Intranasally administered oxytocin affects how dogs *(Canis familiaris)* react to the threatening approach of their owner and an unfamiliar experimenter

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Abstract

Fear and aggression are among the most prominent behavioural problems in dogs. Oxytocin has been shown to play a role in regulating social behaviours in humans including fear and aggression. As intranasal oxytocin has been found to have some analogous effects in dogs and humans, here we investigated the effect of oxytocin on dogs' behaviour in the Threatening Approach Test. Dogs, after having received intranasal administration of oxytocin (OT) or placebo (PL), showed the same reaction to an unfamiliar experimenter, but OT pretreated dogs showed a less friendly first reaction compared to the PL group when the owner was approaching. Individual differences in aggression (measured via questionnaire) also modulated dogs' first reaction. Moreover, subjects that received OT looked back more at the human (owner/experimenter) standing behind them during the threatening approach. These results suggest that oxytocin has an effect on dogs' response to the threatening cues of a human, but this effect is in interaction with other factors such as the identity of the approaching human and the 'baseline' aggression of the dogs.

Keywords:

aggression; dog; oxytocin; social behaviour; Threatening Approach Test

Highlights

Dogs' behaviour towards a threatening human is influenced by intranasal oxytocin.

The familiarity of the threatening human (owner/experimenter) has a modulating role.

Owner-rated aggression of the dogs affects their reaction to a threatening human.

1. Introduction

Dogs are the most commonly kept pets in the western world (Hart, 1995) and are present in almost every human society worldwide (Serpell, 2003). Thus dogs pose severe economical expenses to our societies not only through veterinary care and the pet food industry, but also because of problem behaviours. Aggression towards and fear from people are among the most commonly reported dog behavioural problems (Diesel et al., 2008; Stephen and Ledger, 2007; van der Borg et al., 1991), and the two are often interrelated (Guy et al., 2001; Klausz et al., 2014; Landsberg et al., 2003; O'Sullivan et al., 2008).

It is plausible to assume that oxytocin might have an effect on behaviours related to fear and aggression as this has already been shown in humans. Evidence suggests that oxytocin reduces fear responses to social stimuli in humans (Kirsch et al., 2005) through the attenuation of amygdala activation (Domes et al., 2007), that encourages social approach and affiliation. Thus the oxytocin induced reduction of social fear may have an impact on aggressive behaviour given the link between anxiety levels and aggression. Decreased levels of oxytocin in the cerebrospinal fluid of adult men and women have indeed been associated with higher levels of reported aggressive behaviour (Lee et al., 2009). Oxytocin is generally thought to promote positive behaviours (e.g. trust – Kosfeld et al. 2005; Baumgartner et al. 2008, and generosity – Zak et al. 2007; Barraza et al. 2011) and thus is assumed to reduce agonistic behaviours. However, other studies have found that oxytocin can also increase aggression and competition, especially towards out-group members (De Dreu, 2012; De Dreu et al., 2010; Stallen et al., 2012) and it also might increase anxiety to unpredictable threat (Grillon et al., 2013). Importantly it has also been suggested that oxytocin can have differential effects according to subjects' baseline level of aggressive responding (Alcorn et al., 2014).

In case of dogs, however, relatively little is known about the effect of oxytocin on social behaviour. Recent research has found that polymorphisms in the oxytocin receptor (OXTR) gene are related to human directed social behaviours in dogs (Kis et al., 2014a) and that intranasally administered oxytocin promotes positive social behaviours toward both humans and conspecifics (Romero et al., 2014). It has also been reported that intranasally administered oxytocin induces positive expectations about ambivalent stimuli in dogs, especially in a social context (Kis et al., 2015), enhances dogs' use of human pointing gestures (Oliva et al., 2015), and increases gazing behaviour of female dogs to their owners in a neutral situation (Nagasawa et al., 2015). However, no study has yet investigated the effect of intranasal oxytocin on dogs' behaviour in negatively valenced situations.

Based on these previous results our aim in the current study was to investigate the effect of intranasal oxytocin on dogs' response to human behaviour cues of threat. In order to do that we applied the Threatening Approach Test (validated by Vas et al., 2008, 2005) previously used to test coping styles in police dogs (Horváth et al., 2007), aggression in shelter dogs (Kis et al., 2014c) and to assess the dogs' ability to use the owner as a secure base (Gácsi et al., 2013). We also aimed to test the effect of group-belonging (familiarity of the interactants), thus dog were approached threateningly by both the owner (in-group partner) and an unfamiliar experimenter (out-group partner). Subjects' baseline aggressiveness towards people was assessed by means of a questionnaire (Jones, 2008).

2. Subjects and methods

2.1. Ethical statement

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

2.2. Subjects

Thirty-six pet dogs (older than a year, mean age±SD: 4.7±2.6 years; 12 intact & 8 neutered males, 6 intact and 10 spayed females) from various breeds (16 mongrels and 20 purebreds from 14 different breeds: Belgian Shepherd, Black Russian Terrier, Border Collie, Boxer, Bulldog, Central Asian Shepherd Dog, Golden Retriever, Norwitch Terrier, Nova Scotia Duck Tolling Retriever, Schnauzer, Shipperke, Scottish Terrier, Siberian Husky, Stafforsihre Terrier) participated in the study. Subjects participated in two study occasions 1–13 days apart receiving oxytocin and placebo pretreatments in a balanced order (N=18 dogs starting with each of the two treatments). On the first occasion the Threatening Approach Test (see later) was performed by an unfamiliar female experimenter (E), on the second occasion the same test was performed by the owner (O), 33 of the 36 owners were females.

2.3. Pretreatment

The pretreatment was performed according to a protocol previously validated by confirming the physiological effect of oxytocin on electrocardiogram (ECG) measures (Kis et al., 2014b); please note that other studies (Nagasawa et al., 2015; Oliva et al., 2015) have used slightly different intranasal oxytocin administration methods. Subjects received 3 puffs, 12 IU (International Unit) oxytocin (Syntocinon, Novartis) or placebo (0.7% NaCl solution) in a within-subject design. Nasal spray was administered by an unfamiliar female (who had no other role in the experiment) while the dogs were gently held by the owner. This was followed by a 40 minute waiting period (that is presumed to be necessary for the central oxytocin levels to reach a plateau based on the vasopressin measurements of Born et al., 2002). During this time dogs spent the first 30 minutes with an on-leash walk at the University Campus (avoiding any contact with other dogs or humans) during which the experimenter ensured that the owner did not make any social contact with the dog either (e.g. did not pet it, did not talk to it) and kept the length as well as the speed of the walk as standard as possible. Dogs spent the remaining 10 minutes resting in a quiet room with their passive owners present. During this last phase owners were asked to fill in an aggression questionnaire, the *Aggression towards people* scale form Jones (2008). The questionnaire (Table 1) consisted of six items and composed of one single factor (1–5 scale).

2.4. Behaviour testing

Subjects participated in the *Threatening Approach* Test developed by Vas et al. (2005) (figure 1.; supplementary video). During the first test occasion an unfamiliar female experimenter (one of three experimenters randomly selected for each dog) played the role of the approaching human while the owner stood motionless and silently 0.5 m behind the dog (*'Experimenter Approaching'* condition). During the second test occasion they switched their roles; the owner was the approaching human while the E was standing behind (*'Owner*

Approaching' condition). Owners received detailed instructions in order to behave in a way as similar to the experimenters as possible.

Dogs were tethered on a 1.5 m long leash tied to a hook fixed to the floor. The approaching human (AH) entered the testing room and stood 3 m away from the dog and, if necessary, made some noise to get the dog's attention. When the dog looked at the AH, she/he began to approach it. The AH was moving slowly and haltingly (one step in every 4 s) with slightly bent upper body and she was looking steadily into the eyes of the dog without any verbal communication with the hands behind his/her back.

The behaviour of the AH was determined and standardized across subjects according to the following 'If ...then...' rules:

1) If the dog kept looking at the AH, then he/she continued to approach the dog until reaching the dog.

2) If the dog interrupted the eye contact with the AH (moving away and/or turning head away), she/he stopped and waited motionless for about 4 s and then tried to attract the dog's attention by making some noise (e.g. coughing or scratching the ground with the foot). If the dog continued to avert its gaze the AH attempted to call the dog's attention two more times (with 2 s in between attempts). Whenever the dog looked at her/him again, the AH continued the approach. If, however, the dog did not look at her/him after the third attempt, the Threatening Approach was terminated.

3) If the dog showed active avoidance, that is, it moved away to the back of the owner/experimenter from the AH while keeping eye contact, she/he stopped and the Threatening Approach was terminated.

4) If the dog showed signs of aggression or fear, e.g. barked repeatedly or growled continuously (more than 4 s) and/or tried to attack the AH (moving ahead and stretching the leash), the Threatening Approach was terminated.

After terminating the Threatening Approach the AH stepped back, crouched down and started calling the dog in a friendly voice. At the same time the dog was released and encouraged to go to the AH who petted it.

2.5. Data analysis

Behaviour coding was based on Vas et al. (2008, 2005). The *First reaction* of the subjects was coded on an ordinary scale from the moment of looking at the approaching human, until the end of the first step. Dogs received *score 1 – Friendly* if they approached the human in a friendly way (with the tail wagging, ears up and no signs of aggression and/or fear), *score 2 – Approach* if they approached or gazed at the human without tail wagging or wagging the tail between the legs and/or with the ears down, *score 3 – Neutral* if they behaved neutrally (e.g. standing still or sniffing around), *score 4 – Avoid* if they avoided the human (retreating, stepping back) and *score 5 – Threatening* if they moved towards the human in an unfriendly way (barking or growling without any signs of play – e.g. play bow).

Additionally the number of times the dog looked back at the human standing behind it was also coded.

Inter-rater reliability was calculated by double coding of 10 dogs (28% of the sample) and resulted in a substantial agreement (0.61 – 0.80 according to the categorization of Landis and Koch, 1977) for both *First reaction* (κ=0.73), and *Looking back* (κ=0.78).

Generalized Linear Mixed Models were used to analyse the data with multinomial logistic in case of the *First reaction* variable, and negative binomial identity function in case of the *Looking back* variable. In case of both dependent variables we tested the main effect of two factors: pretreatment (OT/PL), identity of the approaching human (O/E), and one covariate:

aggression questionnaire score; as well as the two- and three-way interactions. In case of the *First reaction* variable two separate follow-up models (GLMMs) were run for the experimenter approaching and the owner approaching conditions. In case of the *Looking back* variable separate models could not be run (as only one placebo pretreated dog looked back at the experimenter when approached by the owner), thus we applied pairwise post hoc comparisons (SPSS 22 default option) in the original model in order to confirm the OT/PL effect. SPSS 22 was used for all data analysis.

3. Results

3.1. First reaction

Dogs showed a *Friendly* first reaction in 32% of the cases, an *Approach* reaction in 25% of the cases, a *Neutral* reaction in 22% of the cases, an *Avoid* reaction in 10% of the cases and a *Threatening* reaction in 11% of the cases. The GLMM model showed no significant main effect of oxytocin/placebo (OT/PL) pretreatment (F=2.977, p=0.087) or identity of the human (O/E) approaching (F=0.673, p=0.413) on dogs' first reaction. The main effect of the questionnaire aggression score was, however, significant (F=4.049, p=0.046) with dogs that were rated more aggressive by their owner, receiving higher scores for their first reaction. Also there was a significant pretreatment (OT/PL) × identity (O/E) interaction (F=7.938, p=0.006; figure 2). The pretreatment (OT/PL) × questionnaire score (F=3.289, p=0.072) and the identity (O/E) × questionnaire score (F=0.088, p=0.767) interactions were non-significant. The three-way interaction (pretreatment × identity × questionnaire score) was significant (F=7.979, p=0.005; figure 3.).

Our follow-up analysis showed that in case of the *Experimenter Approaching* condition there was no significant effect of OT/PL pretreatment (F=0.698, p=0.406) or questionnaire score (F=2.886, p=0.094, with a tendency for the questionnaire score to be positively related to the first reaction score) and their interaction was also non-significant (F=0.627, p=0.431). In case of the *Owner Approaching* condition there was a significant OT/PL pretreatment effect (F=7.426, p=0.009) with OT pretreated dogs showing a less friendly first reaction. The main effect of the questionnaire score was not significant (F=1.130, p=0.293), but there was a significant pretreatment × questionnaire score interaction (F=7.550, p=0.008).

3.2. Looking back at Human (Owner/Experimenter)

The looking behaviour of dogs was influenced by both the pretreatment (OT/PL, F=5.007, p=0.029) and the identity of the human standing behind the dog during the threatening approach (O/E, F=6.152, p=0.016, figure 4). Pairwise post hoc analysis confirmed that OT pretreated dogs looked more at the human standing behind them compared to PL pretreated dogs (p<0.001), and dogs looked back more at their owner (i.e. when the experimenter was approaching) compared to the reversed condition (p<0.001). Dogs' baseline aggression (questionnaire score) had no effect (F=2.451, p=0.122) and all interactions were non-significant (p>0.05).

4. Discussion

We have found evidence that oxytocin has the potential to modulate dogs' behaviour in a situation involving threatening behaviour signals by a human. Importantly, however this effect is in interaction with other factors such as the identity of the humans involved in the situation (owner or a stranger) and the baseline aggression of the dogs. This is in line with previous results (Kis et al., 2014a) showing that two OXTR polymorphisms (rs8679684 and 19131AG) affect dogs' Friendliness, a behavioural score mainly composed of their reaction to a threatening stranger. Our results are also in line with human studies that indicate a modulating role of baseline aggression on the effect of oxytocin (Alcorn et al., 2014) and others showing differential effects of oxytocin on conflict behaviour towards in-group versus out-group partners (De Dreu, 2012; De Dreu et al., 2010). However, as in our study dogs were tested in a fixed order (first in the *Stranger Approaching* and then in the *Owner Approaching* conditions) we cannot exclude the possibility of order effect (though previous research has shown that dogs' reaction in the Threatening Approach Test is consistent across test occasions except for immediate re-testing – Klausz et al., 2014; Vas et al., 2008).

Our results showed that contrary to our expectations oxytocin did not decrease aggressive responses in dogs, but they showed a less friendly first reaction towards their owners and behaved in the same way towards the experimenter as in the placebo group. This is in line with other recent research suggesting that oxytocin is not a magical "trust elixir" (Mikolajczak et al., 2010), and that despite increasing prosocial behaviours, it does not make people blind to negative social stimuli, but on the contrary in some cases it even increases the salience of negative social stimuli (Theodoridou et al., 2013). But because of these results the direct applied relevance of our findings is questionable, as a "desirable" outcome would be to use a treatment that decreases unwanted aggression. However in case of some working dogs (e.g. police dogs) sensitisation to threatening social stimuli might also be beneficial. Furthermore we find that dogs look back at the human (owner or experimenter) standing behind them during the threatening approach more often after oxytocin pretreatment. This finding is in line with the study of Guastella et al. (2008) showing that oxytocin increases looking towards the eye-region of faces in humans and corresponds with more recent studies (Nagasawa et al., 2015) demonstrating that dogs look more at their owners in a neutral situation after oxytocin administration. Note, however that in our previous study (Kis et al., 2014a) we could not find any effect of OXTR polymorphisms on looking at humans during a problem-solving task in dogs. Thus in a more naturalistic situation when the owner is allowed to communicate with the dog when it looks back at him/her upon detecting a threat (see e.g.

Merola et al., 2013, 2011 for social referencing about threatening stimuli) this might make a difference in the controllability of their fear and/or aggression response.

It is also important to note that clinical/veterinary practice may not benefit from the research findings on the behavioural effects of a single dose of intranasal oxytocin. Chronic oxytocin treatment has been for example proved to be less effective in improving the symptoms of young patients diagnosed with autism (Guastella et al., 2015) as it could have been expected based on the promising results of single-dose studies.

In the present study oxytocin only influenced dogs' first reaction to the owner, but not to the experimenter. This might suggest that the effect of oxytocin is specific and/or more pronounced towards socially more relevant partners (see e.g. Kis et al., 2014b for the modulating role of social task context on the oxytocin effect in a cognitive bias task). An alternative explanation is that similarly to Alcorn et al. (2014), who found that human subjects with low levels of baseline aggressive behaviour showed an increase in aggressive behaviour, but subjects with high baseline aggressive responding did not, our data might merely reflect that the level of aggression towards the owner is lower than to the experimenter.

In sum our results provide evidence for the effects of physiological (exogenous oxytocin) and contextual (owner/stranger) factors as well as individual differences (baseline aggression) on dogs' behaviour in the Threatening Approach Test. Clearly, these phenomena deserve further investigation in order to determine the possible applied relevance of these results as well as shed light on the role of other factors such as the gender of the approaching human or training history of the dogs.

Acknowledgement

Financial support was provided by the Hungarian Scientific Research Fund (OTKA K12138 & K100695). We thank Ádám Miklósi for his support.

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Figure captions

Table 1. Items of the aggression questionnaire. Owners scored their dogs on a 1–5 scale.

Figure 1. Photograph showing the Threatening Approach Test

Figure 2. First reaction of the dogs (until the first step of the approaching human) towards their owner and an unfamiliar experimenter after oxytocin or placebo pretreatment; median, quartiles, whiskers and outliers

Figure 3. Relationship between dogs' baseline aggression (questionnaire score) and their first reaction to the approaching owner or experimenter after oxytocin and placebo pretreatment

Figure 4. Frequency of looking back at the human (owner/experimenter) in the placebo and oxytocin pretreated groups in the *Owner Approaching* and *Experimenter Approaching* conditions; median, quartiles, whiskers and outliers

Table 1.

Questionnaire item	**Multiplier**
Dog behaves aggressively towards unfamiliar people.	+1
Dog is friendly towards unfamiliar people.	-1
Dog shows aggression when nervous or fearful.	+1
Dog behaves aggressively in response to perceived threats from people (e.g., being cornered, having collar reached for).	+1
Dog behaves aggressively when restrained or handled (e.g., groomed).	+1