Divergent effects of oxytocin on eye contact in bonobos and chimpanzees

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Abstract

Oxytocin has drawn significant research attention for its role in modulating mammalian social behavior. Despite generally conserved roles, oxytocin can function differently even in closely related species. Previous studies have shown that bonobos and chimpanzees, humans' two closest relatives, demonstrate considerable behavioral differences, including that bonobos look more at others' eyes than chimpanzees. Oxytocin is known to increase attention to another's eyes in many mammalian species (e.g. dogs, monkeys, and humans), yet this effect has not been tested in any nonhuman great ape species. This study examined how intranasally-administered oxytocin affects eye contact in bonobos and chimpanzees using eye tracking. Following administration of either oxytocin or saline control with a nebulizer, chimpanzees (n = 6) and bonobos (n = 5) were shown images of conspecific faces while their eye movement was recorded. Oxytocin changed the eye-looking behavior of bonobos and chimpanzees differently. We found that oxytocin increased eye contact in bonobos but not chimpanzees; while one chimpanzee showed an increase, interestingly, 5 out of 6 chimpanzees showed decreased looking to the eyes compared to the mouth, suggesting moderate eye avoidance. Given the importance of eye contact in their social interactions, our results suggest that oxytocin may play modulatory roles in bonobos' and chimpanzees' species-specific social behavior and underscore the importance of oxytocin in hominid social evolution.

Keywords: Oxytocin, eye contact, bonobos, chimpanzees, species differences, social attention

1. Introduction

Oxytocin (OT) has gained growing research interest in past decades due to its diverse regulatory roles in mammalian social behaviors. The function of OT is largely conserved across mammals, specifically that it regulates essential reproductive needs, such as maternal attachment and pair bonding (Anacker and Beery, 2013), as well as nuanced socio-cognitive behavior and cognition, such as non-kin social bonding (Crockford et al., 2013; Romero et al., 2014; Wittig et al., 2014) , outgroup mentality (De Dreu et al., 2010; Samuni et al., 2017), social attention (Dal Monte et al., 2014; Guastella et al., 2008), and empathy (Burkett et al., 2016). However, despite its largely conserved roles, previous studies have demonstrated that the role of OT on behavior differs even between closely related mammalian species, partly due to differential distributions of OT receptors in their brains (Anacker and Beery, 2013; Insel and Shapiro, 1992). Studies have also demonstrated that genes encoding the receptors of OT (OXTR) and the structurally similar neuropeptide arginine vasopressin (AVP) can change rapidly in evolution (Hammock and Young, 2005), and that polymorphisms in these genes are related to the expression of social behaviors across individuals and species (Hopkins et al., 2012; Rodrigues et al., 2009; Staes et al., 2014; Staes et al., 2016). These pieces of evidence offer a potential mechanism by which species-typical social behaviors can rapidly evolve in closely related mammalian species (Insel and Young, 2000). In a similar vein, some researchers suggest that animal domestication is facilitated by changes in the OT and AVP systems and the associated reduction of stress sensitivity and aggression (Herbeck and Gulevich, 2019) – thought to be essential changes for nonhuman animals integrating into a human society.

Given this accumulating evidence, researchers also suspect that neuropeptides may have played a key role in the evolution of species-typical behaviors in bonobos and chimpanzees, humans' two closest relatives (Staes et al., 2014). Bonobos and chimpanzees diverged only recently

in evolution, (~1-2 million years ago; Prufer et al., 2012), but differ in social organization and a number of important social behaviors (Hare and Yamamoto, 2017). Critically, bonobos and chimpanzees differ in tolerance and aggression, particularly to outgroup individuals (Samuni et al., 2017; Tan and Hare, 2013; Tokuyama et al., 2019), socio-sexual behaviors (De Waal, 1990b) and social attention (Herrmann et al., 2010; Kano et al., 2015), all of which are known to be regulated by OT in human and nonhuman mammals (Anacker and Beery, 2013; Bartz et al., 2011; Bauman et al., 2018). Moreover, bonobos and chimpanzees differ neuroanatomically in brain areas related to socio-emotional behavior, such as the amygdala (and its connection to the anterior cingulate cortex) and insular cortex (Hopkins et al., 2015; Issa et al., 2019; Rilling et al., 2012; Staes et al., 2018; Stimpson et al., 2016), which are known to be modulated by OT (Burkett et al., 2016; Gamer et al., 2010; Rogers-Carter et al., 2018). Studies have also shown that bonobos and chimpanzees differ in OT and AVP receptor genes (Staes et al., 2014), that polymorphisms in these genes are linked with personality in bonobos and chimpanzees (Anestis et al., 2014; Hopkins et al., 2012; Staes et al., 2016; Wilson et al., 2017), and that urinary oxytocin increases after engaging in species-typical behavior, such as non-copulatory sex in bonobos (Moscovice et al., 2019) and intergroup conflict in chimpanzees (Samuni et al., 2017). One theory proposes that bonobos may have undergone a domestication-like process which may have reduced aggression in their evolution (Hare et al., 2012), a process which might be related to the changes in OT/AVP systems (Herbeck and Gulevich, 2019). Therefore, these studies suggest that species-typical behaviors of bonobos and chimpanzees may have coevolved with the OT/AVP system. However, despite this accumulating evidence, no comparative study has been conducted to test whether OT affects the behavior of these species differently under the same experimental conditions. To this end, intranasal administration of OT seems most effective.

Several previous studies have demonstrated that intranasal administration of OT causes diverse effects on behavior and cognition in human and nonhuman primates. In studies with humans, OT has been found to be associated with alleviation of social anxiety (Macdonald and Macdonald, 2010), enhancement of prosocial behavior such as increased trust and donation (e.g. Israel et al., 2009; Kosfeld et al., 2005), and enhancement of attention to certain social stimuli such as eyes (Andari et al., 2010; Auyeung et al., 2015; Guastella et al., 2008). OT has also been found to be associated with derogation of the outgroup (De Dreu et al., 2010), enhancement of negative emotion such as envy and schadenfreude (Shamay-Tsoory et al., 2009), and reduced avoidance of negative (non-social) stimuli (Harari-Dahan and Bernstein, 2017). These studies and others have stressed multifunctionality of OT, namely that OT has both prosocial and antisocial as well as social and non-social effects, which has given rise to the social-salience (Shamay-Tsoory and Abu-Akel, 2016), social approach-withdrawal (Kemp and Guastella, 2011), and general approach-avoidance hypotheses (Harari-Dahan and Bernstein, 2014). In studies with macaques, the effect of OT on social orientation and attention is well studied. In addition to enhancing social proximity (Simpson et al., 2014), nebulized intranasal OT increases attention to eye gaze (Dal Monte et al., 2014; Ebitz et al., 2013; also see Kotani et al., 2017 with marmosets), enhances gaze-following (Putnam et al., 2016), and attenuates attention to both negative facial expressions (Parr et al., 2013) and threat staring to others (Jiang and Platt, 2018a). One study also found antisocial OT effects (e.g. increase in threat staring to males) in female macaques following OT administration (Jiang and Platt, 2018b), highlighting the complex effects of OT depending on subjects' biological differences and social contexts.

There have been fewer studies about the oxytocinergic system in non-human great apes and no studies investigating its effect on social attention, though field studies measuring urinary OT suggest an important role of OT in several key social behaviors. In addition to the previously

106 mentioned studies reporting increased urinary OT following non-copulatory sex in bonobos
107 (Moscovice et al., 2019) and intergroup encounters in chimpanzees (Samuni et al., 2017), urinary
108 OT in wild chimpanzees has been found to rise following food sharing (Wittig et al., 2014),
109 grooming (Crockford et al., 2013), group hunting (Samuni et al., 2018) and reconciliation (Preis et
110 al., 2018). Two previous studies (Hall et al., 2019; Proctor et al., 2016) have administered nebulized
111 OT to chimpanzees to test its effects on their real-life social interaction. In particular, in Proctor et
112 al. (2016), one individual was administered OT or placebo, and was then observed for their daily
113 social interaction with individuals who did not receive OT, though they found no statistically
114 significant differences between conditions. In Hall et al. (2019), no consistent patterns in a token
115 exchange task were found with or without OT administration. As authors noted, both studies
116 yielded null results presumably due to several methodological limitations; for example, in
117 optimizing OT administration procedures for great ape species or in detecting subtle changes in
118 social behaviors in complex interactions with non-OT-administered individuals.

119 This study tested whether intranasal administration of nebulized OT affects eye contact
120 behavior in bonobos and chimpanzees similarly or differently using an eye tracking setup. Eye
121 contact plays significant roles in primate cognition and social communication (Emery, 2000) and
122 the function of eye contact is largely similar among primate species, including bonobos and
123 chimpanzees. For example, staring at another's eyes signals threat (Emery, 2000) as well as
124 affiliation (e.g. mutual gaze between mothers and infants Ferrari et al., 2009). Chimpanzees
125 establish eye contact before attempting to reconcile with others after fights (De Waal, 1990a).
126 Bonobos engage in prolonged eye contact during characteristic non-copulatory sex (GG-rubbing;
127 Annicchiarico et al., 2020; Moscovice et al., 2019). On the other hand, however, bonobos and
128 chimpanzees seem to differ significantly in their sensitivity to others' gaze (as among other closely
129 related primates; Thomsen, 1974). Specifically, in eye tracking tests, bonobos viewed the eyes of

conspecific facial images longer than chimpanzees (Kano et al., 2015). This same pattern is also observed in response to a human experimenter's eyes (Mulholland et al., 2020). Relatedly, in a battery of tests examining cognitive differences between bonobos and chimpanzees, bonobos followed the experimenter's gaze more frequently than chimpanzees (Herrmann et al., 2010). This study thus asked whether OT modulates the species differences in eye contact behavior in bonobos and chimpanzees.

We predicted that the use of an eye tracking setup may reveal subtle changes in the effect of OT on eye movement in bonobos and chimpanzees. Regarding the direction of change, our first hypothesis was that OT administration would increase eye contact in both species, consistent with the previous findings with both neurotypical and autistic humans (Andari et al., 2010; Auyeung et al., 2015; Gamer et al., 2010; Guastella et al., 2008), macaques (Dal Monte et al., 2014; Ebitz et al., 2013), marmosets (Kotani et al., 2017), and dogs (Nagasawa et al., 2015). Thus, administration of OT may alter chimpanzees' looking pattern to become more like that of bonobos by increasing attention to the eye region. Our second hypothesis was that OT administration would differently affect eye contact in bonobos and chimpanzees, consistent with the findings that bonobos and chimpanzees differ in neural and genetic structures related to OT receptor systems (Hopkins et al., 2015; Issa et al., 2019; Rilling et al., 2012; Staes et al., 2018; Staes et al., 2014; Stimpson et al., 2016). Given that bonobos are more sensitive to others' eyes than chimpanzees, OT may enhance such pre-existing eye sensitivity, particularly in bonobos but not chimpanzees.

2. Material and methods

2.1 Participants.

Six chimpanzees (*Pan troglodytes verus*) and five bonobos (*Pan paniscus*) living at Kumamoto Sanctuary, Japan, participated in this study (Table 1). One additional bonobo male was tested but excluded from the analyses because he rejected inhaling nebulized mist.

2.2 Ethics statements.

All ape participants were tested in rooms prepared for each species, and their daily participation in this study was voluntary. They received regular feedings, daily enrichment, and had ad libitum access to water. No changes were made to their daily care routine. Research protocol was approved by the institutional review board (WRC-2019-KS013A for chimpanzees, and WRC-2019-KS014A for bonobos). Safety of the OT administration was carefully considered and accepted given the fact that 1) OT is often administered to human children and adults, that 2) OT is active for only a short period of time following administration with no known side effects in humans (MacDonald et al., 2011), that 3) OT is naturally produced in bonobos and chimpanzees following relevant behaviors (Crockford et al., 2013; Moscovice et al., 2019), and that 4) previous two studies administering OT intranasally to chimpanzees did not report any agonistic interaction (Hall et al., 2019; Proctor et al., 2016). In addition, we conducted a pilot test with chimpanzees following the same OT administration procedure and confirmed no irregular behaviors or interactions were observed.

2.3 Oxytocin administration apparatus and procedure.

We modelled our general OT administration procedure on methods commonly adopted in tests with macaques (for a review, see Bauman et al., 2018). In particular, we did not use nasal spray but instead used a nebulizer to administer aerosolized OT to ape participants. This procedure has proven to be effective in a number of behavioral and physiological tests with macaques, including the observation that OT level in cerebrospinal fluid (CSF) increased following administration of

aerosolized OT (Modi et al., 2014). OT or saline placebo were administered with a portable nebulizer (NE-U22-4, Omron, Kyoto, Japan) to apes in a custom-designed box while apes were drinking a dripping of juice thorough a nozzle attached to the box (13 w × 16.5 h × 8 d cm), via a custom-made juice dispenser (Figure 1A; similar to the device used and validated by Parr et al., 2013). Criteria for successful administration was 4 cumulative minutes, counted with a stopwatch, of nebulized mist being projected onto their nose inside the box. We paused counting when the nose of ape was out of the box. All apes (except one bonobo who dropped out) completed this procedure within 10 minutes in each trial. We used a concentration of 40 IU/mL of oxytocin, which was nebulized at a rate of 0.25 ml/minute, meaning roughly 40 IU of oxytocin was nebulized in the cumulative 4 minutes. A dose of 40 IU was chosen because it is well within the range of human and monkey studies (commonly 24-40 IU; Bauman et al., 2018; MacDonald et al., 2011), and a relatively high dose was chosen because certain amount of the mist was expected to evaporate from the box (as in Parr et al., 2013). We visually confirmed that individuals breathed the mist through their nose during administration. The eye tracking test on each day was both started and completed 30-60 minutes after the end of administration procedure, an interval also well within that of previous studies (Bauman et al., 2018).

2.4 Eye tracking apparatus.

Following an established procedure (Kano et al., 2011; Krupenye et al., 2016), ape eyes were recorded by an infrared head-free eye tracker (300 Hz, TX300, Tobii Technology AB). Apes sipped a dripping of juice via a custom-made juice dispenser while they viewed the stimuli. The stimuli were presented with a resolution of 1,280 × 720 pixels at a viewing distance of 70 cm on a 23-inch LCD monitor (43 × 24°) with Tobii Studio software (ver. 3.2.1). Due to apes' relatively short attention span, automated calibration was conducted at two points for each ape by presenting a

small object or video clip on each reference point. Subsequently, we checked the quality of calibrations by presenting small reference icons on the monitor before each recording session and confirming the ape's gaze did not deviate from the icons. We repeated the calibration procedure whenever necessary. Following these procedures, calibration errors in apes are typically within one degree (Kano et al., 2011), an accuracy sufficient to distinguish between eyes and mouth of presented faces in this study.

2.5 Stimuli and procedure.

Stimuli consisted of two 3-minute videos for each species including both movies and slideshows of images. Each image was of conspecific faces and was presented for three seconds each, and there were approximately 30 images in each video. To maximize each species' interest and natural eye movement responses to the faces, we presented complex scenes depicting various natural behaviors (resting, fighting, playing, copulating, displaying, grooming, and tool-using), and faces with varying facial expression (neutral, play, and scream faces, and grimaces), gaze direction (direct and averted), and posture, of individuals of all ages and sexes, and of both ingroup and outgroups (see Supplementary Excel file for more details). Both species viewed only conspecifics (images containing themself were excluded from the analysis). Some individuals appeared in multiple images (maximally in 4 images) with different camera angles and configurations. The contents and configurations of scenes were matched as much as possible between the videos prepared for each species (Figure 1B). Two different videos of 3 minutes each were prepared for each species (Chimpanzee/Bonobo Video 1 and 2) which each individual saw twice (across conditions). Following administration of either OT or saline, each ape saw one video each day (this is a trial). The order of OT and saline administration (Condition) was counterbalanced across individuals, either in ABBA or BAAB order. The first two trials (Block 1) presented Video 1 and latter two

trials (Block 2) presented Video 2. Thus, each ape saw each of two videos twice on consecutive trials (this is a block). There were thus four trials for each ape. Each trial was separated by a minimum of 5 days to avoid any possible lasting effects of OT.

2.6 Analysis.

Eye movement was filtered using Tobii Fixation Filter with default parameters. Areas-Of-Interest (AOIs) were defined for each cut of movies and each picture in slideshows in the Tobii Studio software (see Figure S1 for examples). AOIs included the eyes, mouth, face (including both eyes and mouth), genital, body (including face and genital), and action target (object, food, and tool held by the hands). In each image, AOI was not defined if its minimal diameter was smaller than one degree (e.g. the vertical diameter of the eye AOI), or the target object was moving rapidly or covered (consequently, eye/mouth AOI was removed in movies). Eye and mouth AOIs were defined as a pair in all pictures.

Statistical analyses were performed in R using linear mixed models (LMM) ('lmer' in the package 'lme4') with Gaussian error structure and identity link function. The dependent variables were total looking duration to each AOI in each trial (presenting a 3-min video); eyes, mouth, face (as a whole), genital, action targets, body (as a whole), and screen (i.e. a whole video screen) and separate models were run for each of these dependent variables. The variation in presentation durations of each AOI across videos was minimal for the eye, mouth, and face AOIs (and absent for the body and screen AOIs), but relatively large for target and genital AOIs (Table S1). Thus, to analyze the looking duration to the latter two AOIs, we used the proportion of looking duration (with respect to the total presentation duration of AOI) as the dependent variable (logit-transformed; Warton and Hui, 2011); for the looking duration to the former three AOIs, such a transformation was not necessary because the variation in presentation duration across videos was minimal, and we

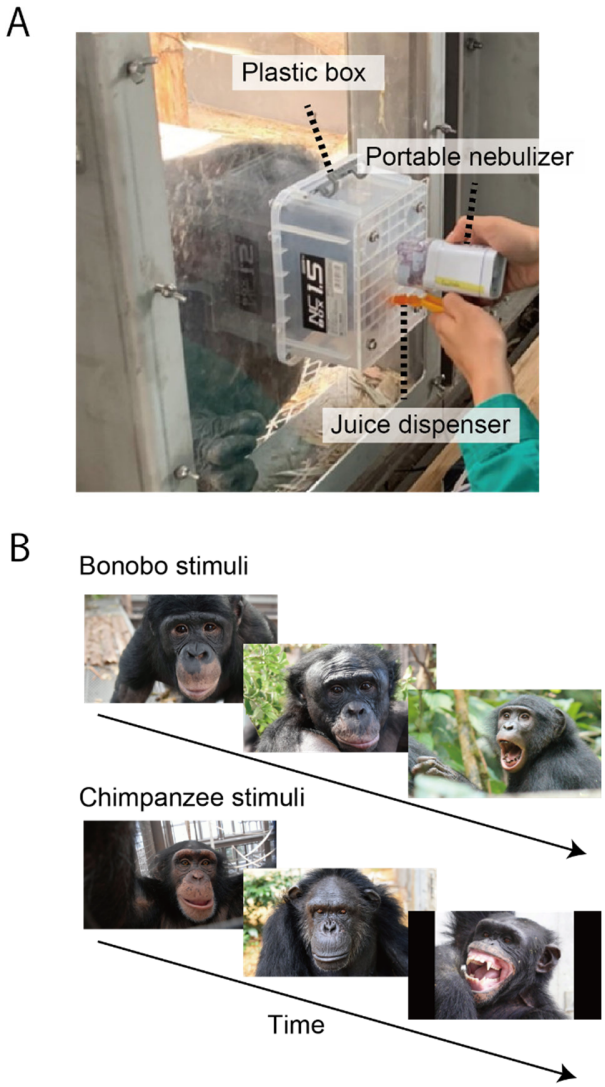
243 confirmed that the same results emerged with or without the transformation. We additionally tested
244 the difference score subtracting eye looking duration from mouth looking duration in a post-hoc
245 model (detailed in Result). In all models, we included Species (bonobos/chimpanzees), Condition
246 (OT/saline), and interaction between the two factors as test predictors. Additionally, we included
247 Block (1/2) and Trial (1/2), which was nested in Block, as control variables. These control variables
248 were standardized (using the 'scale' function) according to the recommendation of Schielzeth
249 (2010). We included participant and video as random intercepts and random slopes of all fixed
250 effects; the random-effects structure was kept maximal to save conservativity of the tests according
251 to the recommendation of Barr et al. (2013), except that we removed the correlation between the
252 intercept (Participant) and slopes (Block/Trial) to keep sufficient random-effect variations. The
253 model syntax in R (used for all models reported in the main texts, including the eye-minus-mouth
254 model) was; Looking duration ~ Species*Condition + Block/Trial + (1 + Condition + Block/Trial ||
255 Participant). The number of observations was 44 in this model (11 participants in 4 trials). We
256 confirmed the assumptions of normally distributed and homogeneous residuals by visual inspection
257 of diagnostic plots (q-q plots and scatterplots of the residuals plotted against fitted values) in all
258 models. We also checked Variance Inflation Factors (VIF) in a R package 'car' and found that
259 collinearity was not an issue in any model (all VIF < 3). To check the model stabilities, we excluded
260 each level of random effects (subject and video) one by one and calculated Cook's distances as
261 measures of influence in a R package 'influence.Me'. When this manipulation suggested any
262 influential cases (Cook's distance > 1), we confirmed that excluding that influential case did not
263 change the main results. We used a likelihood ratio test to examine the significance of an effect in
264 question (using the 'drop1' function). We first tested the interaction term in each model, and if not
265 significant, and then reran the model without the interaction term (Engqvist, 2005). When we found
266 a significant interaction effect in the model, we further examined it by testing simple effects in the

subsets of data including each level of predictors. Finally, to explore whether any of the stimulus properties (e.g. gaze direction, ingroup/outgroup, male/female, facial expressions of the presented faces; see Supplementary Excel file) critically affected the results (eye-mouth difference looking score), we examined the effect of each stimulus property individually (for simplicity, the interaction between stimulus properties was not considered in this analysis) by restructuring the dataset to include three factors, species, condition, and stimulus property, and then testing the three-way interaction effect; the results from these additional analyses were reported in Supplemental Material.

Table 1. Participant information. In the rearing history column, 'Mother' indicates the individuals reared by their biological mothers, and 'Nursery-peer' indicates the individuals reared by human caregivers and conspecific peers.

Species	Sex	Age	Rearing history	Name
bonobo	F	29	Nursery-peer	Ikela
bonobo	F	38	Mother	Lenore
bonobo	F	48	Nursery-peer	Louise
bonobo	F	31	Nursery-peer	Lolita
bonobo	M	16	Nursery-peer	Vijay
chimpanzee	F	12	Nursery-peer	Hatsuka
chimpanzee	F	12	Mother	Iroha
chimpanzee	M	25	Mother	Zamba
chimpanzee	F	21	Mother	Misaki
chimpanzee	F	24	Nursery-peer	Mizuki
chimpanzee	F	15	Mother	Natsuki

*For further information of these apes, visit GAIN (https://shigen.nig.ac.jp/gain/; Great Ape Information Network; the online studbook of Japanese apes) and type the names in the search bar.



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282 Figure 1: Study Design. (A) Oxytocin (OT) administration apparatus. (B) Examples of presented
283 images. Also see the link (https://youtu.be/_LbQ3qtlEcA) for more examples and superimposed eye
284 movements of bonobos and chimpanzees.

285

3. Results

OT differently affected bonobos' and chimpanzees' looking duration to the eye of the images (Figure 2A; a significant interaction effect between species and condition: $\beta = -3.4$, SE = 1.4, $CI_{lower} = -6.2$, $CI_{upper} = -0.4$, $\chi^2 = 4.9$, $p = 0.028$). OT did not significantly affect their looking duration to the other AOIs (see Table S2 for full statistical results). Although not significant, OT tended to affect differently the two species' looking duration to the mouth of the images (Figure 2B: $\beta = 3.6$, SE = 1.9, $CI_{lower} = -0.09$, $CI_{upper} = 7.5$, $\chi^2 = 3.2$, $p = 0.072$); the directions of these OT effects were the opposite of those on their responses to the eye of the images. As the responses to the eyes and mouth are essentially related (viewing mouth is not viewing eyes of the same face, and vice versa), and the two species did not differ in their overall looking duration to the faces of images (Table S2), we additionally ran a model with a difference score which subtracted looking duration to the mouth from that to the eyes in each image (Figure 2C). There was a significant interaction between species and condition ($\beta = -6.9$, SE = 2.4, $CI_{lower} = -12.0$, $CI_{upper} = -2.6$, $\chi^2 = 6.4$, $p = 0.011$). To explore this observed interaction effect further, we tested simple effects in the subsets of data on each level of test predictors. We found that OT increased bonobos' looking duration to the eyes compared to the mouth ($\beta = 3.5$, SE = 0.9, $CI_{lower} = 1.8$, $CI_{upper} = 5.3$, $\chi^2 = 8.2$, $p = 0.004$). On the other hand, OT did not significantly change chimpanzees' looking durations for the eyes compared to the mouth ($\beta = -3.5$, SE = 2.6, $CI_{lower} = -9.1$, $CI_{upper} = 1.6$, $\chi^2 = 1.5$, $p = 0.22$). In neither condition did bonobos and chimpanzees differ significantly in their looking durations for the eyes compared to the mouth (OT: $\beta = -9.0$, SE = 7.3, $CI_{lower} = -23.1$, $CI_{upper} = 5.5$, $\chi^2 = 1.4$, $p = 0.24$; Saline: $\beta = -2.2$, SE = 6.4, $CI_{lower} = -13.7$, $CI_{upper} = 9.8$, $\chi^2 = 0.1$, $p = 0.73$). Figure 2C show that the effect of OT was relatively consistent across individuals in bonobos. One chimpanzee (Iroha) showed the same direction of change after administration of OT as bonobos did, while the other five chimpanzees

showed the opposite direction of change. Figure 2C also showed relatively large individual differences within each species in the eye-mouth looking duration, which explained the absence of a significant effect of species in this study. Finally, an inclusion of each stimulus property (group affiliation, sex, gaze direction, and facial expression of the presented faces) as an additional test predictor into the same model (with the eye-mouth difference score as the response) confirmed that the same interaction effect (species*condition) can be observed in these models. We additionally found the main effects of group affiliation and facial expression of the presented faces (see Figure S3 and Supplemental Results). Notably, we found a three-way interaction effect between species, condition, and gaze direction of faces. This result indicated that the species difference in OT effect can be observed when the two species viewed the faces with direct gaze, but not those with averted gaze, suggesting that OT affected the two species' eye contact behavior (i.e. looks to others' direct gaze).

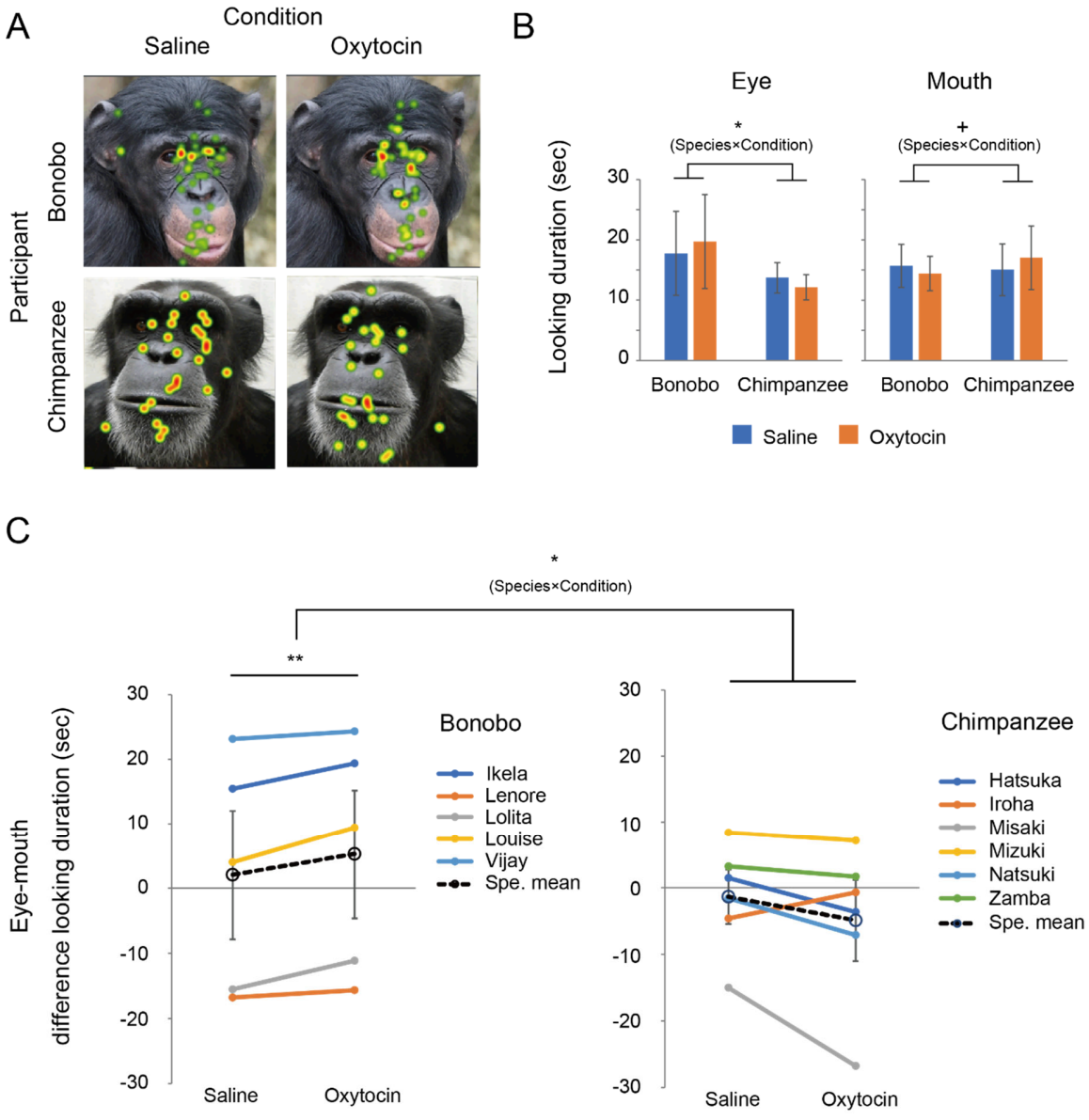

Figure 2: Effect of oxytocin on eye contact in bonobos and chimpanzees. (A) Heatmaps of eye fixations (redder parts indicate greater attention). (B) Mean looking durations (sec) for the eyes and mouth of faces in a trial (presenting a 3-min video). (C) Mean difference looking durations (sec) for the eyes and mouth (eyes minus mouth) by individuals (chimpanzees: n = 6, bonobos: n = 5, in

329 solid lines) and also by each species (in dashed lines) in a trial (presenting a 3-min video). Error
330 bars denote 95% confidence intervals. **p < 0.01, *p < 0.05, +p < 0.1.

331

4. Discussion

Intranasal administration of nebulized OT increased looking duration to eyes of conspecific images in bonobos but not chimpanzees, supporting our second hypothesis predicting differential effects of OT on the eye looking behavior of these species. The increased eye looking observed in bonobos was consistent with a number of previous findings with human and nonhuman primates (Andari et al., 2010; Auyeung et al., 2015; Dal Monte et al., 2014; Ebitz et al., 2013; Gamer et al., 2010; Guastella et al., 2008; Kotani et al., 2017) and also dogs (Nagasawa et al., 2015). OT, however, did not affect chimpanzees in the same way. Interestingly, while one chimpanzee (Iroha) showed the same direction of change as bonobos did, 5 out of 6 chimpanzees decreased total looking duration at eyes relative to mouth. Most likely, looking at the mouth in chimpanzees indicates a moderate eye avoidance; that is, although they attended to faces (like bonobos), they did not look at eyes directly but looked slightly down (i.e. mouth). Thus, OT may not only operate differently but enhance species-typical behavioral tendencies in these species.

Before proceeding to more detailed discussions about the hypotheses, several low-level explanations can be ruled out. First, it is unlikely that procedural differences between species caused the differential OT effects on their behavior because we tested the two species using identical experimental apparatus and procedures. Second, it is unlikely that the dose of OT (40 IU) used in this study was inappropriate to test either species of great ape because this dose was well within the range of previous studies with macaques and humans of respectively smaller and larger body weights compared to bonobos/chimpanzees (Bauman et al., 2018; MacDonald et al., 2011), and we visually confirmed that individuals from both species breathed the mist through their nose during administration. Third, it is unlikely that the observed species difference is explained by a general increase in attention to the face because OT did not change overall attention to faces in either species. Moreover, we found that the observed species difference was particularly evident

when the two species were viewing faces with direct gaze, but not with averted gaze. These results thus suggested that the observed OT effects reflect differential operation of OT on eye contact behavior in bonobos and chimpanzees.

Psychologically, among several hypotheses proposed in literature, the social salience hypothesis seems most parsimonious with our results. This hypothesis was proposed to explain both prosocial and antisocial effects of OT on human social behavior, and assumes that OT enhances pre-existing social sensitivities and ongoing psychological processes under given contexts (Shamay-Tsoory and Abu-Akel, 2016). In this study, therefore, OT may have enhanced pre-existing gaze sensitivity in bonobos. Other hypotheses, such as affiliative/prosocial and anxiety/stress-reduction hypotheses (Ebitz et al., 2013; Macdonald and Macdonald, 2010) may explain the results observed in bonobos (i.e. increase in eye contact) if attending to others' eyes is driven by affiliative motivation or is somewhat stressful (and then this stress was alleviated) in this species. However, these hypotheses do not explain why the same effects were not observed in chimpanzees. Another hypothesis, social approach-withdrawal hypothesis seems to fit our results relatively well because it predicts that OT facilitates the approach behavior of participants (Kemp and Guastella, 2011). Previously, it has been shown that the degree of eye contact between individuals varies as a function of both physical and psychological distances between individuals in both human and nonhuman primates (Argyle and Dean, 1965; Thomsen, 1974). In addition, social distances among individuals between bonobos and chimpanzees seem to differ by default, specifically that bonobos (especially females) are overall more gregarious than chimpanzees (Tokuyama et al., 2019). Therefore, if OT facilitated social approach in both species, bonobos may have perceived the social stimuli as more relevant, leading to an increase in eye contact, while chimpanzees may not; in fact, some of them may have perceived the social stimuli as more stressful or threatening, leading to a decrease in eye contact. Future research should directly test these hypotheses, as well as examine

whether the effect of OT is modulated by social distance. In any case, the most relevant point here is that OT may have acted differently in bonobos and chimpanzees by interacting with certain pre-existing tendencies that each species had (or did not have).

Neurologically, the differential operation of OT on the two species is likely caused by differential distribution of OT receptors in brain (or differential density of OT receptors in particular brain regions) between species, as suggested in previous studies (Anacker and Beery, 2013; Insel and Shapiro, 1992). Unfortunately, much remains to be studied as to the neuroanatomical distribution/molecular structure of OT (and AVP) receptors in bonobos and chimpanzees. However, one study comparing OT- and AVP-synthesizing neurons in the hypothalamus did not find species differences between bonobos and chimpanzees (Hopkins et al., 2015), suggesting that the production of OT does not differ between the species (but the distribution, density, and binding affinity of OT receptors may). Our results thus encourage a comparative study of OT receptors in brain regions related to eye contact and social attention (e.g. amygdala), which likely differ between the species. To speculate the candidate region, a high-resolution fMRI study with humans (Gamer et al., 2010) may be particularly informative, which found that different subregions of the amygdala mediate valence-related and attention-related effects of OT. Of particular relevance to this study, an increase in activation of the posterior amygdala (likely basal nucleus) and a functional coupling of this region to the superior colliculi was observed in response to the eyes of presented faces in the OT compared to placebo condition. This amygdala subregion may be particularly relevant to the species difference in the effect of OT observed in this study because microstructural differences have been found between the bonobo and chimpanzee amygdala, including in this subregion (Hopkins et al., 2015; Issa et al., 2019; Rilling et al., 2012; Staes et al., 2018; Stimpson et al., 2016). Investigation of receptor structure, in particular binding affinity of OT receptors, may additionally

403 reveal important species differences in light of genetic analysis showing polymorphisms in the
404 OXTR region that differ between the two species (Staes et al., 2014).

405 One limitation of this study is the relatively small number of ape participants tested, mainly
406 due to the rarity of opportunity that allowed us to do both OT administration and eye tracking with
407 apes. There are several points to be discussed regarding this issue. First, the relatively small number
408 of participants may have hampered the detection of significant overall species differences in the
409 looking durations towards eyes and mouth in this study. However, we are confident that increasing
410 the number of participants can solve this issue because 1) the previous study which included a
411 larger number of bonobos and chimpanzees (including the ape participants tested in this study)
412 yielded significant species difference in the looking duration to eyes, and 2) the ape participants in
413 this study showed high intra-individual consistency in looking duration to eyes between conditions
414 (Figure 2C) and also in comparison to the previous study (Kano et al., 2015) (Table S3). Second,
415 the relatively small number of ape participants in this study also limits systematic examinations of
416 individual differences. For example, among the six chimpanzees tested in this study, one
417 chimpanzee (Iroha, a 12-years-old, mother-reared female) showed an opposite tendency from the
418 other chimpanzees in her response to OT (Figure 2C). As we found no demographic parameter
419 unique to this chimpanzee (Table 1), this result remains difficult to interpret. Third, sex difference is
420 of potential interest as it is known to influence the effect of OT in human and nonhuman primates
421 (Domes et al., 2010; Insel and Young, 2000; Jiang and Platt, 2018a, b). In this study, most ape
422 participants were females, with one male in each species. It should be noted that these males'
423 responses to OT were not substantially different from the females (they did not show the strongest
424 or weakest responses in either species). Our follow-up test including stimulus sex in the model
425 (with eye-mouth difference score as a response) did not reveal the effect of stimulus sex (or the
426 interaction between this and other factors). However, it is worthwhile to test more directly whether

OT modulates each sex's responses to the same/different sex in bonobos and chimpanzees, particularly because bonobos and chimpanzees are known for distinctive dominance styles; namely, chimpanzee males are generally more dominant over females, while bonobo females are often more dominant than males through coordinating female coalitionary aggression towards males (Tokuyama et al., 2019). It remains unclear how OT might be involved in males' and females' attitudes towards different sexes in these species. Overall, the key contribution of this study is the addition of the knowledge that OT affects eye contact behavior of bonobos and chimpanzees differently, with some caution that such species differences could be female-biased.

Another limitation of our study is that, although we found changes in eye movement in both species after OT administration, we do not have strong external validation of the efficacy of administration procedures from hormonal measurement. Our results generally suggest that the administered OT did reach the central nervous system (and thereby caused a behavioral change), and one of our ongoing studies following the same OT administration procedures additionally found changes in eye movement patterns with a new set of stimuli in chimpanzees (Kawaguchi, personal communication). However, in our preliminary endocrinological tests examining whether OT administration increases urinary OT in chimpanzees (summarized in Supplementary material), we did not find a clear effect of OT administration, although we found some differences in the pattern of changes in urinary OT between the OT and saline conditions (sampling urine 15-90 minutes after OT administration). Our working hypothesis is that OT administered to the nasal cavity reached the central nervous system via direct nose-to-brain routes (Quintana et al., 2015) without necessarily reaching the periphery, while in previous studies with wild populations (Crockford et al., 2013; Samuni et al., 2017; Wittig et al., 2014) endogenous OT trigged by relevant social behaviors was secreted into the periphery from the hypothalamus. Clearly, further studies are necessary to examine administered OT's effects on behavior and physiology in these great ape species.

These potential limitations aside, one interesting implication from our results is that the observed opposite effect of OT on eye contact behavior may affect social interactions of bonobos and chimpanzees through the hypothesized biobehavioral feedback loop (Crockford et al., 2013; Wittig et al., 2014), and thereby promote species-typical patterns of social interaction in both species. That is, previous studies with wild bonobos and chimpanzees showed that urinary OT increased after grooming, reconciliation, food-sharing, and intergroup conflicts in chimpanzees (Crockford et al., 2013; Preis et al., 2018; Samuni et al., 2017; Wittig et al., 2014), and non-copulatory sexual contact (GG-rubbing) in bonobos (Moscovice et al., 2019). Such increases in endogenous OT may lead to different eye contact behavior in each species, as suggested in our experiments. In addition, great apes generally use eye contact to initiate and facilitate social interaction between individuals, such as an establishment of eye contact before attempting reconciliation in chimpanzees (De Waal, 1990a) and the maintenance of eye contact during GG-rubbing in bonobos (Annicchiarico et al., 2020; Moscovice et al., 2019). Therefore, OT-driven changes in eye contact behavior may result in a relatively large difference in social interaction between bonobos and chimpanzees via different biobehavioral feedbacks. It remains unclear whether eye contact per se triggers OT release in bonobos and chimpanzees, as was found in dogs and humans (Nagasawa et al., 2015). Thus, further tests with bonobos and chimpanzees are required.

In conclusion, we demonstrated that OT affects eye contact behavior differently in humans' two closest living relatives, bonobos and chimpanzees. This suggests that OT may have played a modulatory role in the evolution of species-typical behavior of bonobos and chimpanzees. Furthermore, this result underscores the diversified roles of OT through phylogeny. Thus, despite the fact that OT has generally conserved roles across mammals, we should be cautious about

474 generalizing results from one species to another, which echoes a message from the previous studies
475 (e.g. Insel, 2010).

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Table S1. Presentation duration of each AOI in each video (sec). For Body (and Screen) AOI, the presentation duration was maximal (3 min).

Video	Eye/Mouth	Face	Action target	Genital
Bonobo video 1	105.1	165.5	41.8	48.6
Bonobo video 2	109.1	163.6	54.2	48.0
Chimp video 1	105.2	163.8	33.3	39.6
Chimp video 2	114.1	167.3	11.8	59.9

Table S2. The model results for looking durations (sec) to each Areas-Of-Interest. The main effects of each term were examined when the interaction term was not significant (but were kept when they were significant or marginally significant). Intercepts were from the full models (with the interaction terms). Confidence intervals (CI) were from the parametric bootstrapping method with 1000 replicates. See Figure 2 and S2 for the means (and CIs) of looking duration to each Areas-Of-Interest. *$p < 0.05$, +$p < 0.1$.

Response	Term	Estimate	SE	CI_{lower}	Cl_{upper}	χ^2	p
Eye	Intercept	17.8	3.6	10.4	25.2	-	-
	Species:Condition	-3.4	1.4	-6.2	-0.4	4.9	0.028 (*)
Mouth	Intercept	15.9	2.8	10.4	21.6	-	-
	Species:Condition	3.6	1.9	-0.09	7.5	3.2	0.072 (+)
Eye minus mouth	Intercept	2.0	5.3	-8.6	12.6	-	-
	Species:Condition	-6.9	2.4	-12.0	-2.6	6.4	0.011 (*)
Face (as a whole)	Intercept	53.4	5.5	42.3	64.3	-	-
	Species:Condition	1.3	3.9	-6.6	9.6	0.1	0.74
	Species	-2.3	7.3	-17.4	12.9	0.1	0.75
	Condition	-0.7	1.9	-4.8	3.0	0.1	0.73
Action target [#2]	Intercept	-2.4	0.3	-3.0	-1.9	-	-
	Species:Condition	0.4	0.3	-0.2	0.9	2.2	0.14
	Species	1.0	0.4	0.2	1.8	4.4	0.036 (*)
	Condition	-0.2	0.1	-0.5	0.1	2.1	0.15
Genital [#2]	Intercept	-2.3	0.2	-2.8	-1.9	-	-
	Species:Condition	0.1	0.2	-0.3	0.7	0.3	0.56

	Species	-0.4	0.2	-0.9	0.09	2.4	0.12
	Condition	-0.1	0.1	-0.3	0.1	0.7	0.40
Body (as a whole)	Intercept	56.4	5.0	45.7	67.4	-	-
	Species:Condition	1.6	3.4	-5.6	8.5	0.3	0.61
	Species	0.9	6.8	-11.5	12.8	0.02	0.89
	Condition	-0.8	1.7	-3.9	2.2	0.2	0.65
Screen (as a whole)	Intercept	106.7	9.4	87.3	125.7	-	-
	Species:Condition	1.6	5.7	-9.3	13.3	0.08	0.77
	Species	31.7	12.8	4.8	58.6	4.9	0.028 (*)
	Condition	-2.5	2.8	-8.0	3.4	0.8	0.38

#1 All degrees of freedom were 1.

#2 For the action-target and genital AOIs, the looking duration was divided by the total presentation duration, and then logit-transformed.

Table S3. The comparison of participants' difference scores (indicated as 'Diff.') as the total looking duration (sec) for mouth subtracted from that for eyes in all trials (6 minutes in this study, and 4.5 minutes in the previous study) across conditions in this study and between this and previous study (Kano, Hirata, & Call, 2015). Also shown is the rank according to the difference scores to guide this comparison. See the details about participants in Table 1.

Species	Name	Oxytocin condition		Saline condition		Previous study	
		Diff.	Rank	Diff.	Rank	Diff.	Rank
Bonobo	Ikela	38.76	2	30.91	2	99.45	1
Bonobo	Connie-Lenore	-31.33	10	-33.57	11	0.27	9
Bonobo	Louise	18.82	3	7.99	4	62.55	3
Bonobo	Lolita	-22.28	9	-31.05	10	-16.2	11
Bonobo	Vijay	48.67	1	46.31	1	95.94	2
Chimpanzee	Hatsuka	-7.27	7	2.96	6	-7.2	10
Chimpanzee	Iroha	-1.37	6	-9.22	8	23.94	6
Chimpanzee	Zamba	-53.52	11	-30.01	9	8.73	8
Chimpanzee	Misaki	14.29	4	16.76	3	27.81	5
Chimpanzee	Mizuki	-14.19	8	-3.13	7	35.46	4

Chimpanzee	Natsuki	3.37	5	6.46	5	14.22	7

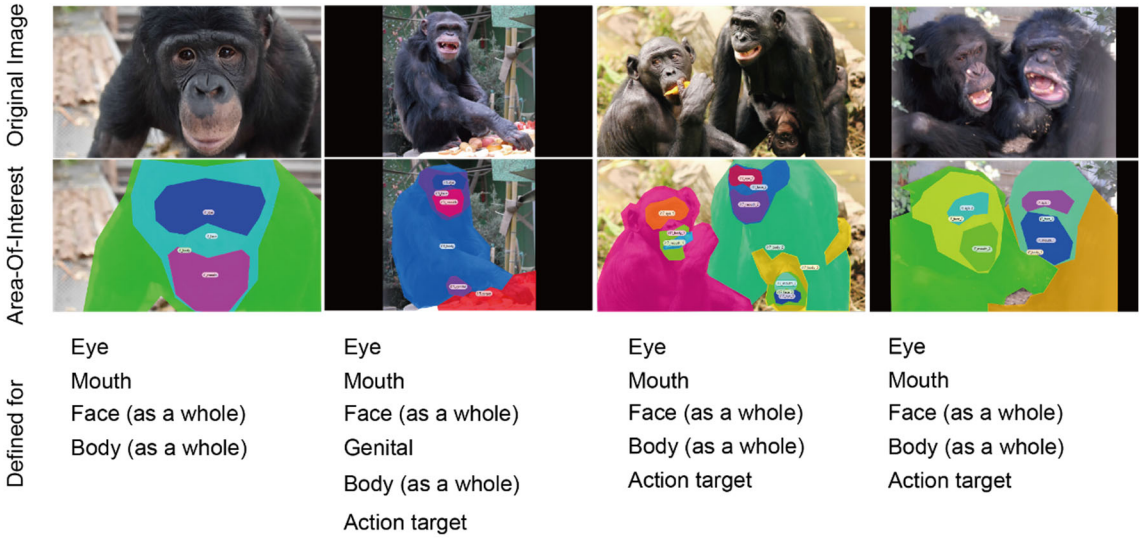

Figure S1. Examples for Areas-Of-Interests (AOIs) defined for the images.

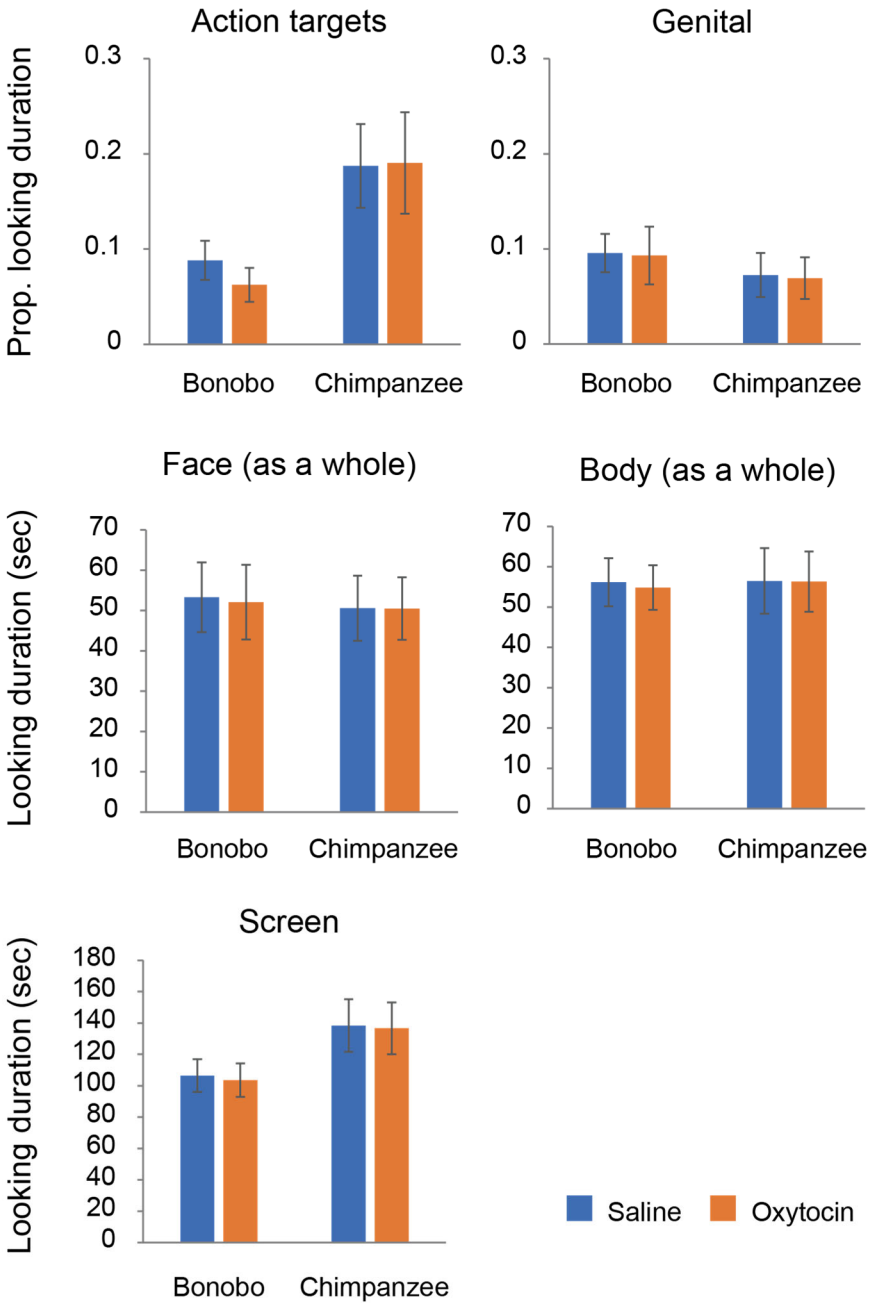

Figure S2. Mean looking durations (sec) for the action targets, face (as a whole), genital, and body (as a whole) in each trial (presenting a 3-min video). Error bars denote 95% confidence intervals. For the action-target and genital AOIs, the looking duration to each AOI was divided by the total presentation duration of AOI (and then logit-transformed in LMM).

Effect of stimulus properties.

We tested the effect of stimulus properties (facial expressions, gaze directions, group affiliation, and stimulus sex) in the model by restructuring the dataset and adding each stimulus property term to the original models: Eye-mouth difference score ~ Species*Condition*Stimulus property + Block/Trial + (1 + Condition*Stimulus property + Block/Trial || Subject). The model checks were performed as described in the main text. We dropped the interaction terms if they were not significant and then tested the lower-order interaction terms or the main effects of respective terms.

Ingroup vs. outgroup face: We included the group affiliation (ingroup, outgroup) into the model as a stimulus property term. The three-way interaction effect, and the two-way interaction effects between group affiliation and condition (this was marginally at $p < 0.1$; $\beta = 2.4$, SE = 1.4, $CI_{lower} = -0.4$, $CI_{upper} = 5.2$, $\chi^2 = 2.7$, $p = 0.098$), and between group affiliation and species were not significant (and thus these were dropped from the model). We found a significant interaction effect between species and condition ($\beta = -2.8$, SE = 1.1, $CI_{lower} = -5.3$, $CI_{upper} = -0.37$, $\chi^2 = 4.7$, $p = 0.030$), confirming the results described in the main text. Additionally, we found a significant effect of group affiliation ($\beta = -4.1$, SE = 1.1, $CI_{lower} = -6.4$, $CI_{upper} = -1.8$, $\chi^2 = 7.8$, $p = 0.0053$). The latter result and Figure S3 indicated that both species viewed the eyes of ingroup faces longer than those of outgroup faces.

Male vs. female face: We included the stimulus sex (male, female) into the model as the stimulus property term. The three-way interaction effect, and the two-way interaction effects between stimulus sex and condition, and between stimulus sex and species were not significant (and thus dropped from the model). We found a significant interaction effect between species and condition ($\beta = -2.8$, SE = 1.2, $CI_{lower} = -5.1$, $CI_{upper} = -0.5$, $\chi^2 = 4.6$, $p = 0.032$), confirming the results described in the main text. The main effect of stimulus sex was not significant ($\beta = -0.7$, SE = 0.9, $CI_{lower} = -2.6$, $CI_{upper} = 1.1$, $\chi^2 = 0.2$, $p = 0.69$). Although we could not test the effect of participant sex due to small number of males, excluding the two males, one male from each species (Vijay and Zamba), from the model yielded similar results.

Direct vs. averted gaze face: We included the gaze directions of faces (direct, averted) into the model as a stimulus property term. The three-way interaction effect was significant ($\beta = -6.7$, SE = 2.5, $CI_{lower} = -11.5$, $CI_{upper} = -1.9$, $\chi^2 = 5.7$, $p = 0.017$). The separate analyses by gaze direction revealed that OT affected bonobos and chimpanzees differentially when they viewed the faces with direct gaze ($\beta = -6.8$, SE = 2.0, $CI_{lower} = -10.6$, $CI_{upper} = -3.0$, $\chi^2 = 8.3$, $p = 0.0040$), but not when they viewed the faces with averted gaze ($\beta = -0.14$, SE = 1.1, $CI_{lower} = -2.5$, $CI_{upper} = 2.2$, $\chi^2 = 0.02$, $p = 0.90$). These results consolidated the idea that OT differentially modulated the two species' eye contact behavior (i.e. looks to direct gaze).

Facial expressions, neutral face, play face vs. grimace: We included the expressions of faces (neutral face, play face, grimace) into the model as a stimulus property term. The three-way interaction effect, and the two-way interaction effects between stimulus facial expression and condition, and between facial expression and species were not significant (and thus dropped from the model). We found a significant interaction effect between species and condition ($\beta = -1.9$, SE = 0.7, $CI_{lower} = -3.4$, $CI_{upper} = -0.5$, $\chi^2 = 5.9$, $p = 0.015$), confirming the results described in the main text. The main effect of facial expression was also significant (neutral vs. grimace, $\beta = -6.8$, SE = 1.9, $CI_{lower} = -10.6$, $CI_{upper} = -3.5$; ; $\chi^2 = 8.4$, $p = 0.004$; neutral vs. play face, $\beta = -6.2$, SE = 1.9, $CI_{lower} = -9.7$, $CI_{upper} = -2.2$; $\chi^2 = 7.1$, $p = 0.008$). These results and Figure S3 additionally indicated that both species viewed the mouths of emotional facial expressions longer than those of neutral faces.

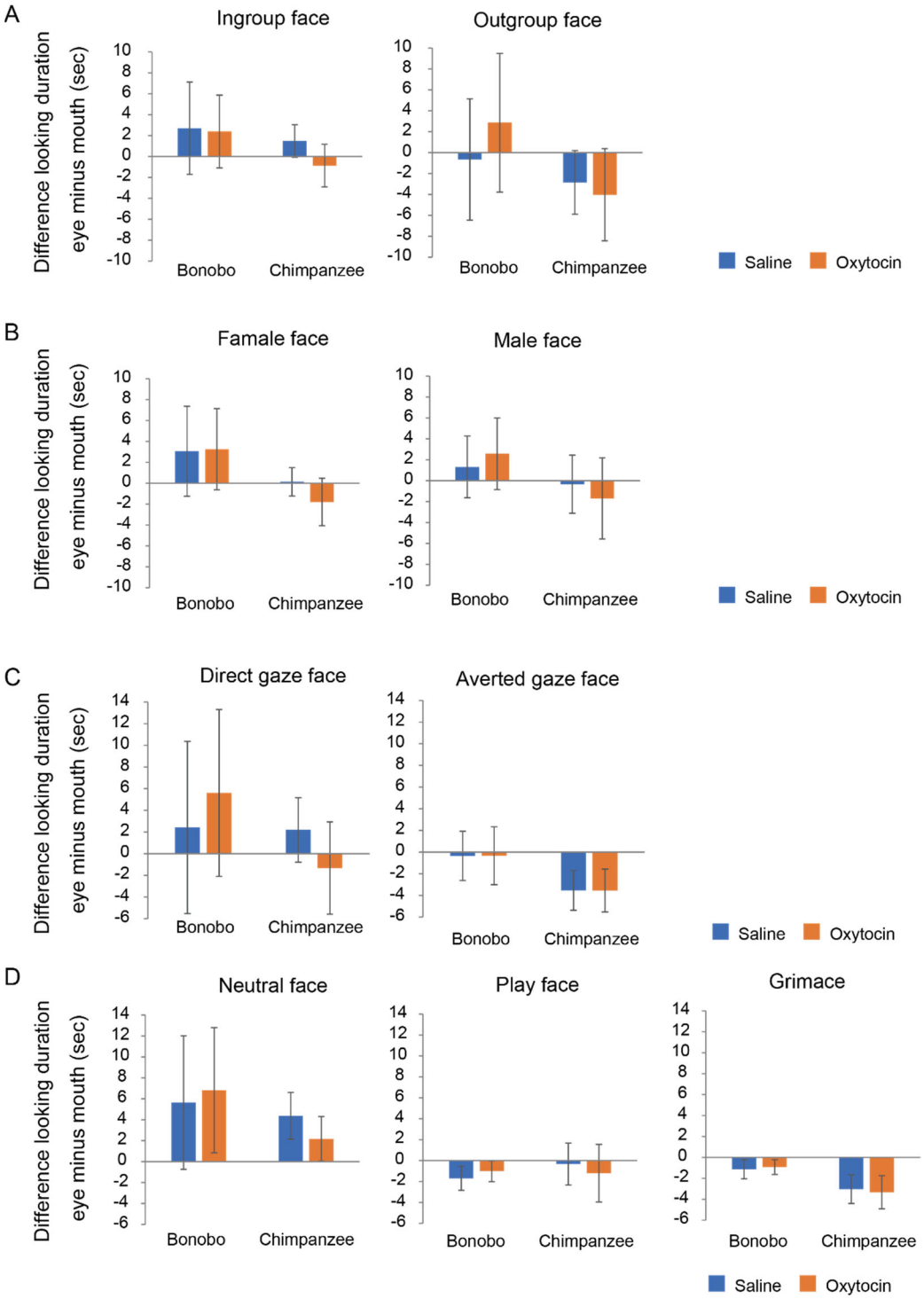

Figure S3. Mean eye-mouth difference looking duration (in sec, per trial) by each stimulus property; group affiliation (A), stimulus sex (B), gaze direction (C) and facial expression (D). Error bars denote 95% confidence intervals.

Checking the results with different model structures

One reviewer questioned whether we should use viewing time per stimulus (picture/scene, ~3s), not the sum of viewing time per each movie (180s), as one data point and then include stimulus ID as an additional random effect in the model, in order to secure a larger dataset and information pertaining to different stimuli. We decided to use the latter unit mainly because there were too many zeros in the data when formatted in the former unit, especially in the viewing times for the eye and mouth AOIs; this violates the assumption of homogeneity of variance in LMM and led us to make more complex models (more specifically, such responses essentially include two kinds of response, looked/non-looked, if looked, how long it was). Additionally, previous eye-tracking studies on which we modelled our study have used the latter unit (Kano et al., 2015; Kano et al., 2018). However, it may be important to check if our results are not confined to any particular model structure. Fortunately, we could use the former unit with one of our key models testing the species difference in the effect of OT on eye contact, namely, the model which used eye-minus-mouth viewing times by excluding cases in which the participant viewed neither eye nor mouth in a given stimulus (excluding 0 minus 0 to be distinguished from X minus X; this could be done because the main purpose of this analysis is to examine the shift of attention from eye/mouth to mouth/eye in the OT compared to the saline condition). In this analysis, we used the model formula: Response (eye-minus-mouth) ~ Species * Condition + Block/Trial + (1 + Condition + Block/Trial | Subject) + (1 + Condition + Trial |Stimulus ID)). The model checks were performed in the same way as described in the main text. We similarly found a significant interaction between species and condition (β = -0.18, SE = 0.08, CI_{lower} = -0.39, CI_{upper} = -0.002, χ^2 = 5.3, p = 0.022), confirming the results described in the main text.

Urinary analysis

Summary: We examined if urinary OT levels differentially increase after administration of OT and saline placebo. We particularly took the sampling timings after administration of OT/Saline with caution and conducted three tests (Test 1-3) which varied the sampling timings (Test 1: 90-120 min,

Test 2: 15-90 min, Test 3: 15-60 min, since the end time of administration). Overall, we did not find a clear OT effect in all three tests, although we found a marginally significant effect suggesting a differential pattern of change over time in urinary OT between conditions when we combined the data from the latter two tests (Test 2-3; i.e. 15-90 min). This unclarity in urinary analysis raised one concern that OT was not adequately administered to chimpanzees. However, given that bonobos and chimpanzees underwent the same administration procedure and most of individuals showed certain changes in eye-movement behavior, we think this possibility unlikely. One potential explanation is that intranasal OT reached the central nervous system and thereby affected behavior, but did not necessarily spread to the peripheral system or spread too little to be captured in our measurement system with chimpanzees. Further studies are necessary to test this idea. It should be noted that in previous studies which measured urinary OT after administration of OT in dogs (Nagasawa et al., 2015; Romero et al., 2014), administered OT led to certain behavioral changes as well as increase in urinary OT, but it is not clear whether the original (administered) OT reached urine; in fact the increase was not found in the absence of social interaction after OT/saline administration (Nagasawa et al., 2015). In previous studies which measured urinary OT in chimpanzees (Crockford et al., 2013; Samuni et al., 2017; Wittig et al., 2014), social interaction led to increase in urinary OT. There are mixed evidences showing that intranasally-administered OT reach to the peripheral system (i.e. blood) in macaques (Bauman et al., 2018). One study with capuchin monkeys showed an increase in urinary OT after intranasal administration of OT (Benítez et al., 2018), but unfortunately, an absence of saline placebo condition made an interpretation of this result somewhat difficult. Critically, no study has confirmed that intranasally administered OT per se reaches urine without behavioral mediations in apes, which we suspect unlikely following our preliminary results described below. Here, we submit these results as supplemental materials to help design future work.

Methods:

Sample collection. We conducted three tests using the same procedures expect the timings of urine sampling (Test 1-3). Six chimpanzees who also participated in our main behavioral study participated

in this series of tests. One chimpanzee (Zamba) did not participate in Test 1 but all six chimpanzees participated in Test 2-3. All chimpanzees had previous experience urinating for sample collection, so urine could be directly caught in a cup while chimpanzees were in a corridor overhead. We also tested bonobos but decided not to use their urinary samples because we collected their urines from the floor (as they did not have previous experience urinating for sample collection); we then found that there were substantial variations in OT and creatinine levels, most likely due to contamination of water and other substances from the floor.

Upon collection of urine samples, urine was pipetted into test tubes, and placed on dry ice in a Styrofoam box exactly 5 minutes after urination. Notes were taken regarding unique characteristics of the samples, including color and volume. Immediately after completion of all samplings in the daily test, the urine samples were brought to a deep freezer and frozen at -80 °C until shipment to a facility for analysis (during shipment, the samples were stored with dry ice in a Styrofoam box and placed in a freezer, -15 °C).

In all tests, prior to administration of either OT or saline, chimpanzees were brought to the overhead corridor and urine was caught in a plastic cup (baseline). Immediately following this baseline urine collection, chimpanzees were given either oxytocin or saline placebo control using the same methods as in the behavioral tests (up to 10 minutes). After a wait period, which differed between the tests (90 minutes in Test 1 and 15 minutes in Test 2-3), chimpanzees were again moved to the overhead corridor for post administration urine collection. Five chimpanzees provided one urine sample between 90-120 minutes following the completion of administration procedure in Test 1, and 6 chimpanzees provide multiple urine samples between 15-90 minutes and between 15-60 minutes following the completion of administration procedure in Test 2 and 3, respectively. The time period between 15-60 minutes in Test 3 was chosen to be consistent with the previous studies (Crockford et al., 2013; Samuni et al., 2017; Wittig et al., 2014). On a given day, half of subjects received oxytocin and half received saline, which was then counterbalanced on the next day of administration (minimum of 1 week later). Food and water intake were not restricted but carefully monitored. Chimpanzees were allowed to leave the corridor if they displayed any signs of discomfort, nervousness, or showed any attempt to leave,

although the most participants provided at least one sample before their leave in all tests.

Urine analysis. Urinary oxytocin was measured using commercially available competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit (ENZO Life Science, NY, UASA) either at a lab in Azabu University (Test 1-2) or its collaborator lab in Tokyo University of Agriculture (Test 3; AIRPLANTS BIO, Tokyo, Japan). Samples were centrifuged to remove dusts/tissues and then diluted by a factor of five. Sample extraction and concentration procedures were conducted following the official kit manual. Following incubation and washing (following kit instructions) results were calculated using a microplate reader 405nm. Final OT concentrations were calculated as pg OT per mg creatinine to control for variation in urine density.

Statistical analysis. As a dependent variable, we calculated the difference OT/cre score; we first divided the measured OT level (pg/ml) by the creatine level (mg/ml) for all samples, and subtracted the post-administration score from the baseline score. In Test 1, we compared between conditions using a paired t-test (one sample was collected from each participant in the post-administration period). In Test 2-3, we ran LMM in 'lme4' with Gaussian error structure and identity link function. We included condition and time of urination (since the completion of the administration procedure; this variable was standardized), and their interaction as test predictors. The model checks were conducted as described in the main text. We included participants as a random intercept. Random slopes were kept maximal. The final model was: post OT/cre ~ Condition*Time of urination + (1 + Condition*Time of urination | Subject). We used a likelihood ratio test to examine the significance of the terms. We dropped the interaction term if not significant, and then reran the model without the interaction term to examine the main effect of each term.

Results:

In Test 1, the difference OT/cre score did not differ between OT and saline conditions ($t(4) = 0.19$, $p = 0.86$). In Test 2, no effect was significant; the interaction effect ($\beta = -17.5$, $SE = 16.7$, $CI_{lower} = -55.1$, $CI_{upper} = 18.9$, $\chi^2 = 0.94$, $p = 0.33$); condition ($\beta = -34.6$, $SE = 29.3$, $CI_{lower} = -99.3$, $CI_{upper} = 31.7$, $\chi^2 = 1.5$, $p = 0.23$); time of urination ($\beta = -13.4$, $SE = 7.7$, $CI_{lower} = -35.2$, $CI_{upper} = 5.7$, $\chi^2 = 1.5$, $p = 0.23$).

In Test 3, no effect was significant; the interaction effect (β = -109.3, SE = 64.6, CI_{lower} = -244.1, CI_{upper} = 28.9, χ^2 = 2.3, p = 0.13); condition (β = 30.6, SE = 64.5, CI_{lower} = -109.6, CI_{upper} = 176.0, χ^2 = 0.2, p = 0.68); time of urination (β = 37.0, SE = 22.8, CI_{lower} = -11.2, CI_{upper} = 97.2, χ^2 = 1.9, p = 0.17). Combining the results from Test 2 and 3 revealed a marginally significant interaction effect between condition and time of urination (β = -91.6, SE = 46.6, CI_{lower} = -187.1, CI_{upper} = -7.4, χ^2 = 3.4, p = 0.065). Accidentally, we detected a larger variation and generally higher values in urinary OT (but not in urinary creatine) in Test 3 compared to Test 1-2. We initially suspected minor technical differences in urinary OT analysis caused such differences because the Test-3 samples were analyzed in a different lab (due to the COVID-19 influence). We thus reanalyzed the Test-3 samples in the same lab where the Test-1-2 samples were analyzed (after the settlement), but confirmed similar OT values. Thus, the change in variation in OT level may be attributed to some other factors that we could not controlled for (e.g. seasonal change). Despite variation in absolute values, in theory, the difference between the conditions (OT/saline) should remain the same.

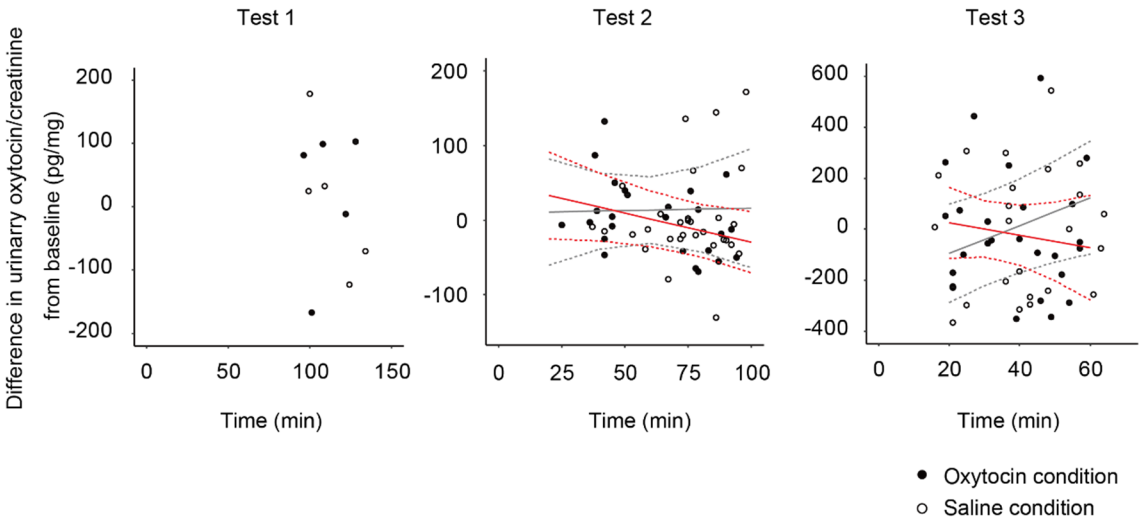

Figure S4. Difference in urinary oxytocin level (pg/ml) corrected for urinary creatine level (mg/ml) from baseline (pre-samples collected just before OT/saline administration). Solid and dotted lines indicate fitted values and confidence intervals, respectively (OT: red, Saline: gray). Regression lines and confidence intervals were drawn on the population level (without random-effects structure).

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