Accepted Manuscript

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PII: S0306-4530(16)30697-7
DOI: http://dx.doi.org/doi:10.1016/j.psyneuen.2016.09.010
Reference: PNEC 3392

To appear in:

Received date: 27-4-2016
Revised date: 13-9-2016
Accepted date: 15-9-2016

Please cite this article as: {http://dx.doi.org/

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Differential effects of oxytocin on social sensitivity in two distinct breeds of dogs *(Canis familiaris)*

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Highlights

Breed-specific effects of oxytocin on dogs' social responsiveness were found

Genetic and epigenetic factors might contribute to differential oxytocin effects

Border Collies are more human oriented than Siberian Huskies

Oxytocin affects some social behaviors irrespective of breed

Sex differences can be found in the effect of oxytocin on dogs

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ABSTRACT

Dogs have been proven to show several human-analogue social behaviors, and recent research raises the possibility that the oxytocin system is related to these. However, despite dogs' general tendency to excel in the domain of social cognition, there is increasing evidence that dogs' ability to utilize human signals may vary with breed. Moreover, breeds may show differences not only in their 'inborn' communicative abilities, but also in their learning skills related to these. The aim of the present study was to explore breed differences and breed-specific effects of oxytocin administration on different aspects of social responsiveness. Dogs from two markedly different breeds, Border Collies (cooperative workers) and Siberian Huskies (independent workers) were tested. After having received intranasal administration of oxytocin or placebo, subjects participated in three behavioral tests measuring social responsiveness. Our results show that there are several behavioral differences between the two breeds and also that there are differential effects of the oxytocin treatment. Border Collies were in general more susceptible to the 'social' effects of oxytocin compared to Siberian Huskies: after oxytocin administration they (1) looked more at the experimenter in the 'Unreachable food' situation, (2) looked more at the owner and shifted their gaze more between the sound source and the owner in a potentially dangerous situation, and (3) looked longer at the experimenter's eyes in the 'Tolerance of prolonged eye contact' test. These findings suggest that selection for enhanced cooperative abilities, possibly complemented by the effect of different social environments the two breeds experience, affects dogs' performance in several behavioral tests and that the neurohormonal background differently modulates social behavior in different working breeds.

Keywords

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Oxytocin; Dog *(Canis familiaris)*; Breed differences; Social responsiveness

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1. Introduction

There is growing evidence indicating that the effectiveness of intranasal oxytocin treatment in influencing human behavior can vary greatly. The effects of oxytocin are indeed constrained not only by features of situations but also by those of individuals (Bartz et al., 2011). In line with these findings, recent research has shown that behavioral responses to intranasal oxytocin treatment can be linked to oxytocin receptor (OXTR) genotype in humans (Feng et al., 2015; Marsh et al., 2012). OXTR polymorphisms, in turn, differentially influence social behavior of both different human populations (Chen et al., 2011) and dog breeds (Kis et al., 2014a). The present study investigates how social behavior is influenced by intranasal oxytocin treatment in two markedly distinct dog breeds (selected for different purposes).

Dogs are highly unusual in their phenotypic variation (Parker et al., 2004) and are thus ideal to study within-species individual differences. Indeed, the more than 400 living dog breeds show extreme morphological and behavioral plasticity (Svartberg 2006) and breed specific behavioral differences are often viewed as a consequence of the past selection during the breeds' origins (Scott and Fuller 1965). It is increasingly assumed that different aspects of social behavior such as enhanced cooperative ability and enduring attention have become a key requirement for the process of breed formation (Gácsi et al., 2009) and the behavioral repertoire of modern dog breeds generally reflects the function for which the dogs were originally used (Coppinger and Coppinger, 2001). Moreover, many of the dog breeds have been selectively bred to perform specific tasks (herding, sledding etc.) that required not only different morphological and behavioral features, but probably also various socio-cognitive capabilities (Hare and Tomasello, 2005).

In addition, some research suggest that types of work for which different dog breeds have been selected may also influence the ways in which they interact with humans (Miklósi

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et al., 2004). For example, Gácsi et al. (2009) propose that dog breeds can be categorized in terms of the communicative role they have been bred to fill in. Namely, there are 'cooperative workers' that were originally developed for cooperative tasks, in frequent visual contact with their human partner (e.g. herding dogs), whereas others, the 'independent worker' breeds, work with no or very little human visual contact (e.g. sled dogs).

Despite the above outlined variability between breeds, some changes that occurred during the process of domestication are common to dogs in general. These have led to a wide range of behavioral analogies between humans and dogs (Topál et al., 2009) and dogs often show human infant-like communicative receptivity in interaction with humans (Miklósi and Topál 2012). For example, dogs often use eye gaze cues in a flexible manner; they show a tendency to make eye contact with their owner in unsolvable tasks (Gaunet, 2008; Miklósi et al., 2005) and alternate their gaze between the potential human helper and the object of desire (Gaunet and Deputte 2011; Merola et al., 2012; Miklósi et al., 2000). Gaze alternation is a three-step sequence whereby the signaler alternates its gaze directly between a target and the partner, and it seems to fulfill the requirements of active information sharing since looking at the human partner − which is subsequently followed by looking towards the target − is functionally referential signaling (Lakatos et al., 2009; Soproni et al., 2001).

Dogs, however, resemble humans not only in their human-analogue social behaviors, but also in that the oxytocin system is related to their social behavior. Evidence suggests that affiliative interactions between dogs and humans have the potential to increase oxytocin in both partners (Handlin et al. 2011; Odendaal and Meintjes 2003). Making prolonged eye contact with a human partner produces intense emotional reactions and a substantial rise of circulating oxytocin in both dogs (Nagasawa et al., 2015) and humans (Gordon et al., 2010). Other studies have shown that polymorphisms in the regulatory region of the oxytocin receptor gene are related to human-directed social behaviors in dogs (Kis et al., 2014a) and

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that intranasal oxytocin administration influences dog's behavior in a wide range of contexts (Kis et al., 2015; Romero et al., 2014).

Concerning the potential breed differences in the domain of social cognition, there is increasing evidence that dogs' ability to utilize human signals may vary with breed. Wobber and colleagues (2009) found that cooperative worker dogs (e.g. shepherds) use human gaze cues more skillfully than independent workers (e.g. sledge dogs). Cooperative worker breeds were also found to be significantly more successful in utilizing the human pointing gesture (Gácsi et al., 2009). Moreover, breeds may show differences not only in their 'inborn' communicative abilities, but also in their learning skills related to these (Jakovcevic et al., 2010).

Based on these findings, the purpose of the present study is to explore the breed differences and the breed-specific effects of oxytocin administration on different aspects of dogs' social responsiveness. Specifically, we investigate (1) tendency to make eye contact with a human partner and gaze alternations between the target object and the human in an unsolvable task, (2) tendency to use referential looking towards the owner, when facing an ambiguous (potentially dangerous) stimulus and (3) tolerance of prolonged eye contact with a human in an emotionally neutral situation. Two markedly different breeds, a cooperative worker (Border Collie) and an independent worker (Siberian Husky) were selected for the study that belong to different genetic clusters (Parker et al., 2004). We predicted that dogs in these two groups differ in sociability, that is, dogs from the breed type that has been selected for cooperation in visually guided tasks would show superior performance in the use of gaze cues as compared to dogs from independent work breed. Moreover we expected that oxytocin administration would increase the use of the communicative signals in both breeds, including breed-specific changes.

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2. Materials and methods

2.1. Ethics Statement

This research was done in accordance with the Hungarian regulations on animal experimentation and the Guidelines for the use of animals in research described by the Association for the Study Animal Behaviour (ASAB). Ethical approval was obtained from the National Animal Experimentation Ethics Committee (Ref No. XIV-I-001/531-4-2012). The owners volunteered to participate and gave written informed consent.

2.2. Subjects

N = 19 adult Border Collies (9 males and 10 females; 6 neutered; mean ± SD age: 3.5 ± 2.0 years) and N = 19 adult Siberian Huskies (8 males and 11 females; 15 neutered; mean ± SD age: 4.8 ± 1.9 years) kept as pet dogs were recruited and tested at the Department of Ethology, Eötvös Loránd University, Budapest, Hungary. According to owner reports there were both similarities and differences between the two breeds in their socialization background and living conditions (for details see Supplementary Table S1). Although Huskies were older when purchased by their owners, the duration of living together with the owner was not different between the two breeds. Moreover, both breeds had similar time activity patterns (time spent indoors vs. outdoors) but more Border Collies have participated in regular training classes.

Subjects were tested in three situations to assess social responsiveness after oxytocin or placebo treatment in a between-subject design. Based on pilot results of 8 dogs (4 Border Collies and 4 Siberian Huskies; 4 males, 4 females, mean ± SD age: 3.0 ± 1.3 years) in a within subjects design, we found a substantial habituation effect of subjects' behavior in all tasks, therefore testing was conducted in a between subject design.

2.3. Procedure

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2.3.1. Oxytocin or placebo treatment

Dogs received a single intranasal dose of 12 IU (3 puffs) oxytocin (Syntocinon, Novartis) or placebo (isotonic sodium chloride 0.9% solution) in a double blind design that has already been proved to have both physiological (decreased heart rate and increased heart rate variability) and behavioral effects in dogs (e.g., Hernádi et al., 2015; Kis et al., 2015; Kovács et al., 2016). The oxytocin or placebo administration was followed by a 40-minute-long waiting period (following the protocol by Kis et al., 2014b) that is presumed to be necessary for the central neuropeptide levels to reach plateau (Born et al., 2002). During this waiting period, dogs spent the first 25 minutes with an on-leash walk at the University Campus (avoiding any contact with other dogs or humans) during which the experimenter ensured that the owner did not make any social contact with the dog either (e.g. did not pet or talk to it) and kept the length as well as the speed of the walk as standard as possible. Then for the remaining 15 minutes the owner and the dog were quietly sitting in an isolated room. During this time the dog was free to move and the owner was sitting and filling in questionnaires about their dog keeping practices while ignoring the dog.

2.3.2. Behavioral tests

All test sessions were conducted in two adjacent experimental rooms (room A: 4.5 × 3.5 m; room B: 3 × 5 m) that were unfamiliar to the dogs. The test series was preceded by a 5-minute-long habituation period when subjects were allowed to explore the rooms freely, while the owner was informed about his/her tasks during the test by the experimenter. All subjects participated in the same three test situations in a fixed order, measuring different aspects of their communicative behavior. After every test, the owner and the dog left the room and waited in the corridor until the room was prepared for the next test (approximately 2 minutes);

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during this time drinking water was offered to the dog. All tests were video recorded for later analysis.

Dogs first participated in the *Unreachable food* task, originally developed by Miklósi et al (2003). This procedure allows us to explore whether, and if so how, dogs change their communication towards a potential human helper when reachable food reward suddenly becomes unreachable to the dog and whether intranasal administration of oxytocin could modify this behavior (for video protocol and photograph see Supplementary material S2). Dogs were presented with four 'solvable' trials (i.e., the dog could reach food reward through an open door of a 64 × 100 × 73 cm wire mesh cage) and this was followed by a single 'blocked' (closed door) trial. Trials were recorded in room A which was empty except for the experimental cage in the middle. At the beginning of each trial, the owner (O) was standing quietly at a predetermined point (next behind the dog, while holding it by its collar) approximately 1.5 m from the cage. The experimenter (E) was standing motionless behind the dog in the opposite corner of the room. Then the E went to the cage, called the dog's attention with a piece of food in her hand (Name! + looking at the dog), and placed it in the center of the cage through a 52 × 52 cm opening. Then she fixed the door in open position and stepped back to her predetermined place (behind the dog). In this moment, the O let the dog free and it was allowed to move freely until the food reward was obtained. After the fourth solvable trial the O and his/her dog left the experimental room, while the E put ten pieces of food in the cage and closed the door so that henceforward the dog could not get the food through the opening. Then the E returned to her predetermined place (in the corner), the O and the dog re-entered the room, and took up their starting position (in the corner – see above). After this, the O released the dog to explore the cage. During this 60 seconds-long 'Blocked trial' both the E and the O were standing quietly at their predetermined place. Importantly, however, both the

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O and the E were watching the dog during the 'Blocked trial' thus giving the dog a chance to make eye contact.

The *Unreachable food* task was followed by the *Potentially dangerous object* task (for a similar task see Merola et al., 2012). This task was used to explore the dogs' tendency to display referential looking (a form of information seeking behavior when facing a potentially dangerous object) towards humans, and, whether intranasal administration of oxytocin could modify this behavior in the two distinct dog breeds (for video protocol and photograph see Supplementary material S2). The test was conducted in room B which was empty except for a speaker (34 × 39 × 22 cm) covered by a blanket in the middle. The O entered the room with the dog on a leash, stepped up to a predetermined point (in the left corner), unleashed the dog and then was standing there motionless. At the moment when the O unleashed the dog, the E who was waiting in the adjacent room played back a 5-second growling sound through the speaker (stimulus from Faragó et al., 2010). The E played back the sound four times in total with 5 s pauses between the repetitions.

Finally, dogs were presented with a *Tolerance of prolonged eye contact* trial (similar to that used by Hernádi et al., 2012) in order to assess whether, after having received intranasal oxytocin, dogs would show a higher tendency to keep eye contact with an unfamiliar human (for video protocol and photograph see Supplementary material S2). Dogs' readiness to establish and maintain eye contact with the E was recorded in room B. The O was sitting on a chair opposite to the E (at a distance of 1 m) while holding the dog by its collar between his/her legs facing the E. The dog was allowed to sit, stand or lay down, but the O had to prevent it from walking away by holding its body, without talking to it. The E sat on a chair quietly facing the dog and looking at it. At the beginning of the trial the E called the dog by its name only once and when the dog made eye contact with her (it happened in less than

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21 seconds for all subjects), she tried to keep continuous eye contact with it. At the moment when the dog averted its gaze from the E, the trial was terminated.

Buccal DNA samples were non-invasively collected after the test from N = 34 of the dogs participating in the study (N = 16 Border Collies and N = 18 Siberian Huskies) for a preliminary gene × behavior analysis (see Supplementary material S3 for details).

2.4. Behavioral variables and statistical analyses

Behaviors displayed by the dog during the three tasks were coded blindly to experimental conditions, frame-by-frame, using a 0.2 second resolution in Solomon Coder (version beta 16.06.26; http://solomoncoder.com/). Inter-rater reliability for dogs' behavior was calculated by double coding 30% of the video recordings by two independent coders (Cronbach's Alpha ≥ 0.796 for all variables). Statistical analyses were carried out using R 3.2.3 (RCoreTeam, 2015).

In the *Unreachable food task* we recorded the latency to approach the food during the four training trials and the blocked trial was analyzed by measuring different aspects of social attention: (1) latency to first looking at the O; (2) latency to first looking at the E; (3) proportion of trial time spent looking at the O; (4) proportion of trial time spent looking at the E; (5) number of gaze shifts between the cage and the O; (6) number of gaze shifts between the cage and the E during the trial. In addition, a non-social looking behavior was coded: (7) proportion of trial time spent looking at the cage. Latencies to approach the food during the training trials as well as latencies to first looking at the O and the E during the blocked trial (response variables) were analyzed in separate Cox Models (R package 'survival'; Therneau, 2015a) with occurrence of looking as terminal event. Dogs that did not look at the human partner (O/E) within 60 seconds were treated as censored observations (N = 5 and N = 10 (of 38) censored dogs, respectively). Proportion of trial time spent looking at the O and the E

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(response variables) were analyzed with Tweedie Generalized Linear Models (Tweedie GLM; R packages 'stats' and 'statmod'; (RCoreTeam, 2015; Smyth et al., 2016) to take into account excess zeros in the dataset due to dogs that did not look at the O or the E. Number of gaze shifts between the cage and the O and the cage and the E were analyzed by using two separate Zero-inflated Negative Binomial GLMs (ZINB GLM, R package 'pscl'; Jackman, 2015). Proportion of trial time spent looking at the cage was analyzed in General Linear Models. Latency to approach food during the four training trials were analyzed in Cox Mixed Models (R package 'coxme'; Therneau, 2015b).

In the '*Potentially dangerous object'* situation we also measured both social and non-social aspects of dogs' behavior: (1) latency to first looking at the O; (2) proportion of trial time spent looking at the O; (3) number of gaze shifts between the speaker and the O; (4) latency to first approaching the sound source (within 10 cm); (5) proportion of trial time spent looking at the speaker. Latency to first looking at the O and first approaching the sound source (response variables) were analyzed in two separate Cox Models with occurrence of looking or approaching as terminal event. Dogs that did not look at the O/approached the speaker within 30 seconds were treated as censored observations (N = 9 and N = 14 (of 38) censored dogs, respectively). Proportion of trial time spent looking at the O (response variable) was analyzed in Tweedie GLMs, number of gaze shifts between the speaker and the O was analyzed using Negative Binomial GLMs (NB GLM; R package 'MASS'; Venables and Ripley, 2002), proportion of trial time spent looking at the speaker was analyzed in GLMs.

In the '*Tolerance of prolonged eye contact*' trial the duration of first eye contact with the E was coded and analyzed in Linear Models (LM, R package 'stats'; RCoreTeam, 2015).

In all above Cox Models, Tweedie GLMs, NB GLMs, ZINB GLMs, GLMs, Gamma GLMs and LMs, the full models included sex (male or female), breed (Border Collie or

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Siberian Husky) and treatment (oxytocin or placebo) as fixed factors with two levels, and all two-way interactions. Model selection was based on AIC values, and the effects of explanatory variables were analyzed by likelihood ratio tests: we provide χ^2 and p values of likelihood ratio tests of models with and without the explanatory variable. For Cox Models, hazard ratio (Exp[β]) between levels of a given fixed effect with 95 percent confidence interval are given. For GLMs, we provide parameter estimates (B) of significant factors with 95% CI.

3. Results

3.1. Dogs' behavior in the Unreachable food situation

During the four solvable (training) trials 100% of the dogs ate the food. Latency to approach the food was not affected by treatment (oxytocin/placebo), breed, sex or their interactions (Cox Mixed Models, all $p > 0.173$), but trial number had a significant effect as dogs in the first trial less likely approached the food after a given time elapsed, compared to the other three trials (Cox Mixed Model, $\chi^2_{(3)} = 30.002$, $p < 0.001$; Exp(β) ± SE: trial 1 → trial 2 = 2.499 ± 0.280, trial 1 → trial 3 = 4.146 ± 0.288, trial 1 → trial 4 = 3.445 ± 0.286). The analysis of the latency to first looking at the O during the blocked (unsolvable) trial showed that oxytocin-treated dogs less likely looked at the O after a given time elapsed than those receiving placebo treatment (Cox Model, treatment: $\chi^2_{(1)} = 6.970$, $p = 0.008$, Exp(β) = 0.386 [0.190; 0.782]; Figure 1a), however, probability of having looked at the O was not different between breeds ($\chi^2_{(1)} = 1.785$, $p = 0.182$) and sexes ($\chi^2_{(1)} = 0.662$, $p = 0.416$).

Moreover, experimental treatment tended to have different effects on probability of having looked at the E after a given time elapsed in the two breeds as reflected by a near-significant interaction (Cox Model, breed × treatment: $\chi^2_{(1)} = 3.752$, $p = 0.053$, Exp(β) = 0.200 [0.039; 1.022]; Figure 1b). The interaction was driven by oxytocin-treated Border Collies (but

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not Siberian Huskies) looking more likely to the E than those treated with placebo. In addition, females (independent of breeds) more likely looked at the E than males ($\chi^2_{(1)}$ = 8.920, p = 0.003, Exp(β) = 3.836 [1.495; 9.841]).

Experimental treatment had no effect on the proportion of time spent looking at the O (Tweedie GLM, treatment: $\chi^2_{(1)}$ = 2.240, p = 0.371). Border Collies spent more time looking at the O than Siberian Huskies (breed: $\chi_{(1)}$ = 4.119, p = 0.006, B = 0.999 [0.281; 1.688]; Figure 2a) and in both breeds, females spent more time looking at the O than males (sex: $\chi^2_{(1)}$ = 2.287, p = 0.039, B = 0.754 [0.025; 1.446]).

Experimental treatment, however, had sex and breed-specific effects on proportion of time spent looking at the E (Tweedie GLM, sex × treatment: $\chi^2_{(1)}$ = 3.490, p = 0.011, B = -3.106 [-5.603; -0.612]; breed × treatment: $\chi^2_{(1)}$ = 2.691, p = 0.026, B = -2.103 [-3.974; -0.271]). The sex-specific effect was driven by male (but not female) dogs spending more time looking at the E when treated by oxytocin. The breed-specific effect was due to Border Collies (but not Siberian Huskies) spending more time looking at the E when treated with oxytocin (Figure 2b).

Number of gaze shifts between the cage and the O was not different between treatments and sexes (ZINB GLM, treatment: $\chi^2_{(1)}$ = 1.108, p = 0.293; sex: $\chi^2_{(1)}$ = 0.240, p = 0.624). However, more gaze shifts were observed in Border Collies than in Siberian Huskies ($\chi^2_{(1)}$ = 9.707, p = 0.002, B = 0.675 [0.295; 1.055]; Figure 3a). None of the investigated variables had significant effect on the number of gaze shifts between the cage and the E (NB GLM, treatment, breed, and sex: all p > 0.724; Figure 3b).

Duration of looking at the cage was not influenced by any of the factors investigated (treatment, breed, sex), nor by their interactions (all p > 0.097).

3.2. Dogs' response to a 'Potentially dangerous' object

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Probability of having looked at the O in the 'Potentially dangerous object' situation was not different between experimental treatments (Cox Model, treatment: $\chi^2_{(1)} = 0.003$, p = 0.956). Females more likely looked at the O than males (sex: $\chi^2_{(1)} = 4.687$, p = 0.030, Exp(β) = 2.568 [1.059; 6.227]; Figure 4), whereas Siberian Huskies less likely looked at the O than Border Collies (breed: $\chi^2_{(1)} = 8.967$, p = 0.003, Exp(β) = 0.264 [0.106; 0.659]; Figure 4).

Oxytocin had a breed-specific effect on the proportion of time spent looking at the O (Tweedie GLM, breed × treatment: $\chi^2_{(1)} = 3.171$, p = 0.009, B = -2.377 [-4.168; -0.581]; driven by oxytocin-treated Siberian Huskies spending less time looking at the owner than oxytocin-treated Border Collies. In addition, female dogs spent more time looking at the O (sex: $\chi^2_{(1)} = 2.370$, p = 0.025, B = 0.999 [0.127; 1.872]).

Number of gaze shifts between the speaker and the O was also differently influenced by oxytocin treatment in the two breeds (NB GLM, breed × treatment: $\chi^2_{(1)} = 6.599$, p = 0.010, B = -2.154 [-3.761; -0.614]; Figure 5). The interaction was driven by opposite effect of oxytocin in the breeds; in Border Collies, oxytocin increased the number of gaze shifts, whereas in Siberian Huskies, oxytocin resulted in less gaze shifts. Moreover, gaze shift was higher in females than in males (sex: $\chi^2_{(1)} = 5.506$, p = 0.019, B = 1.035 [0.207; 1.911]).

Probability of having approached the sound source was not influenced by experimental treatment (Cox Model, treatment: $\chi^2_{(1)} = 2.338$, p = 0.126). We found sex and breed-specific reactions in this situation (sex × breed: $\chi^2_{(1)} = 8.213$, p = 0.004, Exp(β) = 0.095 [0.018; 0.492]). This interaction was driven by opposite reactions of the sexes in the breeds: in Border Collies females, while in Siberian Huskies males approached more likely the speaker than the opposite sex.

Duration of looking at the sound source was not influenced by experimental treatment (GLM: $\chi^2_{(1)} = 2.415$, p = 0.120), but the breeds showed sex-specific reactions in this situation

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(sex × breed: $\chi^2_{(1)} = 4.461$, p = 0.035, B = 0.319 [0.003; 0.635]) resulting from female Siberian Huskies, but nor Border Collies, looking more at the speaker than males.

3.3. Dogs' response in the Tolerance of prolonged eye contact situation

Oxytocin treatment had a breed-specific effect on the duration of first eye contact with the E in the 'Tolerance of prolonged eye contact' situation. In Border Collies, oxytocin increased the tendency to maintain eye contact whereas in Siberian Huskies, oxytocin resulted in a decrease (LM, breed × treatment: $\chi2(1) = 4.430$, p = 0.035, B = -0.073 [-0.146; 0.001]; Figure 6). Besides this interaction, duration of first eye contact was longer in females than in males (sex: $\chi^2_{(1)} = 7.994$, p = 0.005, B = 0.051 [0.014; 0.088]).

4. Discussion

Increasing evidence suggest a significant effect of intranasal oxytocin on different aspects of social behavior in dogs (for a review see Thielke and Udell, 2015). However, previous studies all included mixed samples of dogs from various breeds. In the present study evidence was found, for the first time, that the neuro-hormonal background relating to the oxytocin system has different impact on dog breeds selected for different work purposes. In all three test situations we found a combined (interactive) influence of breed and oxytocin treatment on some aspects of human-directed social behavior in dogs. Namely, in the *Unreachable food* task Border Collies (but not Huskies) tended to look at the potential helper (i.e. the experimenter who has previously demonstrated her ability to manipulate the apparatus) sooner and they also spent more time looking at the experimenter after receiving oxytocin. Moreover, in the *Potentially dangerous object* situation oxytocin-treated Border Collies spent more time looking at their owners and showed more 'social referencing' (i.e.

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shifted their gaze more frequently between the sound source and the owner) than oxytocin-treated Huskies. This finding adds further details to previous results (Hernádi et al., 2015) that in a threatening approach situation (when the object of the threat is a slowly approaching human) oxytocin-treated dogs looked more frequently at the human (owner or experimenter) standing behind them compared to placebo-treated subjects. The differential effects of oxytocin treatment on behavior is also evidenced in Border Collies' and Siberian Huskies' tendency to maintain eye contact.

It has been recently argued that the field of intranasal oxytocin research would in general benefit from increasing sample sizes and/or conducting replication studies (Walum et al., 2016), and our pioneering results (although using the conventional, relatively low, sample size) suggests that taking into account the specific population in which the study was conducted is also a crucial validity issue. Our study populations (Siberian Huskies and Border Collies) have been selected for markedly different purposes (cooperative versus independent work) and they belong to different genetic clusters (Parker et al., 2004). Thus genetic differences between these breeds in the oxytocin system could be (at least partly) responsible for the differential effects of oxytocin treatment on behavior. This is also consistent with previous reports indicating that the genetic component (polymorphisms in the dog DRD4 gene) is a key factor of looking behavior towards humans in unsolvable task situations (Hori et al., 2013). We should note however, that a recent study investigating single nucleotide polymorphisms (SNPs) in the oxytocin receptor (OXTR) gene has found very similar allele frequency distribution in Border Collies and Siberian Huskies (Kubinyi et al., unpublished). This is in line with the genotype distribution that we found in our preliminary analysis (see Supplementary materials S3). This preliminary gene × behavior analysis also highlights that polymorphisms in the OXTR gene might indeed be a biologically relevant underlying factor

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explaining some variance in IN-OT reactivity, although our preliminary analysis will need to be carried out with an increased sample size in order to confirm the findings.

Although the oxytocin system includes genetic components other than the OXTR gene that can potentially cause behavioral differences between breeds, additional (non-genetic) factors should also be considered. For example, experience during ontogeny has been shown to have an enduring effect on behavior, and the oxytocin system (sensitivity to this neurohormone) might also be modulated through epigenetic effects (Kumsta et al., 2013; Apter-Levy et al., 2013; Feldman et al., 2010). Indeed, subjects of the two breeds included in the present study differed in some aspects of their socialization background (see in Subjects section and Table S1). Despite similar living conditions of Border Collies and Huskies at the time of testing, the two breeds differed in some aspects of early (social) environmental factors. Thus future research should look at the environmental background of dogs (including owner-related factors such as gender and personality) and how these relate to oxytocin-sensitivity. This is especially important from an applied perspective as environmental factors may have the potential to modify oxytocin-related behavioral changes in different dog breeds.

Unfortunately very little is known about the oxytocin system in these two (and other) dog breeds. We have some information about the polymorphic variations in the OXTR gene (Bence et al., 2013; Kis et al., 2014a), but the potential differences in baseline oxytocin levels across breeds – that might contribute to their differential reaction to oxytocin treatment – have not yet been investigated. The potential role of epigenetic factors contributing to breed differences is also raised by Passalacqua et al. (2011) who found that hunting and herding breeds (to which Border Collies belong) looked at a person more than dogs from Mastiff-like and ancient breeds (to which Siberian Huskies belong) at the age of 4 month and when adults, but breed group differences were not seen in 2-month-old puppies. Therefore it is reasonable to assume that markedly different dog breeds (such as Border Collies and Siberian Huskies)

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develop different social behaviors as a consequence of different experiences during ontogeny as they are kept for different purposes, undergo different trainings, etc. In fact, training experience has been shown to affect dogs' performance in a wide range of tasks including independent problem solving as well as human-directed communicative abilities (Marshall-Pescini et al., 2016). Moreover, dogs' relationship with the human participants (e.g. their owner) is another factor that may have differential effects on Border Collies and Siberian Huskies (see Horn et al., 2013 on how dog-human relationship affects problem-solving behavior).

Our results (together with the findings of Kis et al., 2014a) suggest that we should be cautious in concluding that oxytocin uniformly facilitate social behaviors in dogs. Similar claims have been made in the human literature (Bartz et al., 2011), however many researchers still report their findings as generalizable to a wider population.

In addition to breed × treatment interactions, relatively robust main effects of treatment, breed and sex were also found. Results show that after oxytocin administration (compared to placebo treatment), dogs in general were less likely to look at their owner after a given time elapsed in the *Unreachable food* situation. Importantly, the owner was not actively involved in this task and thus looking at the owner may merely reflect task-related anxiety that can be reduced by oxytocin treatment. The finding that oxytocin treatment does not affect dogs' looking and approach behavior towards nonsocial targets (cage containing food in the *Unreachable food* task and speaker in the *Potentially dangerous object* situation) also supports the idea that the oxytocin system is involved in the regulation of social (but not non-social) attention in dogs.

Further, in accordance with previous studies on breed differences (e.g. Gácsi et al., 2009) our results also suggest that Border Collies appeared to be more human oriented as indicated by an increased duration of looking at the owner and more gaze-shifts when faced

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with an unsolvable problem which may reflect the dog's tendency to interact with the owner in order to 'ask for help'. This is also supported by Border Collies' shorter latency to look at the owner in the presence of a potentially dangerous object. Although a potential confound in the investigation of breed effects is that behavioral coding cannot be blind to this factor (e.g. the coder will always see the breed of the given subject), the fact that we found high consistency across two coders is a hint that no such bias was included in the present dataset.

Another important factor that needs to be considered in oxytocin research is the effect of sex. It has been shown in both humans (e.g. Herzmann et al., 2013) and dogs (Kovács et al., 2016; Nagasawa et al., 2015) that oxytocin can have differential effects on males and females. Sex differences may be rooted in the differences in oxytocin receptor affinity because steroid hormones, such as estradiol and progesterone, have the potential to modulate the OXT receptor (estradiol enhances OXT receptor affinity, while progesterone has been shown to decrease receptor binding — Choleris et al., 2008; Gimpl et al., 2002). In line with this a combined effect of treatment and sex was found in the unsolvable task (on the duration of looking at the experimenter). A potential confound to sex × oxytocin treatment interactions in this and previous research (on both dogs and humans) is that as males and females significantly differ in body weight, the per-kilogram doses of intranasally administered oxytocin systematically differ between sexes. The effect of sex on behavior was also found to be in interaction with breed in the social referencing test (*Potentially dangerous object* situation, latency to approach the sound source). This might be due genetic differences in the estradiol and progesterone system of the two species and/or due to sex differences in proneness to epigenetic modification of the oxytocin system during ontogeny. Sex differences were also found regardless of oxytocin administration and breed: female dogs looked longer into the eyes of the owner (*Unreachable food* and *Potentially dangerous object* situation) and they looked more likely to the experimenter (*Unreachable food* task) and longer (*Tolerance of*

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prolonged eye contact test) compared to male dogs. Previous studies (Duranton et al., 2015; Muller et al., 2011) have also documented sex differences in dog behavior, although these investigations have focused on physical and not social cognition. However, sex differences in social behaviors can also be expected because social motivation — which appears to differ between the sexes — may be a driving force behind the development of sex differences in social skills (Christov-Moore et al., 2014). Furthermore sex × breed interactions in non-social behaviors were also found in the present study (*Potentially threatening object* task, probability of having approached the sound source and duration of looking at the sound source).

In conclusion our study provides experimental evidence that oxytocin administration can have differential effects on social responsiveness of two dog breeds selected for independent and cooperative work respectively. Also, the present results show that oxytocin can have disparate impact on the performance of male and female dogs and that these effects can be in interaction with dog breed.

Acknowledgement

The authors are grateful to Ádám Miklósi (Eötvös Loránd University) and Zsolt Rónai (Semmelweis University) for their support. Financial support was provided by the Hungarian Science Foundation (OTKA grants K-112138 and K-109337).

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Role of the funding source

The founders had no role in the design of the experiment, analysis of the data and publication of the manuscript.

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Figure captation

Figure 1 Probability of having looked at the owner (a) and the experimenter (b) in placebo and oxytocin-treated Border Collies and Siberian Huskies after certain time elapsed in the 'Unreachable food' task.

Figure 2 Proportion of trial time spent looking at the owner (a) and the experimenter (b) in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Unreachable food' task.

Figure 3 Number of gaze shifts between the cage and the owner (a) and the cage and the experimenter (b) in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Unreachable food' task.

Figure 4 Probability of having looked at the owner after a certain time elapsed in male and female Border Collies and Siberian Huskies in the 'Potentially dangerous object' situation.

Figure 5 Number of gaze shifts between the sound source and the owner in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Potentially dangerous object' situation.

Figure 6 Duration of the first eye contact with the experimenter in placebo and oxytocin-treated Border Collies and Siberian Huskies in the 'Tolerance of prolonged eye contact' situation.

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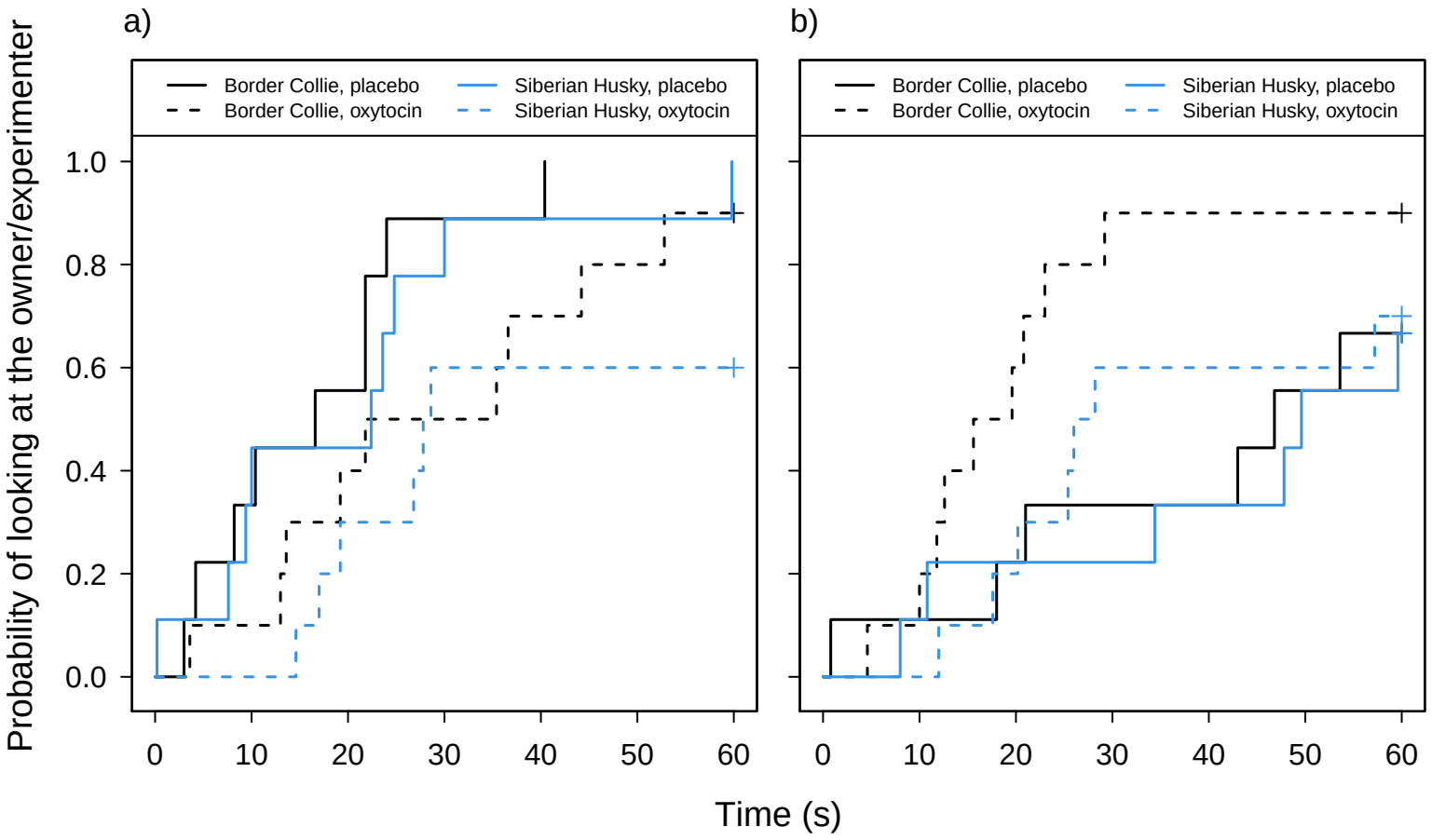

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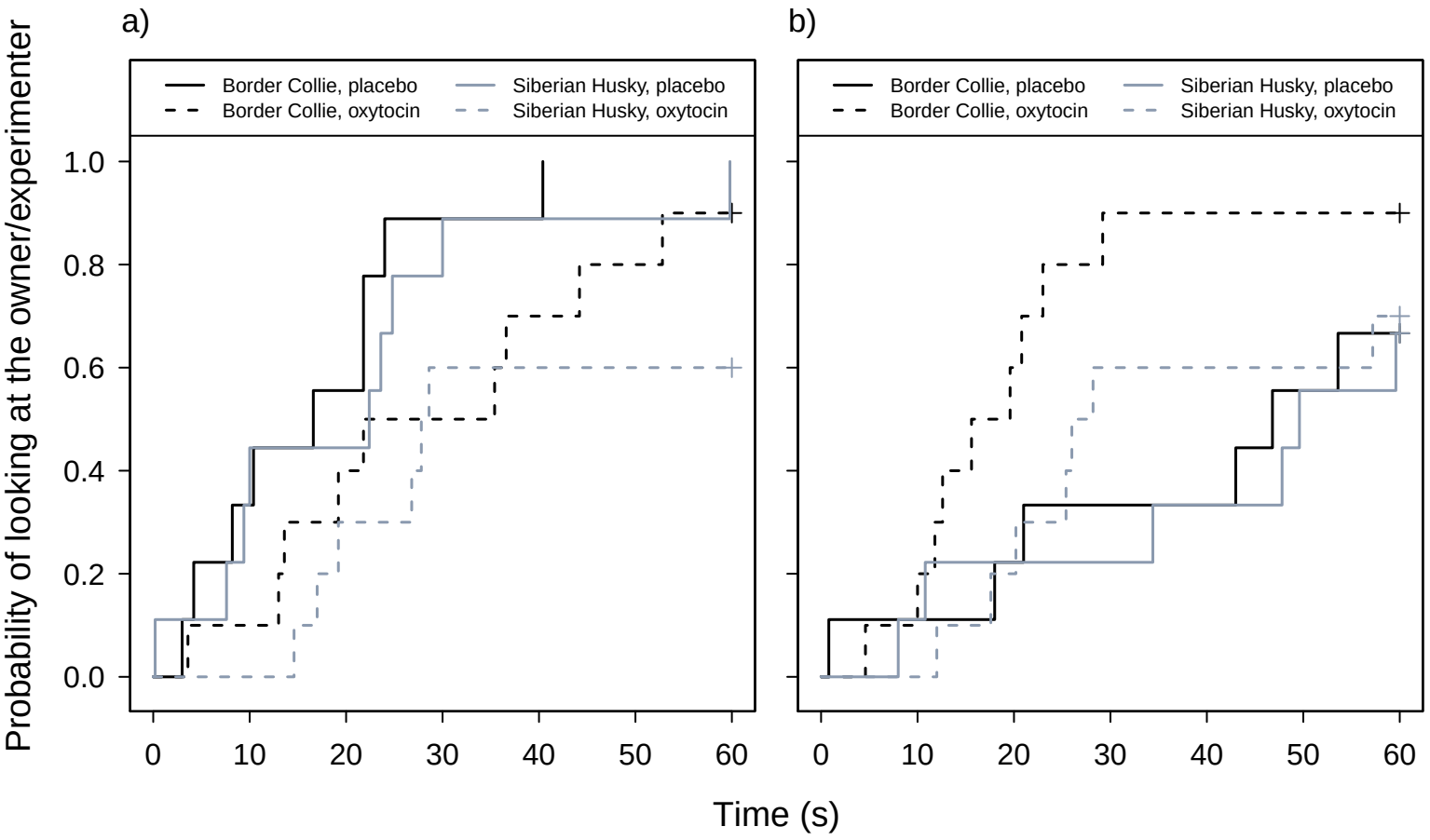

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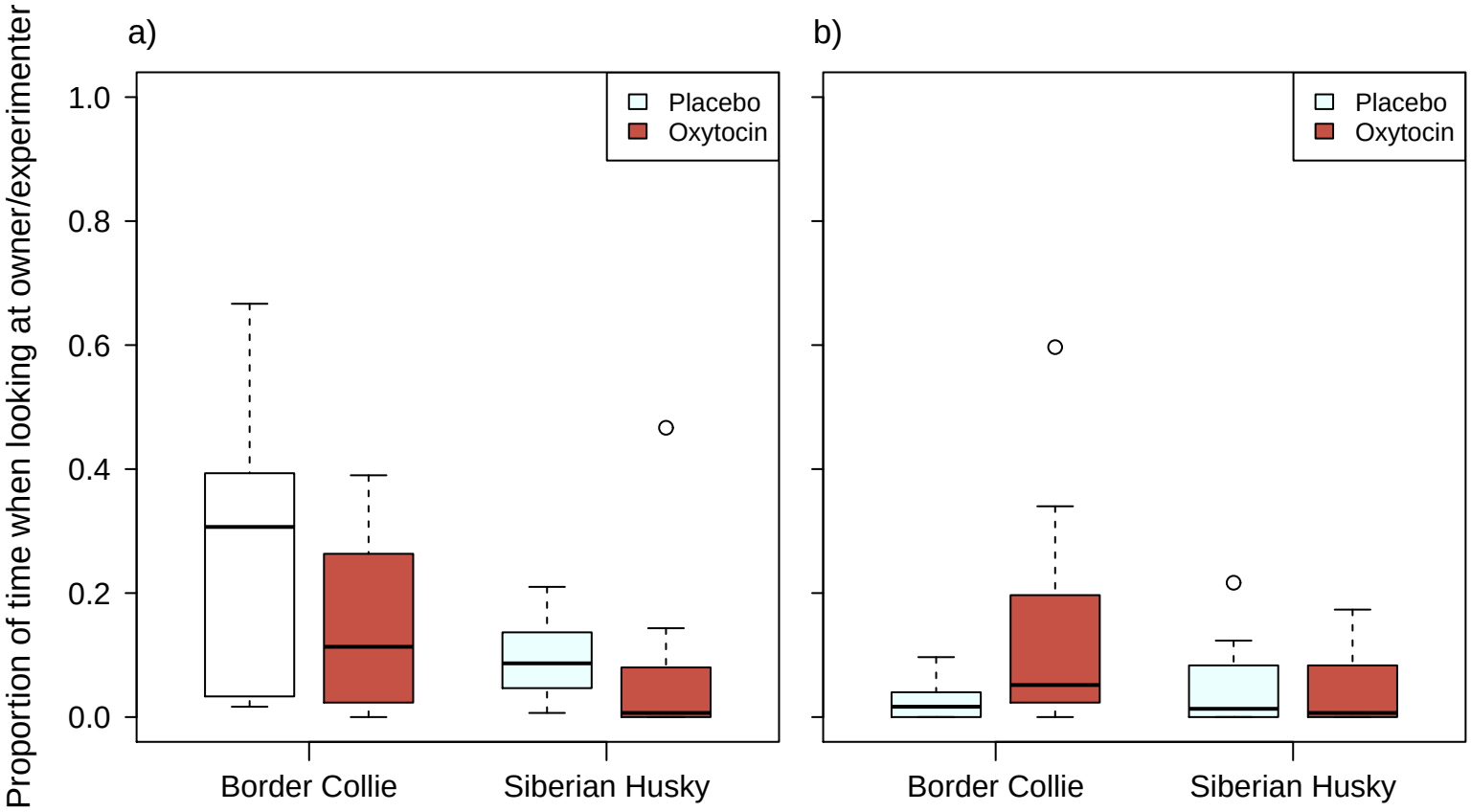

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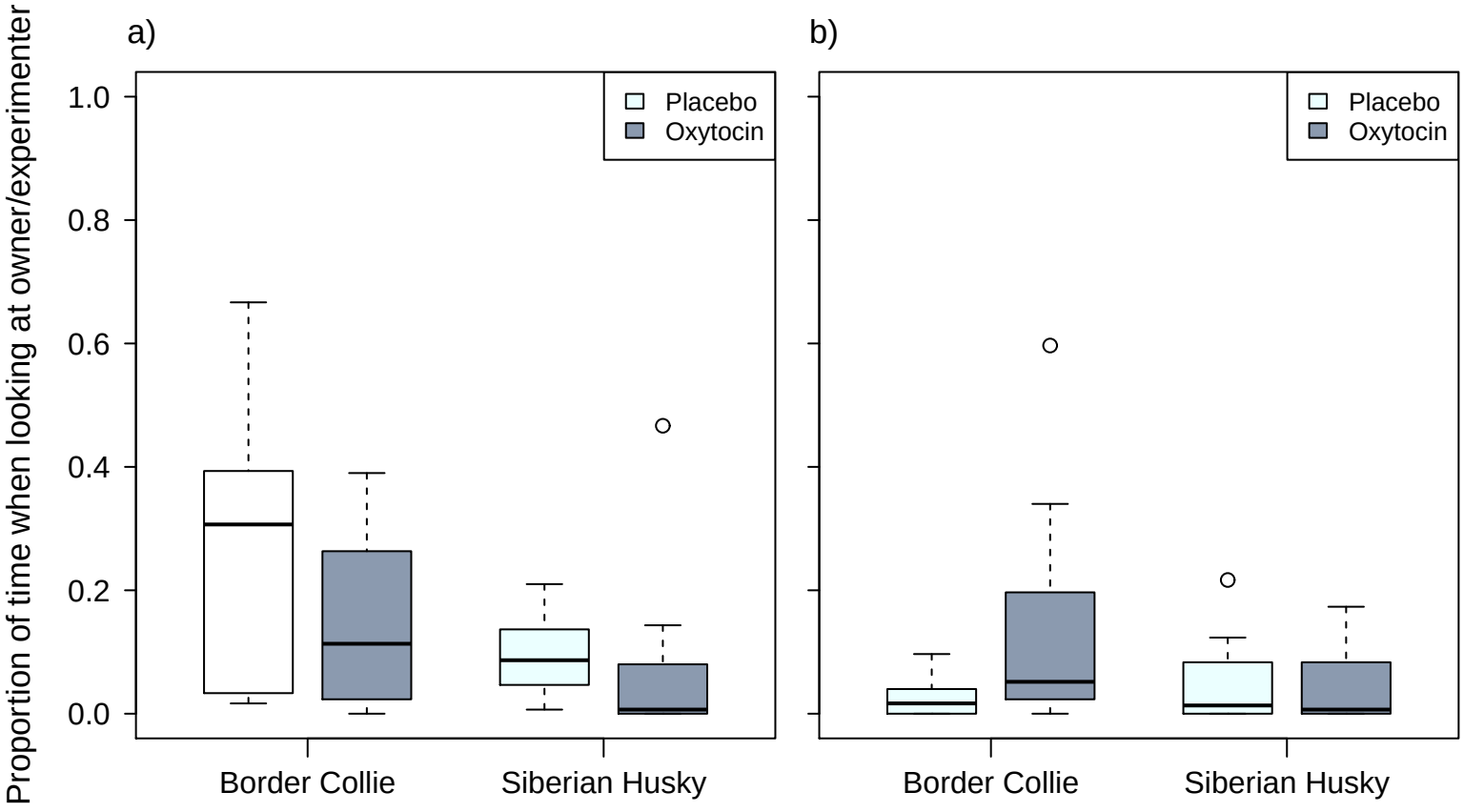

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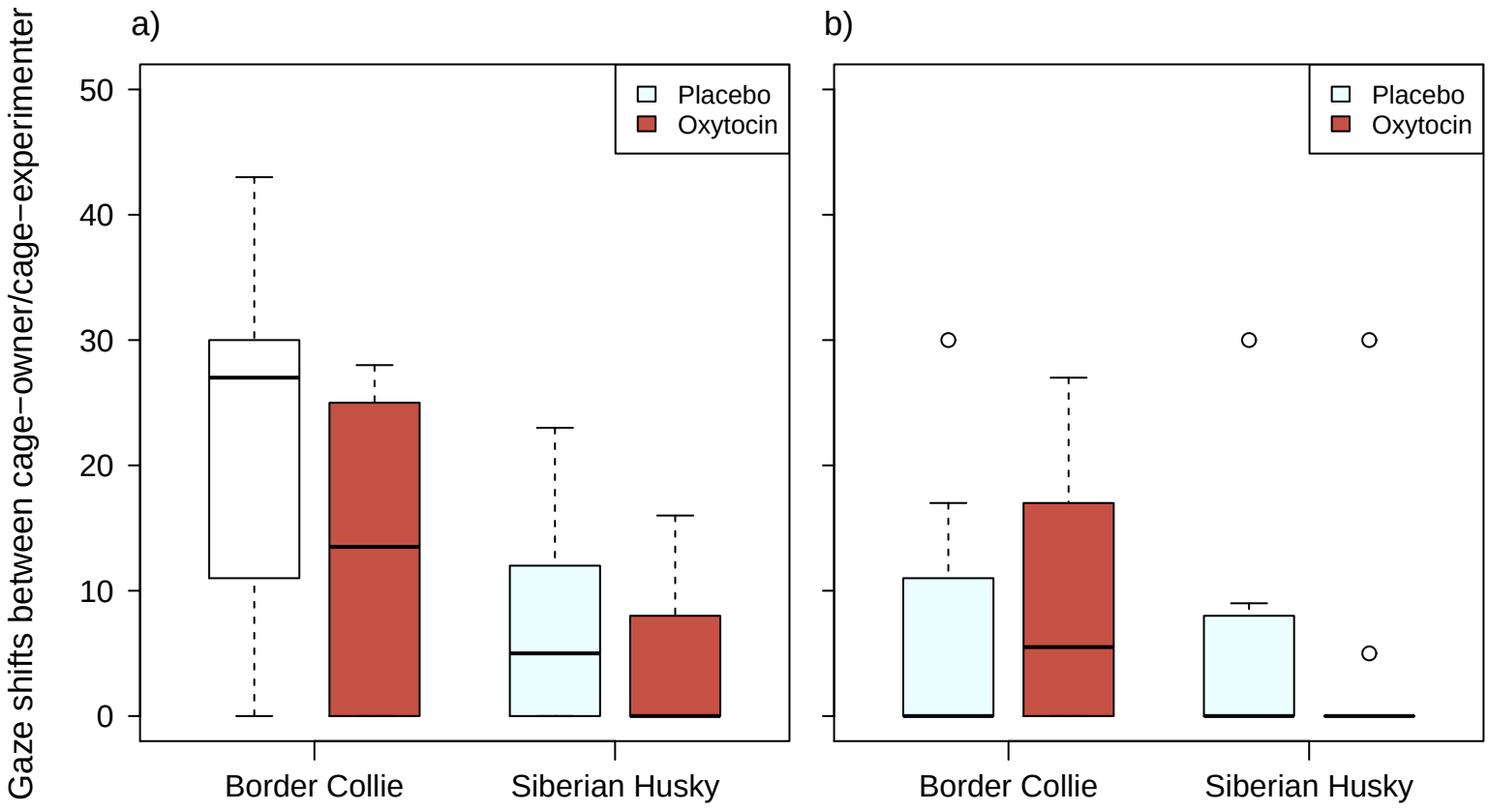

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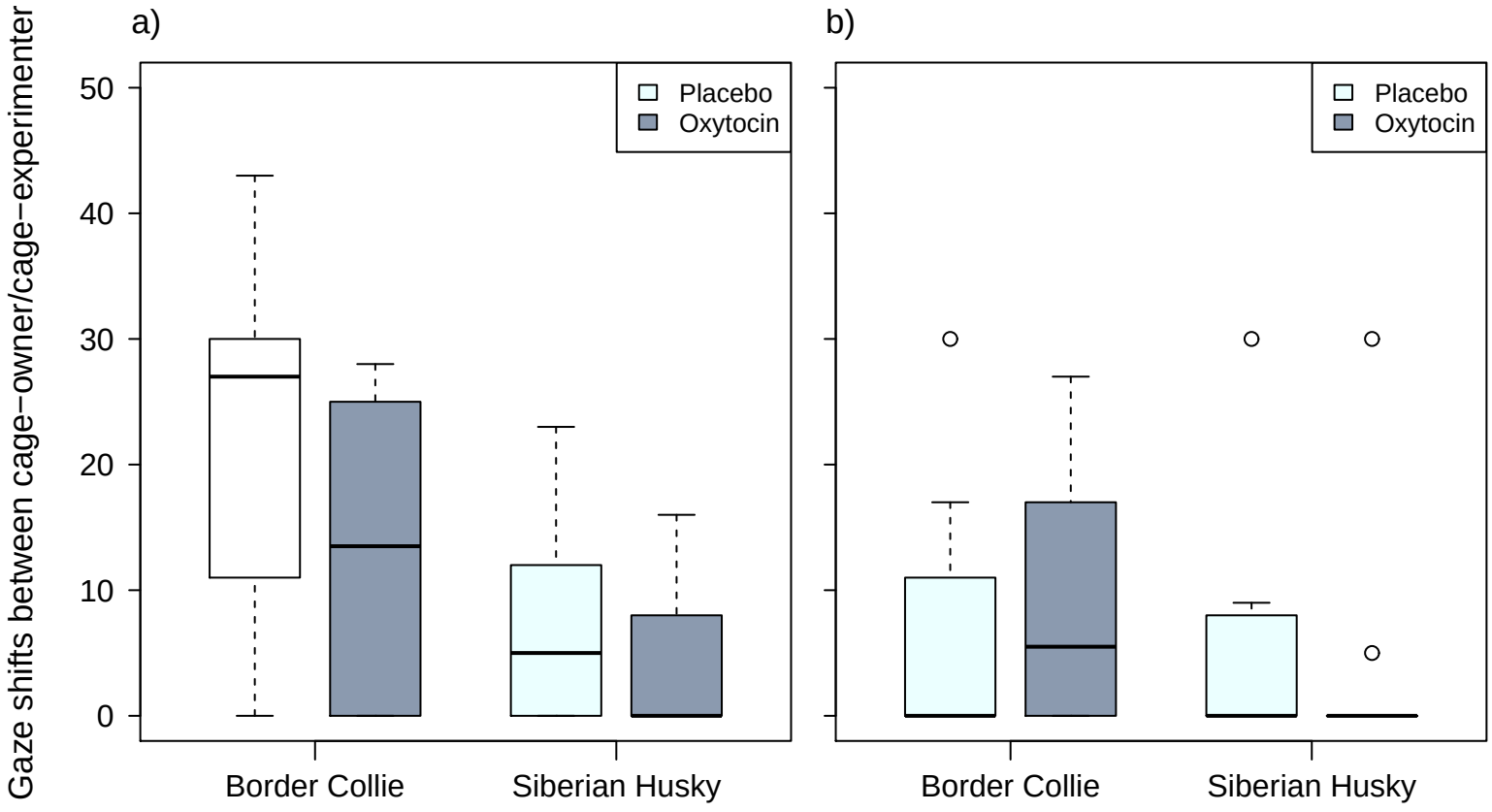

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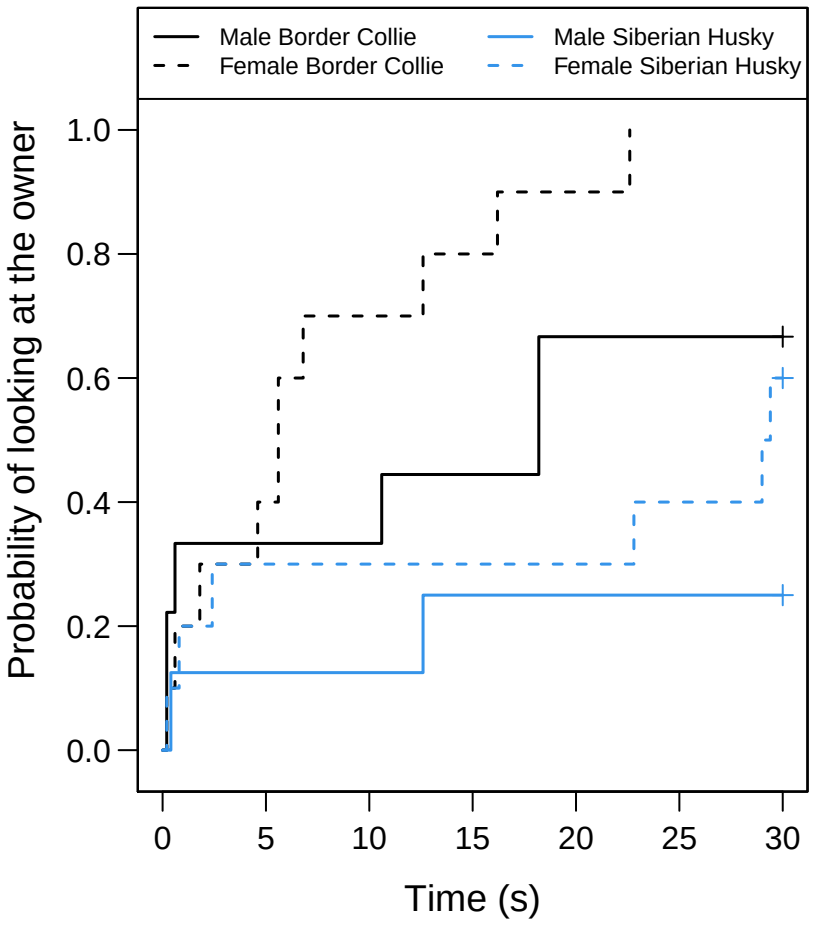

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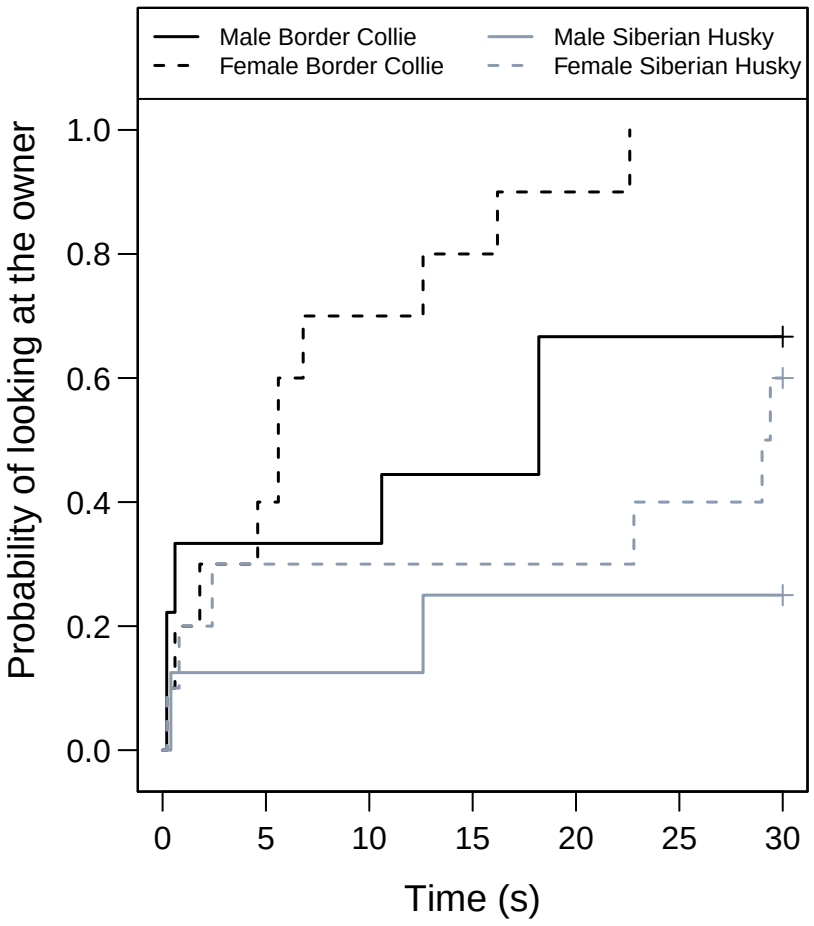

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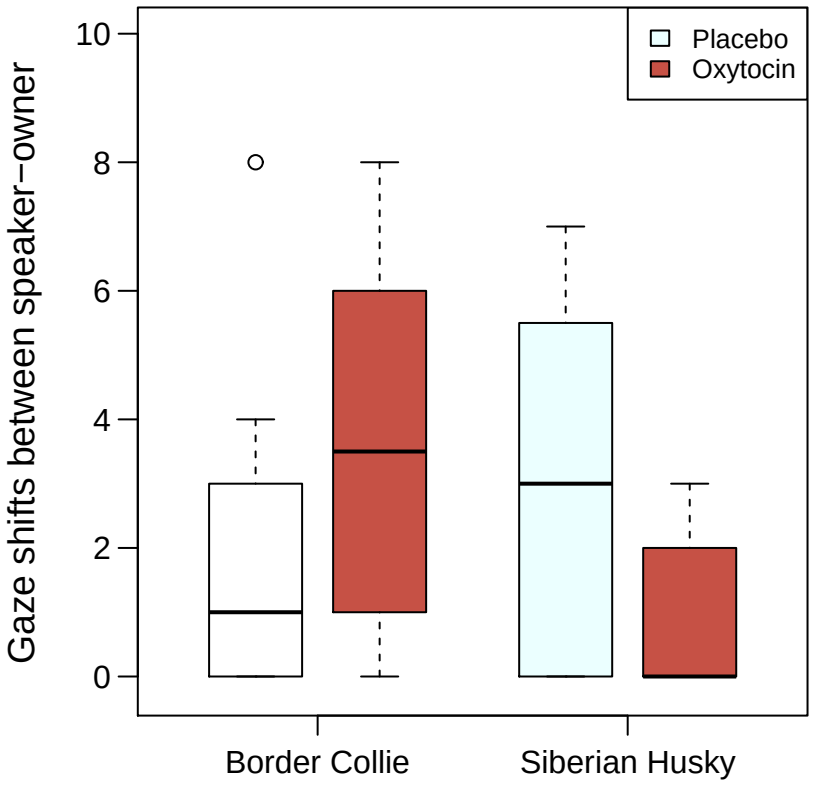

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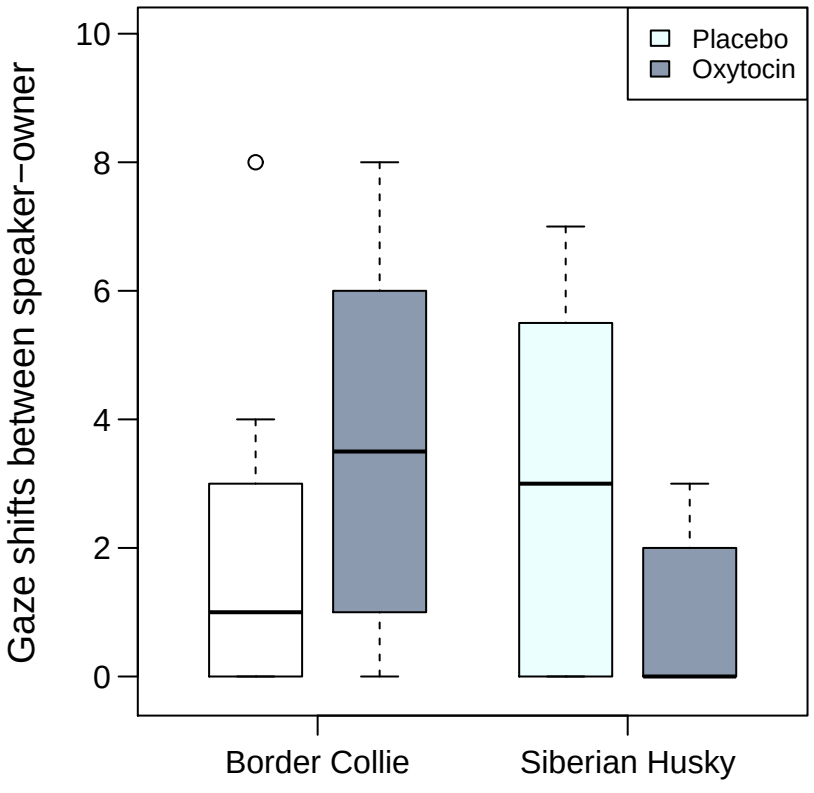

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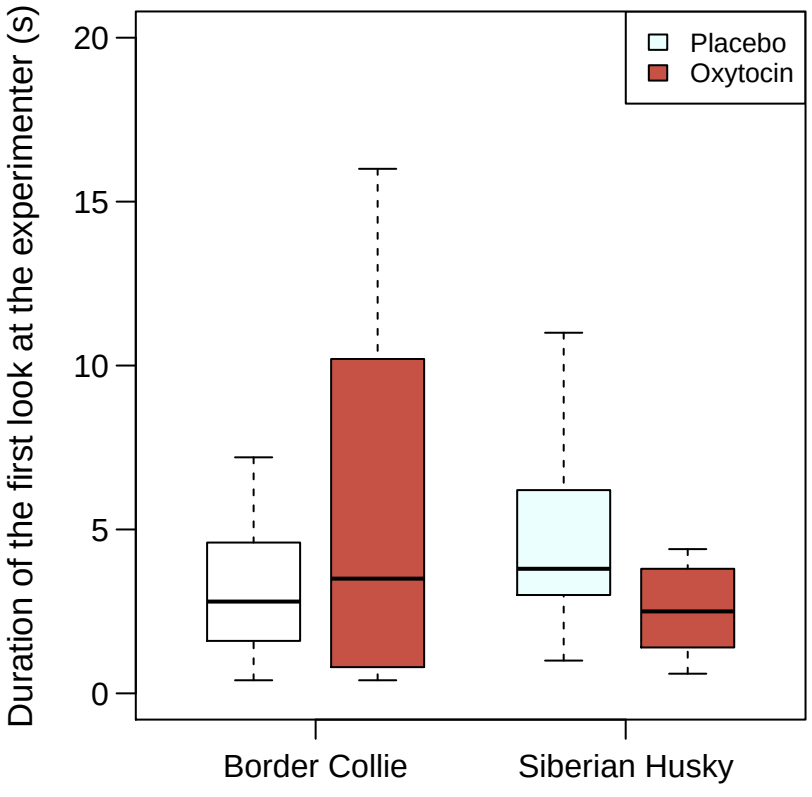
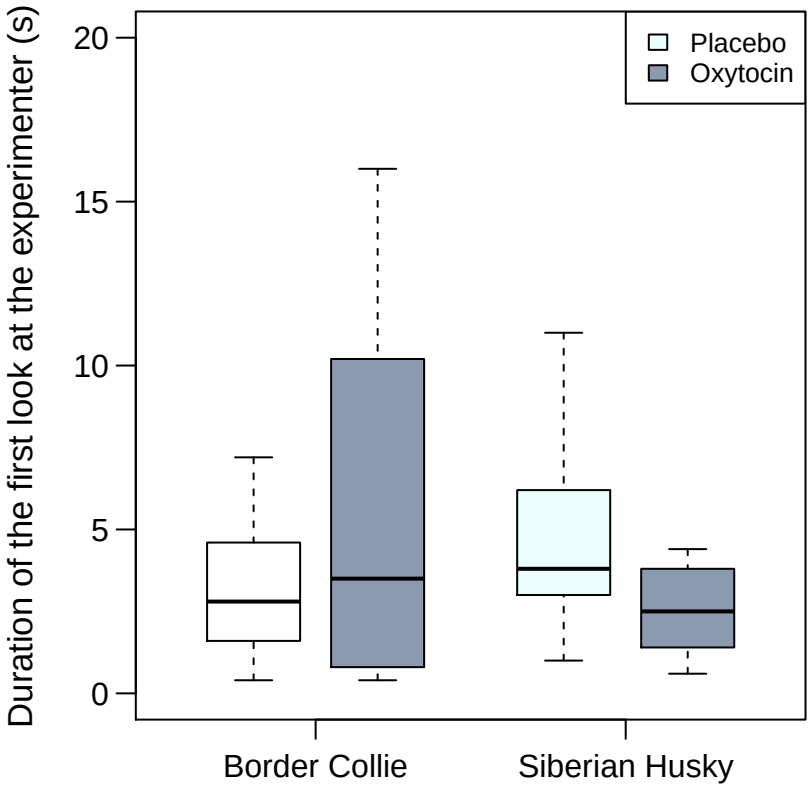
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