Effects of an Extended-Use ThunderShirt™ Pressure-Wrap Protocol on Chronic Stress in Shelter Dogs

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Abstract

There is growing interest in studying shelter dog welfare, specifically how to the stress of the shelter environment. Human interaction, food enrichment toys, and synthetic dog appeasing pheromones have all been found to reduce some stress-related behaviors and cortisol in shelter-housed dogs. Several studies have found pressure wraps reduce stress-related behaviors and some physiological signs of stress for homed dogs during acutely stressful situations. This study investigated the efficacy of pressure wraps for use in treating chronic stress in shelter dogs. Ten shelter dogs were randomly selected to be in either the treatment group (wearing a ThunderShirt™) or control group. During the treatment phase, dogs wore their shirt for one hour, twice per day for 14 consecutive days. Control group dogs experienced an interaction designed to simulate applying and removing the shirt to coincide with the same hour-long window as the treatment group dogs. Behavior observations and salivary cortisol samples were evaluated at multiple time points pre-, during, and post-treatment to look for differences across the study both within each group as well as between groups. We found no indication that there is a sustained reduction of stress-related behaviors or cortisol levels after the ThunderShirt™ had been removed. This may mean that while the shirt is useful when on, it does not induce any long-lasting effects. Or, more likely, our sample size was too small to detect changes. Future research should include a larger sample size for more robust results. It may also be beneficial to use a passive control group who does not have increased human contact throughout the study.

Keywords: ThunderShirt™, shelter dogs, chronic stress, welfare

1. Introduction

Over the last two decades, there has been increasing interest in research related to shelter dog welfare (see for example: Beerda, van Hooff, de Vries, & Mol, 2000; Hennessy, Davis, Williams, Mellott, & Douglas, 1997; McCobb & Dowling-Guyer, 2016; Protopopova, 2016; Wells, 2004). Dogs living in shelter environments are particularly prone to stress, fear, anxiety and frustration (Beerda, van Hooff, de Vries, & Mol, 1999; Denham, Bradshaw, & Rooney, 2014; Hennessy, 2013; Hennessy et al., 1997; Tuber et al., 1999). Even in well-run shelters, dogs are exposed to various psychological stressors that range from isolation from any former attachment figures to unpredictable, loud noises, loss of a sense of autonomy, and even simply the novelty of the environment (Hennessy, 2013; Herron, Kirby-Madden, & Lord, 2014; Protopopova, 2016; Rooney, Gaines, & Bradshaw, 2007; Tuber et al., 1999). Some interventions shown to reduce the impact of stress in shelter dogs include human interactions (Dudley, Schiml, & Hennessy, 2015; Hennessy et al., 2002; Hennessy, Williams, Miller, Douglas, Voith, 1998; Shiverdecker, Schiml, & Hennessy, 2013), food enrichment toys (Schipper, Vinke, Schilder, & Sprujit, 2008), playing classical music (Wells, 2004) and using synthetic dog appeasing pheromones (Tod, Brander, & Waran, 2005). Each of these interventions has demonstrated at least some reduction in either observable stress-related behaviors or cortisol measures over the short-term. These interventions are particularly useful in a shelter environment as they are relatively inexpensive and easy to implement such that many dogs could benefit from their use. Another possible tool might be an anti-anxiety pressure wrap such as the ThunderShirt™ (ThunderWorks, Durham, NC, USA) which has been used to reduce stress in dogs in other settings. The ThunderShirt™ is a washable and reusable product designed to provide comfort and stress reduction. Pressure wraps designed for dogs have been demonstrated to reduce observable signs of stress in homed dogs during acutely stressful events (Cottam & Dodman, 2009; Cottam, Dodman, & Ha, 2013; Pekkin et al., 2016; Damon, Rozanski, Spagnoletti, & Sharp, 2014). Based on these findings, we hypothesize that pressure wraps may also provide a sustaining reduction in stress-related behaviors and cortisol measures,

even at times when the wrap is not on, for dogs suffering from chronic stress. While pressure wraps have been studied in owned dogs (Cottam & Dodman, 2009; Cottam, Dodman, & Ha, 2013; Pekkin et al., 2016; Damon, Rozanski, Spagnoletti, & Sharp, 2014), and some shelters use them with resident dogs, there has not yet been any published research investigating their efficacy in the shelter environment. Therefore, the current study aims to assess the use of the ThunderShirt™ for chronic stress in shelter-housed dogs.

Clinical studies have found that firm pressure over a large area of the body may have a calming effect in animals (Grandin, 1992; Pekkin et al., 2016). Specifically, it has been shown that pressure wraps on dogs can reduce some stress-related behaviors including panting and hiding during naturally occurring loud noise events such as thunder storms (Cottam & Dodman, 2009; Cottam, Dodman & Ha, 2013), and reduce the amount of time spent lying down during contrived noise events such as fireworks (Pekkin et al., 2016). When pressure wraps have been used with dogs displaying stress-related after admission to an animal hospital, it was found that after wearing a ThunderShirt™ for just one hour, vocalizing, pacing and chewing were reduced in frequency to nearly zero (Damon et al., 2014). King, Buffington, Smith and Grandin (2014) found that using a ThunderShirt™ with dogs diagnosed with separation anxiety resulted in a lower heartrate for dogs in the treatment group compared to those in the control group when left unattended for 15 minutes in a novel location. More specifically, they found that heartrate for those dogs in the treatment group decreased over the 15-minute study period while the heartrate of dogs in the control group increased over the same period. Due to the prolonged confinement and over stimulation experienced at many shelters, there is an ongoing effort to provide the best tools possible for mitigating the impact of that stress for the residents. To that end, pressure wraps such as ThunderShirt™ may be particularly useful in providing both immediate and long-lasting comfort and stress reduction to dogs in the shelter environment.

Stress can be described as a response to any stimulus requiring the individual to adjust their behavior (Lazarus, 2000). An individual experiences *distress* (referred to simply as stress) when they have assessed

a stimulus as unpleasant or negative, while *eustress* is the result of assessing a stimulus in a positive manner (Colligan & Higgins, 2006; Lazarus, 2000). Some behaviors may manifest with the experience of either kind of stress – anxiety or excitement (Protopopova, 2016). Behaviors that are considered to be associated with stress in dogs have been studied with growing interest in recent years (Beerda, Schilder, van hooff, de Vries, & Mol, 1998; Beerda et al., 1999; Herron et al., 2014). These behaviors include, but are not limited to, panting, pacing, barking, whining, howling, lip-licking, yawning, drooling, spinning, escape attempts and inappropriate elimination. Trainers, behaviorists and veterinarians utilize these behaviors as a means to monitor the emotional status of dogs during interactions such as obedience training, behavior modification, and medical procedures (Handelman, 2008; Neilson, 2015; Overall, 2013; Rugaas, 2005; Yin, 2009).

Reducing behaviors related to stress is not the only goal when trying to improve the welfare of dogs in a shelter environment; we also hope to see a clear increase in behaviors associated with relaxation and comfort as these may be signs of successful adaptation and coping (Protopopova, 2016). Behaviors related to successfully coping with the environment (eustress) include affiliative and alert behaviors in response to the presence of a novel person, facing the front of the kennel, standing and gazing into the environment, tail wagging, offering play bows, playing with toys, barking and whining. As there are some behaviors that may indicate either stress or successful coping skills, context must be considered when interpreting the observed behaviors (Protopopova, 2016). Barking and whining, for example, may reflect a state of discomfort with the environment or may result from anticipation of human interaction or other enjoyable activities.

While behaviors are an easily observable method for monitoring stress, the conflicting motivations for some behaviors leaves open the possibility of observer misinterpretation (Protopopova, 2016). That potential for misidentifying some behaviors as caused by distress when it could be eustress makes behavior observation by itself a less than complete measure of a dog's state of stress. For this reason, physiological measures such as cortisol play an important role to our fuller understanding of the effects of

stress in dogs. Cortisol is a hormone secreted during acutely stressful circumstances and has been found to be a valid measure of an individual's stress in a number of species from monkeys (Boinski, Swing, Gross, & Davis, 1999) to birds (Jayson, Williams, & Wood, 2014; Palme, Rettenbacher, Touma, El-Bahr, & Mostl, 2005) to cats and dogs (Schatz & Palme, 2001; Rooney et al., 2007). Cortisol has been measured in many animals through a variety of methods including plasma, urine, feces, hair and saliva (Hekman, Karas, & Dreschel, 2012; Jayson et al., 2014; Palme et al., 2005; Schatz & Palme, 2001). Due to the relative ease and non-invasiveness of the collection process, salivary cortisol is frequently used to capture a snapshot of an individual's current state of stress (Beerda et al., 1998; Cobb, Iskandarani, Chinchilli, & Dreschel, 2016; Hennessy, 2013). Although some mild restraint is necessary, most dogs tolerate the collection process quite well (Cobb et al., 2016; Kobelt, Hemsworth, Barnett, & Butler, 2003), making it a good option to use when efficiency of collection is a priority.

Damon et al. (2014) and King et al. (2014) both found that dogs experienced a reduction in some stress-related behaviors during acute stress situations while wearing a ThunderShirt™. For this study, we were interested in interventions that may result in a sustained reduction in stress-related behaviors and salivary cortisol measures, even when the intervention is not actively being used; therefore, we assessed the effects of an extended use ThunderShirt™ protocol on both stress-related behavior and salivary cortisol in shelter dogs. We hypothesized that for chronically stressed shelter dogs, wearing a ThunderShirt™ twice per day, for 14 consecutive days, would show a significant reduction in stress-related behaviors and salivary cortisol, as compared to pre-treatment baseline values, which cannot be accounted for by habitation over time. We chose treatments of one hour, twice per day to provide frequent acute-stress relief. We further hypothesized that reductions in stress-related behaviors and salivary cortisol measures would sustain for one week after the intervention had been discontinued. If frequent use of the ThunderShirt™ is effective at reducing stress such that those reductions sustain between each wearing, it could become an important tool for addressing the impact of chronic stress in shelter dogs.

2. Material and methods

The purpose of this study is to assess the effects of wearing a ThunderShirt™ pressure wrap for one hour, twice daily, for 14 days on the stress-related behaviors and salivary cortisol measures in shelter dogs who may be experiencing chronic stress. We included a control group of dogs who experienced a handling interaction designed to simulate the experience of applying and removing the shirt in order to more precisely control for human interaction and habituation over time as a potential cause for reduction of stress-related behaviors. Dogs were not held in the shelter if they were adopted prior to completion of the protocol. This study meets all ethical guidelines for working with live animals and was conducted with the approval of the Tufts University Institutional Animal Care and Use Committee.

2.1. *Dogs and housing*

Using a convenience sample of shelters located in the New England region of the United States, 35 dogs across three shelters met our eligibility criteria. Dogs were considered eligible to participate in the study if they met the following inclusion criteria: either sex, intact or neutered/spayed, any breed or breed type, between 6 months and 9 years of age, not currently being treated for major illness or injury, not currently taking any behavioral medication, unlikely to bite while being handled, and in the shelter for at least 14 days prior to the first day of the treatment phase of the study but not more than 365 days prior to the first day of the treatment phase. We based the minimum length of stay requirements on research that indicates dogs experience a spike in cortisol measures upon arrival, with salivary cortisol at its highest during the first three days in the shelter, and then gradually declining (Hennessy, 2013; Hennessy et al., 1997). Because this study is focused on chronic stress, rather than acute, we chose to include dogs after their initial adjustment period. Research has also shown that ongoing exposure to a stressor, such as a prolonged stay in a shelter, may result in an immune-suppression response which leads to a dysregulation of the production of cortisol in relation to other stress-related hormones (Hennessy, 2013; Protopopova, 2016). For this reason, we excluded dogs who had been in the shelter system for more than one year. Other exclusion criteria included signs of stress or aggression during the handling procedures either for the shirt/simulation or the saliva collection process. One dog was excluded from the study for showing

increased stress by complete disengagement with humans and refusal to take treats while wearing the ThunderShirt. Two dogs were excluded from the study for demonstrating increased stress via avoidance behaviors and stiffening body posture during the practice saliva collection. Therefore a total of 32 dogs were qualified to participate in the study.

Of the 32 dogs initially enrolled, nine were adopted prior to the start of the study and one was reclaimed by his owner. Twenty-two dogs began the study, and 12 of these were adopted prior to completing the 26-day protocol. This paper will focus on the 10 dogs who were shelter residents long enough to complete the 26-day protocol. Of these 10 dogs, five were male and five female. Staffordshire terrier mix was the most represented breed type (n = 7). There were two Chihuahua mix breeds and one Cairn Terrier/Cocker Spaniel mix (Table 1). All breeds were determined by shelter staff report, based on visual appearance. Eight dogs were spayed or neutered prior to the start of the study while two dogs, one female and one male, were intact for the duration of the study. The mean age was 35.7 months (median: 25.5; range 12 – 72 months). Ages were determined either by shelter staff based on visual appearance, or by veterinary dental check. The mean weight was 100.03 Kg (median: 130.9 Kg; range 17.6 – 145.6 Kg). Dogs had been in the shelter for a mean of 26.1 days (median 23.5 days, range: 14 – 61 days) on the first day of the treatment phase of the study.

The 10 dogs who completed the study resided in one of three New England shelters. Two dogs were housed at the New Hampshire Society for the Prevention of Cruelty to Animals (NHSPCA) in Stratham, NH, USA. Three dogs were housed at the Thomas J. O'Connor Animal Control and Adoption Services (TJO) in Springfield, MA, USA. Five dogs were housed at the Worcester Animal Rescue League (WARL) in Worcester, MA, USA. All dogs were single-housed at all three shelters. Kennel sizes varied both by shelter and within shelter. At NHSPCA, the kennels on the main adoption floor range from 1.53m^2 to 9.19m^2. One small dog was housed in a back room in a standard, stacked veterinary kennel approximately 0.23m^2 on the first day of the pre-treatment phase, but was then relocated to the main

adoption floor for the duration of the study. Kennels at TJO are 2.23m^2, and kennels at WARL range from 3.07m^2 to 4.59m^2.

NHSPCA kennels are indoor spaces with a raised platform at the rear of the kennel, with either a bed or blanket and at least one toy. Dogs are walked for toilet opportunities several times per day by staff and volunteers. TJO kennels are indoor divided spaces with a bed or blanket and toys on one side and bare floor on the other side. The two halves of the kennel are equal in size and can be separated by closing a guillotine door. Dogs had solo yard time at least twice every day (after meals) but were often able to interact with other dogs through fencing that separates the yards, and volunteer walks as available. WARL kennels, like TJO, are divided with bed or blanket and toys on one side and bare floor on the other side, and each side can be isolated by closing a guillotine door. Each kennel at WARL has a large side which ranges from 2.23m^2 to 3.34m^2, and a smaller section which ranges from 0.84m^2 to 1.25m^2. Dogs had solo yard time twice per day (after meals) as well as staff/volunteer walks as available. Each shelter moved dogs from one kennel to another per their usual protocol based on several factors including the level of stress the dog appeared to be experiencing, health of the dog, quarantine status, and if the shelter required a particular kennel space for another dog. All three shelters played soft classical music throughout the day in all kennel areas. During the course of the study at WARL, the staff began using lavender aroma therapy for a dog who was not part of this study. It was in the same kennel room as two participating dogs (one treatment group and one control group, both male), but not in the same aisle.

2.2 *Design and equipment*

The study was arranged in three phases: Pre-Treatment (Day -5 – Day -1), Treatment (Day 1 – Day 14) and Post-Treatment (Day 15 – Day 21). During the pre-treatment phase, video observations and saliva samples were collected on Day -5 and Day -2 from each dog in order to determine baseline measures for behavior and cortisol. Three data collection points during the treatment phase occurred on Day 3, Day 8 and Day 14. A final data collection point occurred on the last day of the post-treatment phase – Day 21. Videos were collected using three digital video cameras – an iPhone 6S (Apple Inc., Cupertino, CA

USA), an iPhone 4S (Apple Inc., Cupertino, CA USA) and a Sanyo Xacti mpeg-4 avc / h.264 digital video camera (Sanyo, Moriguchi, Osaka Prefecture, Japan). The two cell phone cameras were used with a GripTight GorillaPod Stand™ (Joby DayMen US Inc., Petaluma, CA USA) while the Sanyo camera was used with a Manfrotto 3047 tripod (Monfrotto Distribution, Upper Saddle River, NJ USA). Cameras were placed between 0.76 m and 0.9 m from the kennel door, up against the wall or near the kennel door on the opposite side of the aisle from the subject dog. Cameras were set as close to centered in front of the kennel as the environment would allow in order to maximize the view within the kennel.

SalivaBio Children's swabs from Salimetrics were used to collect saliva from each dog. Forty ThunderShirts™ were provided by the manufacturer (ThunderWorks™, Durham, NC, USA). The ThunderShirt™ is made of a stretchy jersey with Velcro™ closures. It includes a neck strap which helps to hold the shirt in roughly the correct position and wide body straps which wrap around the dog's chest such that it is snug, but not tight. It is meant to create a constant, subtle contact over a large area of the body, which has been found to create a calming effect (Grandin, 1992; Pekkin et al., 2016).

All data were collected between June 27, 2016 and August 31, 2016, and all videos and saliva samples were collected between 13:00 h and 14:30 h outside of treatment windows so that no dogs were wearing a shirt during data collection.

2.3 *Experimental groups and procedures*

Participating shelter staff were trained per study protocol for how to apply and remove the ThunderShirt™ as well as how to conduct the interaction exercises which simulated the application and removal of the ThunderShirt™. The staff then practiced these procedures on each dog who met the inclusion criteria as we assessed the dogs to determine if they were comfortable being handled as required for the application of the shirt/simulation exercise and saliva collection process. During the assessment, each dog wore the ThunderShirt™ for 5-7 minutes and also experienced the interaction exercise designed to simulate having the shirt applied and removed (see Appendix A for a detailed description of the shirt application and

simulation exercise procedure). We then conducted a practice saliva collection by pairing a Q-Tip with string cheese several times, and then inserting the Q-Tip into the dog's mouth and holding it there for approximately 30 seconds. During this process, a trained shelter staff member gently restrained the dog in a modified veterinary hold while the researcher inserted the Q-tip into the dog's mouth, holding a bite of cheese such that the dog could continuously smell it, but could not reach it with their tongue. The researcher encouraged the dog to sniff the cheese and chew on the Q-tip to increase salivation. Immediately after the Q-tip was removed from the dog's mouth, they were given the cheese and both the researcher and shelter staff offered praise and pets for cooperating. If the dog became at all agitated, trying to escape the restraint or spit out the Q-tip, we allowed the dog to take a break for 1-2 minutes before making another attempt to hold the Q-tip in the dog's mouth.

To ensure that dogs who were exhibiting more stress and dogs who were exhibiting less stress were equally represented in both the treatment and control groups, we developed a 10-point rating scale where 1 = "not at all stressed" and 10 = "very stressed" based on an overall impression of the dog's behavior since arrival at the shelter. This global stress assessment (GSA) was completed by two shelter staff who were knowledgeable of all enrolled dogs at their shelter. The two scores for each dog were then averaged and dogs scoring less than 4 were categorized as "less stressed" while dogs scoring 4 or higher were categorized as "more stressed." Using a random team generator (https://www.randomlists.com/team-generator?items), we then randomly assigned all dogs in each category to either the treatment group or the control group. This ensured that we had representation of both "less stressed" and "more stressed" dogs (according to shelter staff observations) in each of our experimental groups.

During the pre-treatment phase, on Day -5 and Day -2, all dogs had 12-minute videos taken while in their home kennels. Video collection was followed by a practice saliva collection using a Q-Tip and string cheese to help acclimate the dogs to the handling required to insert and hold the saliva collection swab in place for the recommended 60-90 seconds to achieve full saturation of the swab. After the practice saliva collection, dogs were left unattended for a minimum of five minutes to allow their mouths to begin to

clear of food to avoid unintended contamination of the actual sample when collected (Dreschel & Granger, 2009). This also allowed the dogs to begin to settle from the restraint interaction. After this pause, we conducted an acclimation session for both the treatment and control groups. Dogs in the treatment group (n = 6) had their ThunderShirt™ put on while dogs in the control group (n = 4) experienced the simulation interaction for putting the shirt on. After a minimum of five minutes, each dog had its shirt removed (or simulation interaction for removing the shirt), and this was immediately followed by the actual saliva collection using the SalivaBio Children's swabs from Salimetrics. Efforts were made to keep the swab in each dog's mouth for a total duration of 60-90 seconds, as recommended by Salimetrics instructions for these swabs, to ensure proper saturation (http://www.salimetrics.com/assets/documents/childrens_swab_saliva_collection_instructions.pdf). Swabs were immediately placed into the saliva vials provided by Salimetrics and placed in an insulated cooler with a frozen Blue Ice (Rubbermaid, Atlanta, GA) to begin cooling the samples immediately. Once all data collection was complete for the day at that location, saliva samples were stored in a freezer set to -20° C until shipping on dry ice to Salimetrics labs in State College, PA USA for processing. The primary researcher managed all video equipment and collected all saliva samples with the assistance of trained shelter staff to help with restraint for the collection process.

Shelter staff trained for this study were responsible for conducting the protocol during the treatment phase which consisted of 14 consecutive days during which the treatment group wore the ThunderShirt™ for 60 minutes in the morning and again for 60 minutes in the late afternoon. The control group experienced the simulation interactions to coincide with the application and removal of the shirt for the treatment group. During these twice-daily treatment windows, the dogs were not allowed to engage in dog-dog play for safety, but they were allowed to do anything else that would normally happen during that time including eating, walks, and meeting potential adopters. Shelter staff noted for each dog the time their shirt was applied and removed, or simulation exercises performed, and what activity the dog engaged in during each treatment window. Outside of the treatment window, dogs who would normally be allowed to

engage in dog-dog play were allowed to do so. On Day 3, Day 8 and Day 14, 12-minute videos were recorded of each dog in their home kennel and immediately following the video recording, a saliva sample was collected.

Shelter staff resumed normal activities during the post-treatment phase of the study. The treatment group dogs did not wear the ThunderShirt™ and the control group dogs did not experience the simulation interactions. On Day 21, a final video recording and saliva sample was collected from each dog.

2.3.1 *Behavior coding*

Using Observer XT, version 11.5, by Noldus (http://www.noldus.com), 10 minutes of each 12-minute video was coded for 33 behaviors, although not all of these behaviors were used in the analysis (for a complete list, see Appendix B). The first and last 60 seconds of video were not coded to minimize the effect of the researcher's presence directly in front of the dog while setting up and removing the recording equipment.

Behaviors coded for seconds of duration included howling, panting, pacing, locomoting, sitting, stretching, lying down with head up (alert), and lying down with head down (resting). Each behavior must have occurred for 2 consecutive seconds to be considered as having started, and must have stopped for 2 consecutive seconds to be considered as having discontinued. Behaviors such as barking, whining and lip licking were coded for frequency. Barking and whining were counted as 'syllables' in that each time the vocalization had a start-stop it was counted as a new occurrence. For example, a dog may have been whining for 5 seconds, but had multiple stop-starts, creating a new "syllable". Each new "syllable" was counted as a new occurrence of whining.

2.3.2 *Behavior scores and salivary cortisol*

While we coded 33 behaviors in all, for our analysis we utilized seven behaviors associated with stress and four behaviors associated with successful adaptation/coping as seen in Table 2 (Handelman, 2008; Neilson, 2015; Protopopova, 2016). In order to gauge an overall picture of each dog's level of stress, we

scored the coded individual behaviors and calculated an overall Behavioral Stress Score. Stress-related behaviors such as panting and lip licking received positive scores (e.g., +1) while adaptive/coping behaviors such as stretching and resting received negative scores (e.g., -1). Behaviors were scored in quartiles based on the total percentage of the 10 observed minutes: behaviors occurring for 1% - 25% of the observation period would score a +1 (stress-related behaviors) or -1 (adaptive behaviors). If the behavior occurred for between 25.1% - 50% of the observation, the score would be +2 or -2; 50.1% - 75% would receive a +3 or -3, and behaviors occurring between 75.1% - 100% of the observation period would receive a score of +4 or -4. Scores for the pre-treatment phase (Day -5 and Day -2) were averaged to create a mean baseline behavior score for each dog. Due to human error, the behavior video for one dog in the treatment group for the first day of the pre-treatment phase (Day -5) was not properly uploaded and thus not coded. The behavior score for Day -2 was utilized as that dog's baseline behavior score throughout the analyses.

Cortisol was measured in duplicate at Salimetrics lab in State College, PA, USA. Mean values for the two assays for each sample were used for analysis. Values were reported in µg/dL. When individual samples lacked sufficient saliva to run two assays, the only assay run for that sample was used for analysis. There were four samples with insufficient saliva to analyze – three of which were from a single dog in the treatment group. When data were missing, that dog was not included in the analysis for that day. As with the behavior scores, the two values for the pre-treatment phase were averaged to create a mean baseline cortisol value for each dog. One dog from the control group had a contaminated sample on Day -5, with a value more than 22 times greater than the next highest value. We therefore eliminated that sample and used the Day -2 cortisol sample for that dog's baseline cortisol value for all analyses.

2.4 *Statistical analyses*

All data were collected and stored in Microsoft Excel 2013 (Microsoft, Redmond, WA, USA). Data analyses were conducted in SPSS v. 23 (IBM, Inc., Chicago, IL, USA).

Descriptive statistics were calculated in order to determine frequencies and means of the demographic information including number of males and females, age, weight, breed type and number of dogs in each "stress category" of the GSA. This was done for the entire sample as well as by treatment and control group.

Prior to running any statistical analyses, Shapiro-Wilk tests of normality were performed for both behavior scores and cortisol values. When data were not normally distributed, nonparametric tests were performed when possible. Pre-treatment behavior scores were averaged in order to define a mean baseline behavior score for each dog. Likewise, pre-treatment cortisol values were averaged to create a mean baseline cortisol value for each dog.

In order to look for relationships within each measure, we began with Pearson's correlations for the behavior scores and separately for the salivary cortisol values across each time point of the study: baseline, Day 3, Day 8, Day 14 and Day 21. The mean baseline cortisol values for the control group were the only set of values that were not normally distributed. A Spearman's Rho correlation was performed for control group cortisol values to look for linear relationships between the baseline cortisol value and each day of the study. These correlations were conducted for the entire sample as well as within each group. We then conducted a Pearson's correlation to compare the mean behavior scores at each time point to the mean cortisol values at each time point across the study to look for any relationships between the two measure types. We conducted a Spearman's Rho correlation to look at the control group baseline cortisol values in relation to behavior scores at each time point across the study: baseline, Day 3, Day 8, Day 14 and Day 21.

A series of paired-sample *t*-tests were conducted to identify any differences between the baseline behavior scores and each of the treatment phase scores and post-treatment score for the treatment group as well as for the control group. Paired-sample *t*-tests were conducted to compare the baseline cortisol values to each day of the study in the treatment group. Related-sample Wilcoxon Sign Ranks tests were conducted

to compare the baseline cortisol values to the cortisol values at each time point across the study in the control group.

We then conducted between-group comparisons using an Analysis of Covariance (ANCOVA) test to assess if any changes found in behavior scores for the treatment group were significant compared to the control group after accounting for baseline behavior scores entered as a covariate. ANCOVA tests were also conducted to look for significant differences between the treatment group and control group for salivary cortisol values, entering the baseline cortisol values as a covariate.

We hypothesized that there was a greater chance of significant differences at the final day of the treatment phase than any other time point in the study. Because we were interested in investigating nuanced behavior change, we looked for differences between baseline behavior scores and Day 14 – the last day of the treatment phase – for individual behaviors. Paired-sample *t*-tests were conducted for the treatment group as well as the control group for barking, down (lying down with head up – alert), lip licking, locomoting, pacing, panting, resting (lying down with head down), sitting and whining. To investigate differences between the treatment and control group, we conducted ANCOVA tests for each of these behaviors at Day 14, using the baseline score as a covariate.

Finally, we investigated the relationship between the GSA staff ratings and the baseline behavior scores. A Shapiro-Wilk test for normality was conducted for the GSA ratings for the entire sample as well as each experimental group. Pearson's correlations were conducted to look for linear relationships between the GSA ratings and the baseline behavior scores for the entire sample as well as for the treatment group. A Spearman's Rho correlation was conducted to explore the relationships between the GSA ratings and the baseline behavior score for the control group.

All tests were conducted with a threshold for significance set at $p = .05$.

3. **Results**

3.1 Behavior scores

Shapiro-Wilk tests for normality indicated that behavior scores were normally distributed for the treatment group as well as for the control group for each day of the study. Descriptive statistics for behavior scores for both the treatment group and the control group can be found in Table 3. Pearson's correlations of the behavior scores detected multiple significant correlations between days of the study in the treatment group (Table 4); the correlation between Day 3 and Day 21 was the only combination that did not achieve significance. In the control group, the correlation between Day 14 and Day 21 was the only relationship of significance (r = .98, n = 4, p = .03). There were no other significant correlations within the control group (Table 5).

Paired sample t-tests conducted for the treatment group did not detect any significant differences between the baseline behavior score and any day of the study (Day 3: t (5) = -0.65, p = .55; Day 8: t (5) = - 1.39, p = .22; Day 14: t (5) = -0.72, p = .51; Day 21: t (5) = -0.96, p = .38). Likewise, paired-sample t-tests for the control group failed to show any significant differences between the baseline behavior scores and any day of the study (Day 3: t (3) = -0.31, p = .78; Day 8: t (3) = 0.62, p = .58; Day 14: t (3) = -0.02, p = .98; Day 21: t (3) = 0.10, p = .93).

A series of analysis of covariance (ANCOVA) tests were conducted to look for differences between the treatment and control group at each observation day of the study, using the baseline behavior score as a covariate. There were no significant findings for any day of the study (Day 3: F (1, 7) = 0.063, p = .81; Day 8: F (1, 7) = 1.32, p = .29; Day 14: F (1, 7) = 0.03, p = .87; Day 21: F (1, 7) = 0.09, p = .77).

3.1.2 Specific behaviors

Mean behavior scores for specific behaviors at baseline and Day 14 for both treatment and control group can be seen in Table 6. A paired-sample t-test revealed that treatment group dogs sat significantly more at baseline than they did on Day 14 (t (5) = 2.54, p = .05). Paired-sample t-tests for other individual behaviors detected no significant differences when comparing baseline scores to Day 14 (barking: t (5) = 0.30, p = .78; down: t (5) = 0.00, p = 1.00; lip licking: t (5) = -0.70. p = .52; locomoting: t (5) = 0.26, p =

.81; pacing: t (5) = -1.15, p = .30; panting: t (5) = -0.70, p = .52; resting: t (5) = -0.46, p = .67; whining: t (5) = 1.60, p = .20). In the control group, paired-sample t-tests detected no significant differences for any of the behavior scores between baseline and Day 14 (barking: t (3) = 0.47, p = .67; down: t (3) = 0.00, p = 1.00; lip licking: t (3) = 0.40, p = .72; locomoting: t (3) = -1.73, p = .18; pacing: t (3) = 0.32, p = .77; panting: t (3) = 0.82, p = .47; resting t (3) = 0.00, p = 1.00; sitting: t (3) = -0.37, p = .74; whining: t (3) = 0.12, p = .91).

A series of analysis of covariance (ANCOVA) tests for specific behaviors, using baseline behavior scores as a covariate found no significant differences between the treatment group and the control group (barking: F (1, 7) = 0.92, p = .37; down: F (1,7) = 0.08, p = .78; lip licking: F (1, 7) = 0.71, p = .43; locomoting: F (1, 7) = 1.62, p = .24; pacing: F (1, 7) = 0.37, p = .56; panting: F (1,7) = 0.98, p = .36; resting: F (1, 7) = 0.06, p = .81; sitting: F (1, 7) = 4.94, p = .06; whining: F (1, 7) = 1.22, p = .31).

3.2 Cortisol measures

Shapiro-Wilk tests for normality found that cortisol values were normally distributed for each day of the study for the treatment group and the control group, with the exception of the mean baseline values for the control group. Therefore, non-parametric tests were used when baseline values were used in the analysis. Descriptive values for both the treatment group and the control group can be found in Table 7.

Pearson's correlations of the treatment group revealed a significant relationship between Day 3 and Day 14 cortisol values (r = .95, n = 5, p = .01). No other significant relationships were found within the treatment group (see Table 8). Within the control group, there was a significant correlation between Day 3 and Day 21 cortisol values (r =-.99, n = 4, p = .01). No other combination of days were significant (Table 9). Spearman's Rho correlations for the control group comparisons of baseline cortisol values with each day of the study detected no significant relationships (Table 9).

Paired-samples t-tests for the treatment group did not detect any significant differences between the mean baseline cortisol value and the mean cortisol value of any day of the study (Day 3: t (5) = -0.32, p = .76;

Day 8: t (5) = 0.18, p = .87; Day 14: t (4) = -0.74, p = .50; Day 21: t (3) = 1.50, p = .23). Likewise, related samples Wilcoxon Signed Rank tests for the control group did not detect any significant differences between the mean baseline cortisol value and the mean cortisol value of any day of the study (Day 3: Z = -0.73, p = .47; Day 8: Z = -1.46, p = .14; Day 14: Z = -1.10, p = .27; Day 21: Z = 0.00, p = 1.00).

A series of analysis of covariance (ANCOVA) tests revealed no significant differences between the treatment and control group at each observation day of the study, using the mean baseline cortisol value as a covariate (Day 3: F (1, 7) = 0.90, p = .38; Day 8: F (1, 7) = 0.03, p = .86; Day 14: F (1, 6) = 0.39, p = .56; Day 21: F (1, 5) = 4.35, p = .09).

3.3 Behavior by cortisol correlations

A strong negative correlation was detected between Day 21 cortisol values and the behavior scores of each day of the study within the treatment group; no other significant correlations were seen (Table 10). A Spearman's rho correlation detected no significant relationships between the baseline cortisol values and behavior scores for each day of the study in the control group; neither did Pearson's correlations reveal any significant relationships between cortisol values and behavior for any day of the study for the control group (Table 11).

3.3.1 Global Stress Assessment (GSA)

All dogs were rated as "more stressed" by shelter staff prior to the start of the study (M = 6.5, SD = 1.29, range: 4.5 – 9.0). Shapiro-Wilk tests of normality revealed normal distribution of GSA ratings for the total sample and the treatment group, but not the control group. Pearson's correlations showed no significant relationship between the GSA ratings and the baseline behavior scores of the complete sample or the treatment group (r = -.05, n = 10, p =.90 and r = -.26, n =6, p =.62, respectively). Spearman Rho correlation did not reveal a significant relationship between the GSA ratings and the baseline behavior scores for the control group (r = .21, n =4, p =.79).

Pearson's correlations did not reveal any significant relationship between the GSA ratings and the baseline cortisol values of the complete sample (r = .41, n = 10, p =.25). Pearson's correlations revealed a significant relationship between the GSA ratings and the mean baseline cortisol values in the treatment group (r = .85, n = 6, p = .03). No significant relationship was detected between the GSA ratings and the baseline cortisol values in the control group (r = -.33, n = 4, p = .67).

4. **Discussion**

The purpose of this study was to assess the effects of an extended-use ThunderShirt™ protocol on chronic stress in shelter dogs. This study included a control group which experienced a handling interaction meant to simulate having the shirt applied and removed so that we could better control for human interaction and habituation over time as possible causes for reduction of stress-related behaviors. While this study did not detect a significant effect on stress-related behaviors or salivary cortisol values over the course of the study, likely due to low power because of the small sample size, future research with a larger sample size is needed to investigate the efficacy of a pressure wrap in reducing chronic stress in shelter dogs. However, since prior research (Cottam et al., 2013; Damon et al., 2014; King et al., 2014) has demonstrated that such pressure wraps do reduce some stress-related behaviors as well as heartrate during acutely stressful events, we believe there may still be some benefit to the use of ThunderShirts™ in the shelter environment to help mitigate the impact of stress in resident dogs.

4.1 Behavior

Our results suggest there was little change in stress-related behaviors or coping/adaptive behaviors when the dogs were not wearing the ThunderShirt™. There are several reasons why this may be. It is likely that we did not detect a significant effect due the small sample size. A replication of this study with an appropriate sample size would be better powered to detect any potential differences. Another possible cause for not detecting a significant outcome is that the effect of the pressure wrap does not sustain once the wrap is removed, and thus we were unable to identify any effect of the ThunderShirt™ from our

observations conducted when the dogs were not wearing the shirt. Previous research, however, has noted that while wearing a pressure wrap, dogs have shown significant reductions in shaking (Cottam et al., 2103) and pacing (Cottam et al., 2013; Damon et al., 2014), and near significant reductions in lip licking and yawning (King et al., 2014) during acutely stressful events. It is therefore likely that using a pressure wrap such as ThunderShirt™ can provide acute stress relief for dogs in a shelter environment, even if that effect does not sustain after the shirt has been removed.

While we developed a behavior score scale for the purpose of analysis, there is always the risk that collapsing behaviors into a single score might result in a loss of detail for individual behavior changes. In an effort to look for such nuance, we chose to analyze certain behaviors alone including lip licking, locomoting, pacing, panting, and whining, as these have been consistently considered to be indicators of stress (Handelman, 2008; Neilson, 2015; Pekkin et al., 2016;, Rugaas, 2005; Yin, 2009). While howling and yawning are also consistently included in stress-related behaviors, we did not include them in our analyses because they did not happen sufficiently often. We found no significant differences either within groups or between groups for any of the individual behaviors. Although the difference in whining between treatment group and control group also did not reach significance, Day 14 did show a trend with the treatment group whining less than the control group. A larger sample size would allow us to determine if there is a real effect on whining behaviors or if this was just a random error in the data. Protopopova (2016) has noted that dogs increased affiliative behaviors such as tail wag, barking and whining when humans were present in the kennel area. Separating the occurrence of behaviors based on whether a person is present or not in the kennel area would shed light on how much of the observed behaviors were potentially due to stress compared to the anticipation of possible human interaction. Excluding behaviors that occurred in the presence of humans may also yield clearer results about the effects of the ThunderShirt™ on chronic stress.

The behavior score scale itself must also be assessed to determine if it provides a valid representation of the observed behaviors that we believe are associated with stress in dogs. This study based the behavior

score on quartiles for each behavior for each day of the study and then combined those scores into a global scale of all possible behaviors for each day of the study. By using a quartile system, small but relevant changes in behavior may not be detected during analyses. It would be appropriate to consider using at minimum a quintile or even a decile breakdown of behaviors on each day of the study in order to ensure the analyses can visualize even small changes. More advanced statistical techniques such as regression analysis may be more effective at creating a global behavior stress score. It may further be appropriate to look at all behaviors individually, rather than collapsing them into global scores for each dog. As noted above, combining all behaviors into a single score risks losing the nuance seen in individual behaviors over the course of the study. Future research should focus on creating and validating a behavior stress scale which can then be used for all studies assessing stress and successful coping in dogs in a variety of environments. Such a validated scale would provide consistency in research, allowing for better comparison of interventions across studies.

Along with exploring changes in stress-related behaviors, we also considered behaviors associated with adapting and coping with the environment (Owczarczak-Garstecka & Burman, 2016; Protopopova, 2016). For this comparison, we looked at movement (pacing and locomoting) compared to settled body postures (down, resting, sitting and stretching). Although there were no significant findings between the treatment and control groups, Day 14 results revealed a trend toward control group dogs sitting more than treatment group dogs. Sitting can be viewed as a transitional behavior that may reflect increased arousal and activity if sitting is increasing while lying down and resting behaviors are decreasing. But it could also reflect an increase in settling behavior if movement behaviors such as pacing or locomoting are decreasing as sitting is increasing. In our data, it is unclear what other behaviors in the control group may have reduced such that there was room for an increase in sitting. It is possible that this finding was simply the result of increased pacing in the treatment group, even though pacing itself was not found to be significantly different between the two groups on that day, nor significantly greater from other days of the

study for the treatment group. Future research should look at the pattern of individual behaviors both in isolation and together to determine the overall trend in behavior.

Taking our results in context with previous research (Cottam et al., 2013; Damon et al., 2014; King et al., 2014) suggests that while the ThunderShirt™ appears to be helpful in reducing some stress-related behaviors when worn during acutely stressful events, it may not create a sustained effect of reduced stress-related behaviors or increased coping/adaptive behaviors in between each wearing. Knowing that there is an observable effect when worn, it is reasonable that using such tools may still prove helpful to some dogs in a shelter environment as part of a broader stress reduction program. Future research on the efficacy of ThunderShirt™ use in shelter dogs should compare baseline behavior scores and cortisol values to observations and samples collected while the shirt is on, rather than when the dog is not wearing it. Such observations would allow for the opportunity to determine if there is an effect on stress-related behaviors, coping/adaptive behaviors, and cortisol values while a ThunderShirt™ is in use in a shelter environment. Confirmation of positive behavior changes while wearing a ThunderShirt™ would support the continued use of such tools in the high-stress environment and constant stimulation that is common in most shelters.

4.2 Cortisol

Similar to our behavior results, we did not find any significant differences in salivary cortisol either within groups or between groups at any time point across the study. It is likely that the small sample size was unable to detect any changes in cortisol over the course of the study. As with the behavior observations, a replication of this study with a larger sample size would increase our ability to detect potential differences. It is also possible that cortisol failed to respond to the ThunderShirt™ intervention because there was no sustaining impact on salivary cortisol that could be detected when the shirt was not on. On the other hand, cortisol presents a bit of a quagmire when studying chronic stress. While an increase in salivary cortisol has been noted when dogs are presented with an acute stressor (Pekkin et al., 2016), Hennessy (2013) and Protopopova (2016) have found that exposure to ongoing stressors, such as

residing for an extended period of time in a shelter environment, may impact the hypothalamic–pituitary–adrenal (HPA) axis, resulting in an immune-suppression response which leads to a dysregulation of the production of cortisol. In other words, dogs may experience an initial spike in cortisol levels when first entering a shelter, which may then be followed by a drop in cortisol levels even though they are still experiencing the stressful stimuli. This makes it difficult to determine if low observed cortisol levels reflect true psychological adaptation (habituation) or just physiological (hormonal) adaptation to the environment (Beerda et al., 2000; Hennessy, 2013; Protopopova, 2016). Pekken et al. (2016) found that wearing a deep pressure wrap during a loud noise event positively correlated with both a lower salivary cortisol value and a reduction in stress-related behaviors in a novel environment. Future research should take cortisol samples while the ThunderShirt™ is on as well as mid-day when the shirt is not on. This would allow a direct comparison of the effect of wearing the shirt to the possible sustaining effect between wearing.

4.3 Correlations

Sporadic correlations were found separately for behavior scores and cortisol values, but because of the small sample size it is impossible to know if these correlations reflect true relationships between data points or simply an insufficient sample to detect real significance. If a larger sample found similarly significant correlations between baseline scores and treatment phase scores, it could reflect relationships that do not support our hypotheses of a sustained reduction on the impact of stress as experienced by the dogs. We would hope to see little correlation between baseline measures and other days of the study, along with clearly significant differences between groups at the end of the protocol, as this would support our hypotheses of a sustained reduction in stress-related behaviors as well as salivary cortisol values when compared to baseline measures.

Finding correlations between observable stress-related behaviors and cortisol measures has also proven to be a difficult task (Hekman et al., 2012; Hellhammer, Wust, & Kudielka, 2009; Hennessy, 2013). One potential pitfall of trying to correlate cortisol to stress-related behaviors is the difficulty in teasing out the

differences between distress and eustress from a physiological measure such as cortisol. This is because whether the individual is experiencing distress or eustress, the physiological response is the same (Lazarus, 2000). In other words, whether the dog is feeling anxious or excited, heartrate and cortisol will increase at least temporarily, creating measurable arousal in the dog. Behaviors also occur due to a variety of motivations. For example, while panting is associated with stress and has been found to correlate with cortisol levels (Hekman et al., 2012), it is also a thermoregulatory action and so may not always be indicative of stress (Shiverdecker et al., 2013). Other behaviors which have been associated with stress or stereotypic behaviors such as jumping or wall bouncing, barking, whining, and even tail wagging (Beerda et al., 1998) have also been noted as affiliative behaviors (eustress) exhibited in response to the presence of a novel person at the front of the kennel (Protopopova, 2016). The deeper we investigate the causes of both cortisol fluctuation and observable behaviors, the muddier the field becomes when trying to find consistent correlations between the two.

With so much room for subjective interpretation, the question becomes one of whether or not tools such as the ThunderShirt™ are useful at all, and specifically in a shelter environment. Shelters do use ThunderShirts™ on dogs they feel would benefit, and have observed what they believe to be an improvement in the behavior of those dogs (personal communication, 2016). In our study, staff from two of the shelters indicated that they believed the behavior of at least one of their dogs was noticeably different mid-way through the treatment phase as compared to the beginning. Of course, it is possible this was due to a placebo effect since staff were not blind to condition and could have expected to see a change in behavior. It is also possible, as Hennessy et al. (2002) found, that the brief human interaction four times per day was at the root of observed behavior changes, which would also account for one control group dog being described as much calmer and easier to handle by the end of the treatment phase. Future research should utilize both a passive control group which does not experience a simulation exercise and an active control group which does have the simulation. Our aim in using the simulation exercise was to tease out what effect the shirt had on the dogs as opposed to the effects of human

interaction in general. Rather than discovering that the ThunderShirt™ is more effective than human interactions, it may be that such wraps are as effective in reducing stress-related behaviors as human interactions, which would allow shelters to use shirts when human interaction opportunities are limited either due to reduced staffing or when staff is tending to other shelter obligations.

Another interesting finding, though not the focus of our study, was the lack of correlation between GSA ratings and observed baseline behavior scores. In some cases, these two values were wildly different such that one dog who had the highest baseline behavior score of all 10 dogs (12.5) had the lowest GSA rating (4.5). Conversely, the dog with the highest GSA rating (9.0) fell right in the middle of the group with the fifth lowest baseline behavior score (5.5). It is possible that the shelter staff were not aware of what behaviors are considered to be associated with stress and with coping/adaptive behaviors and so were misreading the dogs in their care. It is also possible that the dogs' behavior was quite different when humans were present compared to when video observations were taken with no humans present. The latter possibility coincides with Protopopova's (2016) observations of increased behaviors including barking and whining in anticipation of humans interaction.

We must also consider the validity of the GSA as a measure of stress. The lack of correlations between the GSA and behavior as well as cortisol suggests that the GSA used in this study may have been inadequate. This 10-point scale may not accurately reflect shelter staff's true opinion of each dog's overall level of stress. It may be that a more detailed measure, which allows staff to rate various attributes such as eating and toileting habits, resting time, arousal when people are present, ability to settle, and ability to focus and engage in training or play would provide a clearer impression of the dogs' level of stress in the shelter environment. Future research should consider a series of rating scales for each of these facets in order to develop a fuller picture of the stress each dog may be experiencing while in the shelter.

4.4 General Discussion

A likely reason for the failure to see significant reductions in stress-related behaviors or cortisol values is the insufficient sample size. The power analysis suggested a sample of approximately 40 dogs would be best. While we did initially enroll 32 dogs, 22 were adopted or reclaimed by their owner before completing the study and so we were only able to analyze results from 10 dogs. This had a severe impact on the results. Repeating this study and running it long enough to achieve a sample size of 40 dogs would likely yield much clearer results. The shelters we worked with had an average length of stay between 7-10 days. Our commitment to allow dogs to be released from the study if they were adopted was ethically sound, but clearly interfered with our ability to collect sufficient data. Any replication of this study should consider enrolling shelters which tend to have a longer length of stay so that there is a greater chance of dogs being in residence long enough to complete the 26-day protocol. This may require looking at smaller rescues with stricter adoption rules, rather than larger shelters with open adoption policies.

In an effort to increase the probability of dogs being in residence for the entirety of the protocol, it may be beneficial to consider shortening the overall length of the study. For example the post-treatment phase could be eliminated altogether, which would shorten the protocol from 26 days to 19 days. This could be justified as the aim of the study was to determine if there is a sustained effect of the ThunderShirt outside of when it is worn. Because our behavior observations and cortisol collection occurred mid-day – between periods of wearing the shirt – we were assessing a sustained effect over a period of hours. Only if such a sustained effect is found in a larger sample size would it then warrant including the post-treatment phase to determine if the effect might last for several days after the shirt is no longer worn regularly. Although we saw the greatest hints of intervention impact on Day 14, our study had the highest drop-off of subjects between Day 3 and Day 8, which continued to fall through the end of the study, with one dog being adopted in the late afternoon of Day 20 – just one day before the final data collection. For this reason, it may be beneficial to reduce the treatment phase from 14 days down to eight in an effort to retain the largest sample size possible. Combining both of these reduction suggestions would further shorten the protocol to just 13 days, a 50% reduction in the total study duration. Finally, because cortisol tends to

spike in the first three days after arrival and then begin to decline (Hennessy, 2013; Hennessy et al., 1997), it may be unnecessary for dogs to be in residence for a 14 days prior to the first day of the treatment phase. Collecting pre-treatment data after the dog has been in residence for 6 or 7 days may be sufficiently long enough to avoid the effect of the spike in cortisol associated with entering the shelter, assuming that a control group is still included to control for both habituation and potential dysregulation over time (Beerda et al., 2000; Hennessy, 2013; Protopopova, 2016).

Another probable cause for our lack of positive results may be in how the data were analyzed. As noted by Protopopova (2016), we found that each dog had a different set of behaviors that appeared to make up the bulk of their personal behavior vocabulary, with some overlap to other dogs. While one treatment group dog licked her lips more than 90 times during the baseline observations, her lip licking had reduced 60% by the end of the treatment phase even though she never sat nor lay down. Another treatment group dog rarely licked her lips, but did increase her down/resting time over the course of the study. We hypothesize that perhaps the behaviors that manifest when a dog is feeling distressed may be quite unique to the individual dog, and this can complicate the efforts to identify stress-related behaviors which consistently correlate with cortisol values. Future research should focus on determining the behavior vocabulary of each dog at an individual level and then analyze changes in behavior and cortisol over time for each dog individually, rather than grouping dogs together which runs the risk of different behavior repertoires cancelling out relevant effects of interventions. One way to systematically study the individual behaviors of dogs would be through a repeated-measures A-B-A design. In such a study, each dog would serve as its own control, and be randomly assigned to begin the study either wearing the shirt or not. Then, a 3-day-on, 3-day-off pattern could be utilized to collect pre-treatment baseline measures as well as data collection at the end of each 3-day period. Two control groups could be included in such a study. One group would experience the simulation interaction that we used in the current study – in the same A-B-A pattern and with a random assignment to begin either with no interaction or simulation. A second, passive control group could also be included to help account for human interaction vs. pressure wrap as a

cause for any reductions in stress-related behaviors or cortisol over time. Thus we would be able to determine each dog's individual behavior vocabulary and then assess changes in behavior and possible correlations with cortisol values over time for each dog.

If we approach assessing stress in dogs similarly to diagnosing mental health issues in humans, we can use the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). as a guide for creating a list of behaviors which, if present, indicate a greater likelihood that the dog is experiencing stress (DSM-V, 2013). For example, when diagnosing a major depressive episode in humans, patients must have 5 out of 9 symptoms, but they need not have all them, and they need not have the same set as other patients with the same diagnosis (Institute for Clinical Systems Improvement, http://tinyurl.com/zs99xkj). Similarly, we may be able to take several behaviors that have been correlated to cortisol in individual dogs and develop a list of co-morbid behaviors and/or frequently seen behaviors such that if a particular set of behaviors is seen, it suggests that the cortisol levels for that dog may be elevated.

With prior research demonstrating a reduction in some stress-related behaviors and heartrate during acutely stressful events in homed dogs (Cottam & Dodman, 2009; Cottam et al., 2013; Damon et al., 2014; King et al., 2014; Pekkin et al., 2016), future research should investigate the possibility of sustained effects in homed dogs who may be experiencing chronic stress. Such a study may show a stronger efficacy for chronic-stress reduction in a home environment, as the shelter environment may be too stimulating for the ThunderShirt to impact the experience of chronic stress. Finally, knowing that most dogs experience a spike in cortisol and stress-related behaviors upon arrival at shelters (Hennessy, 2013; Hennessy et al., 1997), a shorter study of applying a ThunderShirt™ within 1-3 hours of arrival and keeping it on for several hours per day for 1-3 days may show a mitigating effect on cortisol and stress-related behaviors in the dog's first days in a shelter environment. This could prove extremely beneficial as a tool to help prevent the onset of chronic stress – especially in those shelters with a much shorter length

of stay. After all, just because a dog may only be in the shelter for 3 or 4 days does not mean we should not do all we can to minimize their experience of stress while in our care.

4.5 Conclusion

The current study failed to show significant reductions in stress-related behaviors or salivary cortisol in shelter dogs wearing a ThunderShirt™ twice per day for 14 consecutive days. It also failed to show significant increases in adaptive/coping behaviors. It is possible that the lack of positive results is because the ThunderShirt™ does not create a stress-reduction effect that sustains after the shirt has been removed, especially in the stimulating and stressful shelter environment. It is also likely that we simply had an insufficient sample to detect an effect of the intervention. The lack of correlation between observable behaviors and cortisol values in this study highlights similar difficulties of relating behavior to cortisol that other researchers have encountered (Hekman et al., 2012; Hellhammer et al., 2009; Hennessy, 2013), and may be due either to a process of the HPA-axis known as dysregulation (Beerda et al., 2000; Hennessy, 2013; Protopopova, 2016) or possibly due to the unique behavior repertoires of individual dogs (Protopopova, 2016). Future research should focus on replicating this study with a minimum of 40 dogs in order to achieve sufficient power to be able to detect significant changes if there are any to find. Modifications to the current protocol include possibly shortening the treatment phase from 14 days down to eight and eliminating the week long post-treatment phase. It is also recommended to ensure enough researcher resources to be physically available to participate in the daily protocol of applying/removing the shirt to help ease the burden on shelter staff, as this may encourage greater participation from shelters which are otherwise under staffed. Also, it may prove valuable to look at stress-related and coping/adaptive behaviors in connection with the use of ThunderShirt™ or other pressure wraps with individual dogs, assessing their personal behavior vocabulary and then observing changes in behavior over time for each individual subject, using a repeated-measures A-B-A design. Finally, exploring the sustained effects of a ThunderShirt™ in homed dogs may demonstrate a more robust outcome than found in the shelter environment due to the great differences between a high-stress shelter and the relative quiet

of a home. Determining the environments and circumstances under which pressure wraps provide some reduction in stress-related behaviors will allow both shelters and companion dog owners the opportunity to offer comfort to dogs in their care when escape from a stressful stimuli is not immediately an option.

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Conflicts of Interest Statement

This study had no conflicts of interest. While ThunderShirt™ provided shirts for the study and contributed financially toward the costs to complete the study, they had no input on study design, implementation nor analyses.

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Table 1.
Experimental subjects (N = 10). Breed type, sex, age in months, and number of days in shelter on the first day of the treatment phase by experimental group.

Name	Breed Type	Sex	Age (in months)	# Days in Shelter on Day 1 of Treatment phase
Treatment Group				
Dame	Staff Mix	NM	24	29
Finn	Chihuahua Mix	NM	60	17
Lily	Staff Mix	SF	67	21
Raina	Staff Mix	SF	29	19
Twinkle	Chihuahua Mix	SF	24	61
Wrigley	Staff Mix	M	24	27
Control Group				
Arnold	Staff Mix	NM	72	30
Nathan	Staff Mix	NM	18	17
Oreo	Staff Mix	SF	27	26
Princess	Cairn/Cocker	F	12	14

NM = neutered male, M = intact male, SF = spayed female, F = intact female

Table 2.
Coded stress-related and adaptive/coping behaviors.

Stress-Related Behaviors	**Adaptive/Coping Behaviors**
Barking (f)	Lie Down (head down - resting) (d)
Howling (d)	Lie Down (head up) (d)
Lip Licking (f)	Sitting (d)
Locomoting (d)	Stretching (d)
Pacing (d)	
Panting (d)	
Whining (f)	

Behaviors coded from video observations used in analyses.
d= duration behaviors scored in seconds
f = frequency behaviors scored by count

Table 3.
Behavior scores – Descriptive statistics across five time points of the study by experimental group (N = 10).

	Mean	*Median*	*SD*	*Minimum*	*Maximum*
Treatment group (n = 6)					
Baseline Behavior Score	4.00	4.50	7.59	-5.50	12.50
Behavior Day 3	5.17	8.00	8.13	-8.00	13.00
Behavior Day 8	6.33	7.50	9.81	-6.00	17.00
Behavior Day 14	5.33	4.00	10.09	-5.00	18.00
Behavior Day 21	5.67	2.50	9.75	-5.00	19.00
Control Group (n = 4)					
Baseline Behavior Score	5.63	6.00	5.95	-2.00	12.50
Behavior Day 3	7.25	9.50	7.04	-3.00	13.00
Behavior Day 8	3.25	5.50	5.68	-5.00	7.00
Behavior Day 14	5.75	6.50	8.30	-5.00	15.00
Behavior Day 21	5.00	5.00	9.56	-6.00	16.00

Table 4.
Treatment group – Pearson's correlations for behavior scores across five time points of the study (n = 6).

		Baseline Behavior Score	Behavior Day 3	Behavior Day 8	Behavior Day 14	Behavior Day 21
Baseline Behavior Score	*r*	1	.844*	.920**	.906*	.908*
	Sig. (2-tailed)		.035	.009	.013	.012
	n	6	6	6	6	6
Behavior Day 3	*r*	.844*	1	.959**	.891*	.775
	Sig. (2-tailed)	.035		.002	.017	.070
	n	6	6	6	6	6
Behavior Day 8	*r*	.920**	.959**	1	.978**	.854*
	Sig. (2-tailed)	.009	.002		.001	.030
	n	6	6	6	6	6
Behavior Day 14	*r*	.906*	.891*	.978**	1	.894*
	Sig. (2-tailed)	.013	.017	.001		.016
	n	6	6	6	6	6
Behavior Day 21	*r*	.908*	.775	.854*	.894*	1
	Sig. (2-tailed)	.012	.070	.030	.016	
	n	6	6	6	6	6

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 5.
Control group – Pearson's correlation for behavior scores across five points of the study (n = 4).

		Baseline Behavior Score	Behavior Day 3	Behavior Day 8	Behavior Day 14	Behavior Day 21
Baseline Behavior Score	*r*	1	-.272	.122	-.222	-.405
	Sig. (2-tailed)		.728	.878	.778	.595
	n	4	4	4	4	4
Behavior Day 3	*r*	-.272	1	.882	.783	.713
	Sig. (2-tailed)	.728		.118	.217	.287
	n	4	4	4	4	4
Behavior Day 8	*r*	.122	.882	1	.864	.737
	Sig. (2-tailed)	.878	.118		.136	.263
	n	4	4	4	4	4
Behavior Day 14	*r*	-.222	.783	.864	1	.975*
	Sig. (2-tailed)	.778	.217	.136		.025
	n	4	4	4	4	4
Behavior Day 21	*r*	-.405	.713	.737	.975*	1
	Sig. (2-tailed)	.595	.287	.263	.025	
	n	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

Table 6.
Specific behaviors - Mean behavior scores for baseline and Day 14 by experimental group (N = 10).

Behavior		Baseline Behavior Score	Day 14 Behavior Score
Barking	Tx	2.17	2.00
	C	1.25	0.75
Down	Tx	-1.67	-1.67
	C	-1.25	-1.25
Lip Lick	Tx	2.42	2.67
	C	2.13	2.00
Locomoting	Tx	1.67	1.50
	C	2.00	2.75
Pacing	Tx	0.42	1.17
	C	1.00	0.75
Panting	Tx	1.08	1.33
	C	1.50	1.00
Resting	Tx	-1.92	-1.67
	C	-1.25	-1.25
Sitting	Tx	-1.33	-0.17
	C	-2.63	-2.25
Whining	Tx	1.17	0.83
	C	2.63	2.50

Tx = Treatment group; C = Control group
Baseline behavior scores for Tx group (n = 5)
Day 14 behavior scores for Tx group (n = 6)
Baseline behavior scores and Day 14 behavior scores for C group (n = 4).

Table 7.
Cortisol values - Descriptive statistics across five time points of the study by experimental group (N = 10).

	Mean	*Median*	*SD*	*Minimum*	*Maximum*
Treatment group (n = 6)					
Baseline Cortisol Value (n = 6)	0.22	0.21	0.10	0.12	0.37
Cortisol Day 3 (n = 4)	0.21	0.23	0.10	0.07	0.31
Cortisol Day 8 (n = 3)	0.20	0.20	0.07	0.14	0.27
Cortisol Day 14 (n = 4)	0.23	0.23	0.14	0.05	0.39
Cortisol Day 21 (n = 3)	0.19	0.20	0.10	0.09	0.28
Control Group (n = 4)					
Baseline Cortisol Value (n = 4)	0.41	0.35	0.22	0.23	0.72
Cortisol Day 3 (n = 4)	0.34	0.31	0.14	0.19	0.53
Cortisol Day 8 (n = 4)	0.23	0.25	0.07	0.13	0.29
Cortisol Day 14 (n = 4)	0.26	0.25	0.06	0.20	0.35
Cortisol Day 21 (n = 4)	0.36	0.36	0.09	0.25	0.46

Table 8.
Treatment group – Pearson's Correlations for cortisol values across five time points of the study (n = 6).

		Baseline Cortisol Value	Cortisol Day 3	Cortisol Day 8	Cortisol Day 14	Cortisol Day 21
Baseline Cortisol value	*r*	1	.648	.113	.512	.707
	Sig. (2-tailed)		.164	.831	.378	.293
	n	6	6	6	5	4
Cortisol Day 3	*r*	.648	1	.540	.952*	.804
	Sig. (2-tailed)	.164		.269	.013	.196
	n	6	6	6	5	4
Cortisol Day 8	*r*	.113	.540	1	.672	.510
	Sig. (2-tailed)	.831	.269		.214	.490
	n	6	6	6	5	4
Cortisol Day 14	*r*	.512	.952*	.672	1	.783
	Sig. (2-tailed)	.378	.013	.214		.217
	n	5	5	5	5	4
Cortisol Day 21	*r*	.707	.804	.510	.783	1
	Sig. (2-tailed)	.293	.196	.490	.217	
	n	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

Table 9.
Control group - Correlations for cortisol values across five time points of the study (n = 4).

		Baseline Cortisol value	Cortisol Day 3	Cortisol Day 8	Cortisol Day 14	Cortisol Day 21
Baseline Cortisol value	Spearman's Rho *r*	1.000	-.200	0.000	.400	.200
	Sig. (2-tailed)		.800	1.000	.600	.800
	n	4	4	4	4	4
Cortisol Day 3	Pearson's *r*	-.200	1	.609	-.843	-.989*
	Sig. (2-tailed)	.800		.391	.157	.011
	n	4	4	4	4	4
Cortisol Day 8	Pearson's *r*	0.000	.609	1	-.932	-.648
	Sig. (2-tailed)	1.000	.391		.068	.352
	n	4	4	4	4	4
Cortisol Day 14	Pearson's *r*	.400	-.843	-.932	1	.850
	Sig. (2-tailed)	.600	.157	.068		.150
	n	4	4	4	4	4
Cortisol Day 21	Pearson's *r*	.200	-.989*	-.648	.850	1
	Sig. (2-tailed)	.800	.011	.352	.150	
	n	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).
Baseline Cortisol value correlations conducted with Spearman Rho; all other correlations conducted with Pearson's Correlation.

Table 10.
Treatment group - Pearson's correlations between cortisol values and behavior scores for each day of the study (n = 6).

		Baseline Behavior Score	Behavior Day 3	Behavior Day 8	Behavior Day 14	Behavior Day 21
Baseline Cortisol value	*r*	-.201	.062	-.194	-.384	-.381
	Sig. (2-tailed)	.703	.907	.713	.452	.456
	n	6	6	6	6	6
Cortisol Day 3	*r*	-.581	-.182	-.388	-.474	-.616
	Sig. (2-tailed)	.227	.731	.447	.342	.193
	n	6	6	6	6	6
Cortisol Day 8	*r*	-.379	.103	-.047	-.053	-.132
	Sig. (2-tailed)	.459	.847	.929	.920	.803
	n	6	6	6	6	6
Cortisol Day 14	*r*	-.674	-.257	-.403	-.440	-.754
	Sig. (2-tailed)	.213	.677	.501	.458	.141
	n	5	5	5	5	5
Cortisol Day 21	*r*	-.952*	-.980*	-.997**	-.984*	-.977*
	Sig. (2-tailed)	.048	.020	.003	.016	.023
	n	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 11.
Control group - Correlations between cortisol values and behavior scores for each day of the study (n = 4).

		Baseline Behavior Score	Behavior Day 3	Behavior Day 8	Behavior Day 14	Behavior Day 21
Baseline Cortisol value	Spearman Rho *r*	0.80	-0.80	-0.32	-0.80	-0.80
	Sig. (2-tailed)	0.20	0.20	0.68	0.20	0.20
	n	4	4	4	4	4
Cortisol Day 3	Pearson's *r*	-0.71	0.24	-0.24	-0.24	-0.15
	Sig. (2-tailed)	0.29	0.76	0.76	0.76	0.85
	n	4	4	4	4	4
Cortisol Day 8	Pearson's *r*	0.13	0.09	-0.14	-0.54	-0.62
	Sig. (2-tailed)	0.87	0.91	0.86	0.46	0.38
	n	4	4	4	4	4
Cortisol Day 14	Pearson's *r*	0.23	-0.26	0.10	0.38	0.41
	Sig. (2-tailed)	0.77	0.74	0.90	0.62	0.59
	n	4	4	4	4	4
Cortisol Day 21	Pearson's *r*	0.65	-0.10	0.37	0.39	0.29
	Sig. (2-tailed)	0.35	0.90	0.63	0.61	0.71
	n	4	4	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).
Baseline Cortisol value correlations conducted with Spearman Rho; all other correlations conducted with Pearson's Correlation.

Appendix A.

Applying the ThunderShirt™: Research assistant (RA) holds the shirt by the neck straps with the logo facing out. While on the dog's left side, the RA kneels down to avoid bending over the dog and lays the shirt on the dog's back, connecting the neck straps against the front of the chest, using the Velcro® closure. The RA flips the small outer flap with the logo toward the dog's right side, reaches under the dog's chest to retrieve the long girth strap. Pulling the long strap across the dog's chest/belly and secures this body strap on the left side of the body by the Velcro® closure. Finally, the RA snugs the smaller outer flap down over the inner body flaps and secures this with the Velcro® closure. The fit should be snug, but not tight.

Removing the ThunderShirt™: The RA does the reverse of applying the shirt. First, the small outer body flap is pulled free from the Velcro® closure and laid toward the right side of the dog's body. The inner body flap is then pulled free and allowed to hang. The neck strap is pulled free and then the RA lifts the shirt from the back – holding the shirt at the shoulder and the waist.

Simulation of putting shirt on: While standing on the dog's left side, just behind the dog's head and facing the same direction as the dog, the person will kneel to avoid bending over the dog. The RA will first lay both hands gently on the dog's back over the shoulders. While leaving the caudal hand in place at the shoulder, the other hand will apply a constant pressure of roughly 10 g (approximately the weight of two nickels) and stroke down to the bottom of the rib cage/dog's waist. The RA will then place both gently at the starting point – over the dog's shoulders and simultaneously run one hand around each side of the dog's neck from the shoulders, around to the sternum below the chin. Finally, placing both hands on the dog's back, roughly halfway

between the shoulders and waist, the person will run both hands, simultaneously around either side of the dog's body, around the girth, until fingers meet on the sternum behind the front legs.

Simulation of taking shirt off: While kneeling parallel to the dog, just behind the dog's head and facing the same direction as the dog, the RA will lay hands gently on the dog's sternum behind the front legs such that the person has one arm around each side of the dog (hands are in gentle contact with the dog's chest, but arms should not be touching the dog's body – it is not a hug). The person shall run the hands gently (10 g of pressure) laterally, around the dog's chest such that the hands meet on the back. Then the person will place both hands on the dog's sternum under the chin (still kneeling parallel, just behind the dog's head, so that one hand is on either side of the dog's body) and the RA will draw their hands laterally around the dogs shoulders and coming together on the dog's back between the should blades. Finally, the RA will place one hand gently on the dog's back at the shoulder and the other hand on the dog's back just above the waist, and lift the hands straight up – to simulate lifting the shirt off the dog's body completely.

Appendix B.

Behaviors coded (f = frequency; d = duration in seconds)

Barking – staccato partial or full voice vocalization (f)

Howling – drawn out full voice vocalization lasting for at least 2 seconds (d)

Whining – staccato, high pitched voiced or voiceless vocalization (f)

Panting – open-mouthed, rapid breathing lasting at least 2 seconds. When face was not visible, panting determined by rapid chest movements (d)

Lip Lick – tongue protruding from the mouth. Includes darting out and back, licking lips or nose (f)

Yawn – self-explanatory (f)

Head Shake – shaking head as if wet, but not a full body shake (f)

Shake Off – full body shake as if wet (f)

Licking Kennel – licking any surface or object in the kennel (d)

Sniffing – sniffing any surface or object within the kennel, pressing nose through kennel door slats and sniffing air – determined by active nose twitching and rapid inhalation. Behavior lasting at least 2 seconds (d)

Grooming – licking or biting at any part of the body lasting at least 2 seconds (d)

Drinking – self-explanatory (d)

Eating – self-explanatory (d)

Urinating – self-explanatory (f)

Playing With Toy – engaging physically with toy – mouthing or pawing (d)

Tail Tucked – tail pointed toward the floor or between the legs lasting at least 2 seconds (d)

Tail Wagging – movement of the tail in any position lasting at least 2 seconds (d)

Stand Still – all four paws on the floor, includes incidental movement lasting less than 2 seconds and momentary paw raise (d)

Locomoting – moving from one location in the kennel to another includes from one edge of the door to the other or from front to back of kennel. Movement lasting at least 2 seconds (d)

Pacing – moving from one location to another and back without pause or with a pause lasting less than 2 seconds (d)

Turn Around/Circle/Nesting – turning in a circle while staying in the same location or digging at bedding prior to lying down (d)

Paws Up – front paws up on wall or kennel door for at least 2 seconds (d)

Wall Bounce – front paws contacting vertical surface and immediately pushing off (f)

Jumping – all four feet off the floor (f)

Sitting – four feet and bottom on the floor (d)

Stretching – bottom in the air while front legs are stretched out and chest is pushed toward the floor – no chest contact with the floor, not a play bow (d)

Down – lying down with head up and eyes open lasting at least 2 seconds (d)

Resting – lying down with head down, eyes may be open or closed, lasting at least 2 seconds (d)

Front of Kennel – body position in the front 50% of the kennel lasting at least 2 seconds (d)

Rear of Kennel – body position in the rear 50% of the kennel lasting at least 2 seconds (d)

Facing Forward – chest facing full forward to ¼ toward profile; face facing full forward to profile with body more forward lasting at least 2 seconds (d)

Facing Rear – chest facing full rear to ¼ toward profile; face facing full rear to profile with body more rear lasting at least 2 seconds (d)

Person Present – person visible on camera, voices heard while person is not in view on camera; no minimum time requirement (d)