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

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## 1 **Abstract**

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

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

17

## 18 **Highlights**

19 After oxytocin administration dogs form more positive expectations about ambivalent stimuli

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

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

22

## 23 **Introduction**

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

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

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

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

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

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

56

### 57 **Ethical statement**

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

62

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

#### 64 *Subjects and methods*

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

76 effort to keep the circumstances of the period before the ECG measurement as standard as  
77 possible, body posture of the dog was not fully controlled by the owner/experimenter in order  
78 to avoid stress inherent to external restraint. Evidently, this procedure caused slight variations  
79 in the subjects' behaviour during the waiting period and this might have caused some noise in  
80 our data. However we expected the effect of oxytocin to be strong enough to manifest even  
81 under these semi-natural conditions. When the 40 minutes waiting period elapsed a 5-10  
82 minutes on-leash exploration and familiarization followed in the ECG measurement room,  
83 after which the owner took a seat on the mattress and assisted the experimenter throughout the  
84 process of fixing two surface attached electrodes onto the dog's chest (second rib on both the  
85 left and right side). Gold-coated Ag|AgCl electrodes fixed with EC2 Grass Electrode Cream  
86 (Grass Technologies, USA) were used for the recordings. The electrode placement was  
87 followed by 4 minutes quiet resting, and then by a 1 minute long recording. During this last  
88 five minutes every dog was in lying position because previous research has shown that body  
89 posture has a significant effect on dogs' heart rate (Maros et al., 2008). The length of the ECG  
90 measurement was based on previous dog heart rate studies (Gácsi et al., 2013; Maros et al.,  
91 2008). Signals were collected, prefiltered, amplified and digitized at a sampling rate of 249  
92 Hz/channel by using the 30 channel Flat Style SLEEP La Mont Headbox with implemented  
93 second order filters at 0.5 Hz (high pass) and 70 Hz (low pass) as well as the HBX32-SLP 32  
94 channel preamplifier (La Mont Medical Inc., USA). R peaks were manually detected, and RR  
95 intervals were measured using the Fercio program (© Ferenc Gombos 2012). Heart rate (HR;  
96 1/min) was derived from RR interval averages ( $60/\text{meanRR}$ ), and heart rate variability (HRV;  
97 sec) was calculated as the standard deviation of RR intervals (see e.g. Gácsi et al. 2013 for  
98 similar measures).

99

100 *Results*

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

112

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

#### 114 *Subjects*

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

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

128

### 129 *Procedure*

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

137

### 138 Training

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

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

172

### 173 Pretreatment

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

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

182

### 183 Re-training

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

192

### 193 Test phase

194 The test phase consisted of three trials: a negative (N), a positive (P) and an ambivalent (A;  
195 during which the baited bowl was placed halfway between the positive and negative locations  
196 (17 cm behind the line connecting the two locations, at a 3 m distance from the dog) trial. The  
197 trials were presented in fixed (N, P, A) order administered in the same Com or NCom context  
198 (half of the subjects participating in each context) as described for the re-training. The  
199 training, re-training and test phases were videotaped and the latencies to approach the food  
200 bowl were coded with a frame-by-frame analysis using Solomon Coder

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

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

210

#### 211 *Data analysis*

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

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

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

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

$$230 \quad CBS = \frac{(\text{latency to reach ambivalent location} - \text{latency to reach positive location}) * 100}{\text{latency to reach negative location} - \text{latency to reach positive location}}$$

231 Note, that CBS is the adjusted cognitive bias score previously developed by Mendl et al.  
 232 (2010).

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

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

248

## 249 **Results**

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

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

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

275 Planned pairwise comparisons confirmed these results as OT pretreated dogs achieved higher  
276 PES both in the Com ( $t_{(30)}=6.118$ ,  $p<0.001$ , Cohen's  $d$ : 2.163) and in the NCom ( $t_{(30)}=2.729$ ,  
277  $p=0.011$ , Cohen's  $d$ : 0.965) contexts. Furthermore, OT pretreated dogs achieved a higher PES  
278 in the Com than in the NCom context ( $t_{(30)}=2.884$ ,  $p=0.007$ , Cohen's  $d$ : 1.020), whereas PL  
279 pretreated dogs did not show a context dependent difference ( $t_{(30)}=0.612$ ,  $p=0.545$ , Cohen's  $d$ :  
280 0.216).

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

285

## 286 **Discussion**

287 This study presents new information in the growing debate over whether oxytocin modulates  
288 positive expectation bias in humans (Cornelis et al., 2012; Saphire-Bernstein et al., 2011) or  
289 in nonhuman animals. Our results validate the effect of intranasal oxytocin administration for  
290 dogs at the physiological level (although without uncovering the exact mechanisms) and  
291 provide the first evidence suggesting that oxytocin induces positive expectations in dogs.  
292 Recent research has provided an increasingly coherent picture of the involvement of oxytocin  
293 in the regulation of human and non-human social behaviour phenomena (such as trust  
294 (Kosfeld et al., 2005) and generosity (Barraza et al., 2011) or social memory (Ferguson et al.,  
295 2002; Guastella et al., 2008)), and in our study the judgement bias in dogs about ambivalent  
296 stimuli also appears to be modulated by the social-communicative nature of the task context.  
297 The differential effects of the communicative and non-communicative contexts on dogs  
298 behaviour might be due to several factors such as the presence of the experimenter providing  
299 communicative addressing signals or simply due to the fact that dogs had to approach a

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

304 The present findings extend our previous knowledge about the role of oxytocin in positive  
305 emotions and welfare (Mitsui et al., 2011) and reveal an interesting parallel between dogs and  
306 humans with regard to the connectedness between the oxytocin system and positive  
307 expectation bias. Human optimism as well as the ‘optimistic/pessimistic’ cognitive bias in  
308 animal models (Harding et al., 2004) have been linked to mental health (Scheier and Carver,  
309 1987, 1985) and behavioural problems (such as separation anxiety – Mendl et al., 2010) as  
310 well as to placebo sensitivity (humans: Geers et al., 2005; dogs: Sümegi et al., 2014). Our  
311 results, therefore, may have potential applied and some indirect clinical relevance. We note,  
312 however, that further studies should determine how other factors, such as ‘baseline optimism’  
313 of the subjects and/or polymorphisms in the OXTR gene, modulate the effect we have found.  
314 Recent accounts in the human literature cautioned about the individual differences in the  
315 effects of oxytocin on social behavior (Bartz et al., 2011), and a recent study on dogs also  
316 found that the effect of a polymorphism in the OXTR gene on social behaviour is conditional  
317 to a breed effect (Kis et al., 2014).

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

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

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

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

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

355

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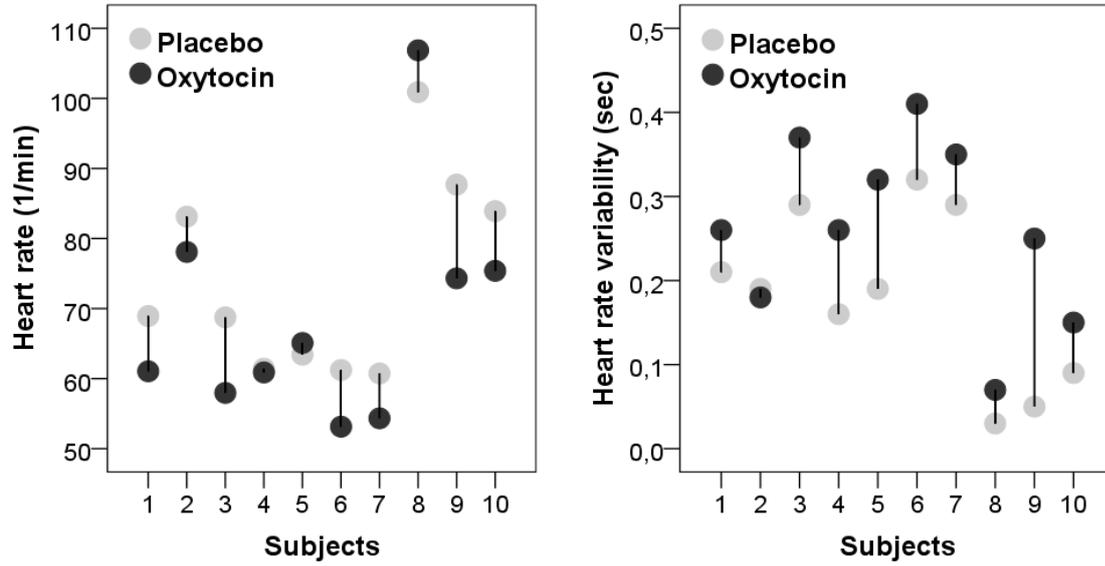
472

473

474 **Figure legends**

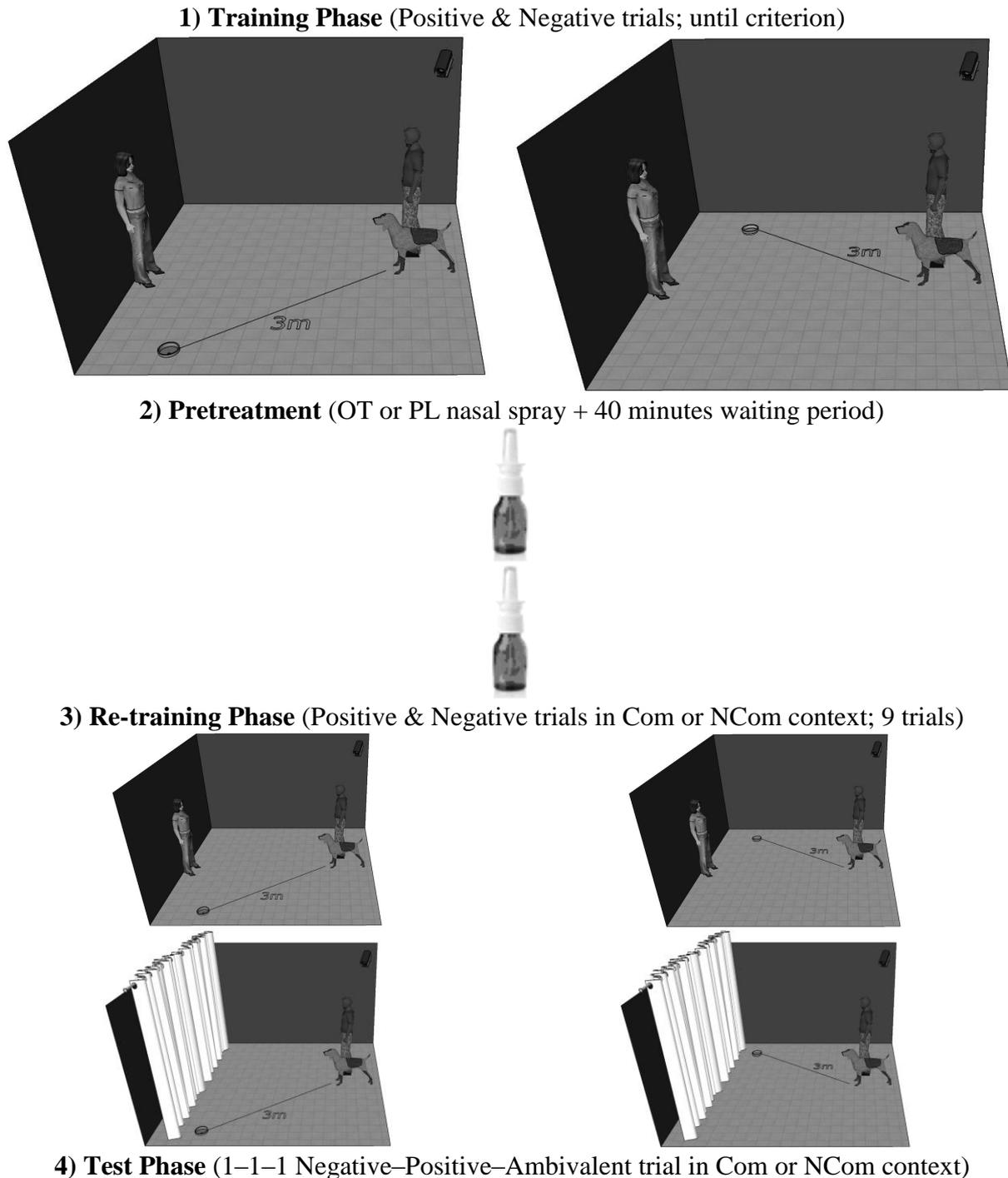
475 **Figure 1.**

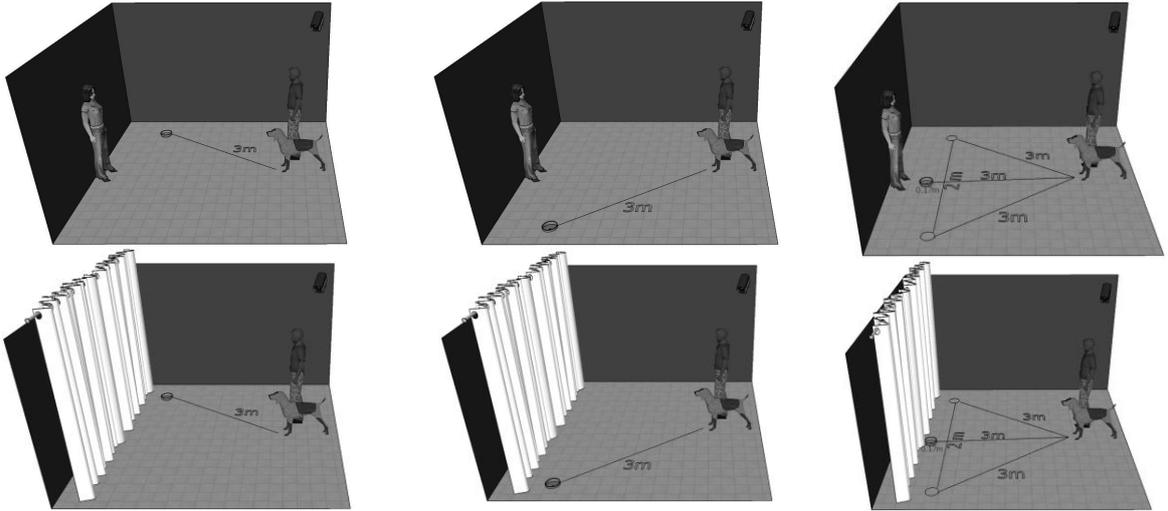
476 The effect of oxytocin on heart rate and heart rate variability in ten individual dogs



477

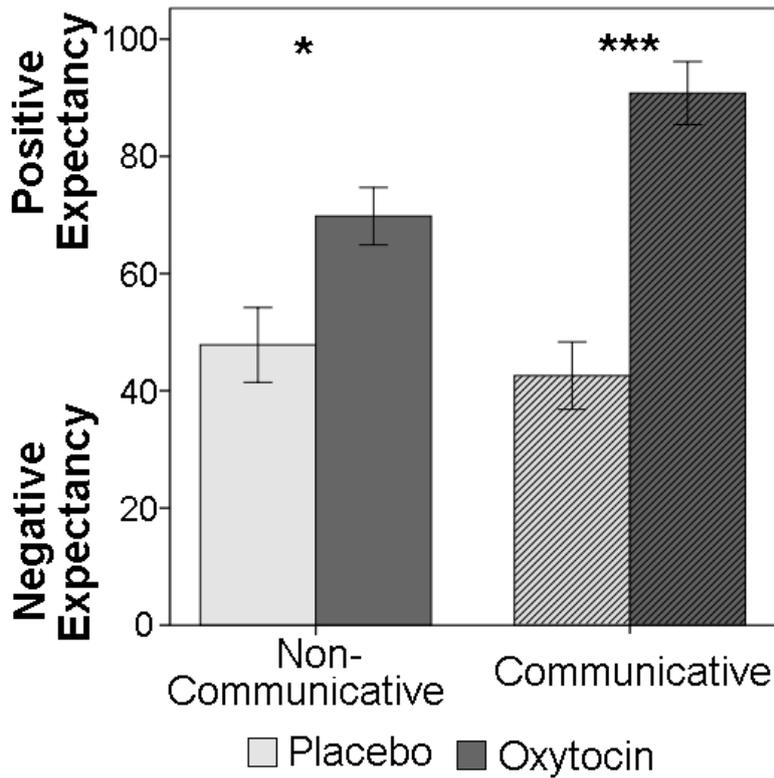
478 **Figure 2.**  
 479 Schematic overview of the different conditions throughout the four phases of the experiment.  
 480 The drawings in one row stand for the different type of trials (e.g. positive and negative) that  
 481 one individual subject received in that phase, while the different rows represent between  
 482 subject treatments (e.g. communicative or non-communicative)





484 **Figure 3.**

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



489

490

491 **Table 1.**

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

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

496