

2015

Factors Determining the Effects of Human Interaction on the Cortisol Levels of Shelter Dogs

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FACTORS DETERMINING THE EFFECTS OF HUMAN INTERACTION ON THE
CORTISOL LEVELS OF SHELTER DOGS

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science

By

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B.A., Miami University, 2012

2015

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WRIGHT STATE UNIVERSITY
GRADUATE SCHOOL

December 13, 2015

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Regina M. Willen ENTITLED Factors Determining the Effects of Human Interaction on the Cortisol Levels of Shelter Dogs BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Willen, Regina M. M.S. Department of Neuroscience and Physiology, Wright State University, 2015. Factors Determining the Effects of Human Interaction on the Cortisol Levels of Shelter Dogs.

Dogs admitted to animal shelters experience psychological stressors resulting in elevated plasma cortisol. We previously found 30 min of human interaction reduced this response. The present study further characterized this effect, with the aim of developing a practical means of reducing stress of shelter dogs. We found that a second day of 30 min of petting reduced cortisol levels as effectively as the first. Further, 15 min of this interaction was as effective as 30 min. During petting, signs of excitation (vocalizations) and anxiety (panting) as well as escaped attempts were reduced, and social solicitation (tail-wagging) increased. However, cortisol levels quickly increased when dogs were returned to the home kennel. Cortisol reductions were pronounced in dogs admitted as strays, but human interaction did not reduce cortisol in a subpopulation relinquished by their owners. We also measured hair cortisol levels to assess stress prior to shelter admittance. Strays and dogs released by their owners showed comparable hair cortisol levels that were intermediate to those of pet dogs living in a home and those of dogs diagnosed with Cushing's disease. The findings show that as little as 15 min of human interaction can moderate cortisol levels of shelter dogs, that

the effect is relatively temporary, that source of the dog is an important variable, and that hair cortisol accumulation may be useful to estimate the condition of the dog prior to shelter admittance.

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INTRODUCTION

Animal shelters provide a sanctuary to thousands of dogs and cats every year. Nevertheless, the environment within even the most state-of-the-art shelters exposes animals to numerous psychogenic stressors known to increase the activity of the hypothalamic-pituitary-adrenal (HPA) axis, the body's primary stress-responsive neuroendocrine system (Mason, 1975). Such stressors include novelty (Ader, 1970; Friedman et al., 1967), uncontrollable and unpredictable events (Beerda et al., 1998; Hanson et al., 1976; Muir and Pfister, 1986), and separation from attachment figures (Hennessy, 1997; Mendoza and Mason, 1986; Smotherman et al., 1979). HPA activation involves a cascade of events that culminate in the release of glucocorticoids into the bloodstream.

Glucocorticoids have a multitude of physiological effects that generally are adaptive (e.g., increasing glucose availability), at least during short-term stressor exposure—minutes or up to a few hours (Sapolsky et al., 2000). However, the prolonged release of glucocorticoids may be maladaptive and negatively impact welfare by disrupting normal physical and behavioral functions (e.g., suppressing immune activity) (McEwen, 2012; Rohleder, 2012). Thus, glucocorticoid levels can be useful as markers of the psychological state and welfare of the dogs housed in animal shelters (Hennessy, 2013; Morméde et al., 2007).

The circulating glucocorticoids of dogs (primarily cortisol) newly admitted to a shelter are greatly elevated over those of dogs sampled at home (Hennessy et al., 1997). It has been suggested that social isolation is the most stressful factor in a kennel environment (Hubrecht et al., 1992). Dogs housed in a shelter avidly seek contact with an unfamiliar person (Gácsi et al., 2001; Pullen et al., 2012), and separation behaviors such as barking and whining are reduced in the presence of a stranger (Prato-Previde et al., 2003). Moreover, our earlier studies found that human interaction reduced the cortisol response of shelter dogs to additional stressors imposed in the shelter environment (Hennessy et al., 1998; 2002).

More recently, several laboratories have found human interaction to reduce the cortisol response to shelter housing itself. Coppola et al. (2006) showed that 30-90 min of playing, grooming, training, and walking on the second day in the shelter significantly reduced the dog's salivary cortisol response to shelter housing on the following day. Menor-Campos et al. (2011) found that 25 min of human interaction in the form of exercise and training on the seventh and ninth day following entrance to the shelter significantly reduced salivary cortisol levels at the end of the sessions. In our laboratory, Shiverdecker et al. (2013) observed that plasma cortisol levels of newly admitted shelter dogs were significantly lower following 30 min of either petting, play, or the mere presence of a woman in a quiet room than they were just prior to the intervention.

These findings are encouraging for the development of programs designed to reduce stress of dogs housed in shelters in that they suggest that human interaction with an unfamiliar person such as shelter staff or a volunteer may be a practicable and effective way of reducing the HPA activity of newly admitted shelter dogs. Nevertheless, there is much to be learned about the nature of the effect and how it might be made more practical for implementation in a shelter environment. The current set of experiments were designed to address some of these issues. Experiment 1 examined whether the effects of petting on plasma cortisol levels persisted for 24 h, whether a second day of petting continued to reduce the cortisol response, and if so, whether the magnitude of reduction due to petting the second day was enhanced by petting the previous day. Behavioral measures during human interaction were collected for comparison with cortisol levels. Experiment 2 examined whether 15 min of human interaction would be as effective in reducing plasma cortisol levels as was 30 min, and whether effects would persist for 1 h after return to the kennel. Finally, Experiment 3 compared stray dogs and dogs relinquished by their owners. We asked whether petting would have comparable effects on the plasma cortisol levels of these two groups. To estimate effects of stressors prior to admission to the shelter, we also evaluated hair cortisol values in both groups.

II. METHODS

Animals

The study took place at the Montgomery County Animal Resource Center (ARC), a large animal shelter in Dayton, Ohio. One hundred and forty-four dogs from the ARC, and seven dogs residing in private homes were included in this study. The dogs were a variety of mixed and pure breeds, intact and gonadectomized males and females. The dogs from the ARC were enrolled in the study within two days of their arrival (Day 1 = day of arrival). All dogs were over the age of 6 months, based on dentition, and weighed more than 9 kg. Dogs were excluded if they were ill, appeared likely to bite, or were pregnant or lactating. The Wright State University Laboratory Animal Care and Use Committee approved all procedures.

Housing

Newly arrived dogs were housed in an area designated as the intake room which contained 75 single, permanent kennels with most being ~1.5 m x 1.2 m x 1.8 m. Kennels were constructed of bricks on three sides with metal mesh for the fourth side, including the door, and arranged in a manner that dogs had visual contact with conspecifics. Dogs were generally housed in kennels proportional to their size, with a raised plastic deck to allow the dogs to remain off the concrete floor. The room was temperature controlled and was illuminated with a combination of natural and artificial lighting that was manually turned off at night

(~ 1800 h). Dogs were fed once daily and water was continually available. The intake room was often nearly fully occupied and extremely noisy due to the barking of multiple dogs.

Testing procedure

Each day, the experimenters assessed the newly admitted dogs. If a dog met the enrollment criteria it was added to the study and randomly assigned to an experimental condition by a coin toss so that testing in all experiments began within about 24 h of arrival to the shelter. However, at the end of each experiment the assignment became quasi-random to balance the sex of the dogs across conditions. Treatment occurred Monday thru Friday between 1330-1730 h.

A pretest blood sample was taken immediately after removing the dog from the home kennel. The dog was then taken to an outdoor enclosure for a 5 min walk to allow for elimination. The experimenter kept the dog on the leash and walked slowly and calmly around the enclosure. Play behavior and running were discouraged, and the experimenter avoided physical contact with the dog. Immediately following the walk, the dog was weighed, and then returned to the home kennel or taken to the treatment room, which was a secluded room in the rear of the shelter. Inside the treatment room was a chain link enclosure (1.5 m x 3.0 m x 1.8 m) that contained a single chair and a soft blanket on the floor. The dog was placed in the enclosure alone or with an unfamiliar woman (i.e., the

petter). Immediately following the treatment, a post-test blood sample was taken. Some dogs were returned directly to their kennel without treatment. These had a second blood sample collected at the same approximate interval from the first sample as did dogs exposed to the treatment room.

Petting technique

The petter did not participate in any other procedures with the dog, and wore green or blue scrubs to differentiate her from the experimenters who wore white lab coats. She sat inside the enclosure and encouraged the dog to lie or sit down next to her. The petter spoke in a soft soothing voice and used long strokes to pet. A deep massaging technique was used with a focus on the dog's lower neck and shoulders (Tuber, 1986). The petting was modified to make the dog as comfortable and calm as possible, and play behavior was discouraged.

Sampling

Blood - Blood samples were collected in a separate room a short distance from the intake and test areas. A steel examination table was used for ease of collection. One experimenter gently placed the dog on the table, restrained the dog, and presented a front leg. Another experimenter collected ~ 1.5 ml of blood from the cephalic vein with a sterile syringe. The blood was dispensed into a heparinized tube for cortisol analysis. Blood samples were gently inverted, placed on ice, and centrifuged at the laboratory to separate the plasma, which was frozen until assayed. A total of 480 blood samples were collected. Four

hundred and forty six of 480 samples were collected within 4 min. Based on data in rodents this was rapid enough to ensure that the samples obtained were not appreciably elevated as a result of the blood sample collection (Coover et al., 1979). Cortisol values for the remaining 34 samples fell within the range of those collected more rapidly. This was not surprising since many of the dogs showed negligible reaction to the actual blood withdrawal and often appeared to be enjoying the close contact with humans.

Hair - Approximately 250 mg (5 x 5 cm section) of 0.5 cm length of hair measured from the skin was collected using a standard pet-grooming tool. The underside right chest area was chosen to keep the dog aesthetically pleasing to potential adopters. The 0.5 cm length was chosen because this was the hair length of the most common dog (i.e., pit bull type) housed in the shelter and because this length provided an estimate of cortisol secretion over an approximate 2-week period (Harkey, 1993). The hair was placed inside a tube and stored at room temperature.

Sample processing

Hormone extraction from hair - The hair was evenly divided into 5 polypropylene tubes and washed twice by adding 1 ml of isopropanol to each tube and gently mixing on a shaker (Scilogex Pro Orbital Digital Shaker, Thermo Fisher Scientific, Model 1126W14, Grand Island, NY, USA) at room temperature for 3 min. The hair was placed in a clean protected area to dry for 5 days

(Davenport et al., 2006). Once dried, the hair was ground to a fine powder using a homogenizer (Precellys[®] 24, Caymen Chemical Co, Model 10011145, Ann Arbor, MI, USA; 2.8 mm metal beads; 2 ml reinforced tubes) for 90 s at 113 Hz. Approximately 50 mg of powder was weighed out and placed in 2 ml tubes with 1 ml of ethanol added to each tube. To extract the steroid, the tubes were placed in a shaker (Multi-Therm Shaker, Benchmark Scientific, Model H5000-HC, Sayreville, NJ, USA) and incubated with gentle shaking at 37°C for 18 h. After the extraction, the tubes were centrifuged for 30 s. Approximately 0.8 ml of supernatant was removed and placed in a clean 2 ml microcentrifuge tube. The supernatant was evaporated to complete dryness with vacuum centrifugation (DNA Speed Vac, Thermo Scientific, Model 20-548-132, Waltham, MA, USA). The dried extract was reconstituted with 0.2 ml of phosphate buffered saline (PBS), and stored at 4°C for future cortisol analysis. To ensure reliability of the preparation, values are based on the mean of three samples, each assayed in duplicate.

Hormone determination - Samples were assayed using a standard radioimmunoassay procedure for cortisol (Coat-a-Count, Siemens) validated for dogs (Reimers et al., 1981) as described in our previous studies (e.g., Hennessy et al., 2006). Intra and inter-assay coefficients of variation were 12% and 11% for plasma, and 23% and 16% for hair, respectively.

Experiments

Experiment 1 – Forty eight dogs were tested on 3 consecutive days at approximately 24 h intervals, as illustrated in Fig 1. Sixteen dogs were tested in each of three conditions. In the Home Kennel condition, the dog was simply returned to its kennel between collection of blood samples. In the Alone condition, the dog was placed alone in the enclosure in the treatment room. In the Pet condition, the dog underwent the standard petting procedure.

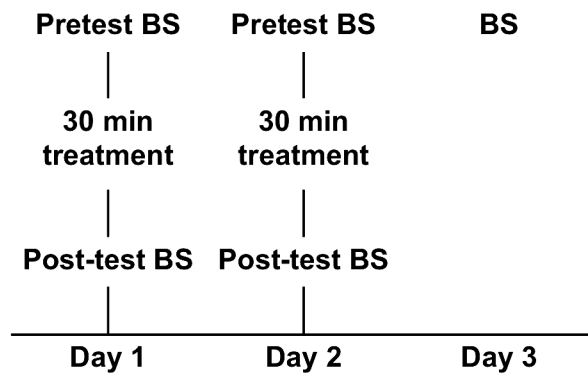


Figure 1. Experimental design used in Experiment 1 (BS = blood sample).

A one-way glass blind was located in front of the enclosure for the observer who recorded the dog's behavior on a laptop computer with a customized data collection program. The frequency of barks and whines, and the duration of tail-wagging were interpreted as measures of excitation and social solicitation, respectively. Barking and whining were combined into a total vocalization score.

The frequency of non-directed licks (the dog's tongue protruding and moving along the upper lip), and the duration of panting were interpreted as signs of anxiety and uneasiness (Beerda et al., 1998; Voith et al., 1996). The frequency of escape attempts (the dog pushing its nose towards the opening of the enclosure, or attempting to jump over the enclosure) also was recorded.

Experiment 2 - Dogs were tested on a single day. Sixteen dogs were tested in each of four conditions (64 dogs total). In the Pet 15 and Pet 30 conditions, the dog was petted for 15 or 30 min, respectively. Blood samples were collected prior to and just following the petting treatment. In the Home Kennel 15 and Home Kennel 30 conditions, the dog was returned to the home kennel for the corresponding length of time prior to collection of the second blood sample. A third blood sample was collected 1 h after the dog had been returned to the home kennel to assess any persistent effects of the petting treatment. Because cortisol values in the Home Kennel and the Alone conditions did not differ in Experiment 1, only Home Kennel controls were included in this experiment.

Experiment 3 - In Experiment 3, we compared dogs that were admitted to the shelter as strays with those that were relinquished by their owners to the shelter. Dogs were tested on a single day. Sixteen stray dogs and 16 relinquished by their owners were tested. In both the Stray and Owner Relinquished conditions, the dogs were petted for 30 min. Blood samples were collected prior to and just following the petting treatment. Hair samples were collected following the final

blood collection. Hair samples were also collected from convenience samples of four healthy pet dogs living in a home environment and three pet dogs diagnosed with Cushing's disease simply for visual comparison and a general indication of the validity of the assessment technique.

Statistical analysis

For all measures, preliminary tests examined effects of sex. If significant, male and female scores were analyzed separately. If not significant, sex was dropped as a variable from the final reported analyses. Analysis of Variance (ANOVA) (repeated measures when appropriate) was the preferred means of analysis as described for individual experiments. Significant effects were followed with tests for simple main effects and multiple paired-comparisons (Newman-Keuls). When data failed to meet assumptions for ANOVA (all behavior measures), nonparametric Mann-Whitney U tests were used. A probability level of $P = 0.05$ (2-tailed) was considered statistically significant. Some samples for analyses of hair cortisol in Experiment 3 were lost due to error resulting in samples sizes of 12 for strays and 10 for dogs released by their owners.

III. RESULTS

Experiment 1

Cortisol - Petting the dog significantly reduced the cortisol response to the shelter. To examine the immediate effect of the manipulations on Days 1 and 2 on plasma cortisol levels, we calculated absolute difference scores (post-test minus pretest). A 3 (Condition) x 2 (Day) repeated measures ANOVA of the difference scores yielded one significant effect, a main effect of Condition, $F(2,45) = 5.56, P < 0.01$ (Fig 2). Neuman-Keuls paired comparison showed that the mean plasma cortisol levels of the dogs in the Pet condition were significantly reduced from pre- to post-test compared to those of dogs in the Alone and Home Kennel conditions, P 's < 0.05 . The lack of a significant interaction of Condition x Day indicates that the magnitude of plasma cortisol reduction was comparable on both days. That is, petting was effective on both days, but there was no cumulative effect of petting on Day 1 on the cortisol response to petting on Day 2 (Fig. 2)

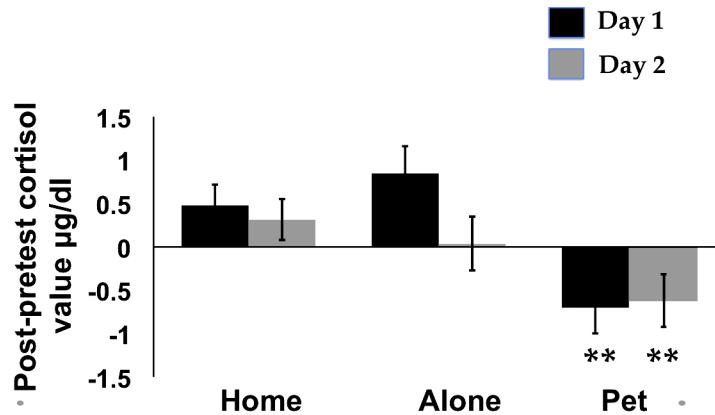


Figure 2. Mean change in plasma cortisol levels from pretest to post-test for dogs in the three treatment conditions of Experiment 1. Vertical lines indicate standard errors of the means. N = 16/condition, ** $P < 0.01$.

There was no evidence of a lasting effect of petting on pretest cortisol levels. To examine if the reduction of plasma cortisol levels persisted to the time of the pretest sample the next day, we calculated absolute difference scores (pretest Day 2 minus pretest Day 1, and pretest Day 3 minus pretest Day 1). A 3 (Condition) x 2 (Day) repeated measures ANOVA of the difference scores yielded only an effect of Day, $F(1,45) = 5.62$, $P < 0.05$ with a reduction in cortisol concentrations from Day 1 to Day 3 across all conditions (Fig. 3).

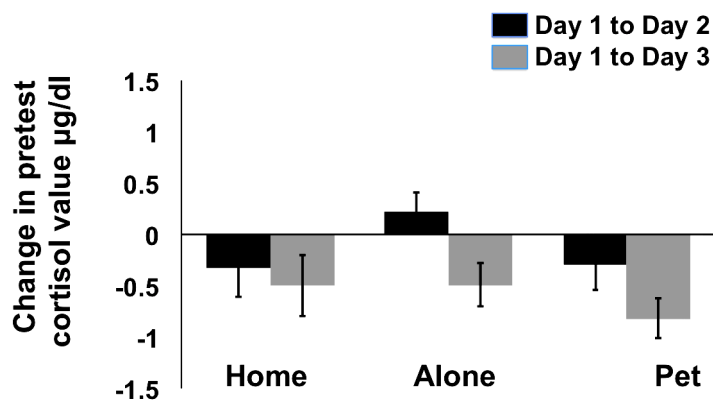


Fig 3. Mean change in pretest cortisol levels from Day 1 to Day 2 and Day 1 to Day 3 for dogs in the three treatment conditions of Experiment 1. Vertical lines indicate standard errors of the means. N=16/condition.

Although there was no significant effect of Condition, 13 of 16 dogs in the Pet condition showed a reduction in cortisol values on Day 3 relative to Day 1 compared to 19 of 32 in the control groups. Nearly all the dogs in the experiment were strays. However of the three dogs in the Pet condition that were relinquished by their owners, two showed an increase in pretest cortisol levels from the first to the last day. That is, 12 of 13 strays showed a reduction with human interaction, whereas only 1 of 3 dogs released by their owners did so. This raises the possibility that the dogs of different sources responded differently to the manipulation. The ANOVAs therefore were repeated with just the strays, but there was no change in the pattern of significant results. Nonetheless, in

Experiment 2 we only used strays to control for the source of the dogs, and in Experiment 3 we directly compared the effects of petting on strays versus dogs relinquished by their owners.

Behavior - Petting decreased excitation (i.e. vocalizing) and increased social solicitation (i.e. tail-wagging). Measures of anxiety (i.e. panting) and escape attempts were reduced, but in a sex specific manner. A Mann-Whitney U test indicated that petting the dog significantly reduced vocalizing and increased tail-wagging on both days relative to the Alone condition (vocalization: Day 1, $P < 0.001$, Day 2, $P < 0.001$; tail wagging: Day 1, $P < 0.001$, Day 2, $P < 0.05$) (Table 1).

Table 1. Median (semi-interquartile range) of behaviors found to differ between conditions.

	Alone		Petting	
	Day 1	Day 2	Day 1	Day 2
Vocalizing (f)	97(118)***	77(200)***	4(8)	3(6)
Non-directed licks (f)	5(5)	4(6)	4(3)	2(3)
Escape Attempts (f)				
Male	19(6)*	2(3)	0(5)	1(1)
Female	22(34)*	73(22)*	0(2)	1(4)
Panting (d)				
Male	1024(493)*	388(324)	333(360)	368(298)
Female	74(213)	71(105)*	70(223)	0(372)
Tail-wagging (d)	0(321)	21(157)	529(995)***	429(933)*

f = frequency, d = duration in sec. * $P < 0.05$, *** $P < 0.001$ for Alone vs Petting in same day.

Because preliminary analysis indicated that escape attempts and panting differed for males and females, separate analyses were conducted for the two sexes. Males in the Alone condition attempted to escape significantly more often than males in the Pet condition on Day 1 of testing, $P < 0.05$, but there was no significant difference in the males' escape attempts on Day 2. On the other hand, females in the Alone condition attempted to escape significantly more times than

females in the Pet condition on Day 1 ($P < 0.05$) and Day 2 ($P < 0.05$) of testing. Further, males in the Alone condition panted significantly longer than males in the Pet condition on Day 1 of testing, $P < 0.05$, but there was no significant difference on Day 2. For females, there was no significant difference in panting on Day 1, but on Day 2 panting was more frequent in the Alone condition, $P < 0.05$. For non-directed licks, there was no significant difference between conditions.

Experiment 2.

Petting for 15 min was just as effective as petting for 30 min in reducing the dogs' cortisol response to the shelter. To examine the effect of the duration of petting on plasma cortisol levels at both post-test time points, we calculated absolute difference scores (post-test minus pretest; post-test + 1h minus pretest). A 2 (Condition) x 2 (Petting Duration) x 2 (Time Point) ANOVA (with the last factor treated as a repeated measure) of the difference scores yielded a main effect of Condition, $F(1,56) = 8.50$, $P < 0.01$ and a Condition x Time Point interaction effect, $F(1,56) = 2.73$, $P < 0.05$. There was no significant main or interaction effect of Petting Duration. Further analysis of the significant Condition x Time Point interaction with simple main effects tests revealed that the cortisol levels of the dogs in the Pet condition were reduced significantly more from pretest to the immediate post-test sample than were those of dogs in the Home Kennel condition, $P < 0.01$. However, cortisol levels in the Pet condition began to

increase when returned to the home kennel so that there was no difference between groups 1h later (Fig. 4).

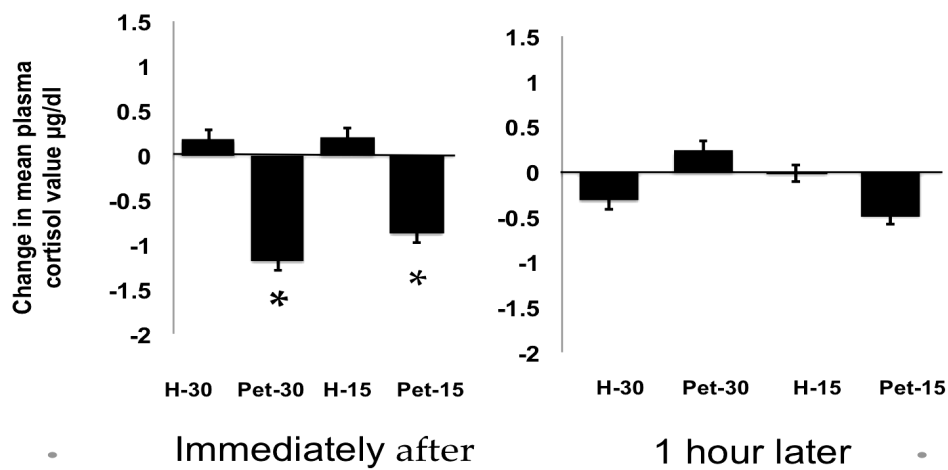


Fig. 4. Mean change in plasma cortisol levels from pretest to post-test for dogs in the four treatment conditions of Experiment 2. Vertical lines indicate standard errors of the means. N = 16/condition, ** $P < 0.01$.

Experiment 3.

The effect of petting varied with the source of the dog. To determine if the response to petting of dogs relinquished by their owners was comparable to that of strays, we examined the cortisol reduction from pretest to post-test immediately following 30 min of petting. A 2 (Condition) x 2 (Sex) x 2 (Pre/Post)

ANOVA (with the last factor treated as a repeated measure) yielded a main effect of Pre/Post, $F(1,28) = 19.17, P < 0.01$; a Condition x Pre/Post interaction, $F(1,28) = 7.00, P < 0.05$; and a Condition x Sex interaction, $F(1,28) = 6.81, P < 0.05$. For the Condition x Pre/Post interaction, tests for simple main effects revealed that the strays' plasma cortisol values were significantly reduced from pretest to post-test, $P < 0.01$, whereas those of dogs released by their owners were not (Fig. 5).

For the Condition x Sex interaction, tests for simple main effects showed that females' plasma cortisol values were elevated compared to those of males in the Stray condition, $P < 0.01$, whereas, males' plasma cortisol values were elevated compared to those of females in the Owner Relinquished condition, $P < 0.05$.

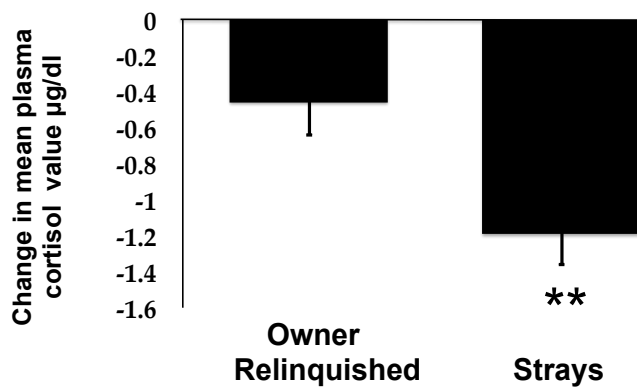


Fig 5. Mean change in plasma cortisol levels from pretest to post-test for dogs in the two conditions of Experiment 3. Vertical lines indicate standard errors of the means. $N = 16/\text{condition}$, ** $P < 0.01$.

Hair cortisol concentrations were measured to provide an estimate of the stress of dogs for the 2-week period just prior to shelter admittance. Cortisol levels of dogs in the Owner Relinquished and Stray groups appeared intermediate to those of the healthy pets and Cushing's dogs (Fig. 6) as one might expect if the dogs admitted to the shelter had been exposed to stressful events prior to admittance. However, a *t*-test found no difference between the cortisol levels of the Owner Released and Stray dogs.

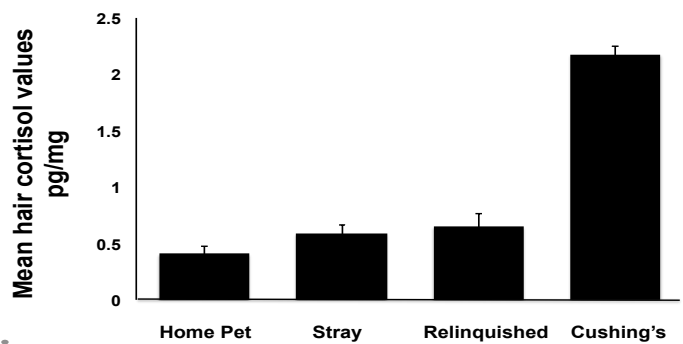


Fig. 6. Mean hair cortisol levels for dogs in the two conditions of Experiment 3. Healthy home pet dogs and Cushing's dogs are included for visual comparison. Vertical lines indicate standard errors of the means. Owner surrender, N = 10; Strays, N = 12; Home dogs, N = 4; Cushing's dogs, N = 3.

IV. Discussion

The current study adds to a growing body of literature indicating that human interaction can reduce the stress and improve the welfare of dogs in shelters. Behaviorally, dogs responded to the petting in a predictable fashion by showing signs of increased social solicitation, and reduced anxiety, excitement, and escape attempts during the petting episode. However, the primary emphasis of the present study was to understand better the impact of human interaction on the HPA system. We once again demonstrated that relatively brief periods of human interaction can reduce the cortisol response of dogs to a shelter environment. In addition, we showed that a second day of petting was as effective as the first. There was no sign of habituation to the petting procedure, but neither was there a cumulative effect of repeated petting on circulating cortisol levels. In another study conducted at about the same time as the work reported here, shelter dogs petted 7-8 times over a 10-day period showed a cortisol reduction on the tenth day that was comparable to that seen on the first (Dudley et al., 2015). Together, these data indicate that this form of human interaction can continue to effectively reduce neuroendocrine stress responses when applied on a daily or near daily basis. Moreover, the results of Experiment 2 suggest that similar effects can be obtained if the petting time is reduced from

30 to 15 min, which may be a more reasonable length of time to expect a volunteer at a shelter to devote to a single dog.

Nonetheless, cortisol levels rapidly returned to pre-treatment values when the dogs were placed back in their kennels in the intake area. In Experiment 1, there was no lasting effect on cortisol concentrations 24h after one or two 30-min interaction sessions, and in Experiment 2 the reduction did not persist even 1h following a 15- or 30-min session. In contrast, Coppola et al. (2006) found that 30-90 min of interaction on the second day in the shelter reduced cortisol levels the following day. That study differed from the present one in a number of ways that may account for the discrepancy in results. These include a longer average duration of interaction, different types of interaction (e.g., walking on leash, grooming, providing treats), less crowded kenneling areas, and the population of dogs examined (e.g., inclusion of younger dogs, smaller breeds, and a seemingly larger variety of breeds overall).

In Experiment 3, we found that a 30-min petting procedure that effectively reduced the cortisol elevations of dogs admitted to the shelter as strays produced only a nonsignificant reduction in the cortisol levels of dogs released to the shelter by their owners. Previously, Hiby et al., (2006) found that owner-relinquished dogs exhibited more-protracted urinary cortisol elevations following shelter admittance than did strays, suggesting that entrance to a shelter has a greater impact on dogs coming directly from a home. For dogs released by their

owners, entrance to the shelter may entail an assortment of stressors not experienced by strays, such as disruption of attachment and an abrupt change in regular routines of feeding and walking. As a result, it may require more than a single half hour of interaction to have a measureable effect on HPA activity.

Further, measurement of hair indicated comparable cortisol secretion by strays and owner-relinquished dogs during the previous 2-week period. This initially was surprising to us in that dogs coming from a home would seem likely to experience fewer threats and aversive circumstances than stray dogs. Yet, a dog's level of attachment is not necessarily reciprocated by the owner (Rehn et al., 2014), and Kwan and Bain (2013) found that most owners of relinquished dogs did not have a significant attachment to their dogs, and reported that unwanted behavior affected their decision to release them to a shelter. Thus, while the transition to the shelter may be more difficult for dogs coming from a home – as reflected in the urinary cortisol levels reported by Hiby et al (2006) and the inability to reduce plasma cortisol levels in the current study – the home environment prior to relinquishment may still be about as stressful as the environment experienced by a stray.

In the present study, we observed sex differences in escape attempts and panting in Experiment 1, and in overall cortisol levels of stray dogs and those released by their owners in Experiment 3. However, because the population of dogs included here—as in other published studies of shelter dogs—were a

heterogeneous assortment of intact and gonadectomized males and females of various breeds, it is difficult to draw any firm conclusion regarding the meaning or reliability of these outcomes.

In recent years, increasing attention has been paid to how the welfare of dogs in shelters can be improved. Human interaction is a simple intervention that can have salubrious effects on the physiology as well as behavior of shelter dogs. This interaction can take various forms. Whereas the current study focused on petting, we and others have incorporated play, training, walking, providing treats, and mere passive presence of a human in procedures that effectively reduced cortisol elevations (Coppola et al., 2006; Menor-Campos et al., 2011; Shiverdecker et al., 2013). In a recent review of procedures at eight European shelters, taking a dog for a walk was associated not only with lower levels of stereotypic and displacement behaviors, but also with a much greater level of antioxidant capacity (Cafazzo et al., 2014). Whatever form of human interaction that might be used should be feasible in the day-to-day operations of the shelter. Our results show that 15 min of petting can reduce cortisol elevations to shelter housing, and that the beneficial effect of petting can be repeated on consecutive days. Developing procedures that have outcomes more persistent than those seen here remain a challenge. Our findings also point to how the source of the dog can determine the effectiveness of a procedure. Finally, the measurement of cortisol accumulation in hair may prove to be a useful means of assessing

aspects of the past experience of the dog, which may make it possible to individualize stress-reduction treatments accordingly.

Acknowledgements

The author would like to thank Alexandra Mutwill, Lauren MacDonald, Amanda Schreibeis, Ann Mitchell, Alex Hensley, Cheryl Brinkman, Nicholas Kovar, Kate Berberich, Darci Gallimore, Adam Koraym, Fatima Vakil, Sara Lacy, Kellyn Wagner, E.J. Loewer, and Mahentikaa Gunasakera for assistance in data collection. Fran Linden for help training students, and Mick Sagester, Mark Kumpf, and the staff of the Montgomery County Animal Resource Center for their continued support. This work was supported by the WALTHAM Foundation.

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