Physiology & Behavior 237 (2021) 113451

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Artificially elevated oxytocin concentrations in pet dogs are associated with higher proximity-maintenance and gazing towards the owners

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ARTICLE INFO

Keywords:
Domestic dogs
Oxytocin
Affiliation
Social behaviour
Behavioural synchrony
Shared attention

ABSTRACT

The relationship between dogs and their owners is characterized by an affective and enduring bond. It has been suggested that oxytocin might be the underlying mechanism driving this relationship, however evidence is mixed. In this study we tested whether intranasally administered oxytocin (compared to saline) would influence dogs' behavioural synchrony and shared attention towards their owners. Each individuals' pre and post administration oxytocin concentrations (measured in urine) were included in the analyses. Urinary oxytocin concentrations after administrations were positively associated with dogs' duration of social proximity and looking behaviours towards their owners supporting the role of oxytocin in modulating dogs' human-directed social behaviours.

1. Introduction

Oxytocin is a neuropeptide and hormone synthesized in the hypothalamus and released into the systemic circulation by the posterior pituitary gland [60]. It has been widely studied in relation to its effects on animals' behaviours in reproductive as well as non-reproductive contexts (for a review see: [1]). For example, oxytocin is associated with affiliative behaviours in a number of species (voles: [4]; rhesus monkeys: [88]; marmosets: [77]), and increases social recognition (mice: [33]) and gazing behaviours towards social partners (humans: [35]). The effects of oxytocin are not always consistent across studies and some even report anti-social effects such as an increase in envy responses (humans: [75]) and social aversion towards out-group members (humans: [89]), underlining how the effects of oxytocin are dependant on the context and the valence of the stimuli involved [8].

Based on both human and animal studies and the potential underlying physiological mechanisms at work, oxytocin has been suggested to modulate social behaviours in different ways. Oxytocin may interact with the dopaminergic system during social interactions [81], increasing the individual's intrinsic social motivation [9]. It may dampen the HPA axis [63], reducing the physiological response to stressors, consequently influencing behaviour [61, 85]. Finally, based on the social salience theory, the interaction between the oxytocin and the dopaminergic system could enhance the salience of both negative and positive social cues, leading to different behavioural effects of oxytocin, dependant on the context and the stimuli involved [8, 75].

Domestic dogs are a good model to investigate the hormonal correlates of social behaviours and social bonds since they form affective and enduring bonds both with conspecifics [14, 23, 84] and humans [69, 83]. An increasing body of data supports the role of the oxytocinergic system in the modulation of dog-human social interactions ([47]; for a review see [17]). Both dogs and their owners show an increase in oxytocin levels after positive interactions (i.e. mutual gaze, stroking) (plasma oxytocin concentrations measured: - [37, 42, 64]; plasma and salivary oxytocin: - [50]; urinary oxytocin: - [56]). Oxytocin administration enhances prosocial behaviours of dogs towards both conspecifics and human partners and mutual gaze with their owner (urinary oxytocin: - [57]). Furthermore, oxytocin-treated dogs outperformed placebo-treated dogs in tasks involving interpretation of human communicative signals (no measure of pre-post administration oxytocin concentrations - [49, 66]) and in a communicative learning task, involving gazing at the experimenter to get a reward (no measure of pre-post administration oxytocin concentrations - [7, 31]), suggesting that this peptide could increase dogs' attention towards human social

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https://doi.org/10.1016/j.physbeh.2021.113451
Received 12 January 2021; Received in revised form 18 April 2021; Accepted 30 April 2021
Available online 8 May 2021

cues. Taken together, these results suggest that oxytocin enhances affiliation and social orientation of dogs towards humans.

However, neither Marshall-Pescini and colleagues (urinary oxytocin: - [51]) nor Powell and colleagues (urinary oxytocin: – [67]; salivary oxytocin: – [68]) found an increase in dogs' endogenous oxytocin after a positive social interaction with the owner. Furthermore, oxytocin administration did not increase social behaviours (social proximity and physical contact) towards humans in pet and shelter dogs during a sociability test (no measure of pre-post administration oxytocin concentrations - [7]) and it actually decreased friendly reactions of dogs towards their owner during a threatening approach test (no measure of pre-post administration oxytocin concentrations - [41]). Taken together, results suggest that the role of oxytocin in dog-human interactions still needs clarification. In particular, a standardized methodological procedure for the administration and the measurement of the actual hormonal levels would be desirable. In fact, studies investigating the effects of oxytocin administration often presuppose the efficacy of the administration, using the treatment as test predictor for the statistical analysis, without measuring subjects' actual change in oxytocin's concentrations [57, 66, 70]. However, it has been shown that different methods of administration lead to variable intake of oxytocin (urinary oxytocin - [74]) and that each individual's endogenous oxytocin concentrations can influence the subsequent intake of the administered oxytocin and thus the potential behavioural effects (urinary pre administration oxytocin - [70]). These aspects highlight the importance of accurately measuring each individuals' endogenous oxytocin concentrations both before and after the administration. The current study aims to further investigate the putative effect of oxytocin administration on dogs' social behaviours towards their owner using a recently validated method for oxytocin administration [74] and including the actual pre and post administration urinary oxytocin concentrations of each individual in the analysis.

In order to study the effects of oxytocin on dogs' behaviours towards their owners we used two behavioural phenomena which have been studied in the context of social interactions: behavioural synchrony [13, 22, 24] and shared attention [21, 32].

Behavioural synchrony is the ability of agents to efficiently coordinate their actions [30]. Various studies confirm that behavioural synchronization is linked to affiliation in both humans and animals (in humans: [22, 44, 54]; in bottlenose dolphins: [72]; in black railed godwit: [36]; in free-ranging dogs: [14]). Recent studies showed that dogs synchronize their location and behaviours (walking or staying still) with those of their owners both in an indoor room [28] and in an outdoor space [29]. Since there is a bidirectional relationship between behavioural synchrony and affiliation and oxytocin has an effect on dogs' affiliative behaviours towards the owner [70], we aimed to investigate whether oxytocin plays a role in dog-owner behavioural synchronicity. This phenomenon includes different components: 'activity synchrony', which consists of exhibiting the same type of behaviours at the same time [22], 'temporal synchrony', which involves switching actions at the same time [27], and finally 'local synchrony' (or proximity-maintenance) which measures the time individuals spend close to each other [13]. We tested dogs' tendencies to synchronize their behaviour with the owner after administration of either placebo or oxytocin. If oxytocin has a prosocial effect on dogs' behaviour towards the owner, we expect dogs to synchronize more with their owner after being administered oxytocin compared to placebo. More specifically, we predicted that in the oxytocin condition dogs 1. will spend more time carrying out the same behaviours as the owner (activity synchrony), 2. will be faster at switching actions in response to the owner's change of state (temporal synchrony) and 3. will generally stay longer in proximity to the owner (local synchrony).

The second behavioural phenomenon we adopted to investigate the effects of oxytocin on dogs' social behaviours was shared attention. Shared attention is a triadic interaction in which two individuals coordinate their attention towards an object [3]. In this study we adopted Emery's [32] definition of shared attention. Emery [32] distinguishes between gaze following, i.e. when an individual follows the direction of attention of another individual (without having a specific target of attention), mutual gaze, i.e. when two individuals direct their attention to one another and joint attention, i.e. when two individuals look at the same object. Shared attention is thus a combination of mutual gaze and joint attention, where the focus of the two individuals is both on an object and the partner (for the debate regarding the 'conscious' engagement of animals in shared attention with humans, see [21] and the 'discussion' below). Thus, at the operational level, shared attention is characterized by gaze alternation i.e. the observation that both individuals alternate their gaze between the object of attention and the partner [20, 43].

Domestic dogs can follow human ostensive gaze cues [28], they are sensitive to humans' attentional focus [18, 38, 45, 86] and can use theirown gaze alternation behaviour to direct their owners' behaviours [39]. Based on these results, we assumed that dogs would engage in shared attention with their owners, and, more specifically, that they 1. will synchronize their looking behaviours with that of their owner by directing it at the object of the owner's attention more compared to at the control object, 2. will gaze alternate more often between the owners and the object of their attention compared to between the owners and the control object, and 3. when allowed to do so, they will preferentially explore the object of the owners' attention rather than the control object. Administered oxytocin increases joint attention in humans [75], and in dogs it enhances their performance in tasks involving the interpretation of human social cues [49, 66]. We therefore hypothesized that oxytocin may play a role in the dog-human synchronization of looking behaviour towards an object (i.e. shared attention). More specifically, we predicted that after oxytocin administration dogs would engage in more gaze alternation behaviour between their owner and the object of the owner's attention than after placebo administration. Furthermore, considering previous studies showing oxytocin's effect on owner-directed looking behaviour [31, 57], we further expected a general increase in the time dogs spent looking at the owner after oxytocin administration compared to placebo.

However, in a number of studies it has been suggested that a human's direct gaze could be perceived by the dog as a mildly threatening behaviour or, if directed towards an object, as an indication of 'ownership', rather than a cooperative/attention-directing signal [28, 66, 79]. To take this possibility into account, we measured the frequency of the dog's self-directed behaviours (e.g. head shaking, yawning, lips licking), which are thought to be linked to stress/distress [11, 12]. Considering oxytocin's alleviating effects on stress [40, 63, 85], we predicted that oxytocin administration would reduce (compared to placebo) dogs' display of self-directed behaviours, which would hence indicate a decrease in dogs' aversion to gazing cues (in line with [66]).

2. Material and methods

2.1. Ethical statement

All the procedures applied in the study were approved by the ethical committee of the University of Veterinary Medicine of Vienna (approval numbers: REF ETK-042/02/2020; ETK-17/01/2019).

The owners were informed about the experimental procedure and signed consent forms before the start of the test. The experimental procedure was completely non-invasive. Training and handling of the dogs were conducted using positive reinforcement only (see - [74] for method used to deliver oxytocin or placebo intranasally). The experiment was interrupted if a dog showed any signs of discomfort.

2.2. Subjects

We trained and started testing 33 pet dogs. However, three of them dropped out after the first test session due to unavailability of the

owners. We tested thirty pet dogs in both sessions but for five of them we could not get the complete pre and post administration urine samples and we had to exclude two of them from the final dataset due to sample analysis problems. Thus, the data analysis included twenty-three dogs [females=13, 1 intact and 12 neutered, and males=10, 5 intact and 5 neutered; mean age: 6.25 (SE = 3.06)] of various breeds as well as mongrels (see Table 1- Supplemental material). The minimum age of the dogs was one year. Since oxytocin's concentrations can be influenced by the female's reproductive cycle, the intact female was tested at least 70 days after their last heat and only if showing no signs of pseudo-pregnancy. Of the dogs' owners, 22 were females and one was male. Because of that, we did not take into account the sex of the owner in the further statistical analyses. Most of the owners participated in the study with only one dog: only one owner participated in the study with two dogs. We asked for additional information, relating to the dog-owner relationship in a brief questionnaire (i.e. the level of training of the dogs, the time spent interacting each day etc.). We found that, overall, our study population was quite homogenous in terms of the training experience (they all had basic training and performed at least one activity such as agility, cani-cross, search tasks etc.) with their owners and owner-reported rate of affiliation ("How much do you feel attached to this dog?" ranged from 8 to 10 in a scale from 1 to 10, indicating that all the owners considered themselves highly attached to their dogs).

2.3. Experimental design and setup

All the experiments were conducted at the Clever Dog Lab (CDL) of the University of Veterinary Medicine of Vienna, Austria.

We adopted a within-subject design in which each dog-owner dyad was tested twice: once after the oxytocin administration and once after placebo treatment. The order of the placebo and oxytocin sessions was counterbalanced across subjects and the two test sessions were separated by at least 7 days to exclude possible carry-over effects. The experiment was double-blind. Two experimenters were involved in the procedure. Every dog-owner dyad was assigned one experimenter who performed the administration of the treatments and another experimenter who performed the behavioural tests. The experimenter responsible for treatment administration was not involved in conducting the behavioural tests nor the video-coding and analyses of the dogs' behaviours. Furthermore, the owners did not know whether the dog was administered oxytocin or placebo. Three different locations were used for administration and testing. A small indoor room was used for the administration of the treatment and the waiting period, a bigger indoor room was used for the shared attention task, and an outdoor fenced area for the behavioural synchrony test.

2.4. Hormonal measures and urine sampling

An individual's endogenous oxytocin (OT) concentration may affect the responsiveness to exogenous oxytocin administration [70], therefore we collected a urine sample from each subject prior to each test to statistically account for pre administration OT concentrations. Furthermore, based on previous results by our own group [74], showing that administration of oxytocin can result in considerable inter-individual variability in its assimilation, we collected urine samples after each test to account for the actual post administration OT concentrations of each subject.

The urine was collected during leashed walks on the university campus using a metal stick with a plastic cup attached (polypropylene, Carl Roth, CEN 7.1). The first urine sample was collected upon arrival of the owners and their dogs at the campus (pre-sample). Each dog was then walked for about 10 min in order to empty the bladder and in order to let the experimenter store the first urine sample and set up for the administration. The second urine sample was collected on average 71 min (min:59 min, max:90 min) after the end of the administration procedure. Urine samples were aliquoted in Eppendorf vials to 1 mL each within 5 min after collection. To each aliquot, 0.1 mL 0.5 N phosphoric acid (PA) was added [25, 73]. The vials were manually shaken for several seconds and labelled before being stored in a −20 °C freezer.

2.5. Oxytocin and placebo administration

The administration of the placebo or oxytocin treatment was performed in an indoor room of the CDL. The room was equipped with a mattress, a chair, and a water bowl.

The administration of the treatment was performed with a vaporizer mask. A recent study by Schaebs et al. [74] validated this procedure, showing that the mask administration leads to more reliable OT uptake and less stressful reactions in the dogs compared to the nasal spray method.

All the dogs were previously trained to voluntarily enter the mask with their snouts and inhale the vapour. The training procedure took between 1 and 6 30-minute sessions. The training was done using only positive reinforcement. The dogs were rewarded with pieces of sausages initially for putting their snout inside the mask, and afterwards for staying inside for an increasing amount of time. The dog was considered ready for testing when it was able to voluntarily keep its snout in the vaporizer mask for at least 30 s. During the administration the owner sat on a chair, not interacting with the dog or the experimenter. The experimenter sat next to the dog on the mattress and administered the substance to the dog with the vaporizer mask (see- [74- for details of the procedure).

For the test, the dogs were administered either 24 international units (IU) of OT (600 μl Syntocinon, Novartis + 400 μl sodium chloride (NaCl) 0.9% Braun) for the oxytocin condition or 1 ml NaCl 0.9% (Braun) for the placebo condition. There is no standard dose in oxytocin administration studies in dogs. Published studies used doses ranging from low doses such as 12 IU ([82]: 12 IU; [31, 41, 46], 16 IU; [48]) to relatively high administration doses of 40 IU ([[58], 70, 71]; [90]). We decided to administer a dose that was intermediate between these two doses, i.e. 24 IU. This dose was also used by other studies reporting effects of OT on dogs' social behaviour [7, 66]. After the administration, a waiting period of 40 min started (time needed for intranasally administered oxytocin to reach peak levels in the brain - [62]). During the waiting period, the owner was allowed to read a book or entertain herself with music or with her phone but was not allowed to interact with the dog.

2.6. Experimental procedure

2.6.1. Behavioural synchrony test

The behavioural synchrony test was performed in an outdoor fenced area behind the CDL, inside the university campus. The test area measured 40×20 m, including some trees. Two chairs were placed on opposite sides of the test area. The owner of the dog was provided with wireless (Bluetooth) earphones so she/he could follow instructions from the experimenter. The experimenter stood outside the area and recorded the entire procedure with a hand-held camera. The test started with a habituation phase (1 min) during which the dog was free to explore the area and the owner was instructed to behave naturally as if in a normal dog zone. After the habituation phase, the owner called the dog to the starting point of the test (entrance of the area) and held it until the experimenter gave the start signal. The owners were instructed to behave naturally during the test. They were told that they could look and smile at their dogs if the dogs sought eye-contact/attention but they were asked not to pet or actively interact with them.

Once the experimenter gave the start signal, the owners released his/her dog. The owner was instructed to walk around the perimeter of the area at normal speed for 1 min, change direction in the middle of the area, and continue to walk until the end of the first minute. Then, the owner sat down in Chair 1 for 1.5 min (see Fig. 1). After this first sitting phase, the owner was instructed to walk around the perimeter of the

Fig. 1. Set up for the behavioural synchrony test.

area again, cross the centre of the area and walk around the perimeter in the opposite direction for another minute. In the last part of the test, the owner was asked to sit in Chair 2 for another 1.5 min. After Phase 4, the test ended and the dog and the owner could leave the fenced area (see S1 video, Supplemental material).

2.6.2. Shared attention test

For the shared attention test the room was equipped with a leash attached to the wall and tape marks to signal to the owners where they had to sit (in front of the dog on the floor) and to the experimenter where the two attention objects had to be placed (1 m on the left and on the right of the owner) (see Fig. 2). Two objects were used for each test, one was assigned as the focus of the owner's attention and the other one as a control object. In total, four different objects were used in a counterbalanced way within the dog-owner dyads and the placebo/oxytocin sessions. The objects were of neutral valence to both the dogs and the owners, and all approximately of the same size. They consisted of a white tissue box, a colourful cardboard box, a plastic bottle, and a transparent plastic bowl (boxes: 25×20×15 cm; plastic transparent bowl, diameter: 20 cm; plastic transparent bottle, height: 25 cm) (see Fig. 2).

The owners were instructed via Bluetooth earphones from outside the room and the whole test was recorded by the CDL's camera system. The dog and the owner were given one minute of habituation inside the room before every test. After the habituation phase, the owner was instructed to attach the dog to the leash and sit on the marked spot in front of the dog (~1 m from the dog). Once the dog and the owner were in place, the experimenter carried the two objects into the room and placed them on the marked spots. The experimenter left again and gave instructions to the owner from outside the room. The owner was instructed to gaze alternate between the dog and one of the two objects (object of attention) for 10 s (gazing phase 1). The other object was completely ignored by the owner during the entire duration of the test. Then, the owner was instructed to pick up the object of attention, saying "Oh this is beautiful!" and manipulate it for 10 s, still engaging in gaze alternation between the dog and the object of attention. In the last phase, the owner had to gaze alternate between the object of attention and the dog for another 20 s (gazing phase 2). Once finished the "gazing phase 2", the owner was instructed to unleash and ignore the dog. In this phase, the dog was allowed to approach and explore the objects ("exploration phase") (see S2 video in Supplemental material).

2.7. Behavioural coding

2.7.1. Behavioural synchrony variables

The behaviours of the dogs during the behavioural synchrony test were coded according to the synchrony components [29]. We measured "temporal synchrony", i.e. the latency for the dog to change activity when the owners changed their activity, "activity synchrony", i.e. the duration of time the dog and the owner spent doing the same activity, and "local synchrony" (proximity-maintenance) i.e. the time dogs spent within 2 m of their owner. Additionally, we created a composite measure which included only the duration of time the dog spent doing the same activity as the owner whilst also being in proximity (<2 m) to him/her ("local plus activity synchrony"). This we considered a more stringent measure of behavioural synchrony since it combines both the location and activity components. The total time the owner spent performing each phase was also coded to account for possible slight variations across tests.

2.7.2. Shared attention variables

A number of different behaviours were coded in the shared attention test. The frequencies of dogs' gaze alternations i.e. a look to the object immediately (within two seconds) followed by a look to the owner, for both the control object and the object of the owner's attention were coded. Furthermore, we coded the duration of the dogs' looking behaviour 1. towards the owner, 2. towards the object of the owner's attention ("looking towards the object of attention") and 3. the control object ("looking towards the control object"). Furthermore, since during the manipulation phase it was impossible to disentangle whether the dog was looking at the object of attention or at the owner, we coded the variable "looking towards the owner/object" for this phase of the test. In the last phase of the test (exploration phase), we noted which object the dog approached first ("object choice") to further evaluate aspects of the animals' interpretation of human gaze (as attention engagement or ownership signal).

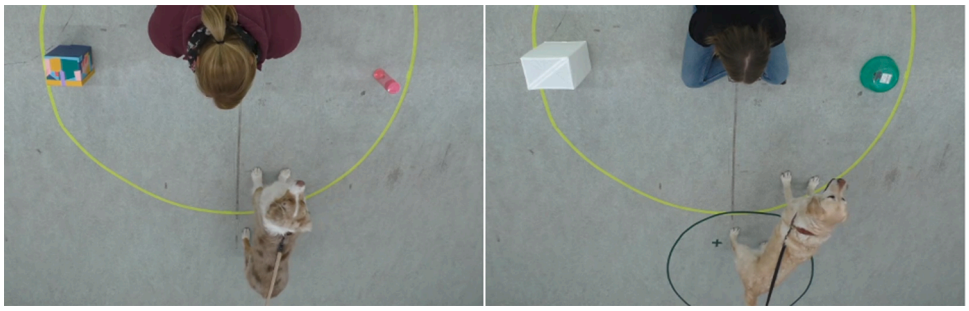

Fig. 2. Set up and objects used in the shared attention test.

We also coded the duration of the owners' activities to control for possible variations across tests and to obtain a more precise measure of dogs' behaviours in response to their owners' actions. Thus, the dogs' behaviours were then expressed as a proportion of time relating to the duration of the owners' activities. Similarly, we measured the actual number of owners' gaze alternations between the object of attention and the dog ("owner's gaze alternations") since this too may vary across subjects and could influence the dog's response.

We further coded the frequency of a number of dog behaviours considered as indicators of stress and/or submissive behaviours during a conflict situation. The behaviours considered to be indicators of stress and discomfort ("self-directed signals") were: yawning [11], body/head shaking [11] and lips licking [11, 12]. Gaze aversion (also referred to as "avoiding eye contact") i.e. looking away from the owner, in response to the owners look at them was also measured as it has been considered a submissive behaviour when observed between conspecifics [14, 76].

All behaviours for both tests were coded from video using the video scoring feature provided by "loopy" (www.loopbio.com).

2.7.3. Interobserver agreement

The first coder coded 100% of the videos and was blind to the treatment the dogs received. In addition, an external coder, also blind to the treatment, coded the 25% of the videos. Pearson correlation coefficients for the behavioural synchrony results indicated a good reliability for all the four variables coded ("activity synchrony": $r = 0.975$, $s = 4$, $p<0.001$; "temporal synchrony": $r = 0.866$, $S = 22$, $p = 0.002$; "proximity": $r = 0.951$, $S = 8$, $P<0.001$; Activity + Local synchrony: $r = 0.966$, $S = 4$, $p<0.001$). For the shared attention test interobserver-reliability was also high ("looking towards the object of attention": $r = 0.821$, $S = 10$, $p = 0.034$; "looking towards the object/owner": $r = 0.842$, $S = 28$, $p = 0.004$; "looking towards the owner": $r = 0.90$, $S = 12$, $p = 0.002$; "dogs' gaze alternations": $r = 0.81$,$S = 1.83$, $p = 0.200$; "lips licking": $r = 0.84$, $S = 26$, $p = 0.004$; "gaze aversion": $r = 0.91$; $S = 12$, $P<0.0001$; "yawning": $r = 1$, $S = 0$, $P<0.001$).

2.8. Laboratory analysis

The urine samples were transported from the CDL to the Endocrinology Laboratory at the Department of Physiology, Pathophysiology and Experimental Endocrinology, University of Veterinary Medicine, Vienna, Austria for processing and analysis.

Urine samples were extracted following the protocol described in Schaebs et al. (2019). In brief, urine samples were thawed, while continuously kept at 4 °C, vortexed and centrifuged for 1 min at 1500 rpm at 4 °C. Solid-phase extraction (SPE) cartridges (Chromabond HR-X, 1 ml, 30 mg) were conditioned with 1 mL 100% MeOH, followed by 1 mL HPLC water. After that, urine samples were mixed with 0.5 mL of 0.1% trifluoroacetic acid (TFA) and applied to the cartridges. Cartridges were washed 5 times with 1 mL 10% (vol/vol) acetonitrile (ACN) containing 1% TFA in water and dried using a vacuum pump. Samples were eluted with 1 mL 80% (vol/vol) ACN and cartridges were dried again. Eluates were evaporated at 50 °C for 35 min using a gentle stream of air. They were reconstituted in 300μL of 100% EtOH, capped, sealed, and stored at −20 °C until analysis. On the day of analysis, eluates were incubated at 4 °C for 1 h to assist protein precipitation and evaporated again at 50 °C for 10 min, or until complete dryness, before reconstitution with 250μL assay buffer (AB) from the assay kit (Arbor Assays, Ann Arbor, Cat.No: K048-H5). Reconstituted samples were gently vortexed, transferred to Eppendorf tubes, and centrifuged for 1 min at 10,000 rpm. The assay was conducted according to the suppliers' instructions.

All samples were measured in duplicates and measurements were kept for statistical analyses only if duplicates' optical densities (OD) differed less than 10%. For three samples, the duplicates differed 13%, 20%, and 25%, respectively. *Re*-measurement was not possible due to time constraints, so these samples were excluded from statistical analyses.

Inter-assay coefficients of variance (CV) were 10.3% for high concentration standard and 14.4% for low concentration standard ($n = 3$ plates). The intra-assay coefficient of variance was 1.4% calculated as the average variability across duplicates of 34 samples measured in one single assay plate. To account for the variable water content of urine samples, we measured urinary specific gravity (SG) for each urine sample using a digital refractometer and expressed urinary OT concentrations as pg/ml SG following the formula used in [55].

2.9. Statistical analysis

To investigate whether dogs actually engaged in shared attention with their owners, we performed preliminary analyses considering just the data from the placebo condition. In particular, we performed two t-tests comparing the time dogs spent looking towards the object of owners' attention and the control object and the numbers of gaze alternations they performed between them. In addition, we performed a binomial test to assess whether, at the end of the test, dogs were more likely to first explore the object of owners' attention compared to the control object.

To investigate whether oxytocin influenced the behavioural synchrony and the shared attention of dogs towards their owners, Generalized Linear Mixed Models (GLMM; [2]) were fitted. The four different behavioural synchrony components ("activity synchrony", "temporal synchrony", "local synchrony" and "local plus activity synchrony") as well as the variables of the shared attention test ("looking towards the owners", "looking towards the object of attention", "looking towards the object/owner", "object choice", gaze alternations, gaze aversions and self-directed behaviours) were analysed separately.

Generalized Linear Mixed Models with beta error structure and logit link function were fitted, using the function "glmmTMB" of the R package "glmmTMB" [16] for duration variables (in the behavioural synchrony test: "activity synchrony", "local synchrony", "local plus activity synchrony"; in the shared attention test: "looking towards the owner", "looking towards the object of attention" and "looking towards the owner/object" during the manipulation phase). The proportion of these responses were calculated dividing the duration of the performed behaviours for the duration of each tests. Beta regression was used because the response was a proportion bounded between 0 and 1. When the response comprised zeros or ones, prior to fitting the model with beta regression the response variables were transformed using the formula (xx(length(x)−1)+0.5/length(x)) [78].

For the "temporal synchrony" variable of the behavioural synchrony test, a Generalized Linear Mixed Model (GLMM) with Poisson distribution error structure [53] was fitted using the "lmer" function of the R package "lme4" (version 1.1–21; [10]). The response for the "temporal synchrony" model was obtained calculating the mean latency of the dog to change activity when their owner changed their activities. To evaluate the effect of oxytocin on the dogs' "looking behaviour towards the object of attention" we ran a Generalized Linear Mixed Model (GLMM) with beta error distribution (since the response was a proportion bounded between 0 and 1) using the function "glmmTMB" of the package "glmmTMB". To model the "object choice" of the dogs in the shared attention test a Generalized Linear Mixed model (GLMM) with binomial error distribution was fitted using the function "glmer" of the R package "lme4" (version 1.1–21; [10]). Finally, for the frequency variables of the shared attention test: "dogs' gaze alternations", "gaze aversions" and "stress behaviours", Generalized Linear Mixed models (GLMM) with Poisson distribution error structure (ideal for count responses) [53] were fitted using the "glmmTMB" function of the package "glmmTMB" [16].

Preliminary investigation of results relating to OT concentrations in urine after administration of OT vs. placebo, revealed large variability across subjects (see Figure 1 in Supplemental Material). Thus, instead of using condition (placebo vs. oxytocin) as the main predictor in our

models, we considered the post-administration OT concentration of each dog after each test as a more accurate measure of the dogs' oxytocin uptake.

Thus, all the behavioural responses were modelled as a function of the dogs' level of post-administration OT concentration, expressed as pg/ml SG and log transformed. We included sex, castration status and pre-administration OT concentration (expressed as pg/ml SG and log transformed) values as fixed control effects in all models, since from previous studies all these variables have been shown to affect dogs' behaviour after OT administration (sex related differences: [48, 66]. Pre-treatment OT concentrations (i.e., endogenous OT concentrations): [70]; castration status: [31]).

Furthermore, for the following variables in the shared attention models: "looking towards the object of attention", "gaze alternations", "object choice", "gaze aversion" and "self-directed behaviours", we included the frequency of the owner's gaze alternations between the dogs and the object of attention as a fixed control effect, since this variable showed considerable variability across owners (ranging from 3 to 14). Finally, since we had repeated observations of the same individuals, we included Dog ID as a random intercepts effect.

As an overall test of the effect of post-administration OT concentration and to avoid "cryptic multiple testing" [34], we compared the full models as described above with null models lacking the test predictor but otherwise identical. We tested the effect of individual fixed effects using likelihood ratio tests comparing the full models with reduced models lacking the fixed effects one at a time [6]. For these tests as well as the full-null model comparison we utilized a likelihood ratio test [26]. Effect sizes (i.e., variance explained by entirety of fixed and random effects, or conditional R2, [59] of the full models were calculated using the function r2 of the R package "performance" (version 0.7.0).

For Beta distribution models we checked for over-dispersion and the dispersion parameters (ranging from 0.77 to 1.17) indicated that the models were not over-dispersed. Model stability was estimated dropping the individuals one at a time from the data and comparing the estimates derived for models fitted to these subsets with those obtained for the full data set. These revealed all the models to be of good stability (for details see Supplemental material).

All statistical analyses were performed in R (version 3.6.1; R Core Team 2019).

Results were considered statistically significant if $p \leq 0.05$.

3. Results

3.1. Behavioural synchrony test results

In the behavioural synchrony test, dogs spent on average 45 ± 20% of the test time performing the same activity as their owners ("activity synchrony"). The model revealed no significant effect of post-administration OT concentrations on the proportion of time dogs spent performing the same activity as their owners (see Table 3 – Supplemental Material).

Dogs took on average 25 ± 21 s to change their activity when their owners changed theirs. The model for the "temporal synchrony" revealed no effect of the post administration oxytocin values on the time dogs needed to change their activity when their owners changed activity. Furthermore, regarding the control predictors, the dogs with higher pre administration (i.e., endogenous) oxytocin concentrations, needed more time to change activity when their owners changed activity (estimate±SE= 0.893±0.040; $\chi 2$=4.190; df=1; $p = 0.040$) (Table 6 – Supplemental Material).

Regarding the "local synchrony" measure, dogs spent on average 33 ± 25% of the test time <2 m from their owners. A positive association was found between dogs' post administration oxytocin concentrations and time spent in proximity to their owners (estimate±SE= 0.612±0.250; $\chi 2$ =5.55, df = 1, $p = 0.018$; Variance explained by the entirety of fixed and random effects: R2c = 0.910; by fixed effects only: R2m = 0.246) (see Fig. 3). Regarding the control predictors, we found that there was an effect of sex and pre administration oxytocin concentrations on the time the dogs spent in proximity to their owners with male dogs spending more time in proximity than females (estimate±SE=1.032 ± 0.492; $\chi 2 = 4.11$, df=1, $p = 0.042$) and dogs with higher pre administration oxytocin concentrations spending less time in proximity to their owners (estimate ± SE = −0.828 ± 0.365; $\chi 2$= 4.8, df=1, $p = 0.027$) (for the detailed results of the model see. Table 4 – Supplemental Material).

The dogs spent on average 24± 23% of the time performing the same activity as their owners whilst being close to them ("local plus activity synchrony"). We found no significant effect of the level of post administration oxytocin on this composite variable but male dogs spent more time in proximity and performing the same activity as their owners ($\chi 2$= 4.64; df=1; $p = 0.031$) (Table 5 – Supplemental Material).

3.2. Shared attention test results

The preliminary analysis on dogs' behaviour when no oxytocin was administered (placebo condition only) revealed that as predicted: 1. dogs spent significantly more time looking towards the object of the owner's attention (mean: 9%; range: 0–41%) than the control object (mean: 2%; range: 0–16%) (t(21)=2.691; $p = 0.014$) and 2. they gaze alternated more between the owner and the object of the owner's attention (mean: 1136; range: 0–4) than the control object (mean:0.318; range: 0–1) (t(21)=3.367; $p = 0.003$). However, differently from our predictions, they were not more prone to first explore the object of owners' attention when released (5 dogs did not explore any object, 9 dogs first explored the object of the owners' attention and 9 dogs first explored the control object).

The models investigating the effect of OT on dogs' shared attention revealed no effect of post-administration OT on dogs' gaze alternations between the owners and the object of attention (mean: 1.2; range: 0–4) (Table 7 – Supplemental material), on the duration of looking at the object of attention (mean: 10%; range: 0–55%) (Table 8 – Supplemental material), on the duration of looking at the owner/object during the manipulation phase (mean: 23%; range: 7 - 38%) (see Table 9 – Supplemental material) nor on the likelihood of first exploring the object of the owner's attention during the final test phase (See Table 10 – Supplemental material). Post-administration oxytocin concentrations affected the time dogs spent looking towards the owners (mean: 35%; range: 3–70%; estimate±SE= 0.512±0.200; $\chi 2$= 6; df=1; $p = 0.014$; variance explained by the entirety of fixed and random effects: R2c = 0.779; by fixed effects only: R2m = 0.536) during the test, with dogs with higher post-administration OT levels spending more time looking at their owners (see Fig. 4). Male dogs spent overall more time looking towards their owners than females ($\chi 2$= 5; df=1; $p = 0.024$) (for detailed

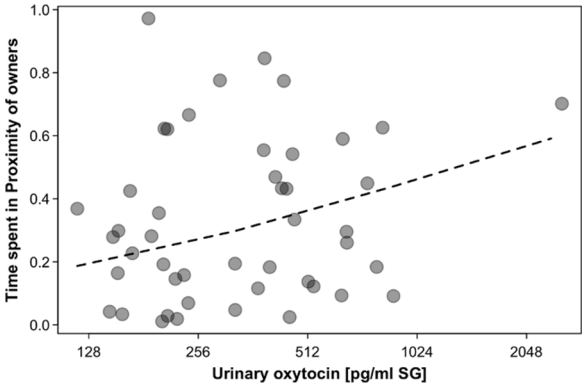

Fig. 3. The association between post-administration OT concentrations and the percentage of time dogs spent in proximity to their owners during the behavioural synchrony test.

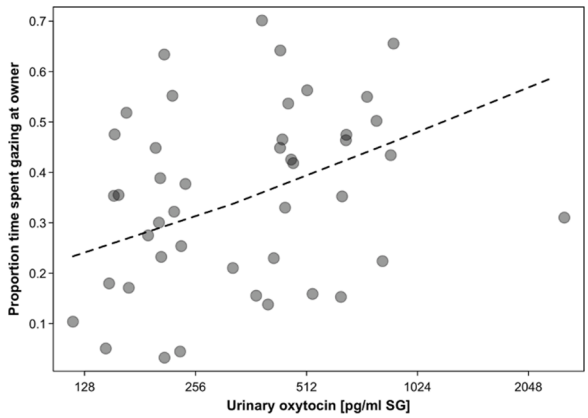

Fig. 4. The association between post-administration OT concentrations and dogs' percentage of time spent in looking at the owner during the shared attention test.

results, see Table 11 – Supplemental material).

Twenty dogs showed at least one gaze aversion (in at least one condition) when their owners were looking at them. In total we observer fifty-eight "gaze aversions" (mean: 1.45; range: 0–6). The model revealed no effect of oxytocin on this behaviour (Table 11 – Supplemental material).

Seventeen out of twenty-three dogs performed at least one self-directed behaviour (in at least one condition). We did not observe any head/body shaking but we observed seven yawns and thirty-six lips licking (considered together: mean: 1.3; range: 0–8). No effect of post administration OT on the performance of these behaviours emerged, however, the number of gaze alternations performed by the owners (included as controlled predictor) was positively associated with the frequency of self-directed behaviours performed by the dogs ($\chi 2 = 6$; df=1; $p = 0.012$) (for detailed results see Table 12 – Supplemental material).

4. Discussion

In this study, we tested pet dogs in a shared attention and behavioural synchrony test after both the administration of oxytocin and placebo. We found that artificially elevated oxytocin concentrations after intranasal administration were associated with the time dogs spent in proximity of their owners during a behavioural synchrony test and with the time dogs spent looking at the owner during a shared attention test. However, oxytocin concentrations were not associated with other behavioural synchrony and shared attention related measures.

Behavioural synchrony has been hypothesized to be bidirectionally linked to affiliation and social cohesion in dogs ([14];[92]) and, since oxytocin enhances social and affiliative behaviours of dogs towards their owners [70], we predicted it would increase the behavioural synchrony of dogs with their owners. We found no association between post-administration oxytocin levels and the more stringent measure of behavioural synchrony (i.e. the time spent doing the same activity but also in proximity of the owner), nor with the dogs' activity synchrony alone, or speed with which they changed activity following their owner's change of state (temporal synchrony component). Our results show high individual variability in the behaviours related to behavioural synchrony which could thus be affected more by each individual's characteristics (i.e. breed) or training experience rather than by their hormonal state and the consequent willingness of the dogs to affiliate with their owners.

We did however find a positive association between post administration oxytocin concentrations and the time dogs spent in proximity to their owners. This result is in line with Romero and colleagues [70], who found that intranasal oxytocin increased dogs' proximity (measured as the proportion of approaches/departures) towards conspecific partners and affiliation towards their owners. Furthermore, previous genetic studies have shown an association between proximity seeking towards humans and nucleotide polymorphisms in the regulatory regions of oxytocin receptor gene in German Shepherds [46]. Thus, our findings add to these results suggesting that oxytocin can affect dogs' proximity-maintenance behaviour with their owners and, considering that proximity-maintenance in many species is used as a proxy for affiliation in many species (dogs: [14]; macaques: [52, 65]; rats: [15]), these results provide some support for the sociability-enhancing effects of oxytocin [81].

Our analysis also showed that there was a negative effect of endogenous (pre administration) OT concentrations on dogs' proximity maintenance and temporal synchrony. Romero and colleagues [70] also found that endogenous OT modulated the effect of intranasally administered OT on behaviour, with dogs with higher pre administration OT concentrations being less responsive to the effects of administered OT. Pre administration endogenous OT slightly influenced individuals' increase of OT concentrations after administration (See Figure 2 – Supplemental Material). However, in contrast to the human OT literature, (e.g. [19, 80]) in dogs there are no dose-response studies investigating the effect of different doses of administered OT. Current results suggest that further studies should carefully consider the interaction between the effects of exogenous oxytocin administration and individuals' endogenous concentrations.

The second task we presented was the first to investigated if dogs engaged in shared attention with their owners towards a neutral object (non-foraging situations) and whether exogenous oxytocin has an effect on this phenomenon. Dogs gazed significantly more at the object of the owners' attention and gaze alternated more between the owner and the object of her/his attention compared to the control object. These results would suggest that dogs engage in mutual gaze (gazing at each other) and joint attention (gazing at the same object) with their owners and thus, following Emery's [32] definition, they engage in shared attention with their owners. However, we did not test whether dogs engage in shared attention with the sole motivation to share attention and no other instrumental goal, nor whether they were 'conscious' or 'aware' of sharing attention with their owner (conditions considered necessary for shared attention by [21]). Thus, further studies would be needed to clarify whether dogs just coordinate their attention with humans due to attention directing gestures or whether there is a level of awareness in the act which would qualify for the more complex/sophisticated definition of 'shared attention'.

Furthermore, when it came to the choice to approach an object, dogs were not more prone to explore the object of the owners' attention, but they also did not avoid it. Other studies, involving dogs' interpretation of human gaze in an object-choice task involving food, have shown that dogs avoided the container the experimenter gazed towards ([79]; [66] ; [5]), unless the gazing cue was preceded by ostensive signals such as calling the dog's name in a positive, friendly tone of voice [30]. These results have led to the suggestion that the dogs may not perceive the humans' direct gaze as a cooperative and an attention engagement signal but rather as an intentional cue indicating the willingness of the partner to approach the object/container [30]. This interpretation is partially supported by our data. Differently from the previous study, dogs did not avoid the object of the owner's attention, rather as a group they chose at random. However, the frequency of self-directed behaviours was positively associated with the number of the owners' gaze alternations, which may partly support the idea that dogs interpret gaze or gaze alternation as a mild threat creating some discomfort. Since the ability of dogs to use human gaze (glancing and head turning) seems to be affected by learning experience ([91]; [87]), is also possible that individuals interpreted human gaze in different ways according to their individual experiences. Further studies should systematically investigate how ontogenetic factors (i.e. training, experiences with humans and conspecifics) influence dogs' spontaneous reactions to human gaze

alternations in a task not involving food.

In contrast to our predictions, we did not find an effect of exogenous oxytocin on dogs' frequency of gaze alternations, on the time dogs spent looking at the object of owners' attention and on their willingness to explore the object of attention when released. These results are in contrast to the positive effects of oxytocin found on dogs' following human communicative cues in an object choice task involving food [49, 66]. In particular, Olivia and colleagues [66] found that oxytocin decreased dogs' aversion to gazing cues: in the gaze cue condition, in which dogs had to follow human gaze in order to find a food reward, dogs avoided the bowl the experimenters' gazed at in the placebo condition but chose at chance level after the administration of oxytocin. We did not, however, find a change in dogs' willingness to approach the object the owners' gazed at following oxytocin administration. But, as highlighted above, our subjects did not avoid the object of attention in the placebo condition, suggesting that not all of them perceived the gaze as a negative cue. We also did not find an effect of oxytocin on the frequency of self-directed behaviours, offering no evidence for oxytocin's influence on dogs' social anxiety or perception of the owners' gaze.

We did find a positive association between oxytocin concentrations after administration and dogs' looking behaviours towards the owner during the test. This result is in line with different studies showing a link between oxytocin and dogs' gaze towards humans. Dogs gazed more at people after receiving intranasal oxytocin instead of placebo in different experiments: when an unfamiliar person approached in a threatening way [41], during an affiliative interaction with owners [58], and during a food communicative task [7, 31]. It is still unclear whether this effect is due to an increase in the willingness of dogs to affiliate with their owners, in line with the social effects of oxytocin [81], or to a decrease in overall stress, in line with the stress-reduction effect of oxytocin [63]. Since in our test oxytocin did not affect the frequency of self-directed behaviours our data would rather tentatively support that the increase in dogs' looking behaviours towards human faces could be due to an increase in the motivation to affiliate with social partners rather than a decrease in social anxiety.

Finally, an important methodological aspect characterized our study: most studies investigating the effects of oxytocin on dogs' behaviour [31, 49, 58, 66, 70] used the treatment (oxytocin or placebo) as predictor for their statistical analyses, assuming that the oxytocin levels of the subjects had uniformly increased after the administration of oxytocin. We measured the actual oxytocin concentrations of each subject before and after both placebo and oxytocin administrations and then used these measures to investigate their association with the behaviours exhibited. In fact, although our administration method had been previously validated and shown to be more effective than intranasal spray administration of oxytocin [74], laboratory analysis of the urine samples nevertheless showed that there was considerable variability in the assimilation of oxytocin across our subject population (see Figure 1 - Supplemental Material). Thus, an important aspect to consider is that the effect of oxytocin administration should not be taken for granted and individual measures of assimilation may be better indicators when investigating their association with behaviour.

In conclusion, in line with the behavioural synchrony results, the shared attention test results suggest that oxytocin may have a "basic" social effect increasing behaviours linked to the willingness to affiliate (such as looking towards the partner and maintaining proximity). We found no evidence for oxytocin influencing more complex behavioural phenomena involving previous learning experiences, such as behavioural synchrony, or the interpretation of social cues, such as gaze alternation (and hence shared attention).

Although the aim of the study was not to explicitly test the different mechanism through which oxytocin can influence social behaviours, our data support the theory that oxytocin may enhance individuals' willingness to affiliate with social partners [81] rather than influencing social stress or altering the perception of social cues [75].

5. Conclusion

This study adds new findings to the poorly studied phenomena of behavioural synchrony and shared attention at the interspecific level and contributes to the knowledge of how oxytocin may influence dog-owner social interactions. We applied a novel paradigm aimed at investigating shared attention between dog and owners, and although we found evidence for shared attention, we did not find that this was modulated by oxytocin. Overall, in line with previous research, we found evidence for the positive effects of oxytocin on dogs' social proximity and looking behaviours towards humans, lending some support to the role of this hormone in increasing social motivation.

Declaration of Competing Interest

The authors declare no conflict of interest.

Funding

The project was supported by funding from the Vienna Science and Technology Fund, Grant Agreement (WWTF CS15–018).

Acknowledgements

We thank dog owners and their dogs for participation in this study, the student Samantha Pot for the help in conducting the test, the research assistant Alina Gaugg for the dogs' administration training and performance, Roger Mundry for statistical advice and Karin Bayer for administrative support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2021.113451.

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