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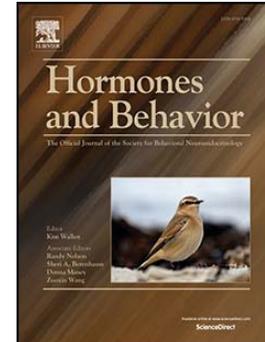
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**Oxytocin induces positive expectations about ambivalent stimuli (cognitive bias) in dogs**

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## Abstract

Expectancy bias towards positive outcomes is a potential key to subjective well-being, and has been widely investigated in different species. Here we test whether oxytocin, suggested to play a role in human optimism and emotional processing, influences how dogs judge ambivalent situations (in a cognitive bias paradigm). Subjects first learned in a location discrimination task that a bowl either contained food (at the 'positive' location) or was empty (at the 'negative' location). Then, after receiving oxytocin or placebo nasal spray, they were presented with the bowl located halfway between the positive and negative positions in communicative or non-communicative contexts (N=4×16). A Positive Expectancy Score was calculated for each subject using the latency to approach this ambivalent location. Compared to placebo groups, subjects that received oxytocin pretreatment showed a positive expectation bias in both contexts, and this effect was more pronounced in the communicative context. Our study provides the first evidence for the impact of oxytocin on dogs' judgement bias and also shows that the social-communicative nature of the task situation modulates the effect of oxytocin.

**Keywords:** Expectation bias; Oxytocin; Dog; *Canis familiaris*

## Highlights

After oxytocin administration dogs form more positive expectations about ambivalent stimuli

The effect of oxytocin is enhanced by the social-communicative context of the task

Dogs parallel humans in that their oxytocin system is related to expectation bias

## Introduction

An increasing body of evidence supports the notion that dogs and humans (infants), in spite of their phylogenetic distance, often show comparable socio-cognitive functioning at the

behavioural level (c.f. Senju and Csibra, 2008; Téglás et al., 2012). It has also been shown that some degree of comparability exists between dogs and humans in ‘dispositional optimism’, a characteristic personality feature of humans which is often conceptualized as positive expectation bias. For example, tendency to form negative (‘pessimistic’) judgements is associated with increased level of depressive symptoms in humans (Strunk et al., 2006) and separation related behaviour problems in dogs (Mendl et al., 2010).

Another line of recent research has provided an increasingly coherent picture of neurohormonal regulatory mechanisms of social life, suggesting that oxytocin is specifically involved in the regulation of human (and non-human) social cognition (Yamasue et al., 2012). Recent findings suggest an association between oxytocin and self-assessed psychological well-being in humans (William et al., 2011). Optimism has also long been investigated due to its role in human health and well-being (Scheier and Carver, 1992) as expectancy biases are known to be influenced both positively and negatively by people’s current mood (Carver et al., 2010). Furthermore, recent research has linked such psychological resources to the oxytocin system (Saphire-Bernstein et al., 2011), although the results are still controversial (Cornelis et al., 2012).

A common way of investigating such questions in humans is by intranasally administering oxytocin (Heinrichs et al., 2009; Van IJzendoorn and Bakermans-Kranenburg, 2012) as there is a tacit assumption in the literature that this method enables direct access of the peptide to the central nervous system. However, there is no evidence yet suggesting that in dogs intranasal oxytocin administration would induce similar physiological changes as in humans.

In the current study we combine these lines of research and investigate – after validation of the physiological effects of intranasal oxytocin administration in dogs – the effects of oxytocin on positive expectations in a cognitive bias paradigm. This paradigm quantifies how subjects react (e.g. in terms of approach latency) to an ambivalent stimulus as compared to the

interval determined by positive–negative stimuli (e.g. Gygas, 2014). As previous research (e.g. Topál et al., 2009) has shown that the social-communicative nature of the task (whether the human experimenter addresses the subjects and makes eye-contact with them) can greatly influence dogs' performance, we decided to test the effect of oxytocin in both communicative and non-communicative test contexts.

### **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).

### **Physiological validation of intranasal oxytocin administration in dogs**

#### *Subjects and methods*

ECG recordings were conducted on ten pet dogs (>1 year; 3 males and 7 females with a mean age $\pm$ SD of 4.33 $\pm$ 2.69 years) following 12 IU oxytocin and placebo administration in a within-subject design. ECG recordings were conducted in the Department of Ethology, ELTE, Budapest. The testing room was equipped with office furniture and a mattress on the floor for the dog and its owner. During a 40 minute waiting period (that is presumed to be necessary for the central oxytocin levels to reach a plateau – Born et al., 2002) dogs spent the first 25 minutes with an on-leash walk at the University Campus (avoiding any contact with other dogs or humans) during which the experimenter ensured that the owner did not make any social contact with the dog either (e.g. did not pet 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 15 minutes resting in a quiet room with their passive owners present. While we made every possible

effort to keep the circumstances of the period before the ECG measurement as standard as possible, body posture of the dog was not fully controlled by the owner/experimenter in order to avoid stress inherent to external restraint. Evidently, this procedure caused slight variations in the subjects' behaviour during the waiting period and this might have caused some noise in our data. However we expected the effect of oxytocin to be strong enough to manifest even under these semi-natural conditions. When the 40 minutes waiting period elapsed a 5-10 minutes on-leash exploration and familiarization followed in the ECG measurement room, after which the owner took a seat on the mattress and assisted the experimenter throughout the process of fixing two surface attached electrodes onto the dog's chest (second rib on both the left and right side). Gold-coated Ag|AgCl electrodes fixed with EC2 Grass Electrode Cream (Grass Technologies, USA) were used for the recordings. The electrode placement was followed by 4 minutes quiet resting, and then by a 1 minute long recording. During this last five minutes every dog was in lying position because previous research has shown that body posture has a significant effect on dogs' heart rate (Maros et al., 2008). The length of the ECG measurement was based on previous dog heart rate studies (Gácsi et al., 2013; Maros et al., 2008). Signals were collected, prefiltered, amplified and digitized at a sampling rate of 249 Hz/channel by using the 30 channel Flat Style SLEEP La Mont Headbox with implemented second order filters at 0.5 Hz (high pass) and 70 Hz (low pass) as well as the HBX32-SLP 32 channel preamplifier (La Mont Medical Inc., USA). R peaks were manually detected, and RR intervals were measured using the Fercio program (© Ferenc Gombos 2012). Heart rate (HR; 1/min) was derived from RR interval averages ( $60/\text{meanRR}$ ), and heart rate variability (HRV; sec) was calculated as the standard deviation of RR intervals (see e.g. Gácsi et al. 2013 for similar measures).

## *Results*

In spite of the considerable individual variation in the effect of oxytocin on HR and HRV (**Figure 1**), at the group level oxytocin significantly decreased HR ( $t_{(9)}=2.810$ ,  $p=0.020$ , Cohen's  $d$ : 0.944) and increased HRV ( $t_{(9)}=4.472$ ,  $p=0.002$ , Cohen's  $d$ : 1.400). These results are consistent with those of previous studies on humans (Gutkowska and Jankowski, 2008; Kemp et al., 2012; Kis et al., 2013; Light et al., 2005) and thus indicate that intranasal administration of oxytocin can be a valid approach to study its effects in dogs. These results do not provide information on the cellular mechanisms nor prove that intranasal administration of oxytocin causes an increase (exclusively) in the central nervous system in dogs, as peripheral increase in oxytocin levels might also lead to changes in heart rate and heart rate variability due the presence of oxytocin receptors in the cardiac tissue (Jankowski et al., 2004).

### **The effect of oxytocin on cognitive bias**

#### *Subjects*

Sixty-four pet dogs (>1 year; 28 males, 36 females; 23 neutered; mean age $\pm$ SD: 4.44 $\pm$ 2.67 years) from various breeds (22 mongrels and 42 pure breeds from 20 different breeds) were tested (21 of small ( $\leq 9$  kg), 33 of medium (10-25 kg) and 10 of large (>25 kg) size based on average standard weight, <http://www.akc.org/> in case of pure breed dogs or based on the inspection of the videos in case of mixed breed dogs). In order to be eligible for the test dogs needed to be motivated by dry food according to their owners. Subjects were randomly assigned into four experimental conditions: receiving oxytocin (OT) or placebo (PL) pre-treatment and participating in the test in either communicative (Com) or non-communicative (NCom) context (N=16 in each). The four groups did not differ in mean age (ANOVA,  $F=0.457$ ,  $p=0.714$ ), sex ratio (Chi<sup>2</sup> test,  $\chi^2=3.089$ ,  $p=0.386$ ), neutered status ( $\chi^2=3.052$ ,  $p=0.384$ ), pure/mixed breed ( $\chi^2=1.140$ ,  $p=0.767$ ), or size ( $\chi^2=0.746$ ,  $p=0.993$ ). Owners were

blind to the aims of the study and details of the experimental procedure as well as to the pretreatment their dogs received.

### *Procedure*

The summary of the procedure is shown in **Figure 2**, the video-protocol can be accessed through the following link: <http://www.cmdbase.org/web/guest/play/-/videoplayer/249>. The whole procedure was carried out on the same day and took approximately 50-60 minutes (training phase: 5-15 minutes, pretreatment: 1-2 minutes, waiting period: 40 minutes, retraining: 3 minutes, test phase: 1 minute) that is presumed to be within the time interval when intranasally administered oxytocin exerts its central effects (see e.g. Ditzen et al., 2009; Heinrichs et al., 2003).

### Training

The training phase was identical for all subjects and was based on the procedure developed by (Mendl et al., 2010). The dog was held by its collar by the owner at a predetermined starting point, at a 3 m distance from the two possible hiding locations placed on the left and right side of the room 2 m apart from each other. The experimenter positioned herself facing the dog, established eye-contact with it, and addressed it (dog's name + "Look!"). Then she placed the food bowl to either of the two locations (i.e. positive – P and negative – N) in a fixed semi-random order (PPNPNN, repeated until criterion – see below), so that at the positive side the bowl always contained a food reward while at the negative side it was always empty. Dogs and their owners had no visual access to the content of the bowl except for the first trial (this was a positive trial for all subjects) when the experimenter, before hiding the food, showed it up in order to motivate the dog to search. The positive and negative side (left/right) was counterbalanced across subjects. The owner was instructed to release the dog at the moment,

when the food bowl touched the ground. If the dog did not start moving when released, the owner was allowed to encourage it with short utterances (e.g. “Go!”, “It’s yours”) or by gently touching it. Apart from this no other forms of communication were allowed. The dog was allowed to approach the food bowl in every trial, while the experimenter was looking straight ahead without maintaining eye contact with the dog. The latency of approach (i.e. the time elapsed between the moment when the food bowl touched the ground and the dog reached the line of the food bowl) was noted in every trial. Although there was some variation in owners’ reaction times releasing their dogs, we decided to use the moment when the food bowl touched the ground as a starting point because this could always be coded reliably, while owners did not always release their dogs with an easily visible movement. In order to exclude the possibility that the owners’ reaction times (e.g. the time elapsed between the bowl touching the ground and the owner releasing the dog) systematically influenced our latency data we coded owner reaction times in case of 32 subjects (50% of the total sample) and found no difference in the test trials among the four treatment groups (OT/PL  $\times$  Com/NCom; ANOVA,  $F=1.707$ ,  $p=0.188$ ) nor among the positive/negative/ambivalent trials ( $F=0.327$ ,  $p=0.722$ ). Successive trials were presented with no breaks in between. Dogs were deemed to have learnt an association between bowl location and food reward when for the preceding five positive trials and the preceding five negative trials the longest latency to reach the positive location was shorter than any of the latencies to reach the negative location (Wilcoxon Test,  $p=0.025$ ). This took on average  $23\pm 6$  (mean $\pm$ SD) trials, with a minimum of 12 and a maximum of 36 trials.

### Pretreatment

After having reached this learning criterion half of the subjects received a single intranasal dose of 12 IU oxytocin (Syntocinon-Spray, Novartis; nasal spray with a nebulizer) (OT,

N=32), the other half received placebo, isotonic natriumchlorid 0.9% solution (PL, N=32). The 12 IU dose was chosen to be half of the 24 IU commonly used in human studies (e.g. Lischke et al., 2012; Perry et al., 2010), and the same dose was administered to all subjects irrespective of their body weight (which is also the common practice in human studies). Then, a 40-minute waiting period followed, divided into a 25 minutes on-leash walk and a 15 minutes quiet resting in the exact same way as described in the ECG study.

### Re-training

After the waiting period had elapsed dogs participated in a 9-trial re-training phase that, in case of the communicative context (half of the subjects, 16 OT and 16 PL), was identical to the training trials, while in the non-communicative context (other half of the subjects, 16 OT and 16 PL) the experimenter acted from behind a curtain, and slid the food bowl under the curtain into position, without providing any communicative cues. (This re-training phase was necessary because our pilot data showed that dogs' latency to reach the food bowl did not differ between the positive and negative sides after a 40 minutes delay that followed the training.)

### Test phase

The test phase consisted of three trials: a negative (N), a positive (P) and an ambivalent (A; during which the baited bowl was placed halfway between the positive and negative locations (17 cm behind the line connecting the two locations, at a 3 m distance from the dog) trial. The trials were presented in fixed (N, P, A) order administered in the same Com or NCom context (half of the subjects participating in each context) as described for the re-training. The training, re-training and test phases were videotaped and the latencies to approach the food bowl were coded with a frame-by-frame analysis using Solomon Coder

(<http://solomoncoder.com/>) blind to OT/PL treatment of the subjects. Inter-rater reliability was calculated for both the start and the end point of the latency based on double coding of 13 recordings (20% of the total sample) and resulted in an almost perfect agreement between the two raters (start:  $\kappa=1.00$ , end:  $\kappa=0.83$ ).

Although one could argue that dogs in this situation can possibly smell whether there is food in the bowl, previous research (e.g. Lakatos et al., 2011) indicates that in similar setups dogs are not able to choose the baited cup based on odour cues alone. (This is further supported by the fact that our subjects did not differentiate in their latency to reach the positive versus negative location (paired samples t-test,  $t_{(65)}=0.553$ ,  $p=0.582$ ) in their first training trials.)

#### *Data analysis*

Training phase. Mean latency to approach the positive and negative locations was calculated for each subject based on the last five positive and the last five negative trials. A Generalized Estimating Equation (GEE) model was used to confirm the effect of location (positive vs. negative; within subject factor) on the latency to approach the bowl and to test the possible differences among the four conditions (between subject factor).

Re-training phase. For each subject the latency for the first positive and the first negative as well as the last positive and the last negative trial was entered in a GEE model with the following factors: positive vs. negative trial (within subject factor), first vs. last trial (within subject factor), Com vs. NCom context (between subject factor), OT vs. PL pretreatment (between subject factor).

Test phase. A GEE was used to test the differences between the latency to approach the positive vs. negative vs. ambivalent location (within subject factor) and the effect of test context (Com vs. NCom; between subject factor) as well as the effect of pretreatment (OT vs. PL, between subject factor). Moreover, in order to assess subjects' judgement bias in the

ambivalent trials and to control for the high individual variation in running speed (which presumably causes a greater variation than the treatment itself), a Positive Expectancy Score (PES) was calculated for each subject using the latency to approach the negative, positive and ambivalent locations according to the following formula:  $PES = 100 - CBS$ , where

$$CBS = \frac{(\text{latency to reach ambivalent location} - \text{latency to reach positive location}) * 100}{\text{latency to reach negative location} - \text{latency to reach positive location}}$$

Note, that CBS is the adjusted cognitive bias score previously developed by Mendl et al. (2010).

Higher PES values thus indicate a more positive expectation bias (the latency for the ambivalent location is more similar to the latency for the positive than for the negative location). In cases when the latency for the ambivalent location is in-between the latency to the negative and to the positive location (with a higher negative latency) the value of the PES falls within the 0-100 interval.

A General Linear Model (GLM) was used to test the effect of test context (Com vs. NCom; between subject factor) as well as the effect of pretreatment (OT vs. PL; between subject factor) on PES. Planned pairwise comparisons (independent samples t-tests) were carried out to assess the effect of OT vs. PL pretreatment in both the Com and NCom contexts; as well as to assess the effects of Com vs. NCom test contexts for both OT and PL pretreated dogs. The effect size (Cohen's D) was calculated using the [www.cognitiveflexibility.org/efficientsize/](http://www.cognitiveflexibility.org/efficientsize/) webpage. The effect of independent variables (sex, neutered status, age, pure / mix breed, size) and the interaction within these factors and with the four groups (OT/PL pretreatment  $\times$  Com/NCom context) was tested with a GLM. All statistical tests were two-tailed with an alpha value of  $\alpha=0.05$ .

## Results

The GEE analysis revealed that by the end of the training phase there was a consistent difference in the latency to approach the positive versus negative location (with a shorter latency for the positive location;  $\chi^2=55.215$ ,  $p<0.001$ ) while the four conditions did not differ from each other ( $\chi^2=3.827$ ,  $p=0.281$ ) and there was no significant condition  $\times$  location interaction ( $\chi^2=3.123$ ,  $p=0.373$ ).

Raw latency data for the different conditions of the re-training and test phases are shown in **Table 1**. In the re-training phase subjects' latencies were higher in the NCom than in the Com context ( $\chi^2=13.089$ ,  $p<0.001$ ), and a significant positive/negative location  $\times$  first/last trial interaction ( $\chi^2=16.361$ ,  $p<0.001$ ) indicated that subjects differentiated between positive/negative locations only at the end of the re-training, but not at the beginning. The effect of OT/PL pretreatment ( $\chi^2=0.159$ ,  $p=0.690$ ) as well as all other interactions were not significant (all  $p>0.05$ ).

During the test phase a similar difference was found between the positive, negative and ambivalent locations (GEE,  $\chi^2=38.353$ ,  $p<0.001$ ). Furthermore, dogs in the NCom context showed higher latencies ( $\chi^2=15.444$ ,  $p<0.001$ ) irrespective of PL/OT pretreatment ( $\chi^2=0.002$ ,  $p=0.963$ ). However there was a significant P/N/A location  $\times$  PL/OT pretreatment interaction ( $\chi^2=8.678$ ,  $p=0.013$ ) indicating that the OT effect was specific to the ambivalent location (see also the results for the PES score) as well as a P/N/A location  $\times$  Com/NCom context interaction ( $\chi^2=8.721$ ,  $p=0.013$ ). The PL/OT pretreatment Com/NCom context interaction did not reach significance ( $\chi^2=2.732$ ,  $p=0.098$ ), and neither did the three-way interaction ( $\chi^2=1.780$ ,  $p=0.411$ ). More importantly, dogs receiving OT pretreatment achieved a higher Positive Expectancy Score (PES), than dogs receiving PL pretreatment (GLM,  $F=38.818$ ,  $p<0.001$ ) and this difference was more pronounced in the communicative context as reflected in a significant pretreatment  $\times$  context interaction ( $F=5.434$ ,  $p=0.023$ , **Figure 3**). There was no main effect of Com/NCom contexts ( $F=1.952$ ,  $p=0.167$ ).

Planned pairwise comparisons confirmed these results as OT pretreated dogs achieved higher PES both in the Com ( $t_{(30)}=6.118$ ,  $p<0.001$ , Cohen's  $d$ : 2.163) and in the NCom ( $t_{(30)}=2.729$ ,  $p=0.011$ , Cohen's  $d$ : 0.965) contexts. Furthermore, OT pretreated dogs achieved a higher PES in the Com than in the NCom context ( $t_{(30)}=2.884$ ,  $p=0.007$ , Cohen's  $d$ : 1.020), whereas PL pretreated dogs did not show a context dependent difference ( $t_{(30)}=0.612$ ,  $p=0.545$ , Cohen's  $d$ : 0.216).

The PES was not affected by the subjects' sex ( $F=0.231$ ,  $p=0.644$ ), neutered status ( $F=0.158$ ,  $p=0.701$ ), age ( $F=0.032$ ,  $p=0.862$ ), breed ( $F=0.652$ ,  $p=0.443$ ) or size ( $F=0.099$ ,  $p=0.761$ ), and there was no significant interaction among these factors or with the pretreatment group (all  $p>0.05$ ).

## Discussion

This study presents new information in the growing debate over whether oxytocin modulates positive expectation bias in humans (Cornelis et al., 2012; Saphire-Bernstein et al., 2011) or in nonhuman animals. Our results validate the effect of intranasal oxytocin administration for dogs at the physiological level (although without uncovering the exact mechanisms) and provide the first evidence suggesting that oxytocin induces positive expectations in dogs. Recent research has provided an increasingly coherent picture of the involvement of oxytocin in the regulation of human and non-human social behaviour phenomena (such as trust (Kosfeld et al., 2005) and generosity (Barraza et al., 2011) or social memory (Ferguson et al., 2002; Guastella et al., 2008)), and in our study the judgement bias in dogs about ambivalent stimuli also appears to be modulated by the social-communicative nature of the task context. The differential effects of the communicative and non-communicative contexts on dogs behaviour might be due to several factors such as the presence of the experimenter providing communicative addressing signals or simply due to the fact that dogs had to approach a

human (especially in the ambivalent trial when the food bowl was placed in the middle position). Interestingly, however, this effect was selectively associated with oxytocin pretreatment which may indicate an interspecific (dog–human) social-tuning effect of this neuromodulator in the dog.

The present findings extend our previous knowledge about the role of oxytocin in positive emotions and welfare (Mitsui et al., 2011) and reveal an interesting parallel between dogs and humans with regard to the connectedness between the oxytocin system and positive expectation bias. Human optimism as well as the ‘optimistic/pessimistic’ cognitive bias in animal models (Harding et al., 2004) have been linked to mental health (Scheier and Carver, 1987, 1985) and behavioural problems (such as separation anxiety – Mendl et al., 2010) as well as to placebo sensitivity (humans: Geers et al., 2005; dogs: Sümegi et al., 2014). Our results, therefore, may have potential applied and some indirect clinical relevance. We note, however, that further studies should determine how other factors, such as ‘baseline optimism’ of the subjects and/or polymorphisms in the OXTR gene, modulate the effect we have found. Recent accounts in the human literature cautioned about the individual differences in the effects of oxytocin on social behavior (Bartz et al., 2011), and a recent study on dogs also found that the effect of a polymorphism in the OXTR gene on social behaviour is conditional to a breed effect (Kis et al., 2014).

It is also important that previous studies (e.g. Mendl et al. 2010) found that their ‘treatment’ had a similar effect on both the latency to reach the ambivalent location and the adjusted score calculated based on latency to positive/negative/ambivalent locations. In the current study, however, we found a main effect of PL/OT treatment only in case of the PES score, while in the analysis of the raw latency data the effect of treatment was in interaction with other factors. This seemingly contradictory finding might be explained by the fact that the subjects of the present study were very heterogeneous (randomly selected from a pet dog database vs.

dogs were from two animal re-homing centres in the Mendl et al. 2010 study) and thus their running speed varied greatly, causing a greater variation in the latency data, than the effect of the treatment. These individual variations were controlled for in the PES score that was calculated based on the latency to positive/negative/ambivalent locations and thus used an individual negative–positive scale for each dog. It can be argued that because dogs received the test trials in a fixed negative, positive, ambivalent order, and the ambivalent trial was always preceded by a positive trial, this might have biased the PES score towards the positive direction. However we found no such deviation from the chance level in the placebo groups. Also, even if such a bias had existed, it would have been the same for all subjects, not affecting the revealed effect of oxytocin and social-communicative task context.

Contrary to expectations (e.g. Herzmann et al., 2013), in the present study dogs' sex, neutered status, age, breed (pure / mix breed) or size did not have an effect on dogs' positive expectancy nor was in interaction with pretreatment. The most parsimonious interpretation of these results is that as the goal of the present study was not to unravel individual differences, we could not find such potential effects due to the limited sample size and the lack of systematic grouping based on these features. We should also note that a further factor that might contribute to individual variations in oxytocin effects in both the present and previous human studies, is the fact that all subjects receive the same dose of intranasal oxytocin irrespective of body weight. Due to the large within-species variation this confound might be more pronounced in dogs.

Previous research has shown that the dog is a promising model species to study human psychiatric conditions (Overall, 2000) as well as the genetic background of certain illnesses (Parker et al., 2010). The present results extend these notions by showing that a similar neuro-hormonal mechanism (the oxytocin system) might be responsible for a crucial psychological resource, the positive judgement of ambivalent stimuli. Importantly, in addition to ample

evidence on the role of oxytocin in regulating social behaviour in humans and rodents (Donaldson and Young, 2008), this is the first evidence of the effect of intranasally administered oxytocin on dog behaviour, and thus our results open up the way for further research to use the dog as a model of human socio-cognitive competences (Miklósi and Topál, 2013) at the neurohormonal level as well.

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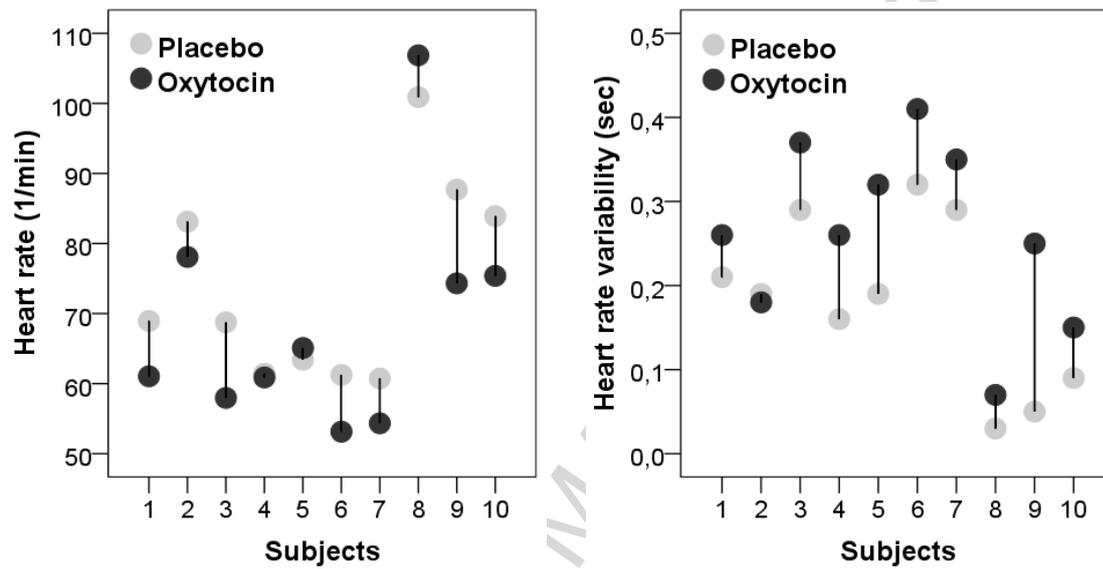
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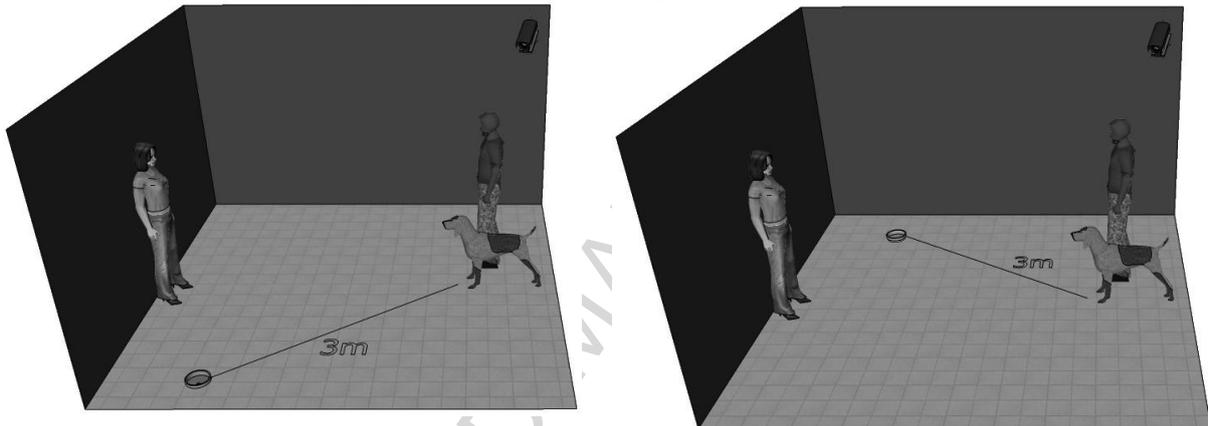
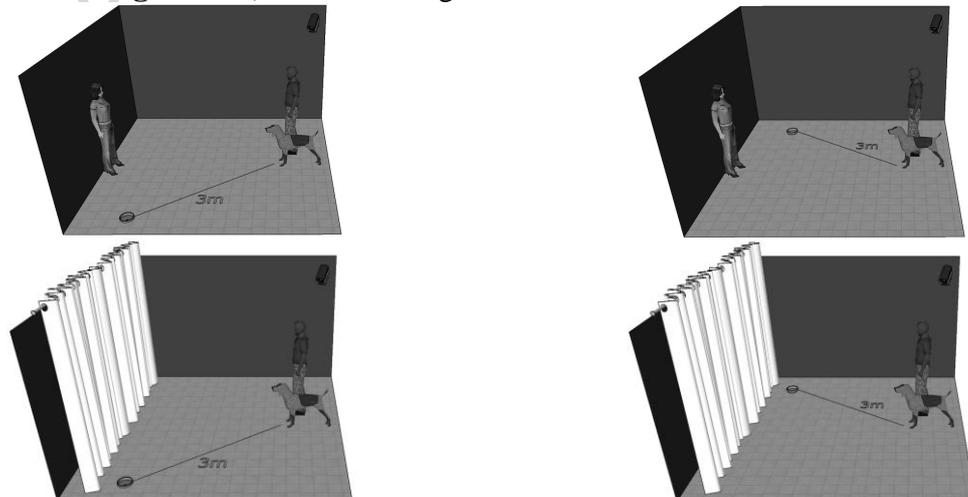
**Figure legends****Figure 1.**

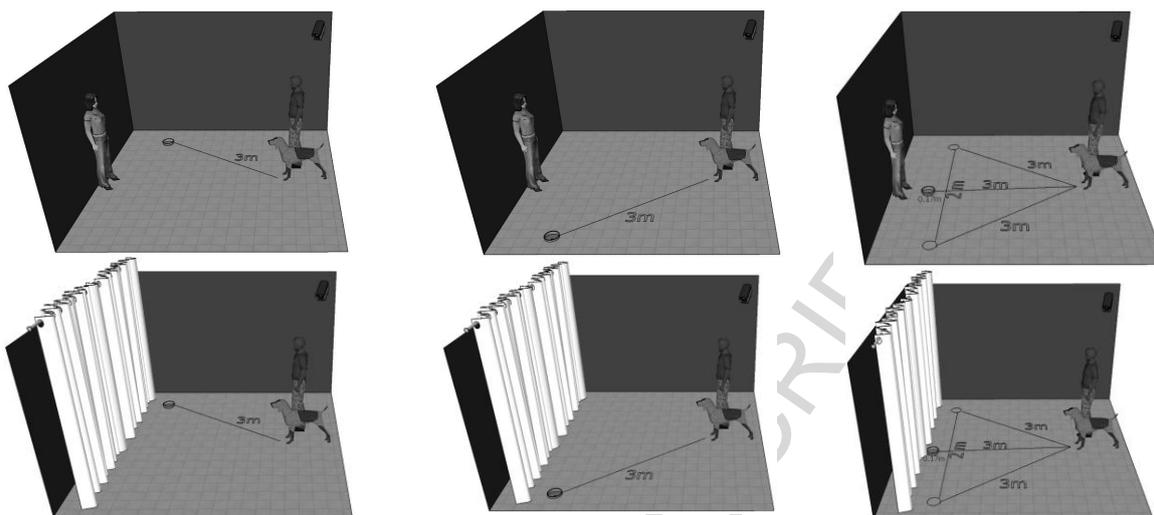
The effect of oxytocin on heart rate and heart rate variability in ten individual dogs



**Figure 2.**

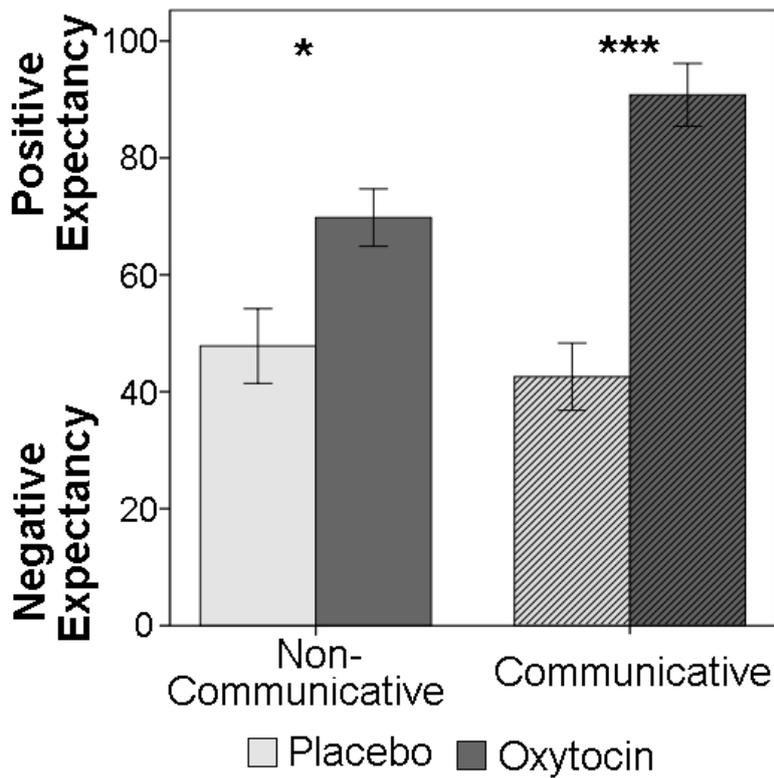
Schematic overview of the different conditions throughout the four phases of the experiment. The drawings in one row stand for the different type of trials (e.g. positive and negative) that one individual subject received in that phase, while the different rows represent between subject treatments (e.g. communicative or non-communicative)

**1) Training Phase (Positive & Negative trials; until criterion)****2) Pretreatment (OT or PL nasal spray + 40 minutes waiting period)****3) Re-training Phase (Positive & Negative trials in Com or NCom context; 9 trials)****4) Test Phase (1-1-1 Negative-Positive-Ambivalent trial in Com or NCom context)**



**Figure 3.**

The Positive Expectancy Scores (PES) of dogs in the non-communicative and social-communicative versions of the cognitive bias task after placebo / oxytocin pretreatment (mean $\pm$ SE). A higher PES indicates a reaction to the ambivalent location that is more similar to the reaction to the positive than to the negative location. \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$



**Table 1.**

Latency  $mean \pm SD$  ( $min-max$ ) to reach the food bowl in the different conditions of the re-training and test phases (sec). Data for the PL/OT pretreated dogs is pulled together for the re-training phase as the statistical analysis showed no effect of treatment on the positive/negative latencies during this phase

|                    |             |              | Positive                 | Negative                  |                           |
|--------------------|-------------|--------------|--------------------------|---------------------------|---------------------------|
| <b>Re-training</b> | <i>Com</i>  | <i>First</i> | 1.84±0.48<br>(0.92–2.67) | 2.50±1.87<br>(1.33–11.53) |                           |
|                    |             | <i>Last</i>  | 2.02±0.76<br>(0.87–4.78) | 4.03±3.26<br>(0.80–15.16) |                           |
|                    | <i>Ncom</i> | <i>First</i> | 3.33±1.04<br>(1.61–5.60) | 3.37±1.39<br>(1.48–7.00)  |                           |
|                    |             | <i>Last</i>  | 3.17±1.26<br>(1.07–7.30) | 6.48±5.97<br>(2.00–27.00) |                           |
|                    |             |              | Positive                 | Ambivalent                | Negative                  |
| <b>Test</b>        | <i>Com</i>  | <i>OT</i>    | 2.03±0.98<br>(1.00–5.40) | 2.16±0.71<br>(1.00–4.00)  | 2.99±1.11<br>(1.20–5.00)  |
|                    |             | <i>PL</i>    | 1.83±0.49<br>(1.00–2.80) | 2.26±0.57<br>(1.40–3.40)  | 2.68±0.88<br>(1.60–4.40)  |
|                    | <i>Ncom</i> | <i>OT</i>    | 2.78±0.84<br>(1.20–4.40) | 3.61±2.03<br>(1.80–10.60) | 5.91±4.40<br>(1.80–17.80) |
|                    |             | <i>PL</i>    | 3.19±1.19<br>(1.80–5.80) | 4.98±3.04<br>(2.80–15.00) | 6.56±3.20<br>(3.00–15.00) |