

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

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## 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.

## Keywords

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

## Introduction

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

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

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

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

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

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

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

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

## Materials and methods

### 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).

### Subjects

N=39 task-naïve adult (older than 1 year) pet dogs (20 males and 19 females; 18 purebreds from 14 different 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 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) were recruited from the Family Dog Project database that contains over a thousand owners who have volunteered to participate in behavioural experiments with their dogs. The participation requirement for the test was that dogs did not have any type of eye or vision problem

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

## **Stimuli**

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

## **Procedure**

Increasing evidence suggest that the intranasal administration of 12 (or more) IU oxytocin can affect the behaviour of dogs (e.g. Kis et al., 2015; Hernádi et al., 2015; Thielke and Udell, 2015). Based on this dogs received a single intranasal dose of 12 IU (3 puffs) oxytocin (Syntocinon, Novartis) or placebo (isotonic natriumchlorid 0.9% solution) in a double blind design. Subjects participated in the task two times, and each of them received both oxytocin and placebo treatment in a random order. In the first test session subjects watched the non-masked stimuli and in the second test session they were presented with the more complex (i.e. masked) stimuli. The mean±SD break between the two test sessions with different treatments was 5.34±3.66 days. The oxytocin or placebo administration was followed by a 40-minute-long waiting period (following the protocol by Kis et al., 2014b). During this waiting period, 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 or talk to it) and kept the length as well as the speed of the walk as standard as possible. Then for the remaining 15 minutes the owner and the dog were quietly sitting in an isolated room. During this time the dog was free to move and the owner was sitting and filling in questionnaires while ignoring the dog. We used the Neuroticism and Agreeableness scales

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

In order to quantify the physiological effect of oxytocin and to test its relation to the behavioural effects, ECG recordings were conducted immediately following the waiting period. The testing room was equipped with office furniture and a mattress on the floor for the dog and its owner. While we made every possible effort to keep the environmental circumstances during the waiting period before the ECG measurement as standard as possible, body posture of the dog was not 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, but the effect of oxytocin has been shown to be strong enough to manifest even under these semi-natural conditions (Kis et al., 2014b). 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 sides). 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-minute quiet resting, and then by a 1 minute long recording period. During this last five minutes every dog was in a lying position because previous research has shown that body posture has a significant effect on dogs’ heart rate (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).

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

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

## **Data analysis**

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

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

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

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

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

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

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

## Results

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

The Random intercept mixed-effects model showed that HR was significantly affected by the pretreatment, that is, OXT administration decreased HR ( $F_{(1,23)}=12.325$ ,  $p=0.002$ ; Fig2.). Male dogs had higher HR, than females irrespective of OXT/PL pretreatment ( $F_{(1,24)}= 5.012$ ,  $p=0.034$ ) (Fig3.). Subjects with higher 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 effect of the first/second test occasion ( $F_{(1,23)}=0.906$ ,  $p=0.351$ ) was found. All interactions were non-significant (all  $p>0.05$ ).

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

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

*Total looking* was higher in case of the non-masked than the masked stimuli (mean $\pm$ SEM: 72.01 $\pm$ 3.22 in non-masked stimuli and 62.79 $\pm$ 4.02 in masked stimuli);  $F_{(1,29)}=7.157$ ,  $p=0.012$ ). Furthermore, we found that dogs who had higher agreeableness scores looked more at the stimuli ( $F_{(1,26)}=4.589$ ,  $p=0.042$ ). Dogs with a lower HR looked more at the stimuli ( $F_{(1,46)}=4.407$ ,  $p=0.041$ ), and the association with HRV showed a reverse tendency; dogs with higher HRV showed a tendency to look more at the stimuli ( $F_{(1,50)}=3.451$ ,  $p=0.069$ ). Furthermore, there was a significant pretreatment  $\times$  sex interaction ( $F_{(2,31)}=4.385$ ,  $p=0.021$ ): female dogs looked more at the stimuli after oxytocin pretreatment than males (mean $\pm$ SEM: 62.52 $\pm$ 5.93 in male subjects after oxytocin pretreatment, 76.3 $\pm$ 4.51 in female subjects after oxytocin pretreatment, 67.65 $\pm$ 4.74 in male subjects after placebo pretreatment and 65.71 $\pm$ 6.06 in female subjects after placebo pretreatment; Fig5.). All other

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

### *Preference index*

Pretreatment had a significant effect on dogs' Preference index: subjects, after having received OXT, looked relatively less at the biological stimuli (mean $\pm$ SEM:  $-0.06\pm0.92$  after oxytocin pretreatment and  $0.14\pm0.93$  after placebo pretreatment;  $F_{(1,52)}=4.974$ ,  $p=0.03$ ). No main effects of stimulus type (masked/non-masked,  $F_{(1,52)}=2.652$ ,  $p=0.109$ ), sex ( $F_{(1,52)}=0.082$ ,  $p=0.770$ ), and questionnaire scores (Neuroticism:  $F_{(1,52)}=0.094$ ,  $p=0.760$ ; Agreeableness:  $F_{(1,52)}=0.351$ ,  $p=0.556$ ) were found. However, there was a significant pretreatment  $\times$  stimulus type interaction ( $F_{(2,47)}=3.212$ ,  $p=0.049$ ; for mean $\pm$ SEM data see Table 1). Namely, placebo-pretreated dogs showed preference for looking at the non-masked, but not the masked biological stimuli while OXT-pretreated dogs had no preference in either of the conditions (Fig6.). All other interactions were non-significant (all  $p>0.05$ ) and we did not find significant effects of HR ( $F_{(1,56)}=0.166$ ,  $p=0.685$ ) and HRV ( $F_{(1,56)}=0.673$ ,  $p=0.415$ ).

## **Discussion**

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

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

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

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

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

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

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

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

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

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## Figure caption

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

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

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

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

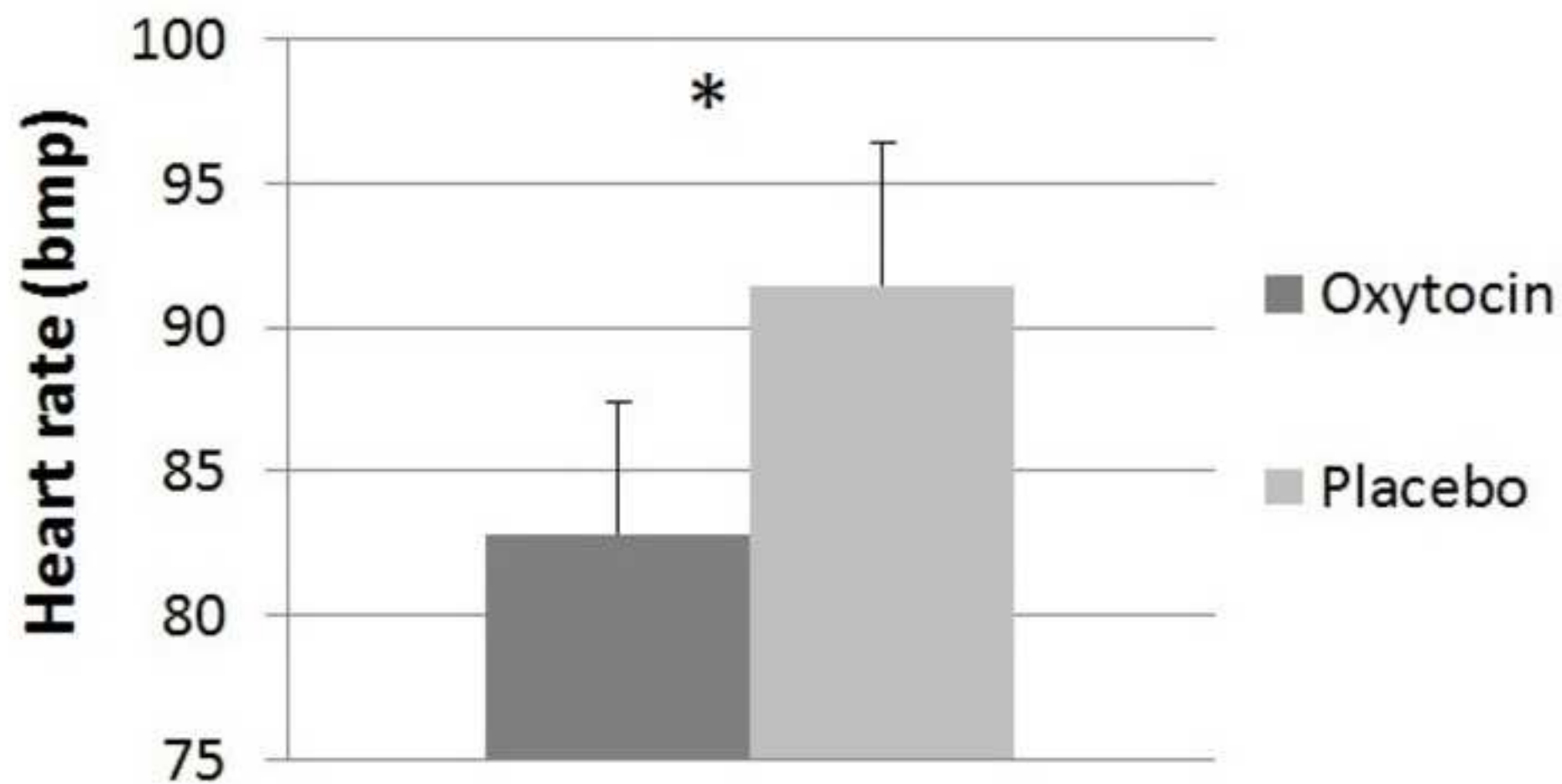
**Fig5.** Differential effect of OXT on the looking behaviour in males and females. Female dogs looked more at the stimuli after oxytocin pretreatment. \*:  $p < 0.05$

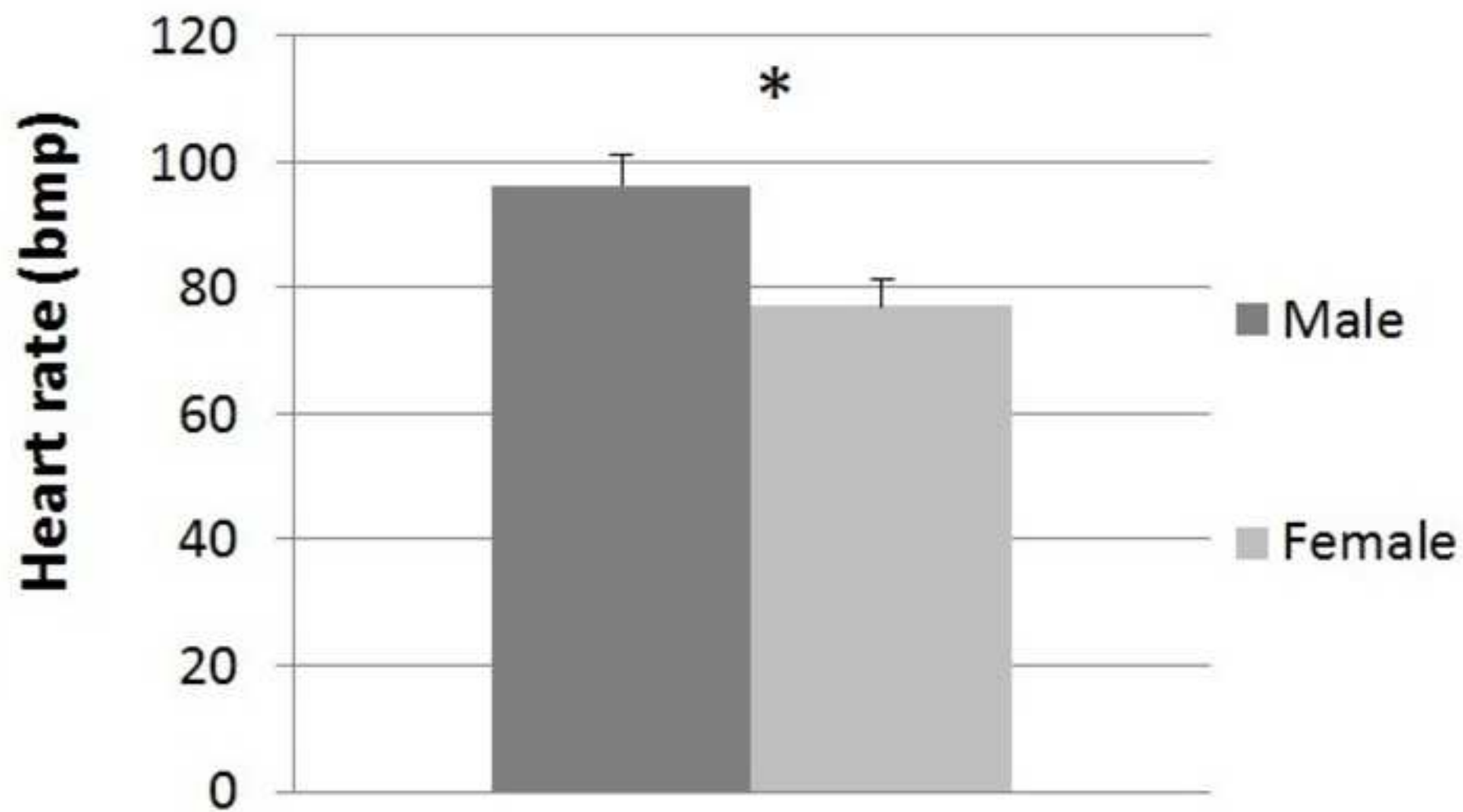
**Fig6.** Placebo-pretreated dogs showed a significant preference for looking at the biological motion stimuli in case of the non-masked, but not in the masked condition. \*:  $p < 0.05$

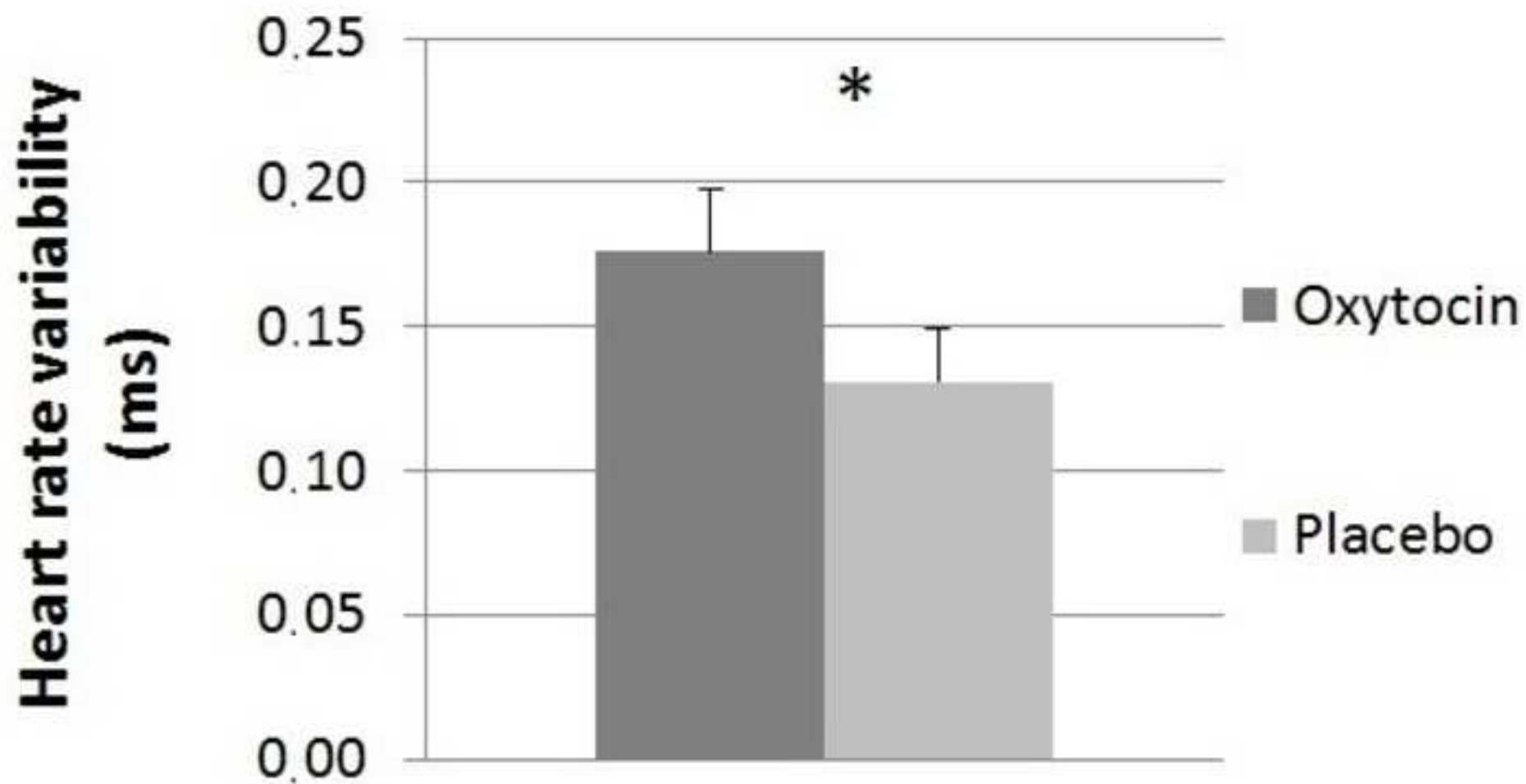
**Table 1.** Mean $\pm$ SEM data of Preference index

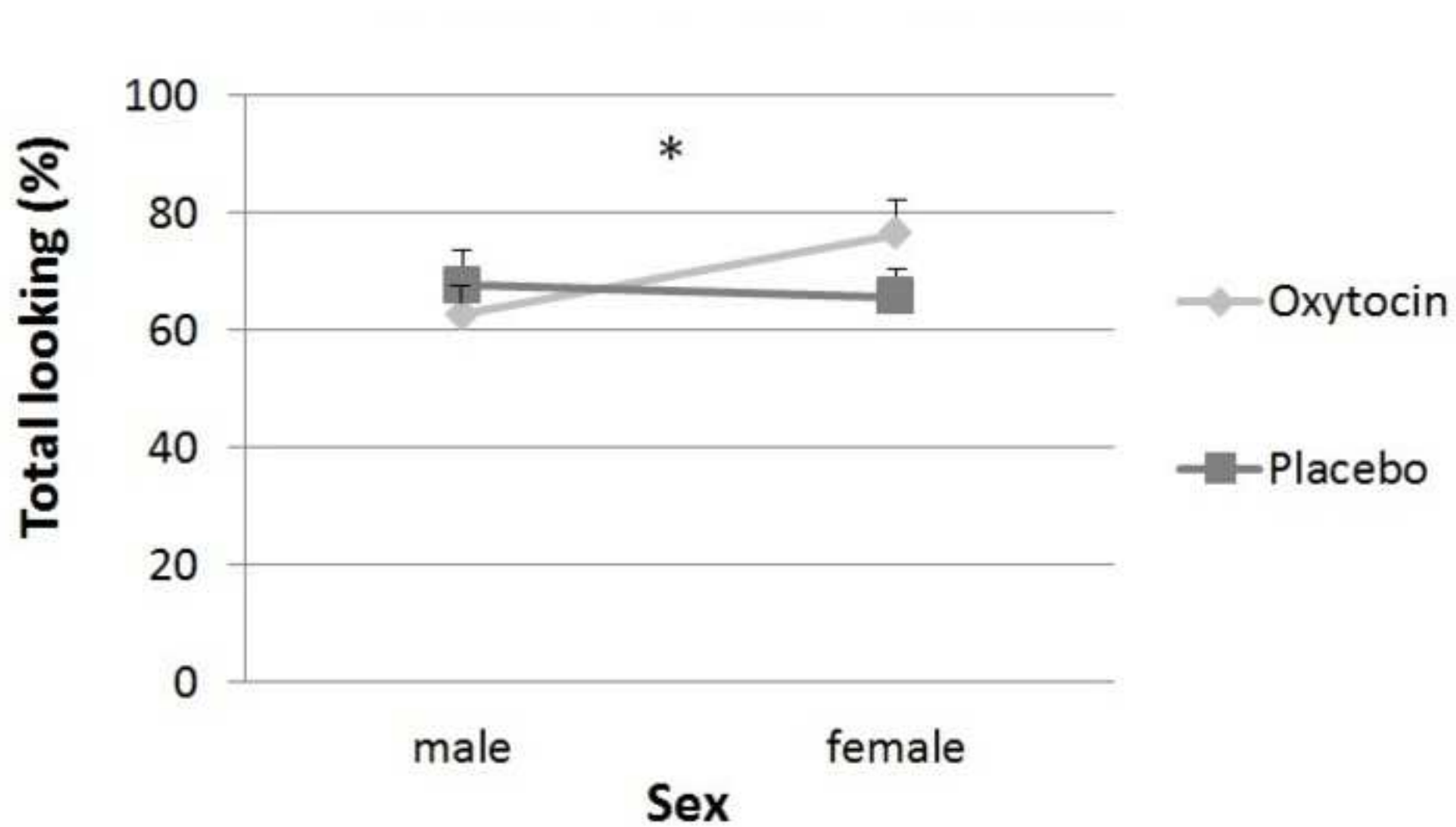
Preference index (mean $\pm$ SEM)	Oxytocin	Placebo
Non-masked	$-0.17 \pm 0.54$	$0.28 \pm 0.27$
Masked	$-0.01 \pm 0.93$	$-0.04 \pm 1.19$

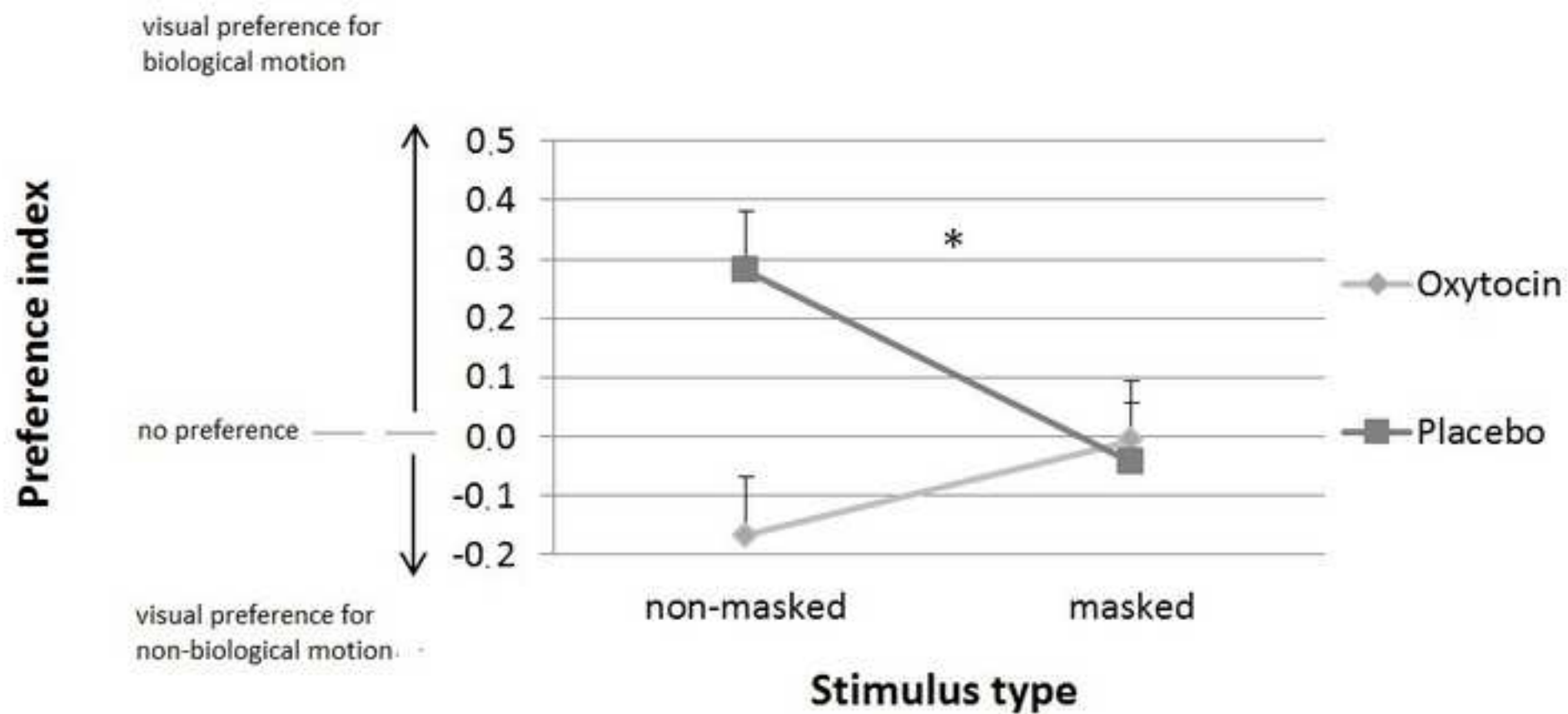














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