romero et al., 2015) and performance in an object choice task relying on human given pointing cues (Oliva et al., 2015). However, oxytocin does not always have pro-social effects and there seems to be both individual variation and contextual components determining the exact effects of oxytocin (Bartz et al., 2011). For instance, in humans it may decrease trust and cooperation directed towards strangers or members of an out-group (De Dreu, 2012, 2010, 2011) and in dogs it may decrease friendliness in a threatening situation (Hernadi et al., 2015). Additionally, breed differences have been described in the effects of oxytocin administration (Kovacs et al., 2016a).

Several studies have also reported sex differences in dogs' responses to oxytocin. In females but not in males, intranasal oxytocin administration increased mutual gazing with humans (Nagasawa et al., 2015), looking time in a social motion perception test (Kovacs et al., 2016a) and performance with following human ostensive cues (Oliva et al., 2015). Hence, it is important to take both breed and sex into consideration when investigating effects of oxytocin in dogs.

The effects of oxytocin depend both on the hormone levels and the receptor activity. Both oxytocin and the oxytocin receptor (OXTR) are highly conserved and present in mammals as well as several other taxa (Gimpl and Fahrenholz, 2001). In humans, variation in the *OXTR* gene have e.g. been associated to autism (Jacob et al., 2007), attachment style (Denes, 2015), temperament (Tost et al., 2010), empathy and stress reactivity (Rodrigues et al., 2009). However, variants of the *OXTR* gene have not yet been as widely studied in dogs.

Among the existing studies, Kis et al. (2014) tested Border Collies and German Shepherds in a battery of social test situations and genotyped them at three different single nucleotide polymorphisms (SNPs) surrounding the *OXTR* gene. Associations were found between specific SNP genotypes on one hand, and human proximity seeking and friendliness on the other. Interestingly, German Shepherds carrying the A allele of the rs8679684 marker and the G allele of the 19131AG marker were more friendly while the opposite pattern was seen in Border Collies. These results corroborate the importance of taking breed into consideration when investigating oxytocin effects. Another study investigated the effects of genetic variation around *OXTR* on object choice task performance in pet and shelter dogs of different breeds (Oliva et al., 2016). Although 10 microsatellite markers surrounding the gene were studied, no significant associations were found with task performance. Twelve wolves were also included in the genetic analysis, revealing two genetic markers with significantly different genotype ratios between species.

The contradictive effects of oxytocin on social behavior in both humans and dogs suggest that we are still far from understanding its proper function and mechanism. One possibility is that individuals with different genotypes associated with the *OXTR* gene respond differently to oxytocin treatment. In humans, *OXTR* genotypes have been shown to affect the behavioral responses to intranasal oxytocin treatment (Feng et al., 2015; Marsh et al., 2012). To our knowledge, the effects of oxytocin depending on genetic variants in the vicinity of *OXTR* have not yet been examined in dogs.

The aim of the present study was to investigate the effects of intranasal oxytocin treatment, genetic variation in association with *OXTR* and interactions between hormone treatment and genotype on human-directed social behavior of dogs in the unsolvable problem paradigm. Additionally, given the differences between dogs and wolves in human communicative skills, we also aimed to examine genotypic differences between the species at the same genetic markers surrounding the *OXTR* gene.

2. Methods

2.1. Ethical note

This study protocol was performed in accordance with the ethical permit approved by the regional ethical committee for animal experiments in Linköping, Sweden (permit number: 51–13) with all owners giving their informed consent for their dogs' participation in this study. The methods were carried out in accordance with the relevant guidelines.

2.2. Subjects

Sixty golden retriever dogs (34 females and 26 males) were tested and genotyped to investigate the effects of intranasal oxytocin and OXTR genotype on human-directed social behavior. Owners were recruited to participate in the study through social media, local advertisement and radio. The dogs were required to be registered by the Swedish Kennel Association as purebred golden retrievers, not to be pregnant or lactating and at least 4 months old (mean age of 5.1 ± 0.47 years ± SE; range 4 months–12 years). Buccal samples were collected from the golden retrievers for genotyping of three genetic markers (SNP) associated with the *OXTR* gene. Additionally, 21 wolf samples (7 females and 14 males) were used for genotyping for the same genetic markers. Eighteen wolf blood samples were supplied from Kolmården Wildlife Park, Sweden and three wolf DNA samples previously isolated from brain tissue were originally supplied from Borås Animal Park, Sweden. All wolves belong to the Scandinavian population (*Canis lupus lupus*) and were either raised at the wildlife parks or wild captives.

2.3. Procedure

2.3.1. DNA Sampling

Buccal samples were collected at the start of the first testing session of each individual, by asking the owner to rub a cotton swab on the inside of their dogs' cheek for approximately 20 s. Samples were genotyped for the same three single nucleotide polymorphisms (SNPs) as previously described by Kis et al. (2014) (Fig. 1). Sequencing primers for pyrosequencing were designed using the software PyroMark Assay Design 2.0.1.15 by QUIAGEN©. All primers were manufactured by Thermo Fisher Scientific (Waltham, MA, USA) and the sequences can be found in Table 1.

2.3.2. Treatment

The same two female experimenters (first and second author, referred to in the following as E1 and E2) carried out all the treatments and experiments. Upon arrival at the testing location, each owner was thoroughly informed about the procedure by E1. At the first visit of