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The Role of Oxytocin in Dog-Owner socio-positive Interactions

Which are the crucial elements inducing an increase of urinary oxytocin levels in dogs and humans?

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I. Introduction

1. Background

Oxytocin (OT), a nine amino-acid neuropeptide, has remained highly conserved among vertebrate taxa throughout evolution (Anacker and Beery, 2013), whereas the distribution of the oxytocin receptor (OTR) varies between species and is probably related to species-specific social behaviours such as affiliation, communication and aggression (Insel and Young, 2000;Anacker and Beery, 2013).

OT can activate its receptor (OTR) in two different ways: centrally, through direct neural release, and also in the periphery via release from the pituitary gland (Anacker and Beery, 2013), which enables the measurement of OT levels in blood, urine or saliva. In addition to its important role in sexual behaviour, parturition and nursing (Gimpl and Fahrenholz, 2001), OT influences the amygdala, a central component of the neuro-circuitry of social cognition and fear (Kirsch et al., 2005). OT has been suggested to be a key mediator of emotional and social behaviours, such as attachment (Insel and Young, 2001), social recognition (Graustella and MacLeod, 2012) and in-group/out-group dynamics (De Dreu et al., 2010;Samuni et al., 2016), and reduces anxiety by inhibiting the stress-induced activity of the hypothalamo-pituitary-adrenal (HPA) axis (Neumann, 2002;Kirsch et al., 2005;Kumsta and Heinrichs, 2013).

OT is involved in the formation of different types of social bonds, ranging from attachment between parents and their offspring and sexual relationships between partners (Anacker and Beery, 2013;Coria-Avila et al., 2014;Crockford et al., 2014) to preferential bonds like “friendship” (Feldman, 2012). For example, a study conducted on wild chimpanzees found that urinary OT levels increased after grooming bouts, whereby this effect was mediated by the quality of the affiliative bond between partners. Regardless of genetic relatedness or sexual interest, the closer the affiliative bond the higher the OT levels following the grooming (Crockford et al., 2013).

Despite the growing number of studies investigating the role of OT in *intra*species social bonds, only a few studied its role in *inter*species bonding. Dogs are a particularly interesting species in which to investigate these potential links between OT and social

bonds, since there is growing evidence that dogs can form preferential relationships to human partners, which have been functionally equated to the mother-infant attachment bond (Topál et al., 1998;Nagasawa et al., 2009b;Handlin et al., 2011;Thielke and Udell, 2015;Buttner, 2016).

Indeed, a number of studies reported that dogs display an owner-specific secure base effect similar to that described in human children: dogs showed an increase in exploration and play behaviour (Topál et al., 1998;Prato-Previde et al., 2003) as well as exhibiting higher success rates in a problem solving task when in the presence of their owner compared to a stranger (Horn et al., 2013). Furthermore, Gácsi et al. (2013) found that dogs reacted with more confidence and showed less signs of stress in a threatening situation when their owner was present, suggesting that the owner functions as a "safe haven" for the dog. Taken together, results indicate that a dog's relationship with its owner is a preferential one, and that similarly to parents with their infants, owners can be a buffer when their dogs are exposed to stressful situations.

Also from the human perspective, a number of similarities to the mother-infant bond have emerged in the relationship between owners and their dogs. Mitsui et al. (2011) conducted functional magnetic resonance imaging studies showing that the presentation of both human and canine family members' faces activated the same brain region. Interestingly, these brain areas are known to interact with the OT system. Furthermore, a more recent study (Stoeckel et al., 2014) also confirmed the activation of a common network of brain regions involved in emotion, reward, affiliation and social cognition both when mothers viewed images of their child and their dog.

These findings raise the question of the mechanisms that enable the formation of such strong preferential relationships between dogs and humans; several studies suggest that OT may play a crucial role (Odendaal and Meintjes, 2003;Nagasawa et al., 2009a;Handlin et al., 2011;Rehn et al., 2014;Romero et al., 2014;Nagasawa et al., 2015).
Nagasawa et al. (2009a) and Nagasawa et al. (2015) measured OT levels in both dogs and owners following social interactions between them. Dogs were clustered in two groups depending on the time they spent looking at their owners (LG - long gaze, or SG - short gaze clusters), and it was observed that following the social interaction with their dog, owners of LG dogs showed an increase in urinary OT concentration compared to

pre-treatment levels. This in turn reinforced owners' affiliative interaction with the dogs (i.e. duration of stroking and talking), which also resulted in an increase in OT levels in dogs compared to pre-treatment levels. The authors conclude that their results support the existence of an OT-mediated positive loop facilitating dog-owner bonding. From the human perspective, several other studies have shown that the plasma OT levels of owners increase following positive social interactions with their dogs (stroking, playing and talking) (Odendaal and Meintjes, 2003;Miller et al., 2009;Handlin et al., 2011), further supporting the potential involvement of OT in the maintenance of the dog-owner bond.

However, more recently a number of authors have criticised the oversimplified view of OT's role in social relationships in general (Grebe, 2016) and in the dog-human relationship in particular (Buttner, 2016). Indeed, findings from the dogs' perspective are inconsistent, with dogs' endogenous OT levels being shown to increase following social interactions with their owner in some studies (Handlin et al., 2011;Nagasawa et al., 2015), but not in others (Romero et al., 2014), despite the same analysis methods being used. Furthermore, in some studies dogs' OT levels increased also after being stroked by a familiar person (Mitsui et al., 2011), calling into question the uniqueness of the dog-owner bond. Additionally, it is not clear which aspects of the dog-human social interaction elicit increased OT levels in dogs. In line with the importance of tactile stimulation for OT levels (see Uvnäs-Moberg et al. (2015) for a review on the importance of sensory stimulation for OT production), stroking has been shown to elicit OT in some studies (Odendaal and Meintjes, 2003;Mitsui et al., 2011;Rehn et al., 2014) but mutual gazing appears to be sufficient in others (Nagasawa et al., 2009a;Nagasawa et al., 2015). Similarly, from the human side, it is not clear whether it is just the sensory stimulation of stroking a dog which may trigger OT release (Uvnäs-Moberg et al., 2015) or whether it is in fact the social interaction, and more specifically the social interaction with ones' own bonded dog, which plays the central role. This would be in line with the hypothesis that OT may be a biomarker of the quality of a relationship (Crockford et al., 2013). Indeed, to date, no study has actually compared whether the production of OT in humans and dogs differs following the positive social interaction with their bonded companion in comparison to interacting with a familiar dog/person.

2. Hypotheses and Predictions

Hence in the current study, I aimed to examine a number of the hypotheses suggested so far in the literature: I tested the "relationship quality hypothesis" by comparing the increase in endogenous OT levels (measured in urine) in both dog and owner following a positive social interaction involving stroking, talking and mutual eye contact (cuddle conditions) with either their bonded partner or a familiar non-bonded individual. Furthermore, to evaluate whether dogs' OT levels would in fact increase more as a result of a social interaction than in its absence („social interaction hypothesis"), the cuddle condition with the owner/ familiar person was compared to an equivalent condition in which the owner/familiar person was cuddling a furry toy dog (fake dog conditions) and ignoring the "real" dog. This condition also allowed us to investigate if the act of stroking itself, regardless of the dogs' identity (own vs. familiar dog), or whether it is real or fake (fake vs. own/familiar dog) leads to changes in OT levels in humans. Additionally, to test for the possibility that in dogs OT levels are simply linked to the aspect of tactile stimulation and not to the social interaction with the person („tactile stimulation hypothesis"), I also conducted a condition where dogs were "stroked" by an artificial hand without social interaction (i.e. talking and gazing) by the human (mechanical cuddle condition). For each condition, urinary OT levels in both dogs and humans were measured before and after the respective interaction and the differences between pre- and post-treatment levels were considered (see **Tab. 1** for an overview on the predicted results regarding the different hypotheses).

If the *"relationship quality hypothesis"* is correct, i.e. in both dogs and humans, changes in OT levels during an affiliative interaction are mediated by relationship quality, then for both dogs and humans I expect a greater increase in OT levels from pre- to post-treatment after interacting with their bonded companion (the owner/own dog) compared to after interaction with the familiar and thus not bonded dog/person.
Handlin et al. (2012) found a positive correlation between attachment, measured by a questionnaire, and plasma OT levels in owners as well as in their dogs. Due to this finding, I also asked the participants to answer an attachment questionnaire and predict to find a higher increase from pre- to post-treatment OT levels in owners with a higher score and their dogs, respectively.

If the *"social interaction hypothesis"* is correct, i.e. affiliative interactions involving stroking, gazing and talking lead to increased OT levels in both dogs and humans, then in both dogs and humans, I should observe a greater rise in OT levels from before to after treatment in conditions in which a human (owner/familiar person) interacted in a friendly way by cuddling the dog (own/familiar dog) compared to non-social control conditions with the owner/familiar person being present but ignoring the dog (fake dog conditions).

Additionally, I collected HR data, since previous studies showed that interaction with their dogs was followed by a decrease in owners' HR (Handlin et al., 2011), whereby it was suggested that decreased HR after human-dog interactions might be facilitated by the OT system (Beetz et al., 2012). I should thus find a correlating effect between a possible increase of OT levels and decreased HR after the treatment, as well as a higher decrease in HR after cuddling a real in comparison to a fake dog.

Finally, if instead tactile stimulation *per se* affects OT levels and social interaction is not a crucial element („*tactile stimulation hypothesis*"), I would predict no significant difference in the change of dogs' and humans' OT levels when cuddling with a bonded or non-bonded partner vs. the respective non-social control conditions; namely the mechanical cuddle condition for the dogs and the fake dog condition for the humans.

Tab. 1: *Overview on the treatment conditions and on the expected results regarding the different hypotheses (HP). ▲ indicates a higher increase in OT levels than ∆; the symbol on the left side of the slash refers to humans and the one on the right side to dogs.*

category	condition	relationship quality HP	social interaction HP	tactile stimulation HP	human sample	dog sample
cuddle conditions	Owner cuddle (OC)	▲/▲	▲/▲	▲/▲	✓	✓
	Familiar person cuddle (FC)	-/∆	-/▲	-/▲	-	✓
	Owner cuddle familiar dog (OF)	∆/-	▲/-	▲/-	✓	-
non-social control conditions	Owner fake dog (OC-)	-/-	∆/-	▲/-	✓	✓
	Familiar person fake dog (FC-)	-/-	-/-	-/-	-	✓
	Mechanical cuddle (MC)	-/-	-/-	-/▲	✓	✓

II. Material and Methods

1. Study Design

In the present study, I tested n = 14 dog-owner dyads (**Tab. 2**). Numbers of female and male dogs were balanced. Since previous studies showed that OT in female participants tended to increase more than in male participants (Miller et al., 2009;Kekecs et al., 2016), I included only female dog owners, who were healthy and neither pregnant nor lactating. Participating dogs had to fulfil the precondition of being used to spending time at the Clever Dog Lab (CDL), and having a comparable relation with the experimenter, who acted as the familiar person in the experiment.

Tab. 2: *Subjects, and relevant information.*

individual	sex	castration	age	breed	owner
Ch	female	yes	8 years 3 months	Australian Shepherd	JS
Cl	female	yes	2 years 4 months	Mongrel	DB
Gu	female	no	11 years 7 months	Border Collie	FR
Hy	female	yes	8 years 3 months	Mongrel	JE
Lo	female	no	3 years 9 months	Mongrel	DB
Ti	female	yes	9 years 8 months	Mongrel	SM
Tu	female	yes	2 years 7 months	Mongrel	JE
Fr	male	yes	2 years 8 months	Mongrel	GC
Li	male	no	2 years 3 months	Australian Shepherd	JS
Ki	male	yes	6 years 9 months	Mongrel	RD
Ma	male	no	11 years 10 months	Golden Retriever	SM
Mo	male	yes	3 years 6 months	Mongrel	AM
Ol	male	yes	10 years 5 months	Mongrel	KG
Sc	male	yes	2 years	Mongrel	SK

I used a within-subject design and conditions were conducted in a randomised and counterbalanced order across subjects. Between conditions there was a gap of at least three days for both dogs and humans.

2. Experimental Setup

All experiments were conducted using the outdoor area of the University of Veterinary Medicine Vienna and an indoor room at the CDL. The outdoor area was used to collect the urine samples of the dogs in the conditions with the dogs being involved (Owner cuddle - OC, Familiar person cuddle - FC, Owner fake dog - OC-, Familiar person fake dog - FC-, Mechanical cuddle - MC); one before (pre-treatment – D1) and one after each session (post treatment – D2).

The bathroom in the CDL was used to collect the human samples in the conditions where the owner was involved (Owner cuddle - OC, Owner fake dog - OC-, Owner cuddle familiar dog - OF, Mechanical cuddle - MC). Pre-treatment urine samples (H1) were collected as soon as the owners entered the CDL and post-treatment samples (H2) before going outside again for collecting the post treatment samples of the dogs.

All urine samples were stored in a -20 °C freezer and sent, on dry-ice, to the Max Planck Institute for Evolutionary Anthropology, Department of Primatology (MPI EVA) in Leipzig, Germany, for analysis.

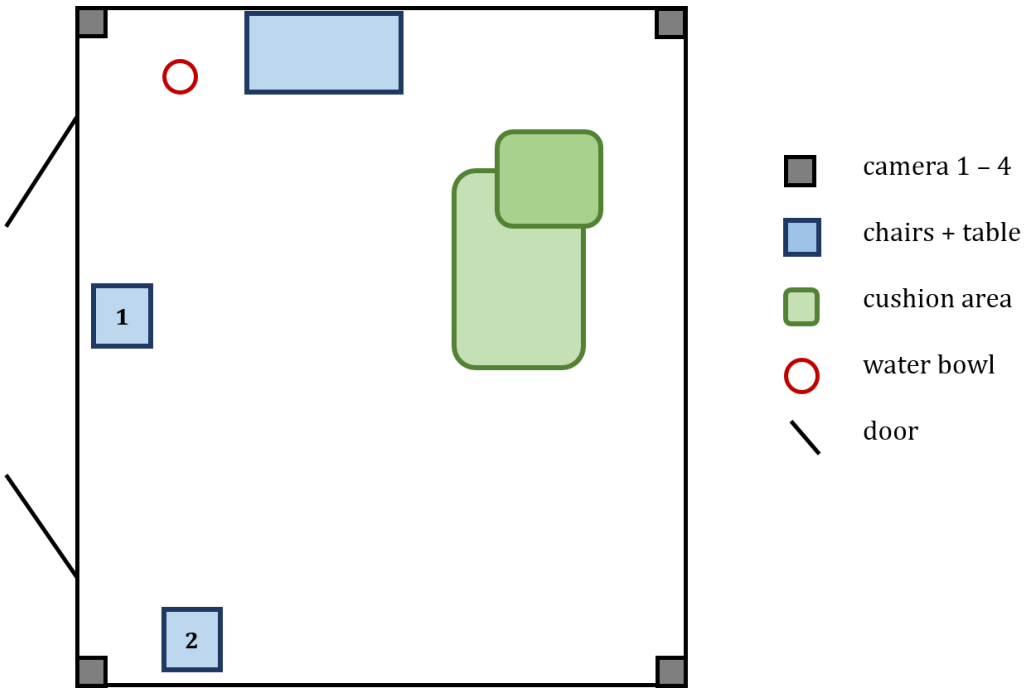

Fig. 2: *Setup of the indoor testing room.*

The indoor room was equipped with two chairs, a table, blankets and cushions on the ground and a water bowl for the dog (**Fig. 2**). Chair 1 was occupied by the owner during the initial 5 minutes of each session, whereas after treatment the owner sat at the desk and worked on her computer. During the whole experiment, the experimenter was present in the room, sitting quietly on chair 2 and interacting neither with the dog nor the owner. In the familiar person condition the positions were reversed. There was an alarm activated by the experimenter to indicate the end of each time phase (**Fig. 3**).

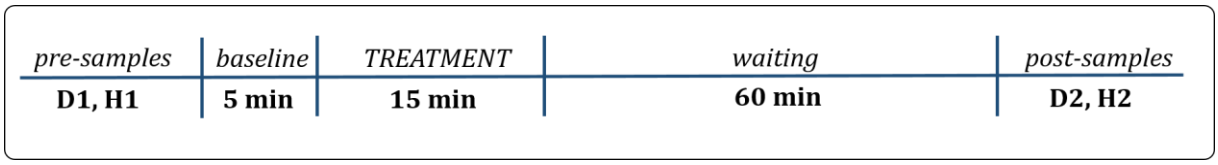

Fig. 3: *Chronology of the experimental phases.*

The familiar person (AM) was known to the dog, but neither lived in the same household nor carried out any activity with it and prior to testing, contact between them was restricted to sporadic interactions no more than approximately 10 minutes per week when meeting in the offices of the university.

The same restrictions applied to the familiar dogs used in the tests. The owners knew the dog, but neither lived nor spent much time with it and prior to testing had only had infrequent contact with it when meeting in the university offices. Thus, the dog was a familiar but non-bonded individual to the owner. The familiar dog was of the same sex as the owner's dog.

3. Pre-testing instructions to owner and pre-sample collection

Preceding the experiment, all dogs and owners were asked to not participate in any social interactions (cuddling, playing), exercise or feeding activities at least 90 minutes before the experiment started, since this could influence the pre-treatment OT level. Owners and their dogs were met infront of the CDL and accompanied to the outdoor area where the D1 urine sample was collected. Afterwards, the experimenter guided the dog and the owner to the CDL indoor area and collected the H1 urine sample. Following

the sample treatment (see 5. Urine Sampling Collection and Treatment) and storing of the samples, the session started.

4. Treatment Procedure

The test consisted of three phases: 1) 5 minutes "baseline", 2) 15 minutes "treatment", followed by 3) a 1 hour "waiting" phase (**Fig. 3**). In all conditions, the baseline phase consisted of a period in which the dog could freely explore the room, whilst the owner/familiar person and experimenter quietly sat in the chairs. Following this, either the owner or the familiar person moved to the cushion area and the treatment phase started. The exact treatment depended on the conditions (**Tab. 1**); each dog-owner dyad participated in each of the six conditions.

Owner cuddle (OC):
Whilst the experimenter remained sitting on the chair reading a book or working on the computer and ignoring both owner and dog, the owner moved to sit comfortably on the cushions. She was allowed to call the dog over to the cushion area and start an interaction in a positive, calm way: gentle stroking or massaging, talking to the dog, looking into the dog's eyes etc. The owner was instructed to have a cuddle session as natural for them as possible, allowing the dog to relax. If the dog moved away, the owner was allowed to gently call it back a maximum of three times. If the dog did not return, the owner did not force it to do so. The owner remained sitting in the cushion area for 15 minutes and spent as much of that time as possible gently stroking the dog, but never forcing the dog to remain there against its will.

Owner fake dog (OC-):
Whilst the experimenter remained sitting in the chair, the owner sat comfortably in the cushion area but in this condition, she stroked a furry toy and did not interact with her dog in any way - no talking, touching or looking at the dog was involved. Instead, the owner was asked to interact with the furry toy as similar as possible to the way of interacting with her dog. If the dog tried to initiate an interaction, the owner just continued stroking the furry toy.

The 5 minutes of exploration before the actual start of the test were used to allow the dogs to get familiar with the toy dog and with the owner holding and stroking it. This was to avoid a surprise effect in the dogs when in the test, the owner starts stroking the furry toy and talking to it. This condition allowed to assess whether tactile stimulation is sufficient to increase OT levels in the humans or if social interaction is a crucial element.

Familiar person cuddle (FC):
Analogous to OC, but whilst the owner remained seated it was the familiar person that got up and sat on the cushions and initiated the cuddle session. The type of gentle interaction was as similar as possible to that of the owner. Hence prior to testing the familiar person asked the owner where and how the dog likes to be stroked.

Familiar person fake dog (FC-):
Analogous to OC-, but carried out with the familiar person instead of the owner.

Owner cuddle familiar dog (OF):
Analogous to OC, but carried out with a familiar dog instead of the owner's dog.

Mechanical cuddle (MC):
An artificial hand was used to "stroke" the dog and provided mechanical stimulation while the social aspect of an interaction with the human was missing. The owner was holding and using the mechanical hand, but she wore sun-glasses to avoid eye-contact, did not talk to the dog and spent most of the time facing away from the dog whilst using the mechanical hand. This condition allowed assessing whether tactile stimulation is sufficient to increase OT levels in the dogs or if social interaction is a crucial element.

In all conditions, in the final waiting phase, the owner/familiar person returned to the chair after treatment and read a book or worked on the computer and no longer payed attention to the dog. After this period, the H2 and D2 urine samples were collected.

5. Urine Sample Collection and Treatment

Dog urine samples were collected using a closable plastic cup (Carl Roth, CEN 7.1) attached to a stick. Human participants were asked to use the bathroom to provide their urine samples in the same kind of plastic cup.
Prior to sampling, Eppendorf vials had been prepared with 100 µl of 0.5 N phosphoric acid. 1 ml of urine was transferred into one of these vials, manually shaken for several seconds and labelled with a unique code, ID of the subject, date and time. Aliquots were created, if the experimenter managed to collect extensive amounts of urine. Additionally, at least 0.1 ml of urine (1 ml, if possible) was transferred into an empty Eppendorf vial for creatinine measurement. The vials were stored in a -20 °C freezer.

6. Heart Rate Data

In the conditions performed by the owner, heart rate (HR) data was collected. A heart rate belt was strapped on the participants. Once a clear signal could be measured, recording was initiated, starting with the 5-minute baseline and continuing throughout the treatment (15 min) and the waiting hour after trial time.
The data was recorded and processed using Polar Pro Trainer 5, which represents the heart rate with two values per second. To correct for outliers, I made use of the software's error correction with moderate filter power. For analysis, I calculated the mean score for the time intervals of each the baseline, the first (test1), middle (test2) and last (test3) 5 minutes of the treatment and the beginning (post) and the end (post_1hr) of the waiting hour. This resulted in six comparable values for each dog-owner dyad.
Due to body movement and vocalisation, humans' HR was found to increase during petting and talking to a dog (Vormbrock and Grossberg, 1988), whereas Handlin et al. (2011) found a significant decrease in owners' HR from the start compared to 55 and 60 minutes after the interaction with their dogs. Since a decrease in HR after human-dog interactions is assumed to be mediated by the activation of the OT system (Beetz et al., 2012), I expected a correlation between an increase of OT levels and a decrease in HR after the treatment.

7. Attachment Questionnaire

The owners were asked to answer an attachment questionnaire before starting the first test session. I used the Lexington Attachment to Pets Scale (LAPS), since it is one of the most validated questionnaires regarding the owners' attachment to their pets (Johnson et al., 1992). Calculating the attachment score should additionally help to illuminate the possible influence of relationship quality on the change in OT levels.

8. Behaviour Coding

For behaviour analysis, the sessions were video-recorded with four cameras located in the corners of the testing room. Dog and human behaviour from baseline and treatment (in total 20 minutes per test session) were coded using the Solomon Coder (András Péter, www.solomoncoder.com).

For the dogs, I coded their posture (lying, lying belly up. sitting, standing, pacing/moving around and exploration), the proximity towards the cuddler (varying from body contact to more than two body lengths), the direction of their gaze (looking to the cuddler or to the experimenter), stress signals (yawning, lips-licking, scratching, whining and barking) and behaviours towards the cuddler (licking and play behaviour).

For the humans, stroking, gazing at the dog, talking to the dog and to the experimenter and calling the dog were coded.

Regarding both humans and dogs, I calculated the occurrence of "mutual eye contact" (owner and dog looking into each other's faces – "gazing at dog" and "looking to cuddler" appearing simultaneously). Additional to the particular behaviours, "duration of interaction" was coded for any form of interaction between cuddler and dog, which was initiated by both interaction partners – i.e. behaviours such as stroking, mutual gaze, licking and body contact initiated by both dog and cuddler were combined here.

9. Laboratory Tests

Analysis of the urine samples for OT levels was conducted in the Endocrinology laboratory at the MPI EVA in Leipzig, Germany. The analyser was blind regarding the conditions the samples belonged to. The methodology for the analysis of urinary OT levels in both dogs and humans was validated beforehand (Schaebs et al., in prep.-a;Schaebs et al., in prep.-b). Urine samples were extracted following Crockford et al. (2013). In brief, after thawing the samples at 4 °C, they were gently vortexed for 10 seconds and centrifuged for 1 minute at 1500 rpm. For all dog samples, 250 µl were mixed with 750 µl of H_2O with 0.1% trifluoracetic acid (TFA) and for humans 1 ml of urine was mixed with 1 ml 0.1% TFA. Following conditioning of the solid phase extraction (SPE) cartridges (Chromabond HR-X, 1 ml, 30 mg) with 1 ml methanol (HPLC grade, 100%) and 1 ml water (HPLC grade), urine samples were loaded on the cartridge and washed with 5 ml 10% (vol/vol) acetonitrile (ACN) containing 1% TFA in water. Samples were eluted with 1 ml 80% (vol/vol) ACN. Extracts were dried down at 50 °C using a gentle stream of compressed air, reconstituted in 300 µl ethanol (100%) and incubated for 1hour at 4 °C after gently vortexing for 10 seconds. Finally, all dried extracts were reconstituted in 250 µl of OT assay buffer (which was supplied with the commercial assay kit), leading to dog urine samples being measured at 1:1 and human urine samples at 4:1 concentration. Reconstituted samples were gently vortexed for 10 seconds, centrifuged for 1 minute at 10000 rpm and measured in duplicates following the instructions given by the assay supplier (Assay designs, Cat. No 901-153A-0001). To compensate for the variation in the volume and concentration of the voided urine, we measured creatinine concentrations in each urine sample and expressed all oxytocin values as pg/mg creatinine. In total 10% of the measurements were outside of the linear range of the assay, but were still kept in the study as it was found that re-measuring urine samples at higher concentrations leads to an underestimation of OT levels (see Schaebs et al., in prep. for further details). Inter-assay coefficients of variation were 11.7% for a high and 9.1% for a low quality control (N=12). Intra-assay coefficient of variation was 10.9% (N=29; following guidelines by Salimetrics). See Schaebs et al. (in prep.-a) for a detailed analytical validation of OT measurements in urine samples of dogs and Schaebs et al. (in prep.-b) for humans.

10. Statistical Analysis

Humans:

To assess the effect of human-dog interactions on human urinary oxytocin levels we fitted a general linear mixed model (Baayen, 2008) with urinary oxytocin levels (log-transformed) as response variable. We included condition (Owner cuddle, Owner cuddle familiar dog, Owner fake dog and Mechanical cuddle) and pre-/post-treatment urine collection as test variables (Mundry, 2014) with fixed effects. To assess whether changes in urinary oxytocin levels were dependent on the condition, we also included a two-way interaction between the respective two main effects. Duration of interaction was included as control variables (Mundry, 2014) with fixed effects and subject and partner ID included as random effects. To keep the type I error rate at the nominal level of 5% (Schielzeth and Forstmeier, 2009;Barr et al., 2013) we included random slopes of all fixed effects within subject and partner ID.

Dogs:

To assess the effect of human-dog interactions on dog urinary oxytocin levels we fitted two general linear mixed models (Baayen, 2008) with urinary oxytocin levels (log-transformed) as response variable. As for two (Owner fake dog and Familiar person fake dog) of the five conditions, there was no interaction between the human and the dog (the owner and familiar person were interacting with the fake dog and not the real one), we fitted one model including the complete data set, but lacking duration of interaction as test variable. A second model comprised only the three remaining conditions (Owner cuddle, Familiar person cuddle, and Mechanical cuddle) including also duration of interaction as test variable.

For the first model (dog model I), based on the complete dataset, we included condition (Owner cuddle, Familiar person cuddle, Owner fake dog, Familiar person fake dog and Mechanical cuddle) and pre-/post-treatment urine collection as test variables (Mundry, 2014) with fixed effects. To investigate whether changes in OT levels from pre- to post-treatment were different across conditions, we also included a two-way interaction between the respective main effects. Sex of the dog and frequency of stress signals were included as control variables (Mundry, 2014) with fixed effects and subject ID as well as partner ID were included as random effects. Random slopes were included for condition,

pre-/post-treatment and daytime within subject and partner ID and for frequency of stress signals within partner ID.
For the second model (dog model II), based on a subset of the dataset only including the conditions Owner cuddle, Familiar person cuddle and Mechanical cuddle, we included duration of interaction, condition and pre-/post-treatment urine collection as test variables (Mundry, 2014) with fixed effects. To test for a differential effect of the conditions in relation to the duration of interaction on changes in OT levels, we included a three-way interaction between the respective main effects. We included sex and frequency of stress signals as control variables (Mundry, 2014) with fixed effects and subject ID as well as partner ID as random effects. We included random slopes for condition, pre-/post-treatment urine collection and duration of interaction within subject ID and partner ID.

For all dog and human models, we z-transformed the following fixed effects to a mean of zero and a standard deviation of one (Schielzeth, 2010): duration of interaction and frequency of stress signals. To allow for their inclusion as random slopes, duration of interaction and pre-/post-treatment urine collection were centered to a mean of zero. By visual inspection of a qqplot and residuals plotted against fitted values, we checked for the assumptions of normally distributed and homogeneous residuals. Model stability was assessed by excluding levels of the random effects one by one and comparing the estimates of the model derived for these fixed effects with the ones obtained for the full dataset. We checked for collinearity using the Variance Inflation Factor (Field, 2009) using the function vif of the R package car (Fox et al., 2016) by fitting a standard linear model excluding random effects and interactions. Collinearity revealed to not be an issue for any of our models (maximum VIF human model: 1.34; dog model I: 1.22; dog model II: 1.34). We fitted all models using the function lmer of the lme4 R package (Bates et al., 2014) using Maximum Likelihood. Likelihood ratio tests were used to establish the significance of the full model when compared to the null model (Dobson and Barnett, 2008;Forstmeier and Schielzeth, 2011); all control factors and the random effects were kept in the respective null models and likelihood ratio tests were used to assess significance of the individual effects (Barr et al., 2013). In case the full-null model comparison revealed to be significant but interactions included in the full model revealed to be non-significant, such interactions were removed to enable easier

interpretation of the main effects (Schielzeth, 2010). All models were fitted using R (R Core Team (2017), version 3.3.3., https://www.R-project.org/).

III. Results

1. Urinary OT levels

Humans:

OT levels increased from a mean of 29.31 pg/mg creatinine (range from 5.78 to 95.06 pg/mg creatinine) in pre-treatment to 42.93 pg/mg creatinine (range from 2.99 to 180.55 pg/mg creatinine) in post-treatment samples.

Increases less than 10% were considered as normal variation. In comparison to dogs, increases in human post-treatment OT levels were generally more pronounced (Owner cuddle condition (N=10) - mean: 156 %; range: 10 % - 346.9 %; Familiar person cuddle condition (N=7) – mean: 206.1 %; range: 17.3 % - 523.1 %; Mechanical cuddle condition (N=8) – mean: 88.9 %; range: 16.6 % - 236.8 %; Owner fake dog condition (N=10) – mean: 77.5 %; range: 15.05 % -271.4 %).

Results showed no treatment by condition interaction (χ^2=1.855, df=3, P=0.603; hence, the interaction between the two respective terms was removed to enable interpretation of the main effects), nor an effect of condition; however a significant main effect of treatment emerged (comparison of the full to the null model showed a clear tendency towards significance: χ^2=13.36, df=7, P=0.064; significant main effect of treatment: χ^2=6.635, df=1, P=0.01), with OT being higher post- compared to pre-treatment regardless of condition. The final model, including both fixed and random effects explained 0.77 of the variance.

Summed up, regardless of condition, there was an increase from pre- to post-treatment human OT levels.

Dogs:

OT levels varied from a mean of 72.73 pg/mg creatinine (range from 23.02 to 218.18 pg/mg creatinine) in pre-treatment to 77.20 pg/mg creatinine (range from 5.27 to 266.77 pg/mg creatinine) in post treatment samples.

A rise of over 10% from pre- to post-treatment OT levels was considered as not being explained by natural variation and thus declared as increase. Whilst in the Owner cuddle condition 7 out of 14 individuals showed an appropriate increase in OT levels from pre- to post-treatment (mean: 52.3%, range: 10.2% – 107.4%), only 4 dogs had higher post-

treatment OT levels in the Familiar person cuddle condition (mean: 61.3%; range: 36.4% – 82.6%). Also in the Owner fake dog condition, 7 dogs showed an increase in OT levels (mean: 67.04%; range: 23.1% – 198.7%). In the Mechanical cuddle condition, 6 dogs showed higher OT levels post-treatment (mean: 44.5%; range: 12.3% – 110.7%).
Stress signals measured by frequency (yawning and lips licking) occurred in average 3.36 times per 15 minutes treatment (range from 0 to 16 times).

Results based on the complete dataset (dog model I) showed no interaction or main effect of condition, treatment or frequency of stress signals on the dogs' OT levels (full model not significantly different to the null model; χ^2=4.28, df=9, P=0.892). Similarly, results of the dog model II, considering only conditions in which an interaction between a human and the dog occurred (Owner cuddle, Familiar person cuddle and Mechanical cuddle), showed no effect of treatment, condition or the duration of interaction (full model not significantly different to the null model; χ^2=9.29, df=11, P=0.595).
Overall, there was no significant difference from pre- to post-treatment OT values in dogs, regardless of condition.

Humans and dogs – conditions compared:
The following plots (**Fig. 4 – Fig. 8**) depict the difference from pre- to post-treatment urinary OT levels; humans (a), on the left side) compared to dogs (b) on the right side) for each condition. Boxplots indicate median and interquartile range.

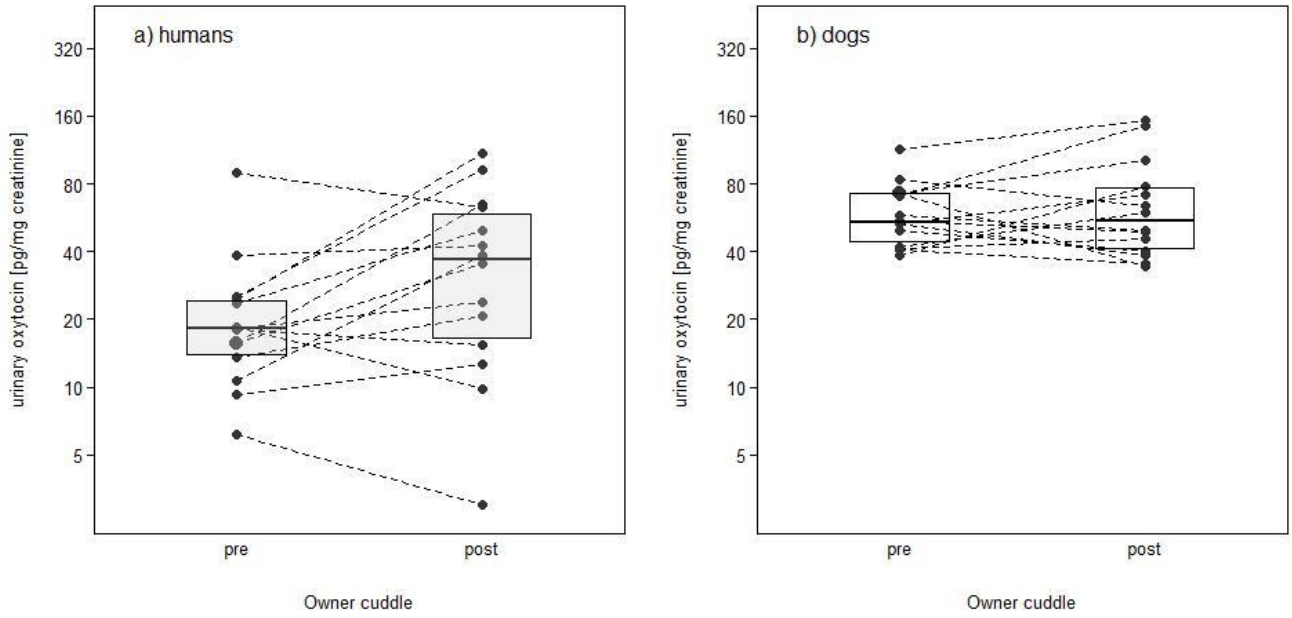

Fig. 4: *Pre- and post-treatment OT levels for the condition with the bonded cuddle partner - "Owner cuddle" (OC).*

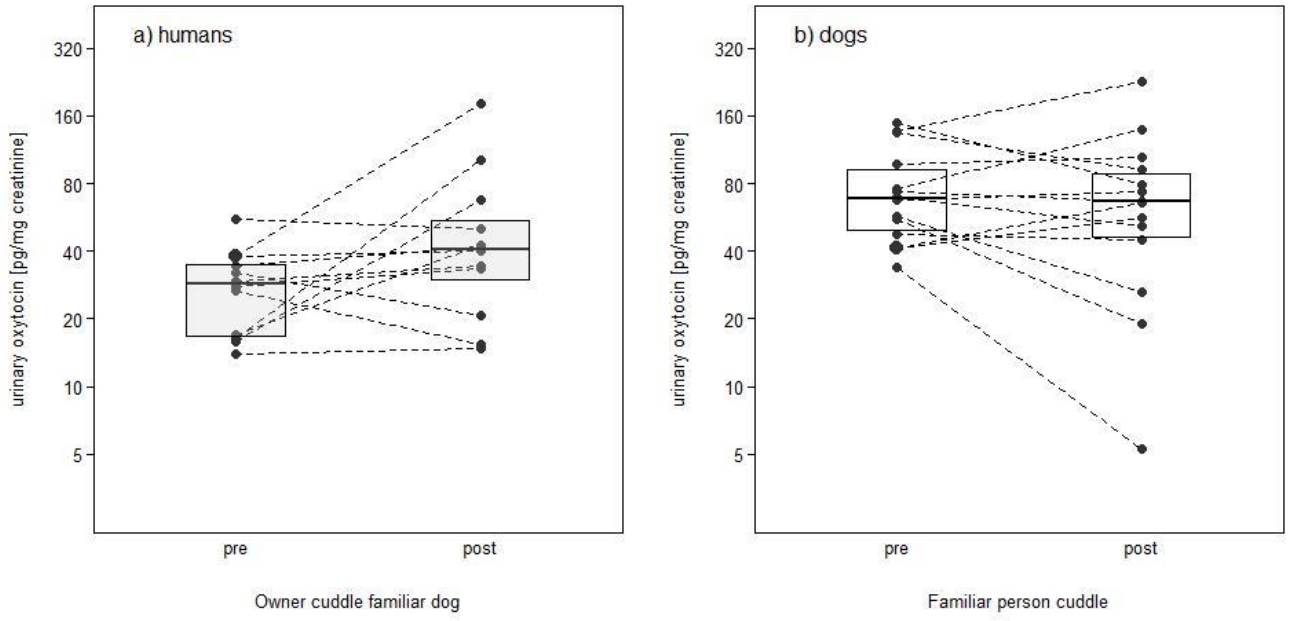

Fig. 5: *Pre- and post-treatment OT levels for the conditions with the familiar cuddle partner – "Owner cuddle familiar dog" (OF) and "Familiar person cuddle" (FC).*

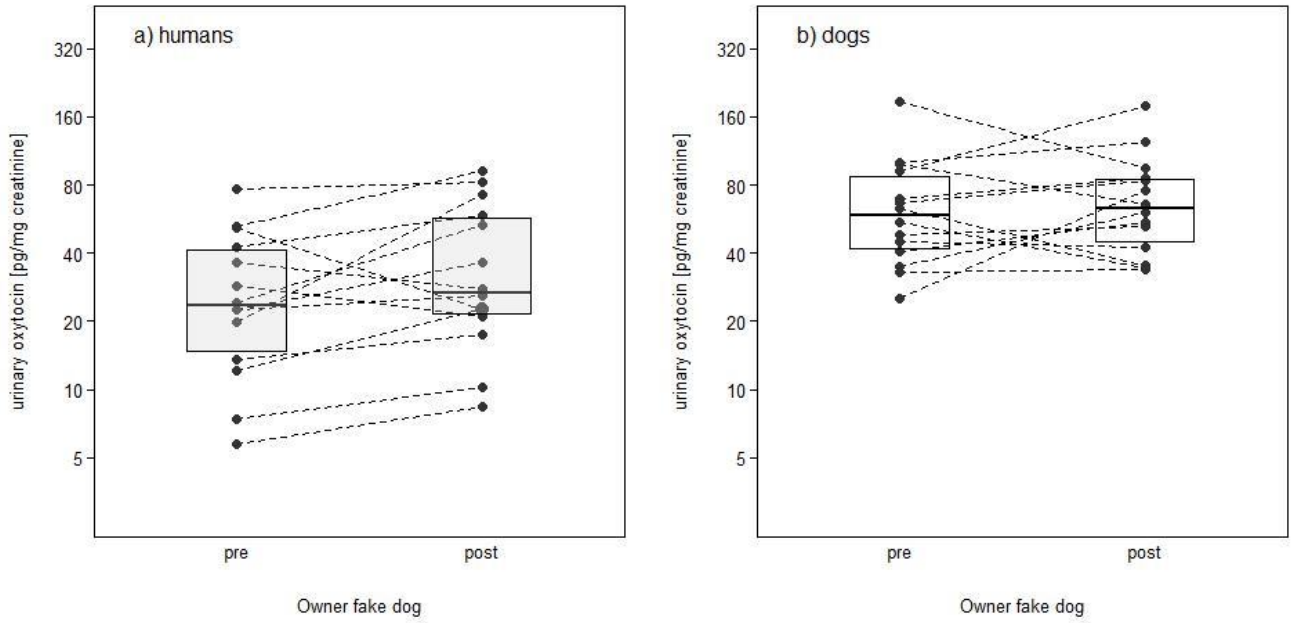

Fig. 6: *Pre- and post-treatment OT levels for the non-social control condition with the owner cuddling a fake dog – "Owner fake dog" (OC-).*

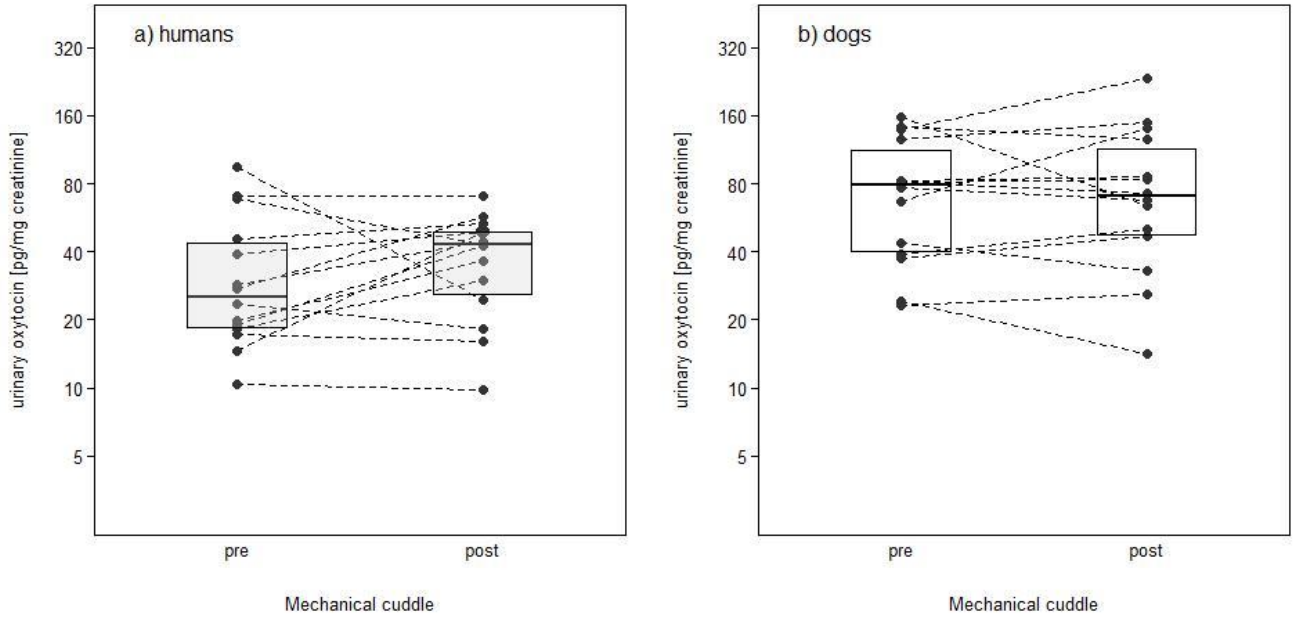

Fig. 7: *Pre- and post-treatment OT levels for the non-social control condition reduced on tactile stimulation – "Mechanical cuddle" (MC).*

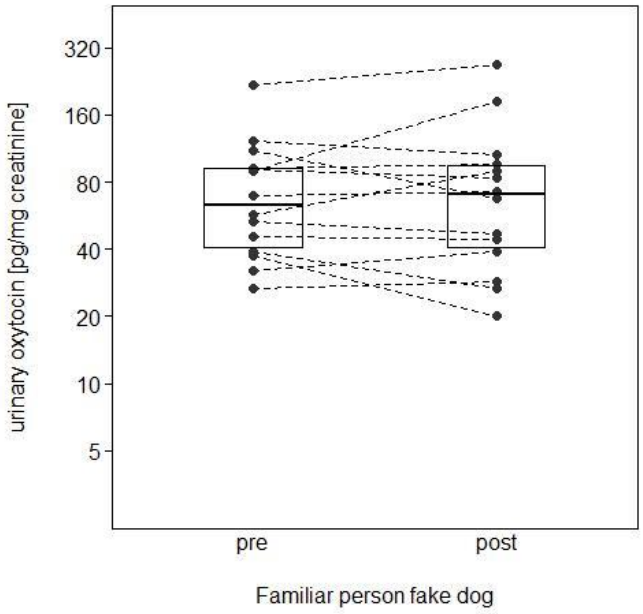

Fig. 8: *Dogs' pre- and post-treatment OT levels for the non-social control condition with the familiar cuddle partner – "Familiar person fake dog" (FC-).*

2. Heart Rate Data

Corrected HR data was pooled to six comparable mean values representing 5 minutes each of the baseline, the treatment (test1, test2, test3) and the beginning (post) and end (post_1hr) of the waiting hour. For each condition, the graphs (**Fig. 9 – Fig. 12**) show the mean values of all participants summed up for the respective points in time. Boxplots indicate median, interquartile range and percentiles (2.5% and 97.5%). General visual inspection as well as individual plotting per participant did not reveal any patterns of correlation with pre- and post-treatment OT levels or condition. Due to the extensive individual variation, HR data was excluded from the statistical model.

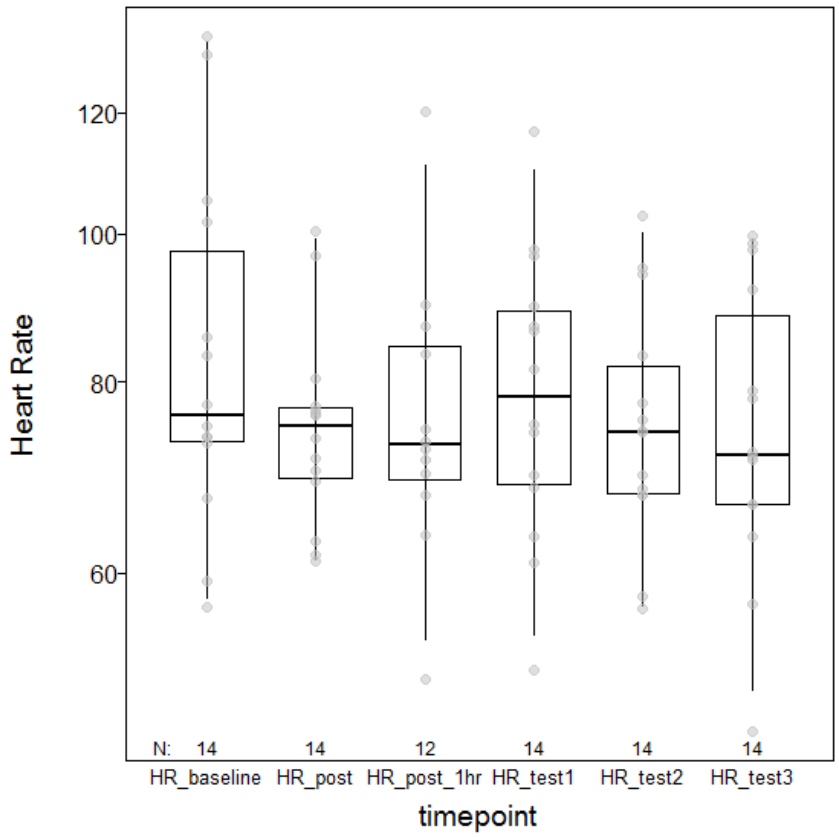

Fig. 9: *Mean HR for the defined points in time for the condition "Owner cuddle" (OC).*

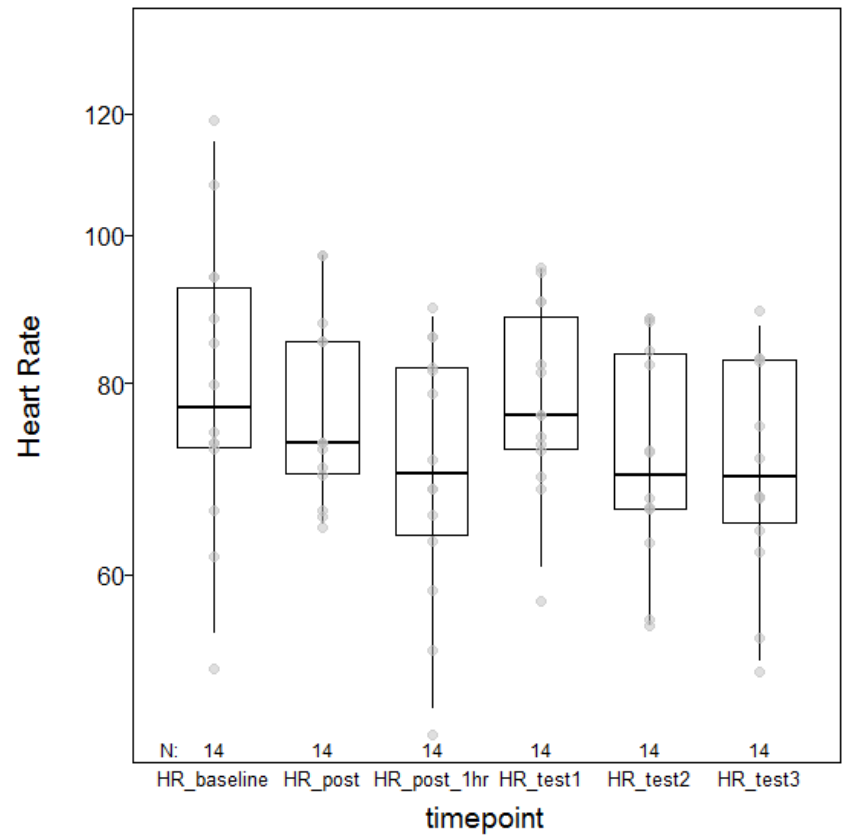

Fig. 10: *Mean HR for the defined points in time for the condition "Owner cuddle familiar dog" (OF).*

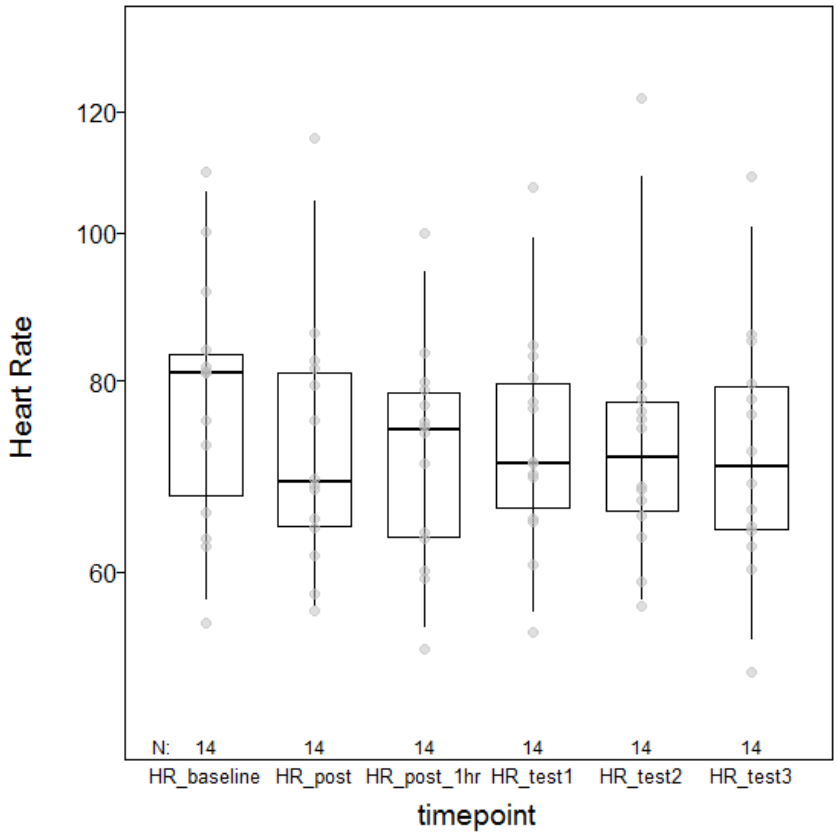

Fig. 11: *Mean HR for the defined points in time for the condition "Owner fake dog" (OC-).*

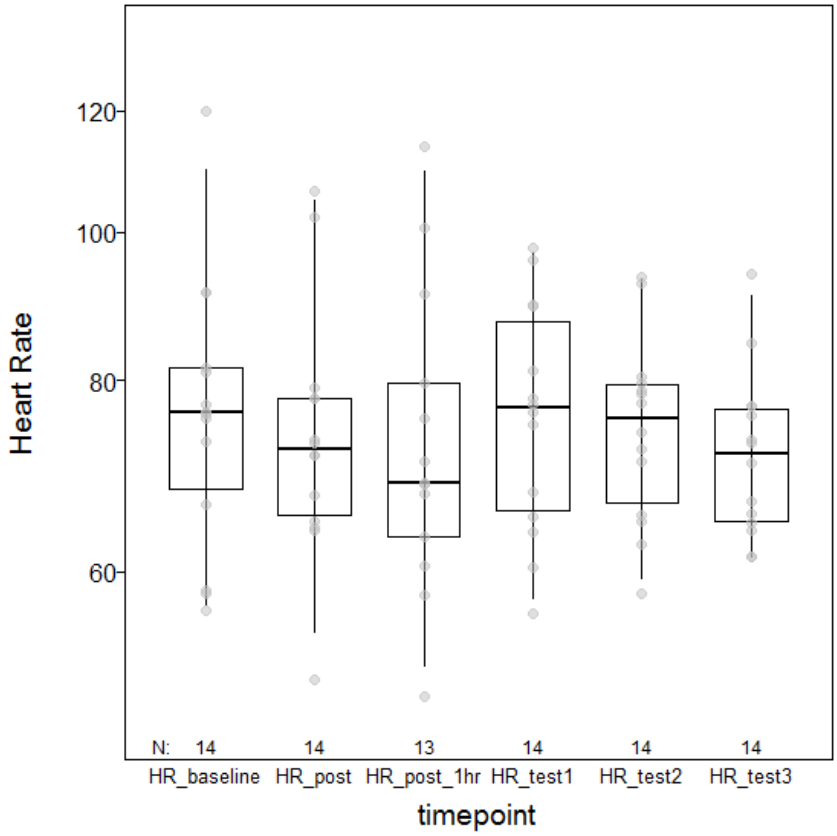

Fig. 12: *Mean HR for the defined points in time for the condition "Mechanical cuddle" (MC).*

3. Attachment Questionnaire

According to the Lexington Attachment to Pets Scale (LAPS), owners were rated with an attachment score between 1 („not at all attached“) and 5 („very attached“). The score varied around a mean of 4.01 (range from 3.00 to 4.52). Neither for the owners (R=0.23; see **Fig. 13**) nor for the dogs (R=0.4) a correlation between the owners' attachment score and the change in the respective change from pre- to post-treatment OT levels in the Owner cuddle condition was detected.

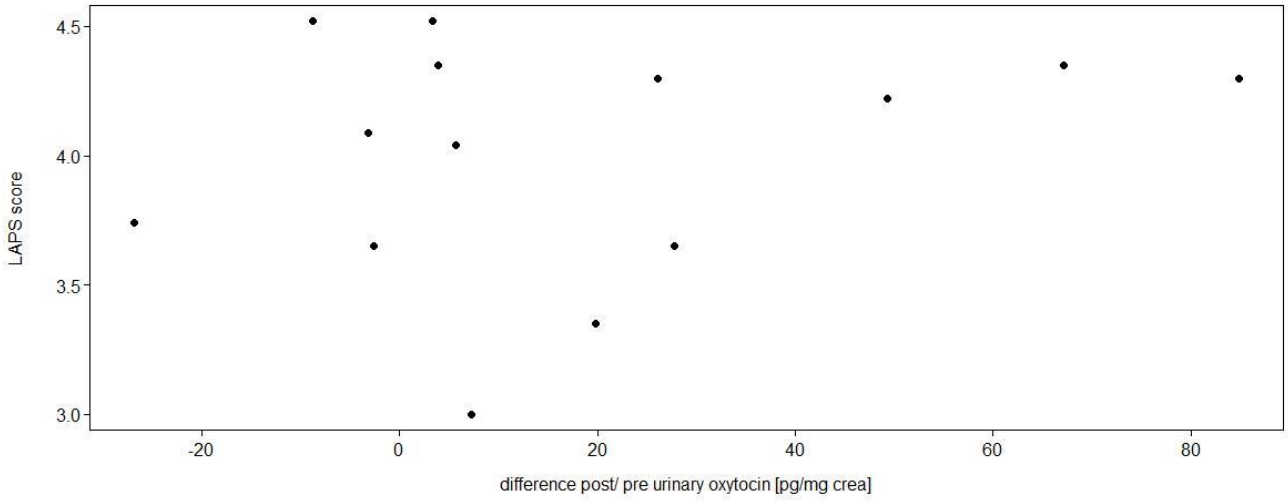

Fig. 13: *LAPS attachment score plotted against the pre to post OT level difference regarding the condition „Owner cuddle“ (OC).*

IV. Discussion

Overall, dogs and humans differed considerably regarding their results. Dogs showed inconsistent variability from pre- to post-treatment OT levels, both in the non-social control conditions as well as in the cuddle conditions. In contrast, human urinary OT levels increased from before to after the treatment, regardless of condition.
Taken together, these results do not support any of my hypotheses neither for the dogs nor for the humans – the kind of condition, respectively the relationship towards the cuddle partner and the type of interaction, could not explain the difference from pre- to post-treatment OT levels. Results for the two species will be discussed separately in the following.

Regarding the *dog* part of the study, results are quite unexpected. The lack of an effect in the dogs could potentially be explained by several factors, including the design of the treatment conditions or aspects regarding the sample characteristics, such as number of participants and individual differences.
Previous studies, which included the same components as my treatment stimuli (stroking, gently talking), found an increase in dogs' plasma OT levels after interacting with their owner or a familiar person (Odendaal and Meintjes, 2003;Handlin et al., 2011). In those studies, the interaction lasted for between 3 and a maximum of 30 minutes; in contrast to a 15-minute interaction in my study. However, Handlin et al. (2011) found an increase of the dogs' OT plasma levels already after only 3 minutes of interaction. This suggests that the interaction time in my study should have been sufficient to induce an increase in urinary OT levels, even with effects on urinary OT levels being delayed in time: Mitsui et al. (2011) showed that increased plasma OT levels in dogs manifest in urine after approximately 60 minutes. Additionally, they found increased urinary OT levels after the dogs had been stroked for 15 minutes (no talking or eye-contact) by a familiar person. The study by Nagasawa et al. (2015) examined the effect of stimuli such as touching, talking and eye-contact in a 30 minute interaction with the owner. They found an increase in urinary OT levels in a subset of the participating dogs which exchanged more eye- and body-contact with their owner (LG – long gaze group); whereas in the short gaze group (SG), no effect was found. However, in the present study, visual inspection of the data did not reveal a correlation between the duration of mutual eye-contact and the reactivity of dogs' urinary OT levels.

Sample size in my study (n=14) outreached the number of individuals which showed an effect in previous studies (Mitsui et al., 2011: n=9; Nagasawa et al., 2015: n=8 out of 30 participants) and even though I used the same stimuli in my experimental setup, I failed to replicate their findings and could not find a general effect on dogs' urinary OT levels.
However, half of the individuals in my study showed an increase from pre- to post-treatment OT levels in the Owner cuddle condition (OC) – together with the results by Nagasawa et al. (2015) with an effect only in a subset of the sample, this could indicate that it is not possible to draw general conclusions about the influence of socio-positive interactions on dogs' urinary OT levels, but that there are strong individual differences.
As a possible explanation for the increase from pre- to post-treatment OT levels in only a part of the participating dogs, one could assume some of them were not feeling comfortable in the test situation. However, the occurrence of stress signals, such as lips licking and yawning, was rare and did not have an effect on urinary OT levels. On top of this, all participating dogs were familiar with the test area and the experimenter, which reduces the probability that they felt uncomfortable. Still, it is possible that some of the dogs did not particularly enjoy the cuddle sessions but tolerated them in respect of pleasing the human.
On the downside, the same number of individuals as in the Owner cuddle condition (OC) showed increased OT levels after the Owner fake dog condition (OC-) with the dogs being ignored – still, the identity of the dogs with elevated OT levels did not coincide over conditions. In some cases, OT levels even decreased during the treatment, regardless of condition - so even if dogs differ in their urinary OT level reactivity, it was not possible to detect a consistent pattern on an individual level.

A clear difference to previous studies is the method we used to analyse samples: in contrast to the studies which found an increase in dogs' urinary OT levels (Mitsui et al., 2011;Nagasawa et al., 2015), we did not use radioimmunoassays (RIA) for analysis, but enzyme immunoassays (EIA). However, partly in contrast to the previous studies, an in-depth validation was performed regarding the extraction and analysis of OT from both dog and human urine samples (e.g. parallelism, extraction efficiency, assay accuracy, immunogram, repeatability – Schaebs et al., in prep.). For both dog and human samples, the results of the validation indicate that urinary OT levels can be reliably assessed using this method.

However, during the process of validation one methodological issue emerged regarding the consistency of OT measurements over time (Schaebs et al., in prep.). OT levels extracted from the same sample did not show a uniform pattern across and within individuals after being analysed repeatedly (after 1, 3 and 5.5 months). The samples of this study were sent to the MPI EVA in Leipzig in bunched shipments, thus the time span from collection to analysis varied between 29 and 182 days (mean: 106.8 days; SD 32.7). This factor might have had an influence on measured OT levels and hence on the overall results – still, respective dog and human samples derived from the same sampling dates and were stored for equal lenths. Regarding the human part of the study, I found an overall increase in OT levels, thus it seems unlikely that these storage issues provide an exclusive explanation for the inconsistent results regarding the dogs.

Additionally, considering that results from previous studies are inconsistent and ranging from positive results (Mitsui et al., 2011) to a partial effect (Nagasawa et al., 2015) to negative results (Romero et al., 2014), my results are hardly surprising. Furthermore, one should keep in mind a possible "publication bias" with negative results being less likely to be published (Lane et al., 2016;Walum et al., 2016).
With regard to the inconsistent variability in urinary OT levels in dogs, in the present as well as in previous studies, an additional factor could be of possible influence: usually, dogs do not empty their bladders completely when urinating – this could cause dilution effects of the post-treatment urine sample and hinder an explicit attribution of the OT levels to before and after the treatment. Forthrightly, the assessment of OT levels in urine may thus be an inadequate method applied in dogs.

While the analysis of the dogs' samples resulted in inconsistent variability, the overall increase from pre- to post-treatment OT levels in the *humans* does also not completely coincide with my predictions.
The rise in OT levels after interaction with a dog (i.e. after the conditions Owner cuddle – OC, and Owner cuddle familiar dog - OF) matches with the results found in previous studies, as measured in blood (Odendaal and Meintjes, 2003;Miller et al., 2009;Handlin et al., 2011) and urine (Nagasawa et al., 2009a;Nagasawa et al., 2015) samples. Nevertheless, most of these previous studies investigated the effect of dog-human interaction on OT levels in owners (Miller et al., 2009;Nagasawa et al., 2009a;Handlin et al., 2011;Nagasawa et al., 2015) or did not distinguish between owners and non-bonded

interaction partners (Odendaal and Meintjes, 2003), but none of these studies compared the change in OT levels after interaction with partners varying in familiarity.

Still, I found no difference in the increase of OT levels after interacting with a bonded in comparison to a non-bonded partner, as it would have been predicted by the "relationship qualitity hypothesis". The lack of correlation between the attachment score and the change in OT levels in both dogs and owners is in line with the rejection of this hypothesis. However, the attachment scores of my participants were in the upper range (mean: 4.01; range from 3.00 to 4.52), thus the lack of a correlation with pre- to post-treatment OT levels could be due to low score variability.

Indeed, the increased OT levels in both the cuddle conditions with bonded and non-bonded partners would be in line with the "social interaction hypothesis" - however, I also found an increase in the control conditions when the owner socially interacted with a toy dog (Owner fake dog - OC-) and even in the Mechanical cuddle condition (MC) where the owners stroked their dog with an artificial hand but without being socially engaged.

The rise from pre- to post-treatment OT levels in the Owner fake dog condition (OC-) could be caused by the sensory component of stroking the furry toy dog and would thus fit the „tactile stimulation hypothesis". Nevertheless, in the Mechanical cuddle condition (MC) the owner was not exposed to any tactile stimulation, still I found increased OT levels. But, while the owners did indeed not directly interact with their dog and eye-contact and talking was prohibited, the dog was still present and they could observe their dog enjoying the stroking (which was the case for most of the participating dogs), which could conceivably be sufficient to be accounted as a form of social interaction and induce an increase in the humans' OT levels. Also in the fake dog condition (OC-), social interaction, even when performed with a toy dog, might be an adequate stimulus to affect human OT levels and alternatively explain my results.

Together, these aspects of social interaction and tactile stimulation might explain the increase from pre- to post-treatment OT levels I found in my control conditions.

The results regarding HR data did not fulfil what I expected; I found neither a correlation with the difference from pre- to post-treatment OT levels nor with the kind of condition. I started to measure HR data at a baseline level before the interaction between owner and dog started and could not confirm a decrease in HR after the interaction, as found in Handlin et al. (2011). However, in this previous study, they compared HR data from the

start of the interaction to the HR one hour after the test and in the control condition, humans just sat in the chair without interacting with a dog. Actually, HR was found to increase during an interaction because of body-movement and vocalisation (Vormbrock and Grossberg, 1988) - due to a missing baseline HR and the comparison of the interaction with an inactive control, this could explain the HR decrease reported by Handlin et al. (2011).

This overall increase of humans' OT levels is not consistent with what I expected; however, previous studies did not conduct comparable control conditions: in Odendaal and Meintjes (2003);Miller et al. (2009) and Handlin et al. (2011) the dog was not present and the participants were either inactive or reading; whereas Nagasawa et al. (2015) did not perform a control at all. Taken together, these previous control conditions did not allow to clarify whether the observed increase in OT levels was influenced by social or tactile aspects.
The results regarding my control conditions, where I found an increase after the owners interacting with a toy dog or stroking their dog with an artificial hand without being socially enganged, require further investigation. A follow-up will repeat the Owner cuddle condition and compare it to two additional control conditions - one with the dog being present and the owner reading or working on the laptop, and another with the dog being absent, will be carried out to clarify the possible influence of the dogs' presence. This will on the one hand allow to compare results to the control conditions of previous studies and on the other hand shed light on the influence of the dogs' presence as a factor of social interaction.

In general, studies on the role of OT in the dog-owner relationship reveal quite inconsistent findings (see Buttner (2016) for a review) and previous conclusions have probably been drawn too fast. Future studies should focus on a proper and well-documented validation process of their analytical methods to ensure the comparability of results. In consideration of the inconsistent findings of this study and previous ones, replication with higher sample sizes is essential to allow for the drawing of valid conclusions.

V. Summary

Oxytocin (OT) is involved in different types of social bonds, ranging from attachment between parents and their offspring to preferential bonds like “friendship”, leading researchers to the suggestion that OT may be a marker of relationship quality.
Dogs are an interesting species in which to investigate the link between OT and social bonds since they form preferential relationships with human partners, functionally equated to the mother-infant attachment bond. Indeed, a number of studies have shown that OT may be at the heart of such relationships, with both dogs and their owners showing an increase in OT levels following socio-positive interactions involving stroking and mutual gazing. However, no direct comparison has been made between increased OT levels following a social interaction with a bonded partner and a familiar but not bonded dog/person, so it is unclear whether it is indeed the relationship quality which mediates increased OT levels during socio-positive interactions or if aspects of social interaction or tactile stimulation are crucial elements.
In this study, I investigated the change of urinary OT levels in both dogs and owners, following a socio-positive interaction with each other and with a familiar partner. A number of control conditions also allowed us to test whether in both dogs and humans, tactile stimulation *per se*, or only in combination with a direct social aspect, would elicit an increase in OT levels. Results showed that in dogs, neither the familiarity with the social partner, nor the type of interactions (social and tactile vs. only tactile vs. no interaction at all) affected urinary OT levels. On the contrary, human urinary OT levels increased from pre- to post-treatment, but did so regardless of whether the socio-positive interaction was with one’s own, a familiar or a fake dog. Results call previous conclusions on the role of OT in the dog-owner bond into question.

Zusammenfassung

Oxytocin (OT) ist an verschiedenen Formen sozialer Beziehungen beteiligt, welche von der Bindung zwischen Eltern und ihren Nachkommen zu Bindungen wie "Freundschaft" reichen. Dies führte zur wissenschaftlichen Behauptung, dass OT als Biomarker der Qualität einer Beziehung fungieren könnte.

Hunde stellen eine interessante Spezies dar, um die mögliche Rolle von OT in sozialen Beziehungen zu untersuchen, da die Bindung, die sie mit ihren Besitzern eingehen, funktionell der Mutter-Kind-Bindung entspricht. Tatsächlich konnte in einigen Studien gezeigt werden, dass OT eine Grundlage dieser Beziehungen sein könnte: sozio-positive Interaktionen, die Streicheln und gegenseitigen Blickkontakt beinhalteten, führten zu einem Anstieg der OT Level bei sowohl Hunden als auch ihren Besitzern. Dem Anstieg der OT Level nach einer sozialen Interaktion mit einem gebundenen Partner wurde jedoch noch nie eine Interaktion mit einem bekannten Hund/einer bekannten Person, zu der allerdings keine Bindung besteht, gegenübergestellt - somit ist unklar, ob der Beziehungsqualität tatsächlich ein Einfluss bezüglich des OT Anstiegs nach sozio-positiven Interaktionen zukommt, oder ob Aspekte der sozialen Interaktion oder der taktilen Stimulation entscheidende Elemente darstellen.

In dieser Studie wurde die Änderung von OT Leveln in Urin bei sowohl Hunden als auch ihren Besitzern untersucht, und zwar jeweils nach einer sozio-positiven Interaktion miteinander und mit einem bekannten Partner. Geeignete Kontrollbedingungen dienten der Klärung der Frage, ob taktile Stimulation *per se* oder nur in Kombination mit direkter sozialer Interaktion einen Anstieg der OT Level hervorruft. Die Ergebnisse zeigten, dass bei Hunden weder die Beziehungsqualität mit dem Sozialpartner, noch die Art der Interaktion (sozial und taktil vs. nur taktil vs. keine Interaktion) einen Einfluss auf die im Urin gemessenen OT Level hatten. Im Gegensatz dazu zeigte sich bei den OT Leveln im Urin der Menschen ein Anstieg von vor zu nach dem Test; allerdings unabhängig davon, ob die Interaktion mit dem eigenen, einem bekannten oder einem Stoffhund stattgefunden hatte. Diese Ergebnisse stellen frühere Schlussfolgerungen über die Rolle von OT in der Beziehung zwischen Hund und Besitzer in Frage.

Ethical Statement

Discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation (University of Veterinary Medicine, Vienna; Approval number: ETK-16/05/2016 for dogs).
For humans, ethical approval was obtained from the Medical University of Vienna (Approval number: 1565/2016).
Owners were also required to sign two respective consent forms prior to testing.

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List of Abbreviations

ACN	Acetonitril
CDL	Clever Dog Lab
D1/D2	Dog pre- and post-treatment samples
EIA	Enzyme immunoassay
H1/H2	Human pre- and post-treatment samples
HR	Heart rate
HP	Hypothesis
HPLC	High performance liquid chromatography
LAPS	Lexington Attachment to Pets Scale
MDORS	Monash Dog Owner Relationship Scale
MPI EVA	Max Planck Institute for Evolutionary Anthropology, Department of Primatology

OT	Oxytocin
OTR	Oxytocin receptor
RIA	Radio immunoassay
SPE	Solid phase extraction
TFA	Trifluoracetic acid

Treatment conditions:

OC	Owner cuddle
OC-	Owner fake dog
OF	Owner cuddle familiar dog
FC	Familiar person cuddle
FC-	Familiar person fake dog
MC	Mechanical cuddle

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