Applied Animal Behaviour Science 159 (2014) 69–81

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

The effect of dog–human interaction on cortisol and behavior in registered animal-assisted activity dogs

Zenithson Y. Ng[a,*], Bess J. Pierce[a], Cynthia M. Otto[b], Virginia A. Buechner-Maxwell[a], Carlo Siracusa[b], Stephen R. Werre[a]

[a] Virginia-Maryland Regional College of Veterinary Medicine, Duck Pond Drive, Mail Code 0442, Blacksburg, VA 24061, USA
[b] University of Pennsylvania School of Veterinary Medicine, 3800 Spruce Street, Philadelphia, PA 19104, USA

ARTICLE INFO

Article history:
Accepted 29 July 2014
Available online 9 August 2014

Keywords:
Animal-assisted activity
Salivary cortisol
Stress
Behavior
Animal welfare

ABSTRACT

The effect of animal-assisted activities (AAA) on the animal participants has been minimally investigated, and the welfare of these animals has been questioned. To enhance our understanding of these animals' welfare, we measured cortisol collected from serial saliva samples of 15 healthy adult dogs registered with an AAA organization. We collected saliva every 30 min before, during, and after a standardized 60-min session across three settings: an AAA session (activity) for college students in the communal area of a residence hall, a novel session located in a novel room without interaction with a stranger, and a home session inside each handler's own home. Each session was videotaped, and specific behaviors during 5-min petting interactions were coded. Salivary cortisol levels were significantly higher in the novel setting (0.397 μg/dL) compared to activity (0.257 μg/dL) and home (0.213 μg/dL) settings at time 30 min ($P = 0.01$ and $P = 0.03$, respectively). Dogs exhibited significantly more standing (59% vs 0%, $P = 0.008$) and ambulating (5.6% vs 0%, $P = 0.001$) behavior in the activity setting compared to the home at time 30 min, as well. Salivary cortisol level was negatively correlated with panting ($P = 0.02$) and standing ($P = 0.02$) at specific time points in the novel and activity settings, respectively. During the 60-min AAA session, salivary cortisol concentration and stress-associated behavior were not statistically different compared to when dogs spent the same amount of time in the home setting, suggesting that they were not distressed when participating in the AAA sessions. The predictability of the environment may be an important consideration when evaluating the effect of AAA on dogs.

1. Introduction

The human–animal bond, as defined by the American Veterinary Medical Association, is "a mutually beneficial and dynamic relationship between people and other animals that is influenced by behaviors that are essential to the health and well-being of both" (AVMA, 1998). Human–animal interaction organizations, providing services in the form of animal-assisted activities (AAA) and animal-assisted therapies (AAT), continue to proliferate globally (Palley et al., 2010). Whereas AAT is a formal therapeutic intervention conducted by a human health professional to meet a specific and measurable goal, AAA is a less formal interaction typically guided by a layperson to broadly enhance quality of life (Kruger and Serpell, 2010). In both circumstances, animals engage with a human recipient.

* Corresponding author. Present address: Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, C247 Veterinary Medicine Building, 2407 River Drive, Knoxville, TN 37996, USA. Tel.: +1 865 974 8387; fax: +1 865 974 3926.
E-mail addresses: zng@utk.edu, zenithson.ng@gmail.com (Z.Y. Ng).

http://dx.doi.org/10.1016/j.applanim.2014.07.009

Although a growing body of evidence supports the rewards and benefits of human–animal interactions for humans, limited evidence exists to document the effects of human–animal interactions on the animals themselves. Specifically, the welfare of dogs used in AAA and AAT has been questioned, as social interactions have been described by some as among the most potent stressors a dog can endure (von Holst, 1998; McEwen and Wingfield, 2003). Social interactions can be unpredictable, requiring the individual to constantly adapt physiologically and behaviorally to maintain homeostasis (Karatsoreos and McEwen, 2011). Iannuzzi and Rowan (1991) also commented on the potential for fatigue and burnout in therapy dogs. Therefore, it is critical that evidence-based research exists to determine if the use of animals for this purpose is detrimental to animal welfare. The study of physiologic and behavioral effects of AAA on registered AAA dogs can enhance our understanding of animal welfare during these interventions, introduce evidence-based applications for handlers, and establish scientific methods for future research.

Animal welfare has been commonly assessed by measuring circulating levels of cortisol, the major physiologic indicator of stress in dogs (Vincent and Michell, 1992; Hennessy, 1997; Beerda et al., 1999; Hennessy et al., 2002), as well as analyzing stress-associated behavior. Cortisol release is activated by a variety of mental and physical stimuli, including extraordinary situations, activities, and emotions (Beerda et al., 1998). Although cortisol is secreted in response to negative events, it can also be secreted in response to situations that are not inherently regarded as distressful, such as courtship, copulation, and hunting (Broom and Johnson, 1993; Handlin et al., 2011). The hypothalamic-pituitary axis (HPA) controls reactions to stress and regulates various physiologic processes. However, since HPA activation is non-specific to the type of change in homeostasis, it is difficult to determine whether a rise in cortisol level is associated with positive or negative emotions (Zorawski and Killcross, 2002, 2003; Boissy et al., 2007). In addition, cortisol response depends on a combination of the individual's perception of the stimulus and other individual factors, including genetic make-up and past experiences (Haubenhofer and Kirchengast, 2007). Therefore, caution should be used to prevent misinterpretation of increased physiologic arousal with negative welfare (Blackwell et al., 2010).

The limited research involving therapy dogs has consistently reported that therapy sessions are associated with subsequent rises in salivary cortisol. Haubenhofer and Kirchengast (2006) reported that salivary cortisol levels of dogs used in AAA/AAT were significantly higher during therapy days than control days. A more recent study showed a significant elevation of salivary cortisol level in therapy dogs between the start of and 1 h after an AAT session (King et al., 2011). Although these studies indicate that therapy work induces an acute rise in cortisol level and that this activity is physiologically arousing, it is unknown if the rise is (a) a result of a stimulating interaction with unfamiliar humans; (b) a result of residing in an environment outside the home; or (c) a result of a combination of these factors and/or others.

Analysis of behavior has also long been used as a research tool to assess stress and welfare in animals. Stress-associated behavior, such as increased locomotor activity, lip licking, yawning, circling, and nosing, have been observed to occur in response to acute stressors in dogs (Beerda et al., 1997, 2000). Although Ferrara et al. (2004) reported the absence of observed stress behavior in dogs *during* AAA/AAT, King et al. (2011) observed multiple behavioral signs of stress (panting, pupillary dilation, yawning, whining, and air licking) in dogs *after* a 2-h AAT session. These discrepancies warrant clarification as to whether activity and therapy sessions alter stress-associated behavior.

Furthermore, the association between stress behavior and physiologic parameters remains inconclusive (Hansen and Jeppesen, 2006); therefore, the robustness of an animal welfare assessment increases by using both stimulating and non-stimulating settings to compare stress-associated behavior in conjunction with cortisol level (Hiby et al., 2006). In this investigation, the objective was to measure and compare salivary cortisol levels and behavior in registered AAA dogs as a function of time in the home setting, a novel setting, and an AAA setting in which college students interacted with dogs in the communal area of a residence hall. This study explored whether AAA dogs exhibited behavioral or physiologic signs of stress in an AAA setting compared to home or novel settings.

2. Methods

All protocols and surveys were approved by the Institutional Animal Care and Use Committee (IACUC Number 11-190-CVM) and the Institutional Review Board (IRB Number 11-998) at the Virginia Polytechnic Institute and State University.

2.1. Participants

Participants were recruited via email sent to active members of the University of Pennsylvania's therapy dog program (Vet Pets) and active members of Therapy Dogs International (Flanders, NJ, USA) living within a 20-mile radius of Philadelphia, PA. The email briefly described the study and eligibility requirements: owner served as handler of the dog; the dog was at least one year of age, was up to date on rabies vaccine, had no evidence of underlying disease; and the dog-handler team was able to attend all three scheduled sessions. The email also explained that a team would be excluded if the dog had been given non-steroidal anti-inflammatory drugs (topical or systemic), or any other medications that could affect systemic cortisol levels within the last 6 weeks (Tanaka et al., 1998; Gottschalk et al., 2011). The first 16 handlers with registered AAA dogs meeting this criteria who emailed the PI (ZN) were sent an online survey with questions regarding demographic information, scheduling availability, and AAA history (Table 1). Owners agreeing to participate in the study were emailed further details about logistics and a textual description and link to an online video demonstrating how to collect a saliva sample from their dog.

Table 1
Descriptive demographics of the population of animal-assisted activity dogs used in this study.

No.	Age (years)	Sex	Weight (kg)	Breed	No. other animals in household	No. humans in household	Years AAA certification	Years owned	No. days dog participates in AAA visits/month	Length of AAA session/day	No. times visited the activity location	Gender of handler
1	4	FS	20.64	Mixed	0	5	2	2–4	7–10	>2 h	0	F
2	3	FS	24.27	Mixed	0	2	1	2–4	4–6	30 min–1 h	0	M
3	2	MN	44.55	Mixed	0	1	1	2–4	2–3	30 min–1 h	1–2	F
4	10	MN	6.82	Mixed	0	2	8	>6	2–3	30 min–1 h	2–5	F
5	3	MN	6.82	Mixed	1	1	1	2–4	2–3	30 min–1 h	1–2	F
6	3	FS	6.82	Mixed	1	1	2	2–4	4–6	30 min–1 h	0	F
7	2	FS	17.27	American Staffordshire terrier	2	5	1	1–2	1	1–1.5 h	1–2	F
8	3	MN	14.09	Mixed	0	1	2	1–2	4–6	1–1.5 h	1–2	F
9	4	FS	9.18	Cavalier King Charles spaniel	3	2	2	4–6	1	>2 h	0	F
10	10	MN	35.18	Golden retriever	2	3	6	>6	>10	30 min–1 h	0	M
11	4	MN	36.36	Akita	1	1	2	2–4	4–6	1–1.5 h	0	F
12	5	FS	11.18	Pembroke Welsh corgi	0	1	3	2–4	2–3	30 min–1 h	1–2	F
13	5	FS	39.55	Rhodesian ridgeback	0	1	3	4–6	2–3	1–1.5 h	0	F
14	3	MN	12.73	Mixed	0	1	1	1–2	2–3	1–1.5 h	0	F
15	8	MN	17.36	Mixed	0	1	2	>6	4–6	1–1.5 h	0	F

F, female; M, male; FS, female spayed; MN, male neutered; AAA, animal-assisted activity.

Eight dog-handler teams were enrolled in the study during each of two, 2-week periods, resulting in 16 different dog-handler teams over 4 weeks from March to April 2012. At the beginning of the study period, each dog-handler team attended an information meeting in a lecture hall at the University of Pennsylvania's School of Veterinary Medicine (Philadelphia, PA, USA). At this meeting, handlers were provided a detailed description of the study and a review on how to appropriately collect saliva samples. Immediately following the meeting, each handler demonstrated his or her ability to collect an adequate saliva sample from the dog using a saliva collection swab. Of the 16 dog-handler teams, one was excluded because the dog resisted having the swab placed in its mouth by turning its head away and retreating when the handler approached it with the swab. Immediately following the saliva collection demonstration, a licensed veterinarian (ZN) performed a physical examination on each dog in the atrium outside the lecture hall. Participants were excluded if the physical examination revealed any significant abnormalities.

2.2. Settings

Collection of saliva (for cortisol analysis) and video recording (for behavior analysis) were performed across three settings: home, novel, and activity. For the novel and activity settings, all dogs arrived 5–10 min early and were transported as they usually arrive to their AAA assignment: in a familiar vehicle with their handlers.

2.2.1. Home setting

To provide baseline cortisol and behavior data, saliva was collected and the dog observed for 60 min in the dog-handler team's residence in the room most regularly inhabited by the dog from 15:00 to 20:00 h. The dog was off-leash, but was directed by its handler to remain near the handler and within the video recording area, which was at least 2.1 m × 2.1 m. Although other familiar animals and humans in the household were permitted to enter and leave the setting, the dog was limited to direct interaction only with the handler during the testing period. The goal of the home setting was to allow the dog to act as it would with its owner in a typical day-to-day living situation for 60 min without stranger interaction.

2.2.2. Novel setting

The novel setting was a room that none of the AAA dogs had seen before in the administrative office area of the University of Pennsylvania School of Veterinary Medicine (Philadelphia, PA, USA). The dog-handler teams waited in this unfamiliar environment for 60 min without stranger interaction. The dog remained in close proximity to the handler on a 1.83-m leash attached to a collar, harness, or head collar (Gentle Leader, Radio Systems Corporation, Knoxville, TN, USA) that the dog was accustomed to wearing at typical AAA visits. The room was 6.1 m × 6.8 m, with tables and chairs arranged to demarcate two, 2.1 m × 2.1 m spaces on opposite sides of the room for each dog-handler team to remain during the session. A maximum of two dog-handler teams used the room simultaneously, but dogs were limited to direct interaction only with their own handlers during the testing period. Three of the dog-handler teams were present in the novel room without another team in the room. Although the room was located in the veterinary school, dog-handler teams did not enter through or have contact with the veterinary hospital facility. The goal of the novel setting was to allow the dog to act as it typically would with its owner when waiting in an unfamiliar environment for 60 min without stranger interaction.

2.2.3. Activity setting

The activity setting was an AAA session functioning as a 60-min study break for college students. To accommodate a large number of students in an efficient and social manner, these AAA sessions require the presence of multiple dog-handler teams in the same venue so that students may circulate between dogs at their leisure. The AAA setting was located in a communal space of an undergraduate dormitory (Rodin College House, Philadelphia, PA, USA) where typical study break activities like this routinely occur. The room was 9.1 m × 9.1 m, with tables and chairs arranged to evenly spread and demarcate eight, 2.1 m × 2.1 m spaces for each dog-handler team to remain during the session. The dog remained in close proximity to the handler on a 1.83-m leash attached to a collar, harness, or head collar that the dog was accustomed to wearing at AAA visits. A maximum of eight dogs used separate spaces of the room simultaneously, but dogs were restricted from interacting with one another. To accommodate all dog-handler teams, two separate activity sessions were held.

College students were invited via email to attend the activity sessions, and announcements posted on dormitory activity bulletin boards advertised the study break sessions 3 weeks prior to the event. The activity functioned as a true study break session during examination week in which students voluntarily attended to interact with any of the dogs at their leisure. Prior to entering the room, each student signed a consent form that stated the participant was a University of Pennsylvania student, at least 18 years old, and would be videotaped for the experiment. Participants were instructed by a research assistant (one assistant assigned to each dog-handler team) with visual aids and verbal descriptions on how to greet and interact with the dogs in a non-threatening manner. Participants were instructed to approach the dog from the side, to extend a hand to allow the dog to sniff, and to pet gently. Participants were instructed to avoid the following: aggressive gestures, making loud noises, leaning over the dog, giving treats, and crowding around the dog. Assigned "petters" were assigned to pet specific dogs during the 5-min petting time (Table 2 and Section 2.2) but participants were permitted to interact with any of the dogs outside of that time.

2.3. Five-min petting procedure

In the home and novel settings, the handler served as the "petter" and was instructed to sit on the floor to the side of the dog away from the video camera. In the activity session, the handler was instructed to sit on the floor to the side of the dog away from the video camera

Table 2
Schedule of events for each setting for all dogs over the course of the 120-min study period.

Time (min)	Location	Setting		
		Home	Novel	Activity
0–2	Outdoors	Saliva collection	Saliva collection	Saliva collection
2–25	Outdoors/indoors	Walk from outside to inside	Walk from outside to inside	Walk from outside to inside
25–30	Indoors	5-Min petting by handler	5-Min petting by handler	5-Min petting by stranger
30–32	Indoors	Saliva collection	Saliva collection	Saliva collection
32–55	Indoors	Remain within video frame as handler sits quietly without interacting with the dog	Remain within video frame as handler sits quietly without interacting with the dog	Remain within video frame as college students visit and interact with dog
55–60	Indoors	5-Min petting by handler	5-Min petting by handler	5-Min petting by stranger
60–62	Indoors	Saliva collection	Saliva collection	Saliva collection
62–85	Indoors	Remain within video frame as handler sits quietly without interacting with the dog	Remain within video frame as handler sits quietly without interacting with the dog	Remain within video frame as college students visit and interact with dog
85	Indoors	5-Min petting by handler	5-Min petting by handler	5-Min petting by stranger
90	Indoors	Saliva collection	Saliva collection	Saliva collection
92–120	Indoors/outdoors	Walk from inside to outside	Walk from inside to outside	Walk from inside to outside
120	Outdoors	Saliva collection	Saliva collection	Saliva collection

and to limit handler interaction with the dog while it was being petted by the assigned student petter. A maximum of eight different students who were unfamiliar to the dogs were randomly assigned to be video-captured petters prior to the session (one student per dog). Each petter was instructed to sit on the floor to the side of the dog opposite the handler, where the petter did not obstruct the video camera view of the dog. In all settings, each petter was instructed to sit next to, rather than facing, the dog and gently stroke, pat, massage, and/or scratch the dog anywhere on the body with at least one hand remaining on the dog at all times. The dog was to be allowed to position itself and behave as it wanted during the 5-min petting procedure, as long as it remained within the assigned space within view of the video camera (accomplished via leash).

All sessions were conducted over 120 min between 15:00 and 20:00 h. Each session began outdoors at time 0. The dog-handler team walked outdoors of the setting for 15–20 min until it entered to sit in its assigned space to start the 5-min petting protocol at time 25, followed by saliva collection at time 30. In the activity setting, each petter was assigned to pet a different dog at each of three, 5-min time points in consecutive order of assigned dog ID numbers. For example, "petter number 2" would pet dog 2 at time 25, dog 3 at time 55, and dog 4 at time 85. Saliva collection was repeated at time 60 and 90. The rationale of the pettings every 30 min was to have a logical flow of events that was standardized to detect a difference in cortisol and behavior. Only behavior observed during the 5-min petting procedure was analyzed to compare the behavioral response to a standard stimulus (petting) across settings and time points. While the 5-min petting intervention was standardized to a single individual petting the dog, there was considerable variation in the number of individuals that visited with the dog in the interim between the three petting interventions (Table 2).

Each assistant used a timer (Accusplit Survivor III Magnum XL, Livermore, CA, USA) to direct the handler where to go, when petting of the dog should start and stop, and when to start and stop collecting saliva samples according to the schedule (Table 2). This schedule standardized the activity of the dog-handler team as well as capture of cortisol and behavior across all settings. Whereas the dog did not interact with strangers between the 5-min petting protocols in the home and novel settings, numerous different strangers interacted with the dog between the 5-min petting protocols in the activity session. The dog-handler team left the assigned space after the saliva collection at time 90 and then walked outdoors until the last saliva collection at time 120.

2.4. Saliva collection and analysis

Prior to the start of each setting, the handler was provided with a belted pouch (Fantasybag 3-zipper fanny pack, Rajaji Nagar, Banglore, India), worn around the waist, containing five saliva collection tubes (Salimetrics, State College, PA, USA), pre-labeled with time and a pre-assigned ID number, and five saliva absorbent swabs (SalivaBio Children's Swabs, Salimetrics, State College, PA, USA).

A saliva sample was collected for each dog at times 0, 30, 60, 90, and 120 min in each setting. The 30-min intervals for cortisol measurement were chosen based on previous studies that detected a significant change in cortisol 15–30 min after a stress event (Vincent and Michell, 1992; Handlin et al., 2011). Prior to each saliva collection, the research assistant gave the handler the pre-determined signal to start collection according to the schedule (Table 2). The handler, who was seated on the floor next to the dog, inserted the swab in the dog's mouth while grasping onto the opposite end of the swab with his or her fingers. The tip of the swab was placed in both cheek pouches and between the teeth to encourage the dog to chew on the swab to stimulate additional saliva production for a total of 90 s (Dreschel and Granger, 2009). After 90 s, the handler removed the swab and folded it into the collection tube, which was immediately placed into a Styrofoam container with an ice pack. After each session, all saliva samples were frozen at −20 °C until they were shipped for final analysis. The research assistant addressed any problems encountered during the saliva collection procedure.

The frozen saliva samples were delivered on dry ice in a Styrofoam container to Salimetrics (State College, PA, USA) for processing. All samples were centrifuged at 3000 × *g* for 15 min and assayed for salivary cortisol using a highly sensitive enzyme immunoassay kit (Salimetrics Salivary Cortisol Immunoassay kit, Salimetrics, State College, PA, USA). Samples were measured in duplicate unless the volume of saliva collected prevented this, and their values were averaged for use in analyses. Samples with insufficient volume were diluted by 50% with assay diluent. Average intra- and inter-assay coefficients of variance were less than 15% and 10%, respectively.

2.5. Behavioral observations

Prior to the start of each setting, the research assistant assigned to the individual dog-handler team set up a tripod connected to an extension cord approximately 0.91 m to the side of where the handler was assigned to sit. The research assistant placed a secure digital memory card (32 GB SDHC Centon card, Aliso Viejo, CA, USA) into the video camera (either Sony DCR-SR68, Tokyo, Japan, or Sanyo VPC-HD2000, San Diego, CA, USA) to record the dog's activity continuously from beginning to end of the 120-min session at 60 frames/s; the camera's focus was zoomed out to capture the dog's entire body at all times. The handler positioned the dog to be facing the camera at all times. The video camera was handheld by the research assistant when following the dog-handler team walking from outside to inside and vice versa; when inside, the video camera was placed on the stationary tripod, which was level to the height of the dog.

All sessions were downloaded in .mpg format. Only behavior observed during the 5-min petting procedure was analyzed to compare the behavioral response to a standard stimulus (petting) across settings and time points. Each 120-min recording was spliced into three separate 5-min video clips to contain only the 5-min petting procedure (at 25, 55, and 85 min) using a video splicing software (OJOSoft Minnetonka, MN, USA).

An ethogram (Table 3) was developed with the assistance of a veterinary behaviorist (CS), from previously recorded sessions in a pilot study. The behaviors included variations in postural state and alertness (mutually exclusive events), and oral behaviors and spontaneous events (start-stop events). Using the Observer XT data recording system (Noldus Information Technology, Wageningen, Netherlands), the video clips were coded by trained users (ZN and DJI) using continuous sampling. Behaviors listed on the ethogram (Table 3) were coded and analyzed according to frequency and duration. Inter-observer reliability exceeded 90% for all behavioral categories.

2.6. Experimental design

Collection of saliva and video recording were performed across the three settings on 3 non-consecutive days in a randomized order within a 2-week period. The schedule was randomized using an online randomization program (Research Randomizer, Version 4.0, Middletown, CT, USA,

Table 3
Ethogram (modified from Beerda et al., 1998) used to code behaviors of registered animal-assisted activity dogs while being petted by the handler in home and novel settings or by a stranger in an activity setting.

Behavior	Description
Postural state	
Sitting	Sitting on ground with pads of front paws in contact with floor and forelimbs straight
Standing	Positioned with just four paws in contact with ground or two in contact with ground and two in contact with wall
Recumbent	Fully positioned, lying with one side in complete contact with ground in lateral, sternal, or dorsal recumbency
Ambulating	Movement from one point to another, with no clear effort to explore, whether pacing, walking straight, or walking in a circle
Exploring	Moving slowly, sniffing, investigating the environment
Crouching	Rapid, pronounced lowering of posture, sometimes in combination with movements that enlarge distance to eliciting stimulus; posture shows lowered position of tail, backward positioning of ears, legs bent
Spontaneous event	
Paw lifting	Raising forepaw into position of approximately 45°
Vocalizing	Any form of vocalization, including barking, growling, whining, yelping
Scratching	Purposeful movement of limb to scratch any part of body
Body shaking	Purposeful shaking of full body
Trembling	Body shaking with small, high-frequency movements, clear shivering of body
Jumping	Springing into air, either to make contact with an object or person or for no apparent reason
Repetitively moving head	Changing head position continuously >3 s
Stretching	Purposeful extension of body and limbs
Oral behavior	
Panting	Increased frequency of inhalation and exhalation often in combination with opening of mouth
Neutral	Mouth closed
Mouth opening	Opening, closing mouth with rapid movements without extending tongue; possibly yawning
Lip licking	Includes tongue out: tip of tongue briefly extended; snout licking: part of tongue shown, moved along upper lip; swallowing; smacking
Licking person	Extending tongue to touch a person's body
Licking object	Extending tongue to touch an inanimate object or floor
Self-grooming	Oral behaviors directed toward dog's own body (licking, chewing skin and coat)
Alertness	
Alert	Eyes kept open
Rest/sleep	Eyes closed, dog inactive >10 s

http://www.randomizer.org) according to availability of handlers.

Thirty-min intervals for cortisol measurement were chosen based on previously published data that show a 15–20-min delay in rising cortisol levels in circulation after exposure to a stress event, as well as a delay in the increase of cortisol in saliva compared to that in plasma (Vincent and Michell, 1992; Kirschbaum and Hellhammer, 1989; Handlin et al., 2011). These studies suggest that a stress event would not result in a change in salivary cortisol until 30 min later.

The dog was not given food after 12:00 h (no later than 3 h prior to collection) on any of the data collection days. Fresh water was available in a bowl within the dog's space. The room temperature in all indoor settings ranged between 20.6 and 23.9 °C. The temperature in all outdoor settings ranged between 11.1 and 21.1 °C. No adverse events occurred during the study period.

2.7. Statistical analysis

Descriptive statistics were used to report demographic data. Salivary cortisol values were log transformed (base e) to achieve normal distribution. A mixed-model repeated-measures ANOVA with Holm–Tukey adjustment for multiple comparisons was used to assess the effect of location, time, and order on salivary cortisol and percentage change of salivary cortisol.

An exact Kruskal–Wallis test with Dunn's procedure for multiple comparisons was used to assess associations between salivary cortisol and sex, AAA organization, duration of ownership, AAA visits per month, and length of AAA session per day. Scatterplots and Spearman correlation coefficients were used to assess correlations between salivary cortisol and number of animals in the house, age, weight, number of years of AAA registration, and behavior.

Friedman's chi-square test was used to analyze differences in behavior between time points and between settings. Scatterplots and Spearman correlation coefficients were used to assess the correlation between each behavior and salivary cortisol level 30 min after the behavior was observed in each setting (e.g., body shaking at time 30 during the activity session was correlated with salivary cortisol at time 60 during the activity). Statistical significance was set at $P < 0.05$. All analyses were performed using SAS version 9.3 (Cary, NC, USA).

3. Results

3.1. Participants

Eight dog-handler teams were enrolled in the study during each of the two, 2-week periods, resulting in 16 different dog-handler teams over 4 weeks from March to April 2012. Of the 16 dog-handler teams, one was excluded because the handler was unable to collect an adequate saliva sample (see Section 2.1). None of the remaining 15 dog-handler teams was excluded based on physical examination findings.

Demographics of the participants are shown in Table 1. There were 13 female handlers and two male handlers, as well as seven spayed female dogs and eight neutered male dogs. There were nine mixed-breed dogs and one of each of the following breeds: Akita, American Staffordshire terrier, Cavalier King Charles spaniel, golden retriever, Rhodesian ridgeback, and Pembroke Welsh corgi. The mean weight of all dogs was 9.14 kg (median 17.3 kg [range 6.4–44.5 kg]). The mean age of dogs was 4.6 years (median 4 years [2–10 years]). The mean years of AAA certification were 2 (median 2 years [1–8 years]). The mode for the average number of days per month the dogs participated in AAA sessions was 4–6 days. The mode for the average duration of the AAA session per day was 30–60 min. Six of the 15 dog-handler teams resided with other animals in the household, and six of the teams resided with other humans in the household.

For the activity sessions, because of the limited size of the available room, up to eight dog-handler teams were present in the room at the same time, and seven dog-handler teams were present during activity 2. Nine of the 15 dogs had never visited the AAA setting used in the study. At the first and second AAA sessions, 45 and 56 undergraduate students, respectively, attended. However, the number of individuals present in the room at any one time varied between 30 and 56 because they were free to enter and exit at their leisure between time 30 and time 90, as they would for a typical AAA study break program.

3.2. Salivary cortisol

From the 15 dogs, 224 salivary samples were collected. Of these samples, 218 yielded sufficient saliva for cortisol analysis. One dog (dog 14, a 5-year-old female, spayed Rhodesian ridgeback) was identified as a persistent outlier in all settings. The salivary cortisol levels of dog 14 were 1.918, 2.668, 1.750, 1.372, and 1.341 μg/dL at 0, 30, 60, 90, and 120 min, respectively, in the activity setting; 2.853, 2.957, 4.615, 2.081, and 3.059 μg/dL, respectively, in the home; and 15.291, >30, >30, 14.911, and 12.810 μg/dL, respectively, in the novel setting. Since this dog was a persistent outlier, its salivary cortisol values were excluded from the data set.

For the remaining 14 dogs, the mean (median) salivary cortisol was 0.305 μg/dL (0.236) in the activity, 0.277 (0.232) in the home, and 0.554 (0.304) in the novel setting. Fig. 1 shows the mean salivary cortisol for all dogs over time in each setting. Salivary cortisol in the novel setting was significantly higher than in the home at time 0 (novel mean 0.423, median 0.325 vs home mean 0.213, median 0.191, Holm–Tukey t value = 4.15; $df = 52.61$; $P = 0.0002$) and time 30 (novel mean 0.397, median 0.332 vs home mean 0.255, median 0.245, Holm–Tukey t value = 2.82; $df = 53.82$; $P = 0.0149$). It was also significantly higher in the novel setting than in the activity setting at time 30 (novel mean 0.397, median 0.332; activity mean 0.257, median 0.291, Holm–Tukey t value = 2.63; $df = 52.61$; $P = 0.0255$), time 60 (novel mean 0.371, median 0.276; activity mean 0.246, 0.29, Holm–Tukey t value = 2.63; $df = 53.49$; $P = 0.0251$), and time 90 (novel mean 0.351, median 0.3065; activity mean 0.229, median 0.197,Holm–Tukey t value = 2.35; $df = 53.85$; $P = 0.0518$).

The geometric mean percentage change of salivary cortisol from time 0 is shown in Fig. 2. There was no significant

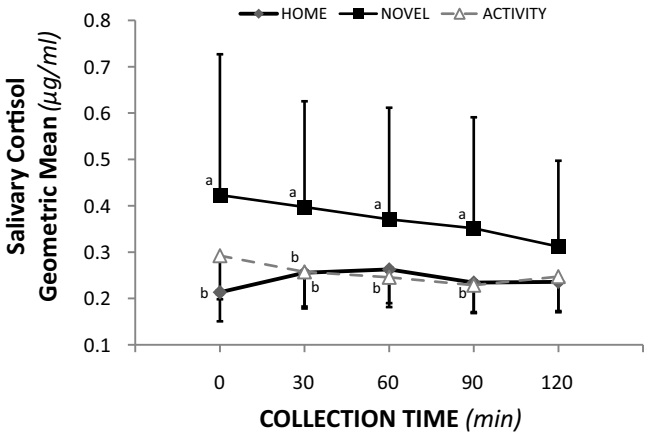

Fig. 1. Salivary cortisol geometric mean for each setting across time. Significant differences between settings are indicated by differing lower-case letters. Bars indicate upper 95% confidence levels for the novel setting and lower 95% confidence levels for home and activity settings.

effect of time on salivary cortisol in each setting (type III test of fixed-effects $F_{4,146.40} = 1.15$; $P = 0.3370$). However, percentage change of cortisol from time 0 to 60 min was significantly higher in the home setting (mean 30.6%, median 36.36%) compared to the activity setting (mean −14.92%, median −23.07%, Holm–Tukey t value = 3.01; $df = 84.50$; $P = 0.009$). Additionally, the percentage change from time 0 to 120 min was significantly higher in the home setting (mean 17.35%, median 2.01%) compared to the novel setting (mean −23.31%, median −23.51%, Holm–Tukey t value = 2.74; $df = 82.82$; $P = 0.0194$).

There was no significant effect of the randomized order of setting on salivary cortisol levels. The difference between the effect of each individual petter on salivary cortisol levels could not be statistically analyzed.

3.3. Behavior

Five-min video clips at time 30, 60, and 90 were coded according to the behaviors listed in Table 3 and analyzed in the home, novel, and activity settings for 15 dogs, 15 dogs, and 14 dogs, respectively. The behavior from dog 1 was not recorded in the activity setting due to technical error.

For each setting, the number of dogs that exhibited each ethogram behavior is shown in Table 4. There were significant differences between settings in percentage of observed behaviors of standing, ambulating, and recumbency. Fig. 3 shows the median percentage standing across time in all settings. The percentage standing was significantly higher in the activity setting (median 58.99) compared to the home setting (median 0) at 30 (Friedman's chi-square = 7.14; $df = 1$; $P = 0.0075$), 60 (activity median 20.21, home median 0, Friedman's chi-square = 6.23; $df = 1$; $P = 0.0126$), and 90 min (activity median 22.43, home median 0, Friedman's chi-square = 7.36; $df = 1$; $P = 0.0067$). Percentage standing was also higher in the novel setting (median 20.59) compared to the home setting (median 0) at time 90 (Friedman's chi-square = 7.36; $df = 1$; $P = 0.0067$). Fig. 4 shows the median percentage ambulating across time in all settings. The percentage of time spent ambulating (Fig. 4) was significantly higher in the activity compared to the home setting at time 30 (activity median 5.6%, home median 0%, Friedman's chi-square = 10.29; $df = 1$; $P = 0.0013$), time 60 (activity median 1.98%, home median 0%, Friedman's chi-square = 6.4; $df = 1$; $P = 0.0114$), and time 90 (activity median 4.04% vs home median 0%, Friedman's

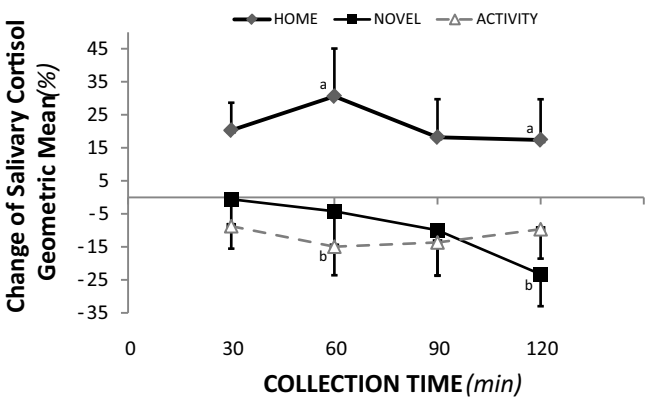

Fig. 2. Geometric mean percentage change of salivary cortisol from time 0. Significant differences between settings are indicated by differing lower-case letters. Bars indicate upper standard error of the means for the home setting and lower standard error of the means for the novel and activity settings.

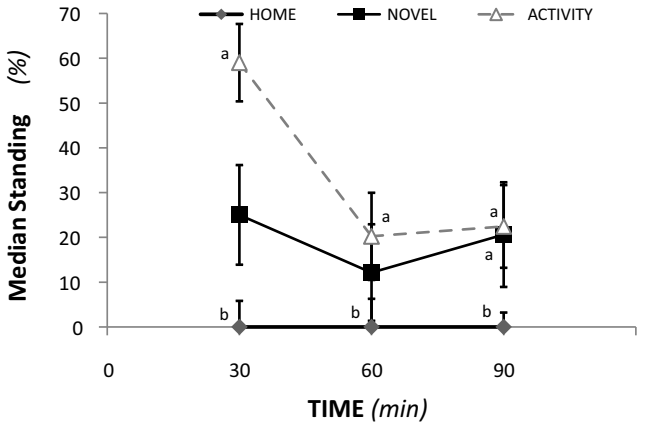

Fig. 3. Median percentage standing in each setting over time. Significant differences between settings are indicated by differing lower-case letters. Bars represent the standard error of the means.

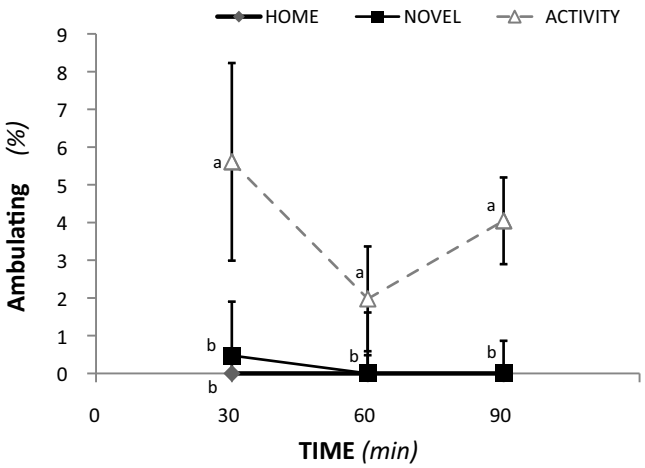

Fig. 4. Median percentage ambulating in each setting over time. Significant differences between settings are indicated by differing lower-case letters. Bars represent the standard error of the means.

chi-square = 10.79; $df = 1$; $P = 0.0016$). Additionally, the percentage ambulating was higher in the activity (median 5.6) compared to the novel setting (median 0.477) at time 30 (Friedman's chi-square = 5.3; $df = 1$; $P = 0.0209$). Fig. 5 shows the median percentage recumbent across time in all settings. The percentage recumbent was significantly higher in the home compared to the novel setting at time 30 (home median 99.18%, novel median 0%, Friedman's

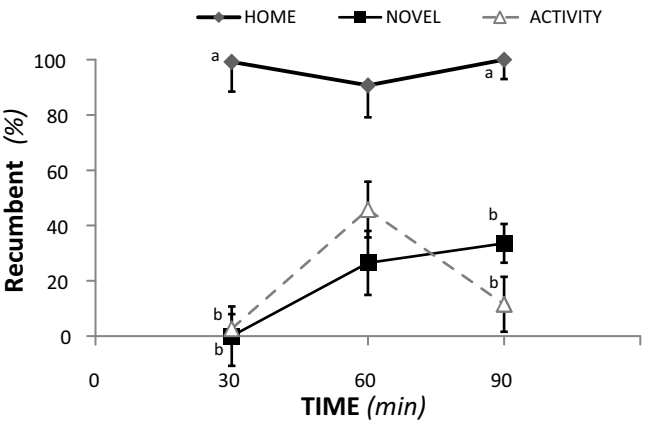

Fig. 5. Median percentage recumbent in each setting over time. Significant differences between settings are indicated by differing lower-case letters. Bars represent the standard error of the means.

Table 4
The number of dogs that demonstrated each behavior at least once during observation in each of the three study settings.

	Home ($n=15$)	Novel ($n=15$)	Activity ($n=14$)
State			
Sitting	9	12	13
Standing	8	14	14
Recumbent	15	12	11
Ambulating	5	7	13
Exploring	3	4	7
Crouching	0	1	0
Event			
Paw lifting	2	3	6
Vocalizing	2	1	3
Scratching	2	2	3
Body shaking	3	7	9
Trembling	0	0	0
Jumping	0	1	6
Repetitively moving head	1	1	0
Stretching	5	2	5
Oral behavior			
Panting	5	8	7
Neutral	15	15	13
Mouth opening	10	9	13
Lip licking	13	15	14
Licking person	6	3	5
Licking object	0	2	4
Self-grooming	2	1	1
Alertness			
Alert	15	15	14
Rest/sleep	10	4	6

chi-square = 4.57; $df = 1$; $P = 0.0325$) and time 90 (home median 100%, novel 33.57%, Friedman's chi-square = 7.14; $df = 1$; $P = 0.0075$). The percentage recumbent was also significantly higher in the home compared to the activity setting at time 30 (home median 99.18%, activity median 2.67%, Friedman's chi-square = 4.57; $df = 1$; $P = 0.0325$) and time 90 (home median 100%, activity median 11.53%, Friedman's chi-square = 7.14; $df = 1$; $P = 0.0075$).

3.4. Relationship between behavior and salivary cortisol

Salivary cortisol was significantly associated with observed behaviors of sitting, standing, panting, and neutral mouth at specific time points in various settings. It was positively correlated with percentage sitting at time 90 in the novel setting (Spearman's correlation coefficient = 0.60; $P = 0.0388$) and at time 30 in the activity setting (Spearman's correlation coefficient = 0.59; $P = 0.0400$). Salivary cortisol was negatively correlated with percentage standing at time 30 in the activity setting (Spearman's correlation coefficient = −0.64; $P = 0.0219$). At time 60 in the novel setting, salivary cortisol was positively correlated with percentage the mouth was closed (neutral) (Spearman's correlation coefficient = 0.78; $P = 0.0019$) but negatively correlated with percentage panting (Spearman's correlation coefficient = −0.62; $P = 0.0284$). There were no significant correlations between any other observed behavior and salivary cortisol at any other time point.

4. Discussion

The 60-min AAA for college students in a dormitory setting did not appear to cause significant HPA activation or increases in stress-associated behaviors in registered AAA dogs. Specifically, there was no difference in salivary cortisol levels between the activity and home settings, and there was no increase in serial salivary cortisol levels over the course of the AAA session. In addition, the frequency of stress-associated behaviors was not different between the activity and home settings. No physiologic or behavioral indicators of stress, fatigue, or exhaustion were present during the AAA, suggesting that this particular AAA with college students did not negatively impact the welfare of these dogs. Furthermore, salivary cortisol was higher in the novel setting, which may be explained by the unpredictable nature of the setting.

An AAA dog-handler team typically consists of a dog with a consistent, non-fearful and non-aggressive temperament and a handler who is trained to minimize interactions that might be perceived as threatening by the dog. The dogs were likely not stressed during the AAA because it was a familiar and predictable situation. A recent study also determined salivary cortisol levels to be no different between home and therapy settings in therapy dogs (Glenk et al., 2013). However, King et al. (2011) reported significant elevations in salivary cortisol levels in therapy dogs from baseline after 1 h of AAT in a hospital environment (King et al., 2011). In addition, Haubenhofer and Kirchengast (2006) found that salivary cortisol levels in registered therapy dogs were higher on days of therapy work compared to control days at home, suggesting that therapeutic work was physiologically arousing. However, Haubenhofer's study did not control for factors such as time, frequency, intensity of interaction, and location of therapeutic work, which could have influenced cortisol levels. We attempted to control for these variables by standardizing each of the experimental settings for all subjects in the current study.

King also noted that their study was limited by the number of missed cortisol samples as a result of the handlers' inability to collect an appropriate saliva sample despite having instruction. Our study addressed the problem of missed samples by providing handlers with careful saliva collection instruction, having the handler demonstrate his or her ability to collect a sample after the information meeting, and having a research assistant to oversee all saliva collections. Adequate samples were obtained by most of the study population, but one dog was excluded because the handler was unable to demonstrate successful saliva collection due to the dog's adverse behavioral response to the swab. Because the dog resisted the swab and required additional restraint, it was unlikely that repeated saliva collections could be achieved. Although salivary sampling is considered a non-invasive method of measuring cortisol, the temperament of the dog determines the perception of degree of invasiveness of the procedure. Despite being a registered AAA dog, there are no requirements for an AAA dog to accept a foreign object in its mouth as is done during saliva sampling. Future studies may require alternative methods of

cortisol measurement in dogs that are not amenable to saliva collection.

Interestingly, cortisol levels were significantly higher at certain time points in the novel setting than in the activity or home settings. This is similar to previous studies which have reported that dogs introduced into a novel environment show enhanced sympathetic activation (Pagani et al., 1991) and enhanced HPA activity (Vial et al., 1979; Beerda et al., 1997), subsequently increasing cortisol levels (Tuber et al., 1996). Additionally, the higher cortisol levels in the novel and activity compared to home at time 0 could reflect HPA activation due to anticipation and transportation to the setting. Since the activity setting was a novel environment for nine of the 14 dogs in the study, cortisol levels for the cohort were anticipated to be higher during the AAA. Despite this bias, cortisol levels in the activity setting were still no different from the home setting. This was likely because the dogs had a predictable and safe interaction during the activity, whereas they did not have a predictable and controlled environment and were furthermore restricted from interaction with others in the novel setting. This inability to predict what will happen induces significant stress in humans (Henry and Stephens, 1977) and likely occurs in dogs as well. In addition, the novel setting, unlike the activity, was located in the veterinary school. Salivary cortisol levels have been found to be significantly elevated in a veterinary hospital compared to the home (van Vonderen et al., 1998). Although the dogs were not in direct contact with the veterinary hospital, the dogs could have detected subtle cues of a veterinary hospital environment. Therefore, the physical environment alone, especially a veterinary hospital setting, may be physiologically stimulating, irrespective of the activity performed.

Although cortisol levels were higher in the novel compared to home and activity settings, the only behaviors that differed significantly between settings at specific time points were in regard to postural state. The dogs stood and ambulated more in the activity setting compared to the home setting, probably because they were stimulated by interaction with strangers. Dogs stood and ambulated more, and consequently were less recumbent, in the novel setting than in the home setting likely because it was an unfamiliar environment and they were hyper vigilant to disturbances outside the room. It is likely that the frequent standing and ambulation at time 30 and subsequent decrease at time 60 and 90 in activity and novel settings were because of the initial stimulation from arriving to the inside of the setting after walking outdoors. Unfortunately, the details of the posture, such as head, ear, and tail carriage were not captured, which may have provided additional behavioral markers of stress. No other traditional behavioral signs of stress, such as increased restlessness, snout licking, paw lifting, yawning, body shaking, nosing, circling, increased locomotor activity, and lowering of body posture (Schwizgebel, 1982; Beerda et al., 1997, 1998, 2000) were increased in the novel setting. This finding is similar to an investigation of the effect of human interaction on canine cortisol level and behavior, which found a significant increase in plasma cortisol in the context of normal behaviors in Labrador retrievers petted by their owners (Handlin et al., 2011). Although King et al. reported an increase in stress-associated behaviors, in that study, only five behaviors were accounted for during a 1-min period after the therapy session was completed: panting, air-licking, yawning, whining, and pupillary dilation (King et al., 2011). It is important to consider that the same behavior can correspond to different emotional states of the dog. For example, ambulating may be strictly a motor behavior, but it can also be an indicator of restlessness or anxiety, depending on how the behavior is performed and the type of concomitant behaviors present at the time of ambulating. Therefore, it is necessary to assess behavior in conjunction with a physiologic parameter such as cortisol.

There were few significant correlations between observed behavior and salivary cortisol: positive correlation with sitting and negative correlation with standing in the activity and novel settings at time 30 and 90 min, respectively. This may be explained by the handler's tendency to instruct the dog to sit during the 5-min interaction so it could remain in view of the video camera. Dogs may be more reactive or frustrated if freedom of movement is restricted (Haug, 2008), and cortisol levels can rise, especially if pulled on leash (Beerda et al., 1998). Similarly, therapy dogs working on-leash were found to have higher cortisol levels than therapy dogs working off-leash during a therapy session (Glenk et al., 2013). The leash physically restricted movement, which may have resulted in an increased likelihood of sitting and simultaneous increase in cortisol in the activity and novel settings.

In addition, in the current study, salivary cortisol positively correlated with the percentage of time the mouth was closed and negatively correlated with the percentage time the dog was panting in the novel setting at time 60 min. This finding contradicts other studies that have associated panting with stress, and thus HPA activation (Beerda et al., 1997; Dreschel and Granger, 2005). Although panting can be associated with negative stress, it may be alternatively associated with positive arousal, such as during anticipation of a desired reward. Few studies have documented a true relationship between elevated cortisol levels and increased panting (Hiby et al., 2006; Hekman, 2012).

The few correlations between cortisol and behavior should be interpreted cautiously because they did not persist through all settings or time points. This inconsistency illustrates the challenge in correlating physiologic and behavioral parameters (Hansen and Jeppesen, 2006). Not all dogs express stress-associated behavior in the same way because temperament and personality are influenced by many variables, including age, breed, and past experience (Hiby et al., 2006). Different dogs often have different responses and coping strategies to the same stimulus (Jones and Gosling, 2005; Rooney et al., 2007). The brain and body develop coordinated biologic mechanisms in response to potent stressors to anticipate and recover from them in the future in an effort to maintain homeostasis (Karatsoreos and McEwen, 2011). Responses are also likely influenced by the type of interaction, as it has been speculated that dogs may not exhibit stress-associated behavior in the context of human–animal interactions (Kuhne et al., 2012) despite being physiologically stressed. Additionally, because cortisol change is on a continuum and not precise to a single

event, it is likely that the cortisol level was influenced by circumstances surrounding the 5-min interaction.

It is important to consider that AAA dogs represent a specific demographic of dogs that were selected for this type of activity because of their temperaments and training to remain calm and relaxed, even in stressful situations (Piva et al., 2008; Viau et al., 2010). Therefore, AAA dogs may not exhibit stress-associated behaviors typically demonstrated by the rest of the canine population when physiologically aroused. This underscores the importance of measuring behavior in conjunction with cortisol, both of which were unchanged in the activity setting compared to the home setting in this study. Although a single AAA session did not induce an acute stress response, it is unknown if there is a limit at which the duration or frequency of AAA sessions may induce a stress response, resulting in a disruption in homeostatic mechanisms and chronic stress (Karatsoreos and McEwen, 2011). Future studies should investigate this limit.

Although the dogs in this study did not appear to be negatively affected by this particular AAA session, the welfare of AAA dogs should be continuously monitored. Until a gold standard measure of stress or distress is clearly established, behavioral observation remains a principal and practical method of evaluating stress and welfare in animals (Hekman, 2012). It is imperative that the handler be properly educated on prevention, recognition, and management of stress-associated behavior in his or her dog (Mariti et al., 2012). It is particularly important that the handler understand normal behavior for the dog in the home setting to be able to recognize behavioral signs of stress when they occur.

By nature, AAA is more variable than AAT because an AAA session typically involves numerous contacts with many different people whereas an AAT session typically involves a continuous interaction with a single or small group of individuals. Currently, there is no single validated model to test the effect of AAA or AAT because they vary greatly in intensity of interaction, duration, objectives, and demographics of recipients. Our study attempted to standardize these variables in an AAA session that was conducted as a conventional 60 min study-break session for numerous college students. Although the college students attending the AAA session in this study were instructed how to interact with the dog in a non-threatening manner, nonverbal cues from the "petter" such as facial expressions, posture, tone of voice, and eye contact were not controlled for, which may have influenced outcomes. These limitations represent the common pitfalls of conducting a validated AAA model, but these variables are typically encountered in the real-world setting, as no two human–animal interactions are ever exactly the same. This underscores the importance of the handler to control the interaction, as the variable that remains consistent throughout all AAA sessions is the handler.

Significant limitations of this study were the small study size and variation in demographic factors of breed, age, and weight. In addition, the level of familiarity with this particular type of AAA varied between dogs, as some had participated in a college break setting previously while it was a novel situation for others. Ideally, dog-handler teams would be randomly selected from an approved AAA organization's registry. In addition, the rise in cortisol in the novel setting would have been more compelling if it took place in the same location as the activity session, suggesting that human interaction can mitigate HPA activation. It is possible that a stress-mitigating effect from being petted was counterbalanced by the stimulating effect of the environment. Our intent was to use the same location for the novel and activity sessions, but the facility was unavailable for use at both times. Future studies should use the same location for both sessions, ideally away from a veterinary hospital or clinic, to test the effect of the physical environment. Although dogs were restricted from interacting with other dogs in the novel and activity settings, the mere presence of another dog in the same room may influence HPA activation and behavioral outcomes. To eliminate this variable, it would be ideal to assess dogs without other conspecifics present, but the practicality of conducting such a study would have been difficult. This study provides a platform for future investigations of animal welfare in AAA, which should explore the effect of different types of AAA and AAT on cortisol and behavior, in conjunction with other physiologic parameters of the stress response. Finally, this investigation validates the process of AAA selection and training since these AAA registered dogs tolerated this particular AAA well without adverse effects.

5. Conclusions

A 60-min AAA session for college students conducted in a safe and controlled manner does not appear to elicit significant HPA activation or observable stress-associated behaviors, and thus is not likely to negatively impact welfare of registered AAA dogs. Although stress-associated behavior did not correlate with salivary cortisol, it is still important to monitor the behavior of dogs in these situations and consider that the physical environment plays a role in their stress response. An appropriately trained handler can influence the dog's perception of the environment and minimize the stress response by facilitating controlled and predictable interactions. This study described a rigorous method of assessing welfare in AAA dogs that can be applied in larger populations of working dogs.

Acknowledgements

The authors would like to thank Daniel Inman, Shavaughn Snipas, Tara Enzweiler, and Courtney Smith for their technical assistance; Misty Bailey for technical editing; Dr. James Serpell, Dr. Nancy Dreschel, Dr. Francis Pau, and Alyssa Bennett for their scientific expertise and advice; and Therapy Dogs International and the University of Pennsylvania housing services for their contributions. This study could not have been possible without the research assistants, handlers, and dogs. The project was funded by Nestle Purina through grant number 457597 (BJP).

References

American Veterinary Medical Association, 1998. Human–animal bond issues. J. Am. Vet. Med. Assoc. 212, 1675.

Beerda, B., Schilder, M.B., Bernadina, W., van Hooff, J.A., de Vries, H.W., Mol, J.A., 1999. Chronic stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological responses. Physiol. Behav. 66, 243–254.

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of chronic and acute stress in dogs. Appl. Anim. Behav. Sci. 52, 307–319.

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., Mol, J.A., 1998. Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. Appl. Anim. Behav. Sci. 58, 365–381.

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., Mol, J.A., 2000. Behavioural and hormonal indicators of enduring environmental stress in dogs. Anim. Welf. 9, 49–62.

Blackwell, E.-J., Bodnariu, A., Tyson, J., Bradshaw, J.W.S., Casey, R.A., 2010. Rapid shaping of behaviour associated with high urinary cortisol in domestic dogs. Appl. Anim. Behav. Sci. 124, 113–120.

Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., 2007. Assessment of positive emotions in animals to improve their welfare. Physiol. Behav. 92, 375–397.

Broom, D.M., Johnson, K.G., 1993. Stress and Animal Welfare. Springer, London.

Dreschel, N.A., Granger, D.A., 2009. Methods of collection for salivary cortisol measurement in dogs. Horm. Behav. 55, 163–168.

Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. Appl. Anim. Behav. Sci. 95, 153–168.

Ferrara, M., Natoli, E., Fantini, C., 2004. Dog welfare during animal assisted activities and animal assisted therapy. In: 10th International Conference of the IAHAIO, Glasgow, Scotland.

Glenk, L.M., Kothgassner, O.D., Stetina, B.U., Palme, R., Kepplinger, B., Baran, H., 2013. Therapy dogs' salivary cortisol levels vary during animal-assisted interventions. Anim. Welf. 22, 369–378.

Gottschalk, J., Einspanier, A., Ungemach, F.R., Abraham, G., 2011. Influence of topical dexamethasone applications on insulin, glucose, thyroid hormone and cortisol levels in dogs. Res. Vet. Sci. 90, 491–497.

Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdeb, ck.M., Jansson, A.U., s-Moberg, K., 2011. Short-term interaction between dogs and their owners: effects on oxytocin, cortisol, insulin and heart rate an exploratory study. Anthrozoos Multidiscip. J. Interact. People Anim. 24, 301–315.

Hansen, S.W., Jeppesen, L.L., 2006. Temperament, stereotypies and anticipatory behaviour as measures of welfare in mink. Appl. Anim. Behav. Sci. 99, 172–182.

Haubenhofer, D.K., Kirchengast, S., 2006. Physiological arousal for companion dogs working with their owners in animal-assisted activities and animal-assisted therapy. J. Appl. Anim. Welf. Sci. 9, 165–172.

Haubenhofer, D.K., Kirchengast, S., 2007. Dog handlers' and dogs' emotional and cortisol secretion responses associated with animal-assisted therapy sessions. Soc. Anim. 15, 127–150.

Haug, L.I., 2008. Canine aggression toward unfamiliar people and dogs. Vet. Clin. North Am. Small Anim. Pract. 38, 1023–1041.

Hekman, J.P., 2012. Salivary cortisol concentrations and behavior in a population of healthy dogs hospitalized for elective procedures. Appl. Anim. Behav. Sci. 141, 149–157.

Hennessy, M.B., 1997. Hypothalamic-pituitary-adrenal responses to brief social separation. Neurosci. Biobehav. Rev. 21, 11–29.

Hennessy, M.B., Voith, V.L., Hawke, J.L., Young, T.L., Centrone, J., McDowell, A.L., Linden, F., Davenport, G.M., 2002. Effects of a program of human interaction and alterations in diet composition on activity of the hypothalamic-pituitary-adrenal axis in dogs housed in a public animal shelter. J. Am. Vet. Med. Assoc. 221, 65–71.

Henry, J., Stephens, P., 1977. Stress, Health, And The Social Environment: A Sociobiologic Approach To Medicine. Springer-Verlag, New York.

Hiby, E.F., Rooney, N.J., Bradshaw, J.W., 2006. Behavioural and physiological responses of dogs entering re-homing kennels. Physiol. Behav. 89, 385–391.

Iannuzzi, D., Rowan, A.N., 1991. Ethical issues in animal-assisted therapy programs. Anthrozoos 4, 154.

Jones, A.C., Gosling, S.D., 2005. Temperament and personality in dogs (*Canis familiaris*): a review and evaluation of past research. Appl. Anim. Behav. Sci. 95, 1–53.

Karatsoreos, I.N., McEwen, B.S., 2011. Psychobiological allostasis: resistance, resilience and vulnerability. Trends Cogn. Sci. 15, 576–584.

King, C., Watters, J., Mungre, S., 2011. Effect of a time-out session with working animal-assisted therapy dogs. J. Vet. Behav. Clin. Appl. Res. 6, 232–238.

Kirschbaum, C., Hellhammer, D., 1989. Response variability of salivary cortisol under psychological stimulation. J. Clin. Chem. Clin. Biochem. 27, 237.

Kruger, K.A., Serpell, J.A., 2010. Animal-assisted interventions in mental health: definitions and theoretical foundations. In: Fine, A. (Ed.), Handbook on Animal-Assisted Therapy: Theoretical Foundations and Guidelines for Practice. Academic Press, San Diego, CA, pp. 33–48.

Kuhne, F., Hößler, J.C., Struwe, R., 2012. Effects of human–dog familiarity on dogs' behavioural responses to petting. Appl. Anim. Behav. Sci. 142, 176–181.

Mariti, C., Gazzano, A., Moore, J.L., Baragli, P., Chelli, L., Sighieri, C., 2012. Perception of dogs' stress by their owners. J. Vet. Behav. Clin. Appl. Res. 7, 213–219.

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.

Pagani, M., Rimoldi, O., Pizzinelli, P., Furlan, R., Crivellaro, W., Liberati, D., Cerutti, S., Malliani, A., 1991. Assessment of the neural control of the circulation during psychological stress. J. Auton. Nerv. Syst. 35, 33–41.

Palley, L.S., O'Rourke, P.P., Niemi, S.M., 2010. Mainstreaming animal-assisted therapy. ILAR J. 51, 199–207.

Piva, E., Liverani, V., Accorsi, P.A., Sarli, G., Gandini, G., 2008. Welfare in a shelter dog rehomed with Alzheimer patients. J. Vet. Behav. Clin. Appl. Res. 3, 87–94.

Rooney, N.J., Gaines, S.A., Bradshaw, J.W., 2007. Behavioural and glucocorticoid responses of dogs (*Canis familiaris*) to kennelling: investigating mitigation of stress by prior habituation. Physiol. Behav. 92, 847–854.

Schwizgebel, D., 1982. Zusammenhänge zwischen dem Verhalten des deutschen Schäferhundes im Hinblick auf tiergerechte Ausbildung (Relationship of behavior of the German Shepherd in regards to appropriate training). Aktuel. Arbeit. Artgemass. Tierh., 138–148.

Tanaka, K., Tanaka, H., Kanemoto, Y., Tsuboi, I., 1998. The effects of nonsteroidal anti-inflammatory drugs on immune functions of human peripheral blood mononuclear cells. Immunopharmacology 40, 209–217.

Tuber, D.S., Sanders, S., Hennessy, M.B., Miller, J.A., 1996. Behavioral and glucocorticoid responses of adult domestic dogs (*Canis familiaris*) to companionship and social separation. J. Comp. Psychol. 110, 103–108.

van Vonderen, I.K., Kooistra, H.S., Rijnberk, A., 1998. Influence of veterinary care on the urinary corticoid: creatinine ratio in dogs. J. Vet. Intern. Med. 12, 431–435.

Vial, G.C., Stabenfeldt, G.H., Franti, C.E., Ling, G.V., 1979. Influence of environment on adrenal cortical response to ACTH stimulation in clinically normal dogs. Am. J. Vet. Res. 40, 919–921.

Viau, R., Arsenault-Lapierre, G., Fecteau, S., Champagne, N., Walker, C.D., Lupien, S., 2010. Effect of service dogs on salivary cortisol secretion in autistic children. Psychoneuroendocrinology 35, 1187–1193.

Vincent, I.C., Michell, A.R., 1992. Comparison of cortisol concentrations in saliva and plasma of dogs. Res. Vet. Sci. 53, 342–345.

von Holst, D., 1998. The concept of stress and its relevance for animal behavior. In: Slater, P., Møller, A., Manfred, M. (Eds.), Advances in the Study of Behavior. Academic Press, Waltham, MA, pp. 1–131.

Zorawski, M., Killcross, S., 2002. Posttraining glucocorticoid receptor agonist enhances memory in appetitive and aversive Pavlovian discrete-cue conditioning paradigms. Neurobiol. Learn. Mem. 78, 458–464.

Zorawski, M., Killcross, S., 2003. Glucocorticoid receptor agonist enhances Pavlovian appetitive conditioning but disrupts outcome-specific associations. Behav. Neurosci. 117, 1453–1457.