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Alicia Buttner
University of Nebraska at Omaha, akphillips@unomaha.edu

Breanna Thompson
University of Nebraska at Omaha, breannathompson@unomaha.edu

Rosemary Strasser
University of Nebraska at Omaha, rstrasser@unomaha.edu

Jonathan Santo
University of Nebraska at Omaha, jsanto@unomaha.edu

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Evidence for a synchronization of hormonal states between humans and dogs during competition

Alicia Phillips Buttner ⁎, Breanna Thompson, Rosemary Strasser, Jonathan Santo

University of Nebraska at Omaha, Department of Psychology — Neuroscience & Behavior, 6001 Dodge St., Omaha, NE 68182, USA

⁎ Corresponding author at: Department of Psychology — Neuroscience and Behavior, University of Nebraska at Omaha, Allwine Hall Room 419, 6001 Dodge St., Omaha, NE 68182, USA.

E-mail addresses: akphillips@unomaha.edu (A.P. Buttner), breannathompson@unomaha.edu (B. Thompson), rstrasser@unomaha.edu (R. Strasser), jsanto@unomaha.edu (J. Santo).

HIGHLIGHTS

- We examined human–dog interactions and hormonal changes during competition.
- Elevations in cortisol levels were associated between dogs and their handlers.
- Male handlers' dogs experienced greater increases in cortisol than females' dogs.
- Handlers' behavior was not associated with changes in dogs' cortisol levels.
- This study provides evidence for coordination of hormonal changes between species.

Keywords:
Interspecies interactions, Dog, Cortisol, Hormonal synchronization, Agility competition

abstract

Social interactions with humans have been shown to influence hormonal processes in dogs, but it is unclear how the hormonal states of humans factor into this relationship. In this study, we explored the associations between changes in the cortisol levels of dogs with humans' hormonal changes, behavior, and perceptions of their performance at an agility competition. A total of 58 dogs and their handlers (44 women, 14 men) provided saliva samples before and after competing. Dogs' saliva samples were later assayed for cortisol and humans' samples for cortisol and testosterone.

Following the competition, handler–dog interactions were observed for affiliative and punitive behavior towards their dogs, and handlers completed questionnaires that included personal ratings of their performance. Structural equation modeling revealed that elevations in handlers' cortisol levels were associated with increases in their dogs' cortisol levels. Handlers' affiliative and punitive behaviors towards their dogs following competition were associated with their ratings of their performance, but these variables were unrelated to changes in their own cortisol levels and their dogs', implying their behavior did not mediate the relationship. These findings suggest that changes in the hormonal states were reflected between humans and their dogs, and this relationship was not due to handlers' perceptions of their performance or the behaviors we observed during post-competition social interactions. This study is one of the first to provide evidence for a synchronization of hormonal changes between species.

1. Introduction

Over the past 18–32 thousand years of domestication [45], humans have directly and indirectly selected for certain traits in dogs that have resulted in a unique predisposition for understanding human social behavior [14,27] and an attachment system analogous to what is seen in human infants [34,46]. The last two decades have seen an upsurge of studies investigating dogs' human-directed social behavior, revealing complex, human-like social skills in dogs, including an exceptional ability to follow human visual, auditory, and gestural cues [22,39], a unique sensitivity to humans' attentional [4,20] and emotional states [30,37, 47], and perhaps even cross-species empathy [6,24]. Recently, Sümegi et al. [42] reported that stress experienced by owners manifested in their dogs' performance on a cognitive task, indicating that emotional states can be transferred from humans to their dogs. Given that humans' affective states are closely associated with their physiological and behavioral states (e.g., psychological stress causes changes in circulating hormones and behavior, [5]), it stands to reason that dogs' perceptive- ness to human behavior could influence their own physiological states, potentially resulting in one that mirrors their humans'. The synchronization of physiological states between species via social interactions is an interesting topic that has received little attention.

Social interactions have a significant influence on underlying hormonal systems. For instance, social interactions can have opposing effects on the hypothalamic–pituitary–adrenal (HPA) axis (i.e., the neuroendocrine system responsible for producing glucocorticoids and mounting the stress response), both causing stress (e.g., aggressive encounters) and also ameliorating it (e.g., grooming) [8]. The effects of social interaction on the HPA axis are often modulated by other neuroendocrine systems that interact with HPA axis activity (e.g., neuropeptides oxytocin and vasopressin from the hypothalamic–neurohypophysial system: [10,15]; testosterone from the hypothalamic–pituitary–gonadal axis: [26]). These effects are not limited to within-species interactions; social interactions between different species can also influence the hormonal systems of each. Several studies have shown that social interactions with humans influence the hormonal states of dogs. Disciplinary behavior exhibited by humans to- wards their dogs has been associated with increased cortisol levels [17, 19], whereas positive social encounters with humans (e.g., petting, playing, talking, passively interacting) generally produce decreases in cortisol levels in dogs [16,41,48] and elevations in oxytocin [38], β- endorphin, phenyl acetic acid, and dopamine levels [31]. Though the positive physiological effects of interspecific interactions are largely mirrored in humans [28,33], only a few studies up to this point have taken into account both humans' and dogs' hormonal states during their social interactions in the same study [12,31]. In these studies, cortisol levels showed a species-dependent pattern following interactions, increasing or remaining elevated in dogs rather than decreasing as they did in humans.

Few studies up to this point have assessed how changes in human hormones directly relate to changes in the physiological states of dogs. Though some have found support for associations in humans' and dogs' oxytocin levels [13], cross-species correlations between cortisol levels have not been found [9,13]. However, a study of handlers' USA Federal Disaster Canine Teams reported positive correlations between handlers' salivary cortisol following certification testing and their dogs' heart rate and body temperature, suggesting a coordination of dogs' and handlers' physiological states [51]. Further, Jones & Josephs[19] found that following a loss at a dog agility competition, losing male handlers' testosterone levels predicted increases in their dogs' cortisol levels after competing, an effect that was mediated by affiliative and punitive behaviors. The authors suggested that these individuals may have been stressed by their loss and transferred this stress to their dog through their behavior, though they did not assess the cortisol levels of the handlers in that study (but see [26]). Whether hormonal states can be transferred across species warrants further investigation in a broader sample that includes both men and women.

The goal of the present study was to assess whether fluctuations in cortisol levels in humans are reflected in their dogs. We examined this question by observing social interactions and hormonal changes in male and female handlers and their dogs during an agility competition. Agility competitions provide a naturalistic opportunity to study cooperative interactions between humans and dogs in which they must work with one another to be successful. In these competitions, dogs complete an obstacle course as quickly as they can without error, relying on their handler's cues to guide them through. Because these events are generally arousing — physically, socially, and as a sensory experience — and potentially stressful for both dogs and humans, agility competitions provide an interesting, dynamic setting for studying the hormonal changes that underlie human–dog interactions.

Structural equation modeling was used to examine the relationship between human handlers' and their dogs' cortisol levels following com- petition and evaluate possible mediating behavioral variables. We anticipated that changes in cortisol levels would be associated between handlers and their dogs (i.e., fluctuations in cortisol levels would be reflected between species). In the original model, we examined whether handlers' perceptions of their performance were associated with changes in their cortisol levels, which were mirrored in their dogs due to their behavior immediately following competition. Since previous studies have found that baseline testosterone levels predict differential behavior [19] and changes in cortisol levels [26] during agility competitions, particularly in men, we assessed if handlers' baseline testosterone levels interacted with handlers' performance ratings to predict handlers' cortisol fluctuations. Additionally, an alternative model was run in which we evaluated whether changes in dogs' cortisol levels predict- ed handlers' perceptions of their performance, which in turn predicted handlers' behavioral and hormonal changes. By evaluating both the predicted model and an alternative model, we were able to assess whether a bi-directional relationship between the variables examined in this study is statistically plausible.

2. Methods

2.1. *Participants and setting*

Data collection took place from June to November 2013 at dog agility competitions around the Midwest. A total of 58 handler-dog teams agreed to participate. Our human sample consisted of 44 women and 14 men, and varied from 26 to 75 years old ($M_{age} = 51.75$, $SD = 10.56$). The advanced mean age of our sample meant that the majority of the female handlers (74%) were menopausal. Our dog sample was comprised of 25 females and 33 males of various breeds, varying from 15 months to 12 years of age ($M_{age} = 5.40$, $SD = 2.49$). Seven males and two females were not neutered or spayed, whereas all other dogs were altered. All parts of this investigation were approved by the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Review Board and Institutional Animal Care and Use Committee. Participants were compensated with a bag of dog treats.

2.2. *Procedure & measures*

2.2.1. *Agility competition*

Handlers were informed of the study during the morning briefing of the agility trial and were asked to sign up with the researchers if they were interested in participating. We observed one of the handler-dog team's runs and their behaviors following their run. Handlers often ran multiple times throughout the day, so researchers asked them to indicate which run they were most looking forward to and observed that run.

2.2.2. *Questionnaire information*

Participants were given a questionnaire to complete during their free time that day. The questionnaire included questions about their dog's age, neuter status, rearing history, competitive history, and their training techniques. Three surveys were included within the questionnaire: a personality questionnaire developed for dogs examining four factors: calmness, trainability, sociability, and boldness [21], the Dog Attachment

Questionnaire [1], and the Interpersonal Reactivity Index [7]. A separate form obtained the handler's age, sex, how many runs they had completed that day already and how many they had qualified, whether they take corticosteroid or testosterone medications, and women's menstrual history and menopausal status.

2.2.3. *Post-competition behavior*

Following the observed run, two researchers followed the handler and dog and recorded their post-competition behavior for approximately 5 min or until the dog was put in its crate. Researchers recorded all occurrences of specific affiliative and punitive behaviors the handler exhibited towards their dogs (see Table 1), which were based on those observed in the Jones and Josephs [19] study. Observations were made in real time, as the researchers were concerned that videotaping would discourage participation or that handlers might change their behaviors if they knew they were being videotaped. Therefore, researchers recorded the occurrences with which the handler engaged in specific affiliative and punitive behaviors. Inter-rater reliability was high (single measures ICC = .81).

Table 1
Frequency of affiliative and punitive behaviors observed between handlers and dogs following competition.

Behavior	Definition	*M*	*SD*
Affiliative behaviors			
Praising	Verbally expresses approval in a warm and enthusiastic tone (e.g., ‘good dog!’)	3.20	2.50
Giving treats	Feeds dog a food treat	2.00	1.50
Petting	Extends hand to physically touch dog by stroking or patting in an affectionate manner	1.80	1.30
Making eye contact	Looks into dog's face and eyes	0.55	0.84
Playing	Attempts to play (e.g., tugging, running, pushing), often accompanied by smiling, laughing	0.05	0.86
Conversing	Speaks to dog in a conversational tone, making positive or neutral comments	0.17	0.43
Embracing	Wraps arms around dog as if hugging	0.09	0.34
Kissing	Puts face close to dogs and kisses or allows dog to lick	0.02	0.13
Punitive behaviors			
Negative comments	Speaks to dog, making negative remarks without raising voice (e.g., ‘that was terrible’)	0.34	1.00
Pulling	Jerks on dog's leash	0.21	0.55
Yelling	Speaks loudly and makes negative comments directed at dog	0.05	0.22
Pushing	Extends hand to push dog in a non-playful way	0.00	0.00

2.2.4. ***Post-competition survey***

Immediately following the observed run, handlers were given a survey asking them to rate statements about their and their dog's performance on a scale from 1 (*strongly disagree*) to 7 (*strongly agree*), such as, “We/I/My dog performed up to my expectations” and, “I/My dog could have performed better”. Internal consistency between the 7 items was high, α = .84. In order to assess handlers' personal ratings of their performance, we used a component score by summing handlers' responses following reverse coding of negatively worded items, with higher values indicating better performance.

We assessed handlers' personal ratings of their performance after the observed run rather than focusing on whether or not teams qualified (i.e., earning a minimum number of points based on completing the course quickly with few faults) because in the events we attended, scores are not posted until after all teams in a category compete, though handlers typically know whether or not they qualified immediately after their run, and waiting for the scores to be posted could potentially mask their initial hormonal response to the performance.

2.2.5. ***Saliva sampling***

Saliva was collected from handlers and dogs to ascertain hormone levels. Salivary sampling is an effective yet noninvasive and well- tolerated means of assessing hormone levels in dogs [9] and is convenient in humans as well. Further, salivary cortisol levels are highly correlated with plasma cortisol levels [49], allowing for collection more easily than blood in settings such as this. Saliva was collected in handlers using Salivettes® (Sarstedt) and in dogs by placing sterile gauze in their cheek pouch and extracting it using a 5 cc syringe until 25 μl was obtained or 3 min elapsed in order to avoid causing stress to the dog, whichever came first. Samples were immediately stored in a cooler with ice and later transferred to a freezer and stored at −20 °C until assay.

Saliva samples were taken from participants between 8 a.m. and 4 p.m. Saliva was collected from handlers at three times: a baseline sample was obtained upon signing up for the study, a pre-competition sample was obtained 5 min after the observed run (taking into consideration the 20 min it takes for hormones to reach saliva, this sample would be reflective of one's physiological state 15 min prior to competing, in anticipation for their run), and a post-competition sample, taken 20 min after the observed run (reflective of one's physiological state right after competing). Post-competition samples were taken on average 104.28 min (*SD* = 53.33) after the baseline sample was collected, and 23.55 min (*SD* = 4.97) after the pre-competitions samples was collected. Saliva was collected from dogs at two time points: a baseline sample was obtained upon signing up for the study and a post-competition was obtained 20 min after the observed run. Because handlers were concerned that experimenters collecting saliva so close in time to the observed run would distract their dogs, only two samples were taken from dogs rather than three. Post-competition samples were taken on average 82.71 min (*SD* = 29.53) after the baseline sample was collected.

Because cortisol levels peak shortly after wakening and gradually decrease during the day [50], we recorded the time at which the sample was collected was associated with cortisol levels. In humans, we calculated the minutes elapsed between the time they reported awakening and their baseline sample. Since the time the dog awoke was not collected, circadian decline was accounted for by calculating time elapsed in minutes when the post-competition sample was collected since 8 a.m., the approximate time of arrival at the agility event.

2.2.6. *Immunoassay*

Upon assay, samples were warmed to room temperature. Salivettes containing human saliva were centrifuged 2400 rpm for 15 min to extract saliva free from particles. Samples obtained from dogs were separated from residuals by centrifuging them at 5000 rpm for 5 min. Samples were analyzed for cortisol levels using an enzyme immunoassay (EIA). Hormone assays were validated for use with both humans and dogs separately by creating displacement curves of halving dilutions from quality control saliva pools for each respective species. The assay is validated when hormone standards are parallel in the 10–90% binding range, such that a difference in dilution results in an equivalent difference in the calculated concentration. Saliva samples were diluted appropriately to fall in this range. To quantify cortisol, microtiter plates were coated with CORT Ab (3.6.07), diluted to 1:25,000 in bicarbonate coating buffer, and incubated for 12 h. CORT standards were diluted in PBS ranging from 1000 to 7.8 pg/well. Labeled CORT-HRP (R4866) was diluted 1:30,000 in PBS. After the 12-hour incubation, 50 ml of PBS was added to each well, followed by 50 ml of the saliva samples or cortisol standards. After 50 ml of HRP was added, the plates were set to incubate for two hours. Free and bound hormones were separated, after which an EIA substrate (ABTS, H_2O_2) was added. Absorbance at 405-nm was measured in a microplate reader. Samples from the same individual were tested together on the same plate in duplicate. The intra- and inter-assay coefficients of variation (CV) were 6.7% and 10.3%, respectively.

2.3. Data analysis

2.3.1. Initial analyses

Data were analyzed using IBM SPSS Statistics v. 21. Analyses used a significance threshold of α b 0.05 (two-tailed). After removing hormonal data from handlers who indicated using T supplements ($n = 2$) and samples from each of the time points that did not contain enough saliva for assay or were contaminated (handlers: $n = 8$; dogs: $n = 14$), changes in hormone levels were available for 48 handlers (36 female handlers, 12 male handlers), and 44 dogs (27 males, 15 females, 2 declined to state). Distributions for hormone levels were positively skewed (i.e., skewness index N 3), and therefore normalized using a square root transformation prior to statistical analysis. Following data transformations, changes in cortisol (ΔCORT) and testosterone (ΔT) levels for each individual were obtained by calculating the percent (%) change in levels (i.e., subtracting the baseline levels from post-competition levels, dividing by baseline levels, and multiplying this value by 100). Multivariate normality was within acceptable parameters and no multivariate outliers were detected using Mahalanobis distance.

Pearson's correlations were utilized to evaluate associations be- tween hormone levels and with other continuous variables (i.e., time of sample collection, affiliative and punitive behavior, handler ratings of team's performance, questionnaire items). Additionally, *t*-tests were used to assess sex differences in changes in hormone levels, affiliative and punitive behavior, and questionnaire items. Repeated measures ANOVAs were used to assess variations in cortisol and testosterone levels across sample times based on handlers' and dogs' sex. Simple main effects were then examined using *post* *hoc* analyses that employed the Bonferroni correction for multiple comparisons.

2.3.2. *Structural equation modeling*

In order to test the proposed hypotheses displayed in Fig. 1, structural equation modeling (i.e., path analysis) was performed using M-Plus version 6.1 [29]. Path analysis is an extension of multiple regression that allows researchers to examine multiple relationships between variables simultaneously. Variables may serve as both independent and de- pendent variables, permitting researchers to examine both direct and indirect (mediating) relationships [29]. Given that our hypothesized models included several variables and predicted mediating relation- ships between them, path analysis provided a comprehensive statistical technique for evaluating this data.

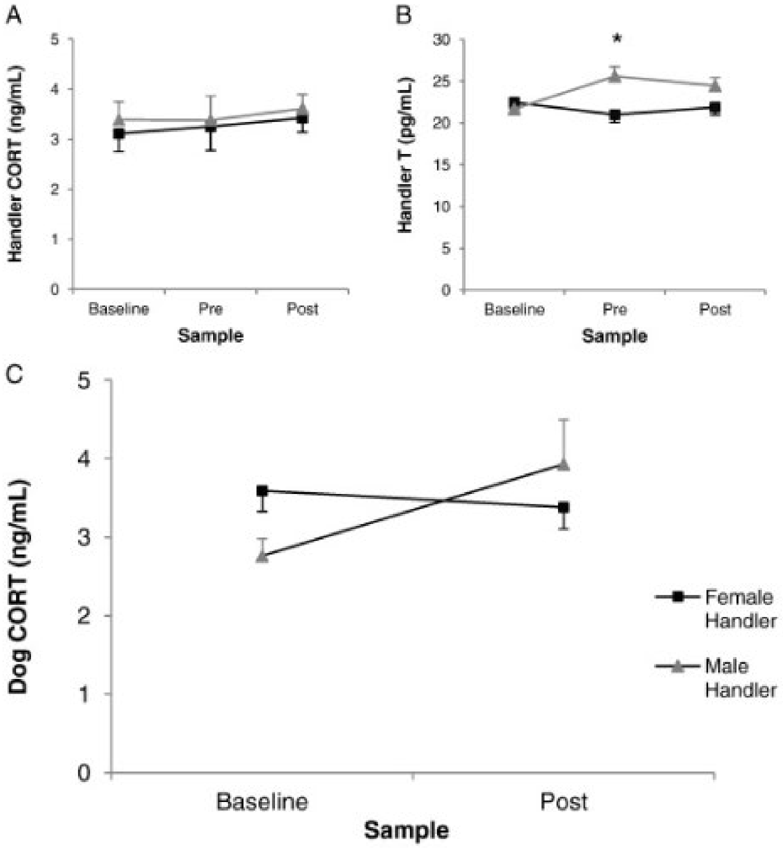

Fig. 1. Handlers' salivary cortisol (CORT) (A) and testosterone (T) (B) levels and dogs' salivary cortisol (C) levels based on handlers' sex. Male handlers' values are depicted by gray triangles and female handlers' values are indicated by black squares. Error bars represent standard error of the mean. Asterisks denote statistically significant differences between groups shown by Bonferroni-adjusted *post hoc* tests following ANOVA. Male handlers' pre-competition T levels were significantly higher than females' levels. Cortisol levels of dogs' belonging to males were significantly higher in post-competition relative to baseline (not marked by an asterisk).

We first tested the original model described in which we regressed dogs' cortisol changes on handlers' cortisol changes and affiliative and punitive behavior. Handlers' affiliative and punitive behaviors were each regressed on handlers' cortisol changes, and each of these three variables were regressed on performance ratings. The model was tested with and without baseline T as a moderator of the relationship between performance ratings and changes in cortisol. The interaction term for baseline T and performance ratings was created by multiplying the standardized form of the two variables. Handlers' change in cortisol was then regressed on baseline T and the interaction term.

We also tested an alternative model that examined the predicted path beginning with dogs' cortisol changes. Specifically, the alternative model tested whether changes in dogs' cortisol levels were the causal factor, such that their cortisol changes predicted their handlers' performance ratings, which in turn predicted handlers' behavior and subsequent changes in cortisol levels. Handlers' changes in cortisol levels were regressed on affiliative and punitive behavior, performance ratings, and dogs' cortisol changes. Affiliative behavior and punitive behavior were each regressed on performance ratings and dogs' cortisol changes. Performance ratings were regressed on dogs' cortisol changes.

Models' goodness of fit was determined by the following: Model chi- square (χ^2) $\alpha \geq .05$, Comparative Fit Index (*CFI*) ≥ .93 (1 indicates a perfect fit), Root Mean Square Error of Approximation (*RMSEA*) ≤ .08 [23], Standardized Root Mean Square Residual (*SRMR*) ≤ .08 [18].

3. Results

3.1. *Preliminary analysis of hormone levels*

Baseline cortisol, post-competition cortisol, and ΔCORT were all significantly correlated in dogs, *p* b .05, indicating stability in hormone levels. For both CORT and T in humans, baseline, pre-competition, and post-competition levels were all associated with one another, as were change values with pre-competition and post-competition levels, *p* b .05. Handlers' T levels and ΔT values not correlated with their CORT levels or ΔCORT values, *p* N .05.

No associations were found between changes in hormone levels in dogs and humans and time since waking/arriving or time between samples, *p* N .05, so these variables were excluded from further statistical analysis. Hormone levels did not differ in handlers or dogs if they had competed in a previous run, *p* N .05. Variables attained through questionnaires, including how long they had been competing in agility competitions, attachment (DAQ scores), empathy (IRI) scores, dog personality scores, and training methods, were not correlated with dogs' or handlers' hormone levels nor did they moderate any relationship, *p* N .05.

3.2. *Sex differences in hormone levels, behavior, and responses*

Hormone levels for handlers and dogs based on handlers' sex are presented are presented in Fig. 1. Overall, CORT levels did not vary be- tween samples, *p* = .337, or based on handlers' sex, *p* = .480, and no interaction between sample and sex existed, *p* = .936. Similarly, ΔCORT values were not different between male handlers and female handlers, Female handlers: *M* = 18.41 (*SD* = 42.11), Male handlers: *M* = 13.37 (*SD* = 32.01), *p* = .708. T levels did not significantly differ between samples, *p* = .427, and overall T levels did not vary based on handlers' sex, *p* = .167. However, an interaction between handlers' sex and sample on T levels was found, Wilks' Lambda = .88, *F*(2, 46) = 3.29, *p* =.046, η^2 = .13. *Post hoc* analyses revealed that pre-competition T levels were significantly higher in male handlers than female handlers, *p* =.029 (see Fig. 1). Differences in ΔT values based on handlers' sex did not reach the level of statistical significance, Female handlers: *M* = −0.16 (*SD* = 26.24), Male handlers: *M* = 19.06 (*SD* = 38.78), *p* = .056.

We also assessed whether the handlers' sex was associated with variations in dogs' CORT across samples. No main effect was found for sample, *p* = .113, or handlers' sex, *p* = .768. However, an interaction between sample and sex was found, Wilks' Lambda = .88, *F*(1, 41) = 5.55, *p* = .023, η^2 = .12. *Post hoc* analyses revealed that CORT levels of dogs belonging to male handlers increased significantly from baseline to post-competition samples, *p* = .029, whereas the CORT levels of dogs' belonging to females did not change, *p* = .449 (see Fig. 1). ΔCORT values were greater in dogs whose handlers were male as op- posed to female, Female handlers: *M* = 0.50 (*SD* = 46.89), Male handlers: *M* = 42.02 (*SD* = 44.19), *t*(41) = 2.48, *p* = .017. Dogs' cortisol levels did not vary based on the dogs' sex, *p* N .05.

We examined whether differences based on the sex of the handler were present in any other measures, including handlers' ratings of performance, affiliative and punitive behavior, or responses to questionnaire items (e.g., attachment to their dog), but no differences were found, *p* N .05.

3.3. ***Structural equation modeling of dogs' and handlers' responses***

Correlations and descriptive statistics for variables of interest are presented in Table 2. In all models, ΔCORT levels were regressed on baseline cortisol levels in order to statistically control for starting levels, since variation in smaller concentrations of cortisol would produce a larger change value than variation in larger concentrations. Additional- ly, dogs' ΔCORT levels were regressed on handlers' sex to account for differences the sex differences reported in Section 4.2. These variables were significant predictors in each of the models tested. All data for each model are included in Supplementary Materials.

In the first model, handlers' ΔCORT was a significant predictor of dogs' ΔCORT, such that greater changes in handlers' cortisol levels were associated with increases in dogs' cortisol levels (displayed in Fig. 2). Affiliative and punitive behavior did not reach the level of significance as predictors of dogs' ΔCORT. However, handlers' ΔCORT positively predicted affiliative behavior but was unrelated to punitive behavior. Handlers' behavior did not mediate the relationship between handlers' and dogs' change in cortisol levels. Further, handlers' performance ratings were significant predictors of affiliative and punitive behavior, such that higher ratings predicted more affiliative and less punitive behavior, but were not associated with handlers' ΔCORT. Overall, the model (displayed in Fig. 3) was a good fit to the data, $\chi^2(11) = 8.90$, *p* N .05, *CFI* = 1.00, *RMSEA* = .08, and *SRMR* = .06, explaining 39% of the variance in dogs' change in cortisol levels.

In a separate model, we tested whether baseline T levels moderated the relationship between dogs' and handlers' changes in cortisol levels, such that poor performance ratings would predict greater elevations in cortisol particularly in individuals with high baseline T. The model was identical to the first model with the exception that baseline T and the interaction between baseline T and performance ratings were also regressed on handlers' ΔCORT. Neither baseline T nor its interaction with ratings of performance was a significant predictor of handlers' ΔCORT, and the model's fit indices were not within the acceptable ranges, $\chi^2 = 21.04$, *p* N .05, *CFI* = .91, *RMSEA* = .92, and *SRMR* = .07.

In the alternative model, we assessed if dogs' cortisol changes were the causal factor, predicting their handlers' performance ratings and subsequent behavioral and physiological changes (displayed in Fig. 4). Dogs' ΔCORT was a significant predictor of handlers' ΔCORT, but not handlers' affiliative and punitive behavior or performance ratings. Handlers' performance ratings predicted affiliative and punitive behavior, but not handlers' ΔCORT. Handlers' ΔCORT did not predict their affiliative and punitive behavior. Overall, the alternative model explained 37% of the variance in handlers' change in cortisol, and aside from a slightly higher than desirable *RMSEA*, other fit indices were with- in an acceptable range, $\chi^2(12) = 14.67$, *p* N .05, *CFI* = .95, *RMSEA* = .09, and *SRMR* = .08. However, a chi-square difference test was performed and indicated that the first model was a better fit to the data than the alternative model, $\chi^2(1) = 5.88$, *p* b .05.

Table 2
Pearson correlations and descriptive statistics for variables of interest.

	1.	2.	3.	4.	5.	6.	7.	8.	*M*	*SD*
1. Dog ΔCORT	—								9.96	48.52
2. Dog BL CORT	−.35*	—							3.36	1.38
3. Handler ΔCORT	.27	−.06	—						17.28	39.03
4. Handler BL CORT	−.11	.12	−.58**	—					3.11	0.98
5. Handler BL T	−.13	−.29	.00	.20	—				22.41	5.45
6. Handler sex	−.36	.22	.06	−.16	−.03	—			—	—
7. Affiliative behavior	−.08	−.13	.25	−.34*	.07	.01	—		8.29	3.70
8. Punitive behavior	−.13	.17	.10	.10	−.10	.13	−.31*	—	0.60	1.35
9. Rating of performance	−.04	.06	−.20	.05	.16	−.12	.37*	−.47**	30.67	10.35

Note. **p* b .05; ***p* b .01.

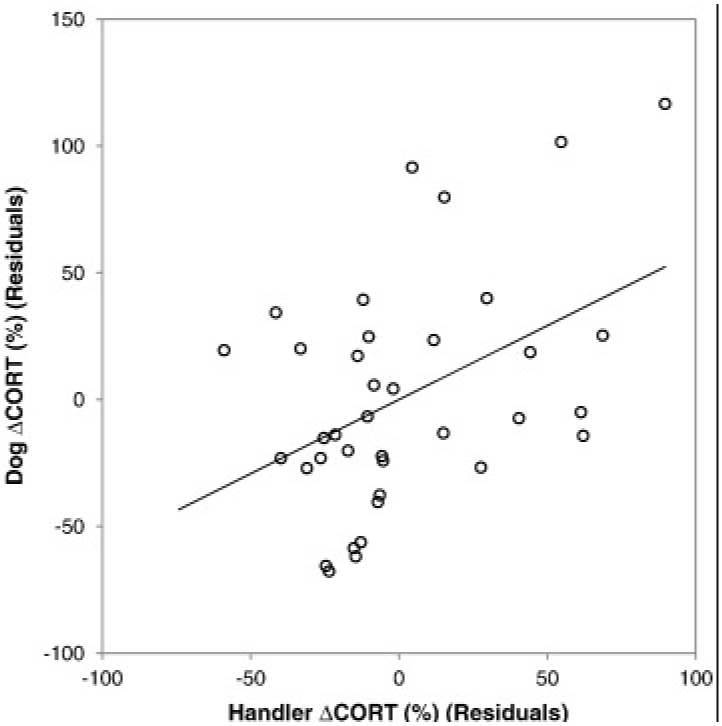

Fig. 2. Partial regression plot in which handlers' change in cortisol levels (ΔCORT) predict dogs' ΔCORT after adjusting for other variables in the model.

4. Discussion

In this study, we aimed to determine associations between changes in humans' and dogs' cortisol levels during an agility competition, and how handlers' behavior and their ratings of their teams' performance were involved in these cross-species hormonal changes. Through the use of structural equation modeling, our findings indicated that following competition, handlers' and dogs' changes in cortisol levels reflected one another. A comparison of two competing models used to evaluate the direction of the relationships between these variables showed that the model in which handlers' hormonal and behavioral changes predict- ed dogs' cortisol levels was a better fit to the data. Further, the predicted associations between handlers' changes in cortisol levels with their behavior and ratings of their performance were not supported in general. In fact, contrary to expectations, greater elevations in handlers' cortisol levels predicted more affiliative behavior. These findings suggest that a synchronization of hormonal states may have occurred between handlers and their dogs, which was not explained by the affiliative and punitive behaviors we observed the handler exhibit towards the dog or how the handler perceived their performance.

4.1. *Human-dog hormonal synchronization*

Our results indicate that during a dog agility competition, handlers' and their dogs' changes in cortisol levels are generally mirrored, and this effect was not accounted for by the humans' behavior towards the dog that we observed. Though correlations have been reported between oxytocin levels in humans and their dogs [13], previous studies have not found a relationship between cortisol levels in dogs and their owners (i.e., during a simulated thunderstorm in their own home: [9]; in an un- familiar testing facility: [13]). However, other studies in more active contexts have provided evidence for a possible coordination of changes in physiological activity (agility competition: [19]; disaster certification testing: [51]), though neither study assessed dogs' cortisol levels. Our study is the first to our knowledge to report that in an active setting such as an agility competition, fluctuations in humans' cortisol levels are associated with changes in their dogs' cortisol levels. These findings suggest that changes in hormonal states may be shared across species boundaries, and complements those of Sümegi et al. [42], in which they found that stress experienced by humans influenced their dogs' cognitive performance similar to dogs that had themselves experienced stress. If emotional states may be transmitted across species from humans to dogs as Sümegi and colleagues suggested, it is plausible that the physiological states underlying these experiences may also be shared. Our findings suggest that this transmission of hormonal states may be possible, though future studies should seek to disentangle the direction of transmission (i.e., whether humans' physiological states in- fluence their dogs' or if dogs' physiological states influence their humans'), or, more likely, if a bi-directional relationship exists, using an experimental design.

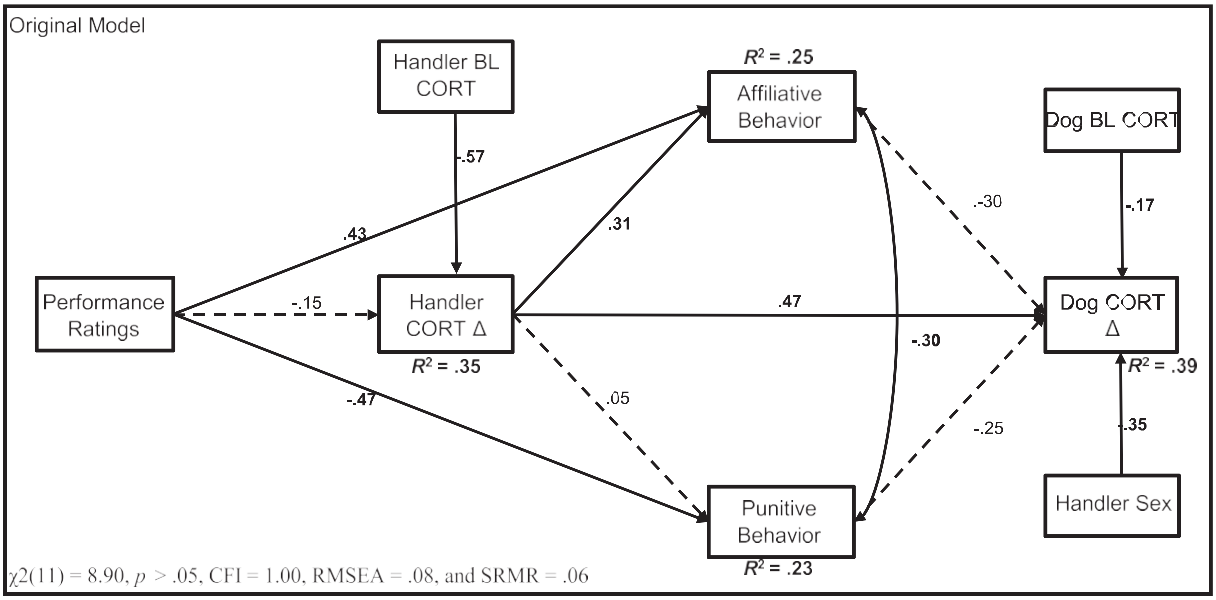

Fig. 3. Original model of dogs' and humans' behavioral and hormonal responses. Significant paths are solid; nonsignificant paths are dashed. Path coefficients are presented in bold to indicate statistical significance.

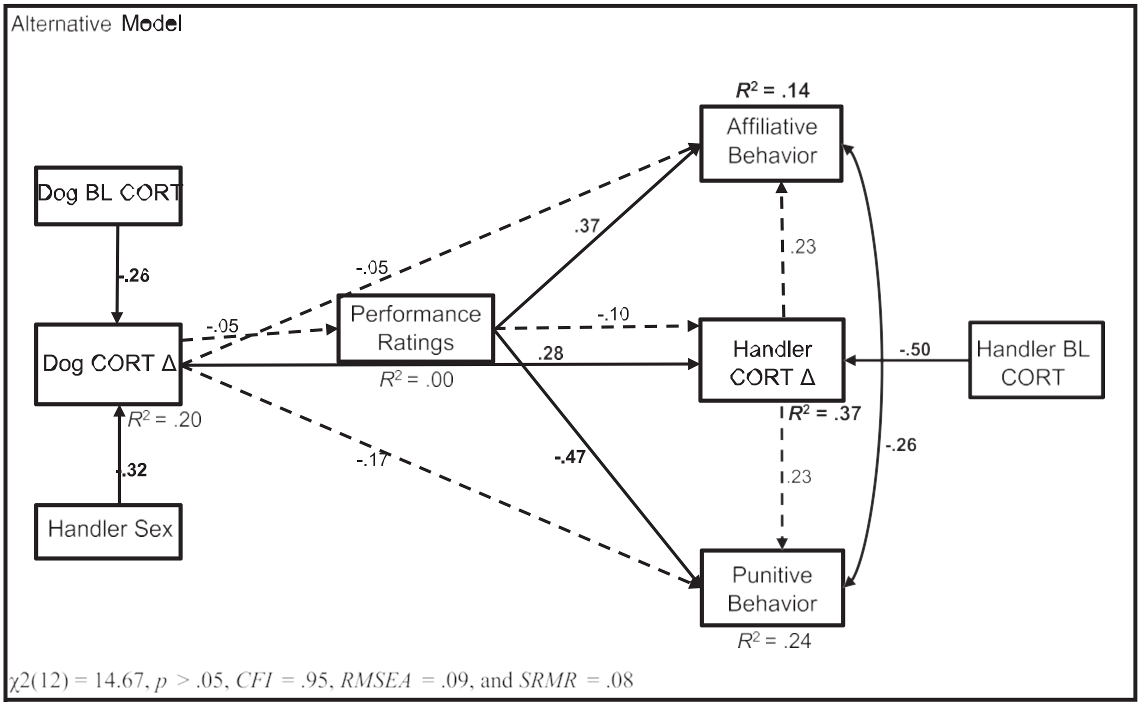

Fig. 4. Alternative model of dogs' and humans' behavioral and hormonal responses. Significant paths are solid; nonsignificant paths are dashed. Path coefficients are presented in bold to indicate statistical significance.

In contrast to the findings of previous studies (e.g., [17,19]), handlers' affiliative and punitive behavior were not associated with their dogs' changes in cortisol levels. One possible explanation for the lack of a relationship between handlers' behavior and dogs' changes in cortisol levels may be related to our behavior categorization procedures. Whether behaviors were deemed as ‘affiliative’ or ‘punitive’ was based in the human perspective of that behavior, whereas dogs may not experience these behaviors the same way. For instance, kissing and embracing may be a negative experience for some dogs, while receiving negative comments without the handler raising his or her voice may be difficult for dogs to distinguish from other speech and therefore not punitive. Further, some types of human behavior may have been more impactful than others on dogs' physiological states than others. For in- stance, food reinforcements have been shown to be more reinforcing for dogs than social interaction [11]. Thus, the consequences of human behavior on dogs' physiological states may be more complicated than the human's intention.

Since handlers' behavior was not associated with their dogs' changes in cortisol levels, the mechanism explaining the observed interspecific coordination of hormonal states is unclear. It is possible that dogs may have picked up on subtle behavioral cues from humans (e.g., body language, facial expressions, tone of voice, type of touch during petting), which served to transmit physiological states between humans and their dogs. Chemosensory signals alone or in combination with such behaviors could also have mediated these hormonal changes between dogs and humans, as chemosensory signals have been found to underlie emotional contagion of anxiety in humans [36]. Future investigations might consider these factors as a possible means of transmitting physiological states between species.

We observed an interesting difference in the cortisol levels of dogs belonging to males compared to those of female handlers. Specifically, male handlers' dogs experienced greater elevations in cortisol levels following competitions relative to their baseline levels, whereas dogs be- longing to female handlers showed no changes. Whether this finding was the result of differences in how men and women interacted with their dogs or some other cue (e.g., chemosensory signals) is unclear. We found no differences in the affiliative and punitive behavior exhibit- ed by male and female handlers towards their dogs, though previous studies have found that men and women interact with dogs differently [3,35]. It is possible that the quality of the interactions may have differed between men and women, which could have differential effects on their dogs. We were limited by the low number of men competing in the observed agility trials, but future studies should explore this finding further in larger samples with an equal number of men and women in different contexts.

4.2. *Handlers' behavioral and hormonal responses to competition*

As predicted, handlers who rated their teams' performance lower engaged in more punitive and less affiliative behavior towards their dogs. However, handlers' ratings of their performance did not predict their own changes in cortisol. We examined whether baseline T levels moderated this relationship but found no such relationship. One explanation for this result may be related to our sample of handlers being primarily female. Mehta et al. [26] found that high basal T predicted an increase in cortisol in male but not female handlers in a dog agility com- petition, perhaps because women engaged in tend and befriend behaviors, which attenuated their cortisol response [43,44]. Though we found no sex differences in the behavior handlers exhibited towards their dogs, it is possible that the quality of these interactions differed between male and female handlers, providing different effects on their own physiological states. Our finding that changes in handlers' cortisol levels were positively associated with affiliative behavior but not punitive behavior towards their dogs during post-competition interactions may support this notion. Though contrary to our expectations, this finding is consistent with the ‘tend and befriend’ hypothesis that when under stress, women may exhibit more affiliative behavior [43,44].

4.3. *Limitations and future studies*

The naturalistic setting of an agility competition provided a dynamic environment for the study of the hormonal changes that underlie human–dog interactions, but also carried limitations regarding the degree of control we had over certain variables. Saliva samples were taken throughout the day, so the effects of the diurnal slope for cortisol were not removed, and the time between samples was not standardized, since some trials went faster than others. However, individuals in this study essentially served as their own controls, since we assessed changes in cortisol levels rather than comparing levels between individuals at individual time points, and time measures did not correlate with changes in cortisol levels. Additionally, behavioral measures of dogs' stress were not recorded, though several previous studies have not found a link between behavioral and physiological responses to stress in dogs [2,9,32].

It is important to consider that although cortisol is typically interpreted as a physiological measure of stress, stress is a subjective phenomenon [40]. Elevated cortisol levels do not necessarily imply that an individual was experiencing distress, but may also indicate excitement (i.e., eustress), or could possibly the result of physical exertion [25]. Further, the same changes in cortisol levels may be ac- companied by different experiences for different individuals. Thus, changes in cortisol levels should be interpreted as fluctuations in general arousal rather than distress. We suggest that this interpretation of elevated cortisol levels be kept in mind when considering if agility competitions pose a threat to dogs' welfare [32], since we currently do not know if acute elevations in dogs' cortisol levels are due to distress, physical exertion, general excitement, or some other explanation. Moreover, future studies should attempt to assess the affective states that accompany fluctuations in cortisol levels, and if they are synchronized along with physiological states between humans and dogs.

5. Conclusions

Throughout domestic dogs' coevolution with humans, an interspecific synchronization of physiological states might have provided an adaptive advantage by facilitating social communication and coordination of behavior between species. In the current study, we found evidence for a correspondence of changes in cortisol levels between humans and their dogs during an agility competition, and this association was unrelated to the handlers' behavior during post-competition interactions with their dog. These new and exciting findings further elucidate the complexity of the hormonal interactions underlying the ancient human–dog bond worthy of further investigation in other con- texts. Experimental studies have the potential to confirm if hormonal states are shared between species in other settings, as well as the mechanisms — behavioral, chemosensory, or otherwise — that play a role in their transmission.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2015.04.010.

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