

1 **The effect of oxytocin on biological motion perception in dogs (*Canis familiaris*)**

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10

11 **Abstract**

12 Recent studies have shown that the neuropeptide oxytocin is involved in the regulation of several complex  
13 human social behaviours. There is, however, little research on the effect of oxytocin on basic mechanisms  
14 underlying human sociality, such as the perception of biological motion. In the present study we investigated the  
15 effect of oxytocin on biological motion perception in dogs (*Canis familiaris*), a species adapted to the human  
16 social environment and thus widely used to model many aspects of human social behaviour. In a within-subjects  
17 design, dogs (N=39), after having received either oxytocin or placebo treatment, were presented with 2D  
18 projection of a moving point-light human figure and the inverted and scrambled version of the same movie.  
19 Heart rate (HR) and heart rate variability (HRV) were measured as physiological responses, behavioural  
20 response was evaluated by observing dogs' looking time. Subjects were also rated on the personality traits of  
21 neuroticism and agreeableness by their owners. As expected, placebo-pretreated (control) dogs showed a  
22 spontaneous preference for the biological motion pattern, however, there was no such preference after oxytocin  
23 pretreatment. Furthermore, following the oxytocin pretreatment female subjects looked more at the moving  
24 point-light figure than males. The individual variations along the dimensions of agreeableness and neuroticism  
25 also modulated dogs' behaviour. Furthermore HR and HRV measures were affected by oxytocin treatment and  
26 in turn played a role in subjects' looking behaviour. We discuss how these findings contribute to our  
27 understanding of the neurohormonal regulatory mechanisms of human (and nonhuman) social skills.

28

29 **Keywords**

30 Oxytocin; Biological motion; Dog (*Canis familiaris*); Heart rate; Individual traits

31

32 **Introduction**

33         Recent studies have provided substantial insights into the neurohormonal mechanisms underlying  
34 human sociality (e.g. Skuse and Gallagher, 2009). Much evidence has accumulated implicating that the  
35 neuropeptide oxytocin (OXT) is involved in the regulation of a variety of human social behaviours. OXT  
36 interacts with the hypothalamo-pituitary-adrenal axis to attenuate the stress response and it induces potent  
37 physiological anxiolytic effects by decreasing cortisol levels, inhibiting cardiovascular responses to stress, and  
38 attenuating amygdala responsivity to emotional stimuli (Rodrigues et al., 2009). Much attention in the human  
39 literature has been devoted to the enhancing effect of OXT on social skills in certain psychiatric conditions such  
40 as autism (Andari et al., 2010). The effect of intranasal administration of OXT on prosocial behaviours and on  
41 higher level cognitive functions has also been in the focus of many recent investigations (see Campbell, 2010 for  
42 a review). There has been, however, few attempts to investigate the effects of OXT on lower levels of  
43 behavioural regulation, such as unconscious visual perceptual processes (e.g. Guastella et al., 2008).

44         Importantly, this latter approach, together with the fact that the OXT system is evolutionarily conserved  
45 (both the hormone and its receptor are present in mammals and other taxa – Donaldson and Young, 2008) could  
46 allow us to use a comparative framework and test the same phenomenon in different species. For example,  
47 targeting relatively basic perceptual mechanisms, a recent study has found evidence that oxytocin enhances the  
48 perception of biological motion in healthy adult humans (Kéri et al., 2009). Furthermore another study (Perry et  
49 al., 2010) has found evidence that oxytocin modulates brain activity during the viewing of biological motion  
50 displays.

51         Many claim that biological motion perception is one of the fundamental aspects of social cognitive  
52 processes (Troje and Westhoff, 2006) that can help distinguish living organisms from other objects in the  
53 environment. It has been shown that human actions presented as a small number of moving dots representing  
54 only the motion of the major joints of the body could be identified by human observers (biological motion  
55 perception – Johansson, 1973). Evidence suggests that human observers can perceive biological motion even  
56 when there are very few points of light (point-light figure or PLF, Troje and Westhoff, 2006), only limited local  
57 motion information is presented (Beintema et al., 2002), and/or the PLF is degraded by masks (Cutting et al.,

58 1988). The perceptual cues of biological motion and the neural mechanisms mediating the perception of  
59 biological motion have been extensively investigated in humans (Giese and Poggio, 2003). The perceptual  
60 ability appears to be functional early in life; even newborn infants show a spontaneous preference for biological  
61 over non-biological motion (Simion et al., 2008). However biological motion perception has been shown to be  
62 impaired in individuals with social disorders (e.g. autism: Klin et al., 2009).

63 A wide variety of non-human species are also capable of discriminating biological from non-biological  
64 motion (e.g. chimpanzees (*Pan troglodytes*) – Tomonaga, 2001; cats (*Felis catus*) – Blake, 1993) and the  
65 preference for biologically patterned motion may have a strong innate component in different species (e.g.  
66 chicken (*Gallus gallus domesticus*) – Vallortigara et al., 2005; marmosets (*Callithrix jacchus*) – Brown et al.,  
67 2010; medaka fish (*Oryzias latipes*) – Nakayasu et al., 2013). It has also been shown that even nonhuman species  
68 are able to extract specific information (e.g. motion direction) from biologically moving point-lights (rats (*Rattus*  
69 *norvegicus*) – MacKinnon et al. 2010). Biological motion preference is not limited to point-light-figures of  
70 conspecifics; newly hatched chicks, for example, exhibit a spontaneous preference to approach biological motion  
71 of both a hen and a potential predator (Vallortigara et al., 2005).

72 Importantly however, biological motion perception has not yet been studied in dogs despite that this domestic  
73 animal, due to its infant-like social-cognitive features, has a privileged status in comparative social cognition  
74 (Miklósi and Topál, 2013). Dogs are exceptionally skilled at reading human behaviour (for review see e.g.  
75 Bensky et al., 2013), and in some cases, their performance is comparable to that of 2- and 3-year-old children  
76 (e.g. Lakatos et al., 2009). Dogs also have different personalities resembling human personality types (Gosling et  
77 al., 2003) that can be characterized along the dimensions of Neuroticism, Extraversion, Agreeableness, and  
78 Openness, but relatively little is known about the effects of oxytocin on dogs' social cognition.

79 It has recently been reported that oxytocin promotes positive social behaviours in dogs toward both  
80 humans and conspecifics (Romero et al., 2014) and polymorphisms in the oxytocin receptor gene are related to  
81 human-directed social behaviours such as friendliness towards strangers, and tendency to seek contact with the  
82 owner (Kis et al., 2014a). Intranasally administered oxytocin also has an effect on dogs' reaction to ambivalent  
83 stimuli (Kis et al., 2015), human pointing gestures (Oliva et al., 2015), human threatening cues (Hernádi et al.,  
84 2015) as well as it increases gazing behaviour towards their owners (Nagasawa et al., 2015). It is not known,  
85 however, whether dogs parallel humans in that oxytocin affects their basic social cue processing such as the  
86 perception of biological motion.

87           The purpose of the present study was to investigate whether dogs show spontaneous preference for  
88 biological motion versus non-biological control stimuli, and how intranasal administration of oxytocin modulates  
89 dogs' reactions. We used point-light display of a human figure as stimulus because humans are highly relevant  
90 social partners for dogs (Nitzschner et al., 2012) to an extent that in some contexts they prefer humans over  
91 conspecifics both at the behavioural (Gácsi et al., 2005) and at the neural (Andics et al., 2014) levels. We also  
92 aimed to study the physiological consequences of intranasal oxytocin administration (changes in heart rate and  
93 heart rate variability) and how the individuals' physiological reaction to oxytocin correlates with the looking  
94 preferences in dogs. Sex differences were also studied as based on the peripheral effects of oxytocin (i.e., to  
95 induce labour and milk ejection), a general difference in its behavioural effects on males and females can be  
96 expected (see e.g. Domes et al., 2010). Moreover, as recent results show that the oxytocinergic system modulates  
97 the *neuroticism* personality trait in humans (Chang et al., 2014), and as the *agreeableness* trait encompasses  
98 different prosocial attitudes such as trust, empathy and altruism that have been shown to be affected by oxytocin  
99 (see e.g. Rodrigues et al., 2009), the canine analogues of these two human personality factors were also included  
100 in the analyses.

101

## 102 **Materials and methods**

### 103 **Ethical statement**

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

### 108 **Subjects**

109 N=39 task-naïve adult (older than 1 year) pet dogs (20 males and 19 females; 18 purebreds from 14 different  
110 breeds and 21 mongrels; mean age  $\pm$ SD: 4.46 $\pm$ 2.51 years; 8 of small ( $\leq$ 9 kg), 23 of medium (10-25 kg) and 8 of  
111 large ( $>$ 25 kg) size based on average standard weight, <http://www.akc.org/> in case of pure breed dogs or based on  
112 the inspection of the videos in case of mixed breed dogs) were recruited from the Family Dog Project database  
113 that contains over a thousand owners who have volunteered to participate in behavioural experiments with their  
114 dogs. The participation requirement for the test was that dogs did not have any type of eye or vision problem

115 (according to the owner). The inclusion criterion was that dogs had to look at the screen more than 30% of the  
116 total time in a session.

117

## 118 **Stimuli**

119 Stimuli consisted of a 4 s attention grabber (sound + moving rattle animation) followed by a 15 s long  
120 stimulus (point-light display) accompanied by a neutral music playback (see Online Resource). The biological  
121 motion stimulus depicted a point-light movie of a side walking human on one side (left/right counterbalanced  
122 across subjects) ('normal point like figure – PLF'), while on the other side the inverted and scrambled version of  
123 the same point-light movie ('distractor') was shown. The projector screen was placed opposite to the door: 2 m  
124 wide, 1.8 m high. The projector was fixed near the ceiling on the other end of the room. The point-light display  
125 was an 11-dot figure with single white dots representing the head, one shoulder, one hip, and each of the two  
126 elbows, wrists, knees, and ankles on a black background. The PLF was shown facing either left or right and  
127 walking in place, as if on a treadmill, with a stride frequency of 0.93 Hz. Both PLF and distractor displays were  
128 presented without mask dots during the first session (Fig1. B,C), while dogs were presented with a masked PLF  
129 during the second session (100 mask dots randomly plotted within the mask area – Fig1. D,E).

## 130 **Procedure**

131 Increasing evidence suggest that the intranasal administration of 12 (or more) IU oxytocin can affect  
132 the behaviour of dogs (e.g Kis et al., 2015; Hernádi et al., 2015; Thielke and Udell, 2015). Based on this dogs  
133 received a single intranasal dose of 12 IU (3 puffs) oxytocin (Syntocinon, Novartis) or placebo (isotonic  
134 natriumchlorid 0.9% solution) in a double blind design. Subjects participated in the task two times, and each of  
135 them received both oxytocin and placebo treatment in a random order. In the first test session subjects watched  
136 the non-masked stimuli and in the second test session they were presented with the more complex (i.e. masked)  
137 stimuli. The mean±SD break between the two test sessions with different treatments was 5.34±3.66 days. The  
138 oxytocin or placebo administration was followed by a 40-minute-long waiting period (following the protocol by  
139 Kis et al., 2014b). During this waiting period, dogs spent the first 25 minutes with an on-leash walk at the  
140 University Campus (avoiding any contact with other dogs or humans) during which the experimenter ensured  
141 that the owner did not make any social contact with the dog either (e.g. did not pet or talk to it) and kept the  
142 length as well as the speed of the walk as standard as possible. Then for the remaining 15 minutes the owner and  
143 the dog were quietly sitting in an isolated room. During this time the dog was free to move and the owner was  
144 sitting and filling in questionnaires while ignoring the dog. We used the Neuroticism and Agreeableness scales

145 of a personality questionnaire adapted for dogs by Gosling et al. (2003). The 17-item questionnaire consisted of  
146 9 statements for agreeableness (e.g. “is sensitive to the needs and feelings of others”) and 8 statements for  
147 neuroticism (e.g. “gets nervous easily”). Owners were asked to score their dogs from 1 to 5 (from disagree  
148 strongly to agree strongly). Both scales contained three reverse scored items.

149 In order to quantify the physiological effect of oxytocin and to test its relation to the behavioural effects,  
150 ECG recordings were conducted immediately following the waiting period. The testing room was equipped with  
151 office furniture and a mattress on the floor for the dog and its owner. While we made every possible effort to  
152 keep the environmental circumstances during the waiting period before the ECG measurement as standard as  
153 possible, body posture of the dog was not controlled by the owner/experimenter in order to avoid stress inherent  
154 to external restraint. Evidently, this procedure caused slight variations in the subjects’ behaviour during the  
155 waiting period, but the effect of oxytocin has been shown to be strong enough to manifest even under these semi-  
156 natural conditions (Kis et al., 2014b). When the 40 minutes waiting period elapsed, a 5-10 minutes on-leash  
157 exploration and familiarization followed in the ECG measurement room, after which the owner took a seat on  
158 the mattress and assisted the experimenter throughout the process of fixing two surface attached electrodes onto  
159 the dog’s chest (second rib on both sides). Gold-coated Ag|AgCl electrodes fixed with EC2 Grass Electrode  
160 Cream (Grass Technologies, USA) were used for the recordings. The electrode placement was followed by 4-  
161 minute quiet resting, and then by a 1 minute long recording period. During this last five minutes every dog was  
162 in a lying position because previous research has shown that body posture has a significant effect on dogs’ heart  
163 rate (Maros et al., 2008). Signals were collected, prefiltered, amplified, and digitized at a sampling rate of 249  
164 Hz/channel by using the 30 channel Flat Style SLEEP La Mont Headbox with implemented second order filters  
165 at 0.5 Hz (high pass) and 70 Hz (low pass) as well as the HBX32-SLP 32 channel preamplifier (La Mont  
166 Medical Inc., USA).

167 The test setup measuring biological motion preference followed the procedure of previous experiments  
168 studying dogs’ responses to projected images (e.g. Faragó et al., 2010). The experiment took place in a dark  
169 room (3 m × 5 m) with a canvas (2 m × 2.2 m) on one of the walls and a chair at a 4 m distance facing the  
170 canvas, as well as a projector on the wall opposite the canvas at a 2.2 m height. During the experiment the  
171 owners were seated on the chair and instructed to keep their dogs between their legs in a sitting position. Infrared  
172 lights and a zero lux camera (optimised for night vision recording) focused on the dog’s head were placed in  
173 front of them at 1 m distance in order to record the head and eye movements of the dogs. An additional camera,  
174 placed above the projector and synchronized with the zero lux camera, recorded the entire room in order to

175 ensure that the looking direction of the dog was only coded during the stimuli projection phase. Dogs were  
176 allowed to look away and owners were asked not to interact with the dog.

## 177 **Data analysis**

178 Agreeableness and Neuroticism questionnaire scores were obtained by averaging the scores of the items  
179 representing each trait using the coding methods of Gosling et al. (2003).

180 Using the ECG recordings R peaks were manually detected (due to the sinus arrhythmia that characterizes dog  
181 heart rate automatic measures are hard to apply – (Schöberl et al., 2014) and RR intervals were measured using  
182 the Fercio program (© Ferenc Gombos 2012). HR (1/min) was derived from RR interval averages ( $60/\text{meanRR}$ ),  
183 and HRV (sec) was calculated as the standard deviation of RR intervals (see e.g. Gácsi et al., 2013 for similar  
184 measures).

185 Looking behaviour during the biological motion preference tests was analysed by frame-by-frame  
186 coding of all experimental recordings (with a 0.2 second resolution, using Solomon Coder,  
187 <http://solomoncoder.com/>), in order to determine the looking direction of the dogs: left side of the screen / right  
188 side of the screen / away from the screen. Coding was blind to subject details and conditions.

189 In order to assess subjects' looking behaviour we measured the relative time (%) spent with looking at  
190 the point-light figure (%PLF) as well as the relative time (%) spent with looking at the Distractor (%DISTR).  
191 Inter-rater reliability for dogs' looking behaviour was calculated by double coding of 30 random frames (on 30  
192 different subjects, 38% of the sample) from the two stimuli by two independent coders (Cohen  $\kappa$ : 0.80).

193 *Total looking* was calculated as (%PLF) + (%DISTR). *Preference index* was calculated as (%PLF –  
194 %DISTR) / Total looking. Linear Mixed Models using restricted maximum likelihood estimation were used to  
195 test the effects of pretreatment (Oxytocin or Placebo; within subject factors) and stimuli type (masked or not  
196 masked; within subjects factors), sex (male or female; between subjects factor) as well as Agreeableness and  
197 Neuroticism scores (covariates) on HR and HRV values. Other Linear Mixed Models were used to test the effect  
198 of pretreatment (Oxytocin or Placebo; within subjects factor), stimuli type (masked or not masked, within  
199 subjects factor), sex (male or female; between subjects factor) as well as Agreeableness and Neuroticism scores,  
200 HR and HRV values (covariates) on Total looking and Preference index.

201 Two dogs from the first session (non-masked) and three dogs from the second session (masked) were  
202 excluded from the analyses because they failed to meet the inclusion criterion (see above). Five additional dogs  
203 did not return for the second test session and in case of five dogs no ECG recordings were conducted (due to  
204 non-compliance of the subject). Moreover we could not analyse the video recording of the first session in one

205 subject because of technical reasons and in case of 5 dogs the questionnaire was not filled. These were all  
206 included as missing data in the analysis. However the statistical methods we used (mixed models) have the  
207 ability to accommodate missing data points (see Krueger and Tian, 2004 for the validity of statistical analysis on  
208 datasets with missing data).

209

210

## 211 **Results**

### 212 *Physiological responses (HR & HRV)*

213 The Random intercept mixed-effects model showed that HR was significantly affected by the  
214 pretreatment, that is, OXT administration decreased HR ( $F_{(1,23)}=12.325$ ,  $p=0.002$ ; Fig2.). Male dogs had higher  
215 HR, than females irrespective of OXT/PL pretreatment ( $F_{(1,24)}= 5.012$ ,  $p=0.034$ ) (Fig3.). Subjects with higher  
216 neuroticism ( $F_{(1,25)}= 4.422$ ,  $p=0.045$ ) and agreeableness ( $F_{(1,24)}=5.256$ ,  $p=0.031$ ) scores had higher HR. No main  
217 effect of the first/second test occasion ( $F_{(1,23)}=0.906$ ,  $p=0.351$ ) was found. All interactions were non-significant  
218 (all  $p>0.05$ ).

219 HRV was also affected by the pretreatment, as OXT significantly increased HRV ( $F_{(1,25)}= 5.796$ ,  
220  $p=0.024$ ) (Fig4.), however, none of the other factors (sex:  $F_{(1,25)}= 1.351$ ,  $p=0.256$ , first/second test occasion:  
221  $F_{(1,25)}= 0.055$ ,  $p=0.817$ , neuroticism:  $F_{(1,26)}= 0.761$ ,  $p=0.391$ , agreeableness:  $F_{(1,25)}= 0.870$ ,  $p=0.360$ ) influenced  
222 the HRV and all interactions were non-significant (all  $p>0.05$ ).

223

### 224 *Total looking (%PLF + %DISTR)*

225 *Total looking* was higher in case of the non-masked than the masked stimuli (mean±SEM:  $72.01\pm3.22$   
226 in non-masked stimuli and  $62.79\pm4.02$  in masked stimuli);  $F_{(1,29)}=7.157$ ,  $p=0.012$ ). Furthermore, we found that  
227 dogs who had higher agreeableness scores looked more at the stimuli ( $F_{(1,26)}=4.589$ ,  $p=0.042$ ). Dogs with a lower  
228 HR looked more at the stimuli ( $F_{(1,46)}=4.407$ ,  $p=0.041$ ), and the association with HRV showed a reverse  
229 tendency; dogs with higher HRV showed a tendency to look more at the stimuli ( $F_{(1,50)}=3.451$ ,  $p=0.069$ ).  
230 Furthermore, there was a significant pretreatment  $\times$  sex interaction ( $F_{(2,31)}=4.385$ ,  $p=0.021$ ): female dogs looked  
231 more at the stimuli after oxytocin pretreatment than males (mean±SEM:  $62.52\pm5.93$  in male subjects after  
232 oxytocin pretreatment,  $76.3\pm4.51$  in female subjects after oxytocin pretreatment,  $67.65\pm4.74$  in male subjects  
233 after placebo pretreatment and  $65.71\pm6.06$  in female subjects after placebo pretreatment; Fig5.). All other



234 interactions were non-significant ( $p > 0.05$ ). No main effects of pretreatment (OXT/PL,  $F_{(1,29)} = 0.218$ ,  $p = 0.644$ ),  
235 sex ( $F_{(1,28)} = 0.806$ ,  $p = 0.377$ ), and score for Neuroticism ( $F_{(1,30)} = 0.007$ ,  $p = 0.934$ ) were found.

### 236 *Preference index*

237 Pretreatment had a significant effect on dogs' Preference index: subjects, after having received OXT,  
238 looked relatively less at the biological stimuli (mean $\pm$ SEM:  $-0.06 \pm 0.92$  after oxytocin pretreatment and  
239  $0.14 \pm 0.93$  after placebo pretreatment;  $F_{(1,52)} = 4.974$ ,  $p = 0.03$ ). No main effects of stimulus type (masked/non-  
240 masked,  $F_{(1,52)} = 2.652$ ,  $p = 0.109$ ), sex ( $F_{(1,52)} = 0.082$ ,  $p = 0.770$ ), and questionnaire scores (Neuroticism:  
241  $F_{(1,52)} = 0.094$ ,  $p = 0.760$ ; Agreeableness:  $F_{(1,52)} = 0.351$ ,  $p = 0.556$ ) were found. However, there was a significant  
242 pretreatment  $\times$  stimulus type interaction ( $F_{(2,47)} = 3.212$ ,  $p = 0.049$ ; for mean $\pm$ SEM data see Table 1). Namely,  
243 placebo-pretreated dogs showed preference for looking at the non-masked, but not the masked biological stimuli  
244 while OXT-pretreated dogs had no preference in either of the conditions (Fig6.). All other interactions were non-  
245 significant (all  $p > 0.05$ ) and we did not find significant effects of HR ( $F_{(1,56)} = 0.166$ ,  $p = 0.685$ ) and HRV  
246 ( $F_{(1,56)} = 0.673$ ,  $p = 0.415$ ).

247

### 248 **Discussion**

249 The present study provides the first evidence that dogs show spontaneous preference for biological  
250 motion. The result that our placebo-pretreated subjects showed a preference towards a human point-light figure  
251 versus non-biological control stimulus is in line with previous findings suggesting that humans (for a review see  
252 Nishida, 2011) as well as non-human animals (e.g. Vallortigara et al., 2005) show positive attentional bias  
253 toward point-light displays representing a biological motion pattern of a conspecific or a relevant heterospecific.  
254 Furthermore, our results show that intranasal administration of oxytocin affects biological motion perception in  
255 dogs, and the effects of this treatment are in interaction with physiological measures such as heart rate and heart  
256 rate variability and different aspects of the dog personality (neuroticism and agreeableness). These findings  
257 support previous suggestions that individual variation in the effect of oxytocin on HR and HRV makes it a good  
258 indicator of the physiological effect of oxytocin and thus can be used as a covariate in behavioural studies (Kis et  
259 al., 2014b). Our results also show (apart from confirming that OXT significantly decreases HR and increases  
260 HRV) that male dogs have higher HR, than female dogs. Moreover, our results further support the notion that  
261 there is a relationship between physiological measurement (e.g. heart rate variability) and temperament in both  
262 human and nonhuman subjects. It has been shown, for example, that children with high, stable heart rates are

263 more shy and fearful in unfamiliar situations whereas children with low and variable heart rates are more  
264 outgoing and relaxed in social situations (Garcia-Coll et al., 1984). In line with this Suomi (1983; 1985; 1986)  
265 found that rhesus monkeys vary in their response to novelty, the ones being slow to explore and avoidant  
266 showing higher heart rate in the face of novelty. However, further studies are needed to investigate how the  
267 aforementioned factors as well as other predisposing factors, such as the subjects' genetic background and  
268 previous experiences (and thus the epigenetic modulation of the OXTR gene) modulate the effects of oxytocin  
269 on dogs' perception of point-like figures.

270           In humans it has been demonstrated that oxytocin enhances the perception of biological motion by  
271 increasing sensitivity for stimuli that represent living objects (a walking character) but does not change the  
272 sensitivity for nonbiological stimuli (a rotating square; Kéri et al., 2009). Based on these results we expected that  
273 oxytocin would increase biological motion preference in dogs, but we found an opposite effect. The two findings  
274 are, however, not necessarily contradictory as the ability to more easily perceive biological motion might lead to  
275 an increased visual attention to the stimuli that are not easily recognizable due to its non-biological motion. If so,  
276 changes in dogs' attentional bias after oxytocin treatment may simply reflect changes in the visual encoding  
277 process (identification) and not changes in the relative attractiveness of PLF versus distractor stimuli  
278 (preference). One way to disentangle the effect of oxytocin on encoding versus preference would be to use active  
279 choice methods (e.g. the touch screen technique, Range et al., 2008) where subjects are rewarded for selecting  
280 either the biological or the non-biological stimuli. The disadvantage of these active choice tasks, however, is that  
281 they require extensive training prior to testing (e.g. MacKinnon et al., 2010), and they also have been criticized  
282 because learning effects could be problematic, as the stimuli become more familiar from trial to trial. Probably a  
283 combination of spontaneous preference tasks and active choice methods could produce valid data for the  
284 evaluation of the effect of oxytocin on processing biological motion.

285 Although most of the human studies examine only males due to practical reasons, there is evidence that oxytocin  
286 has an effect on socio-cognitive behaviours in both genders, but there might be differential effects (Herzmann et  
287 al., 2013). In the present study the total looking time of the dogs was affected by oxytocin only in females, but  
288 not males. Previous studies on the effect of oxytocin on dog social behaviour mostly reported no sex effects,  
289 although Nagasawa et al. (2015) found that oxytocin administration significantly increased the duration of  
290 gazing at the owner in female but not male dogs. Research on other species has also shown that oxytocin can  
291 affect males and females differently. For example, higher binding of oxytocin receptors in the medial prefrontal  
292 cortex have been found in female prairie voles (Smeltzer et al., 2006). Human studies that investigated the effect

293 of oxytocin on amygdala reactivity also reported sex differences in oxytocin effectiveness. Oxytocin selectively  
294 enhances amygdala reactivity to fearful faces in women (Domes et al., 2010), although previous findings showed  
295 that it reduces the amygdala reactivity social and emotial stimuli in healthy men (e.g. Domes et al., 2007;  
296 Petrovic et al., 2008). It is possible that social motivation – which appears to differ between the sexes – may be a  
297 driving force behind developmental sex differences in social skills (Christov-Moore et al., 2014). So female  
298 dogs, who may be more socially motivated, may be more impacted by exogenous oxytocin, compared to male  
299 dogs, who may be less socially motivated. Sex differences may also be rooted in the differences in oxytocin  
300 receptor affinity because steroid hormones, such as estradiol and progesterone have the potential to modulate the  
301 OXT receptor (estradiol enhances OXT receptor affinity while progesterone has been shown to decrease receptor  
302 binding – Choleris et al., 2008; Gimpl et al., 2002). These results add to the growing literature that draws  
303 attention to the importance of including both males and females when investigating the effects of oxytocin. This  
304 line of research might also have some indirect clinical relevance as disorders like depression, autism, and  
305 schizophrenia have been connected to oxytocin innervation and show sex differences in humans (de Vries,  
306 2008).

307 The finding that total looking time was higher towards the non-masked compared to the masked stimuli suggests  
308 that dogs were probably unable to recognize the biological motion when the point-like figures were masked. This  
309 is not surprising, as even 14-year-old children are less accurate than adults in a walker-detection task when the  
310 walking figure is embedded in moving noise dots (Pavlova et al., 2000). In our study we used a relatively high  
311 number of surrounding masking dots, thus it is possible that the supposed enhancing effect of oxytocin on  
312 biological motion perception could have been detected with a stimulus having fewer noise dots.

313 Although it has been argued (Kéri et al., 2009) that oxytocin in humans has an effect on basic social behaviours,  
314 most of the findings support the role of oxytocin in modulating higher level social cognitive functions such as  
315 emotion regulation (Rodrigues et al., 2009) or attachment (Donaldson and Young, 2008). However, the  
316 combination of these two approaches could also be used. It seems that based on motion cues alone, people are  
317 capable of extracting socially relevant information about the figure, such as emotion (Dittrich et al., 1996),  
318 gender (Schouten et al., 2010) or intention (Manera et al., 2010). In order to test this, paradigms that have  
319 already been proven to be suitable for dogs could be adapted using point-light figures – e.g., gender  
320 differentiation of human figures (Takaoka and Morisaki, 2013).

321 In summary, our study presents information about intranasal oxytocin pretreatment affecting biological  
322 motion perception in dogs and its potential connection with physiological measures as well as some aspects of

323 the dog personality (neuroticism and agreeableness). In conclusion we propose that intranasal administration of  
324 OXT may be a valid approach to study mechanisms underlying basic social behaviour and cognition in dogs.

325

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330

331 **Conflict of interest:** The authors declare that they have no conflict of interest.

332

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480

481 **Figure caption**

482

483 **Fig1.** Stimulus displays. A: attention grabber; B: inverted and scrambled version of a side walking human  
484 (distractor); C: normal version of a side walking human; D: inverted and scrambled version of a side walking  
485 human (distractor) with mask dots; E: normal version of a side walking human with mask dots

486 **Fig2.** Effect of oxytocin pretreatment on heart rate. Oxytocin administration decreased heart rate in dogs. \*:   
487  $p < 0.05$

488 **Fig3.** Effect of sex on heart rate. Male dogs had significantly higher heart rate, than female dogs. \*:  $p < 0.05$

489 **Fig4.** Effect of oxytocin pretreatment on heart rate variability. Oxytocin significantly increased heart rate  
490 variability. \*:  $p < 0.05$

491 **Fig5.** Differential effect of OXT on the looking behaviour in males and females. Female dogs looked more at the  
492 stimuli after oxytocin pretreatment. \*:  $p < 0.05$

493 **Fig6.** Placebo-pretreated dogs showed a significant preference for looking at the biological motion stimuli in  
494 case of the non-masked, but not in the masked condition. \*:  $p < 0.05$

495

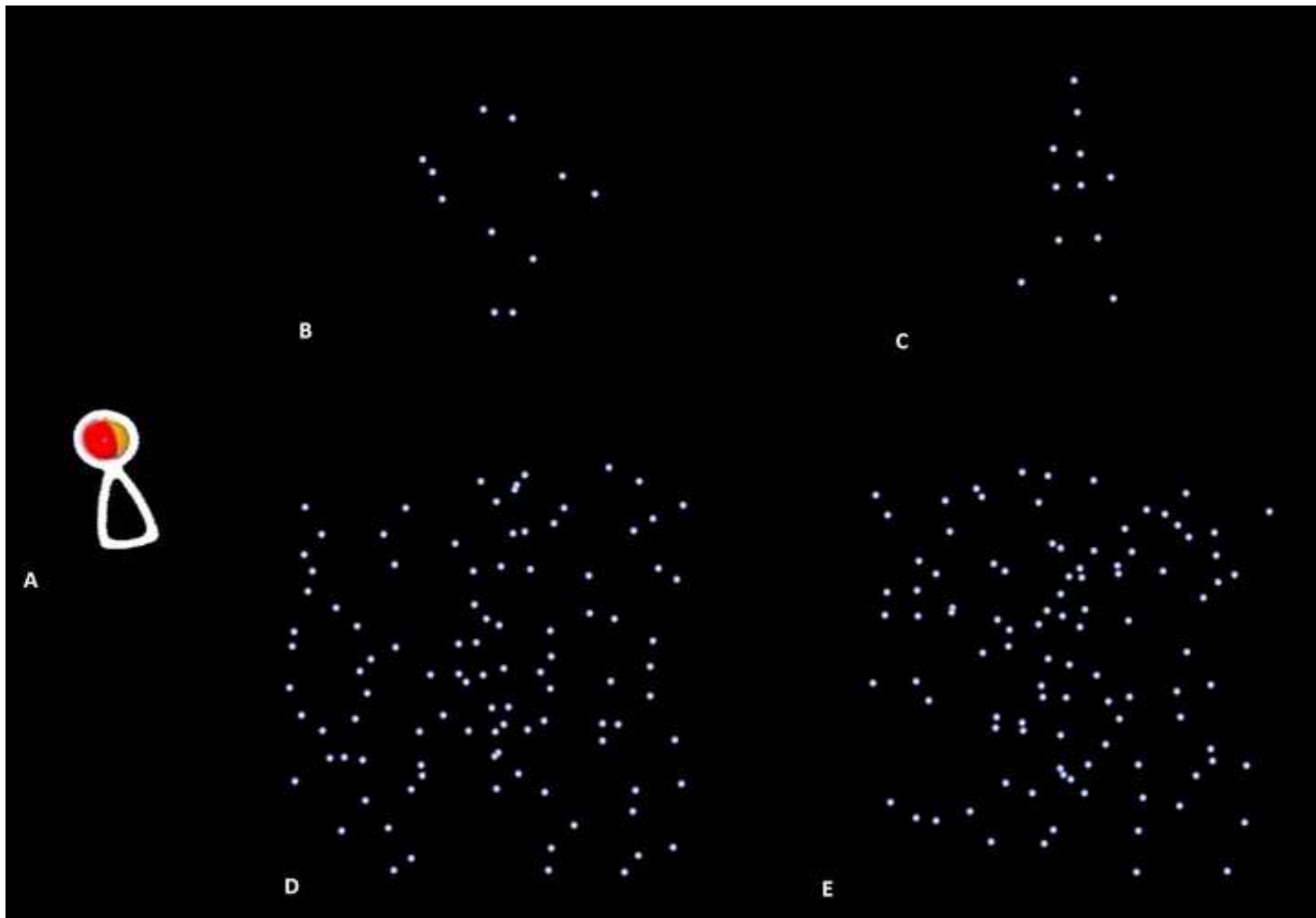
496 **Table 1.** Mean $\pm$ SEM data of Preference index

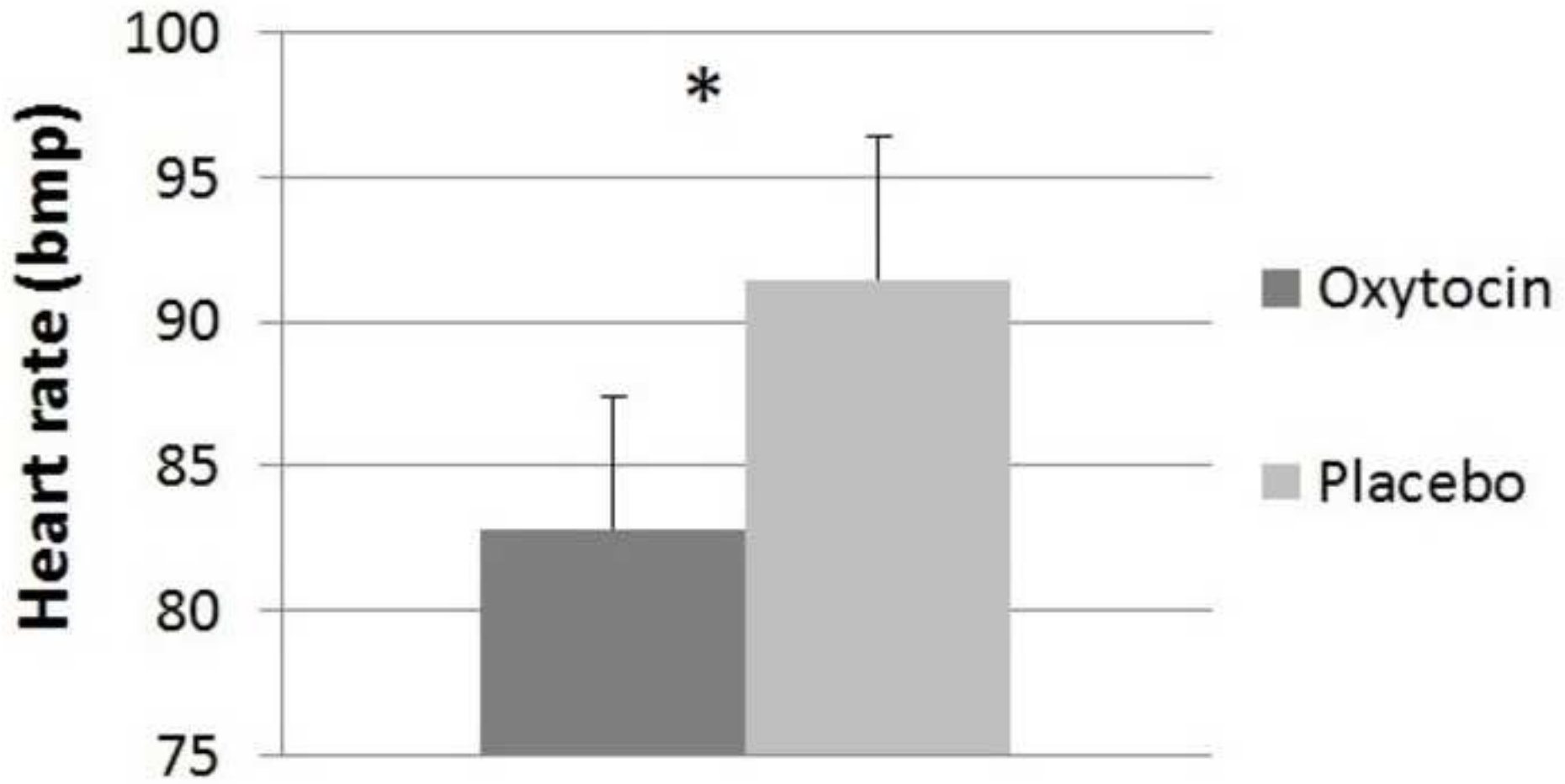
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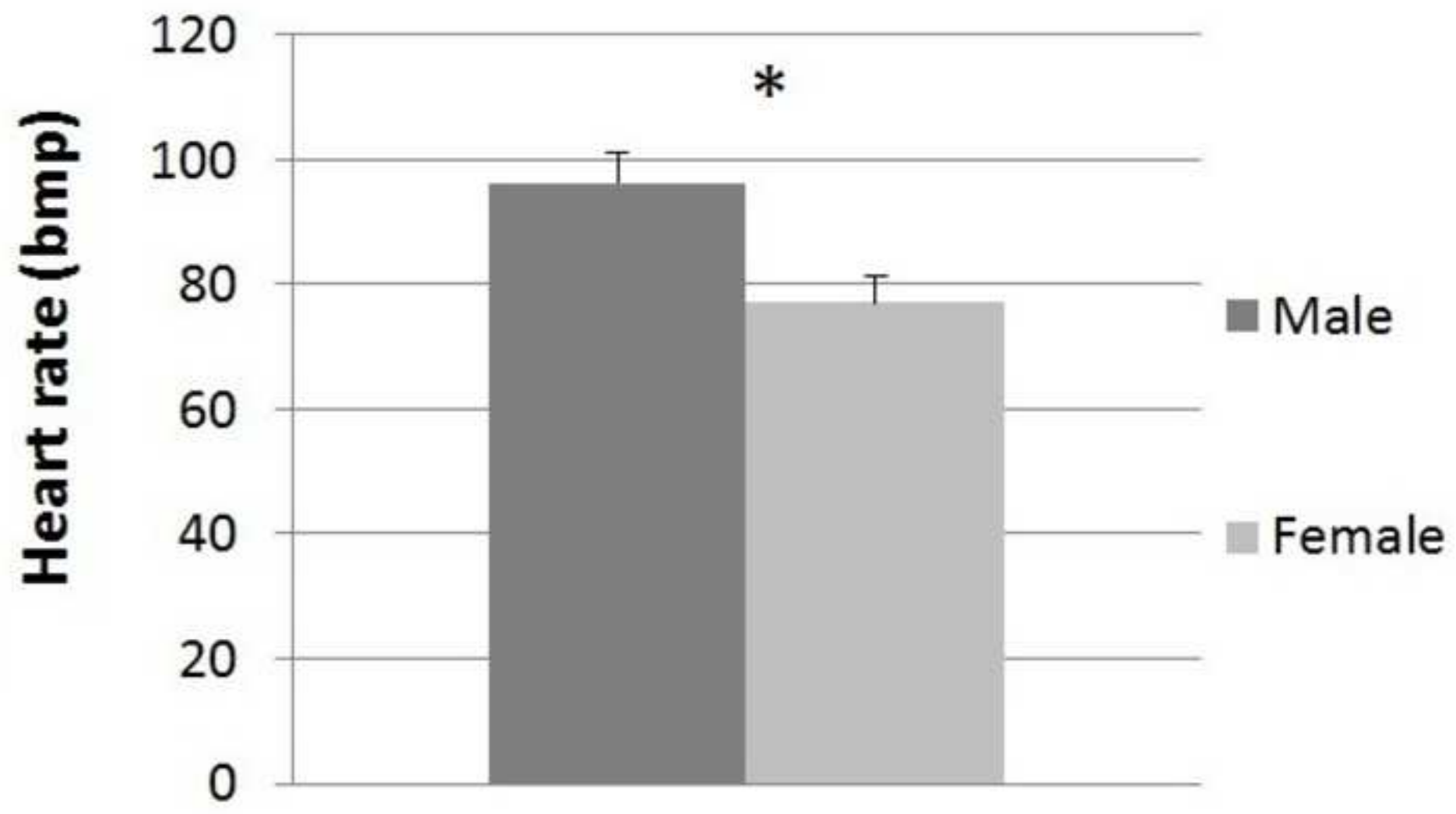
<b>Preference index (mean <math>\pm</math> SEM)</b>	<b>Oxytocin</b>	<b>Placebo</b>
<b>Non-masked</b>	-0.17 $\pm$ 0.54	0.28 $\pm$ 0.27
<b>Masked</b>	-0.01 $\pm$ 0.93	-0.04 $\pm$ 1.19

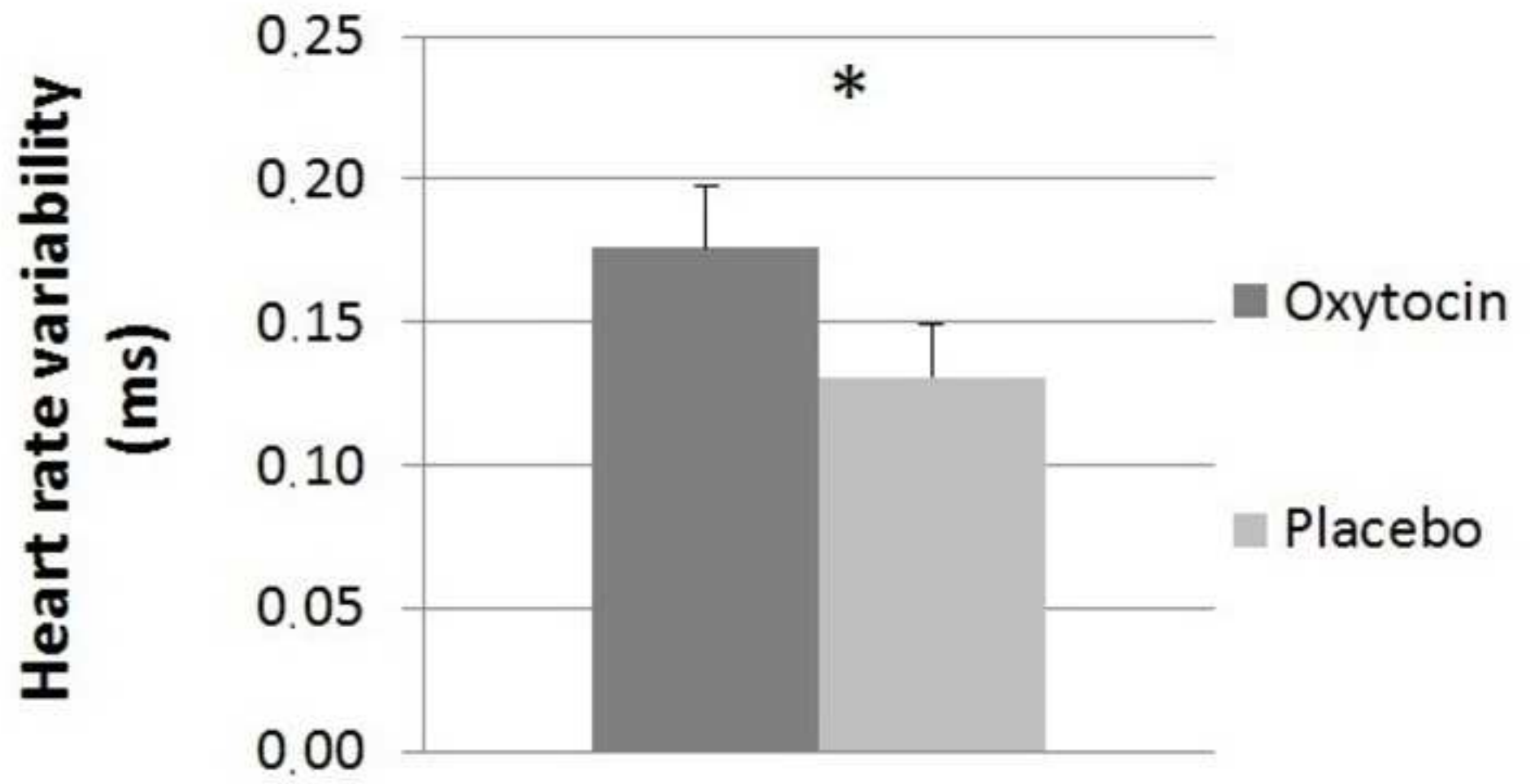
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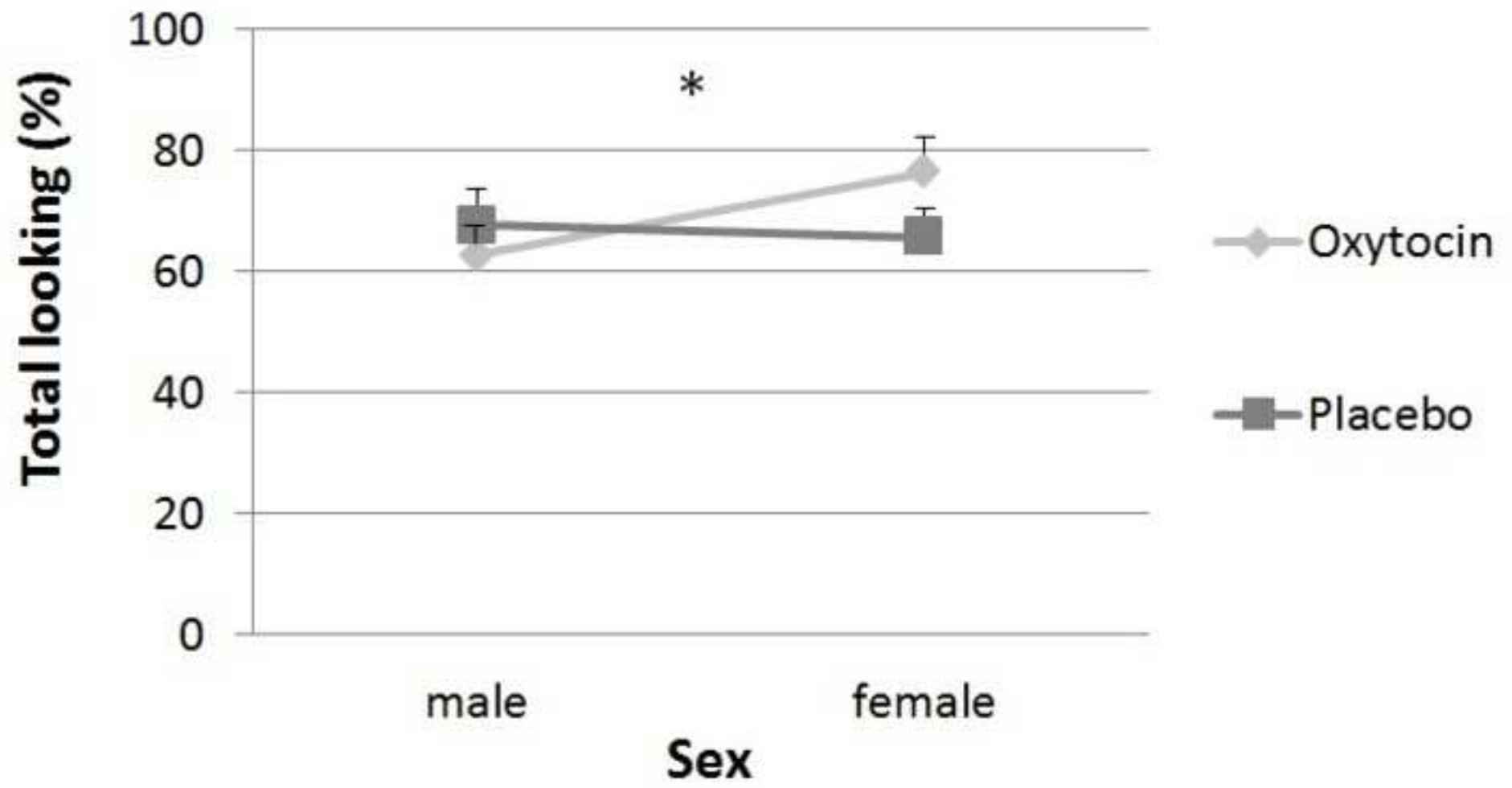


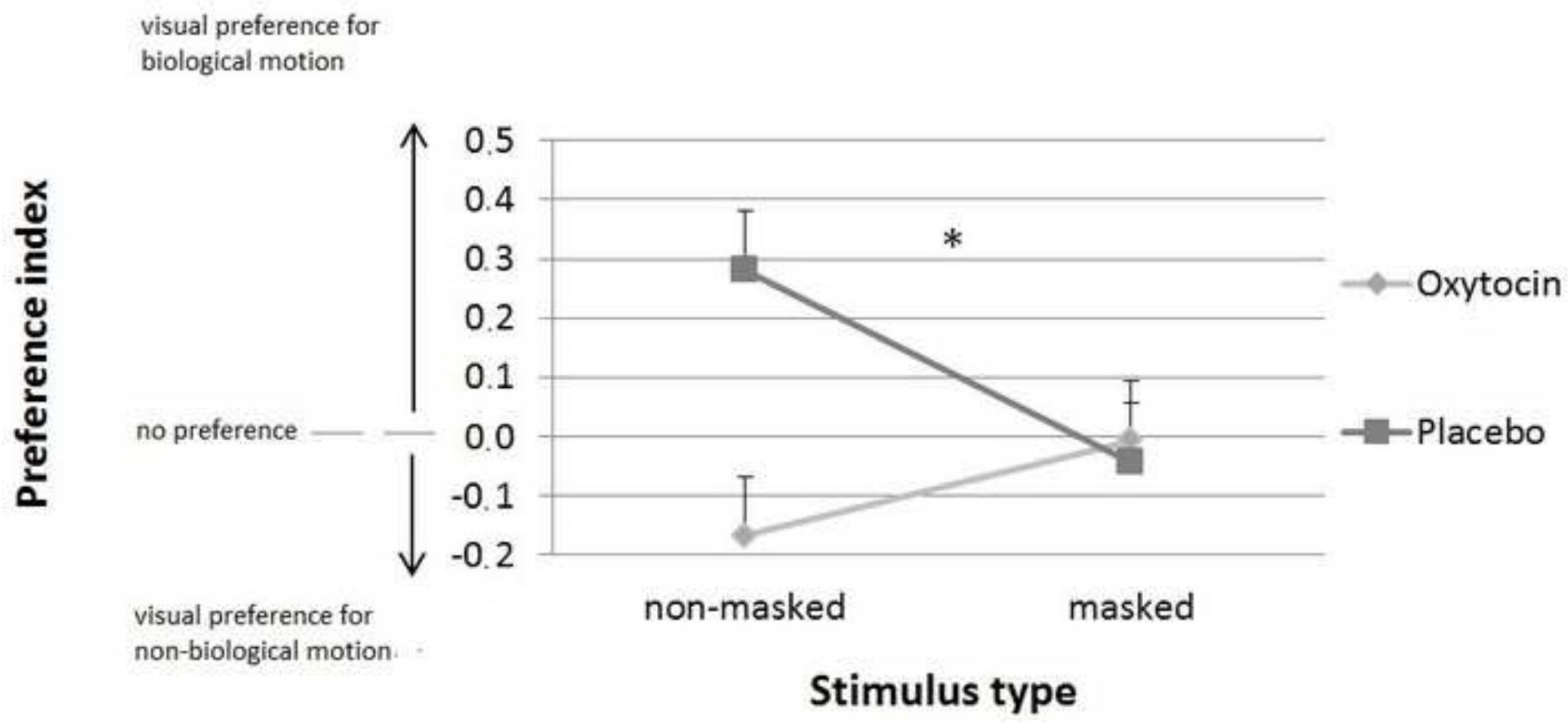














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