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

Zenithson Y. Ng

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
in
Biomedical and Veterinary Sciences

Bess J. Pierce, Chair
Cynthia M. Otto
Virginia A. Buechner-Maxwell

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

Zenithson Y. Ng

ABSTRACT

Background: The effect of animal-assisted activities (AAA) on the animal participants has been minimally investigated and the welfare of these animals has been questioned. Cortisol, in conjunction with stress-associated behavior, has been utilized as an objective assessment of animal welfare.

Objective: Salivary cortisol and behavior in AAA dogs were measured to test the null hypothesis that salivary cortisol concentration and behavior are not different in an AAA environment compared to home or neutral environments. Hair cortisol was measured in AAA dogs to test the null hypothesis that there is no relationship between hair cortisol and salivary cortisol.

Methods: Fifteen healthy adult dogs registered with an AAA organization were recruited. A hair sample was collected from each dog upon enrollment. Saliva samples were collected from each dog every 30 minutes, starting 30 minutes prior to and 30 minutes after a standardized 60 minute session across 3 settings: an AAA session (AS) for college students in the communal area of a residence hall; a neutral session (NS) located in a novel room without interaction with a stranger; and a home session (HS). Each session was videotaped continuously and behaviors were coded at three separate 5-minute intervals while the dog was petted by a stranger in the AS or handler in the NS and HS.

Results: Salivary cortisol levels were not different in the AS compared to HS, but were significantly higher in the NS compared to AS and HS. Dogs exhibited significantly more standing and ambulating behavior in the AS compared to HS. Salivary cortisol level was negatively correlated with panting and standing at specific time points in the

NS and AS, respectively. Hair cortisol level did not correlate with salivary cortisol level at any time point in any of the settings.

Conclusions: During a 60 minute AAA session, salivary cortisol concentration and stress-associated behavior were not different compared to when dogs spent the same amount of time in the home setting, suggesting that they were not stressed when being used as AAA animals. The physical environment may be an important consideration when evaluating the effect of AAA on dogs. Hair cortisol did not correlate with salivary cortisol, suggesting that hair may not be a representative predictor of cortisol in these environments. Additional investigation is required to support cortisol and behavior as measures of stress and welfare in AAA animals.

DEDICATION

I would like to thank my committee members for their essential roles in this project: Dr. Cindy Otto and Dr. Virginia Buechner-Maxwell. Their time, knowledge, and support were invaluable. In particular, I would like to thank my committee chair, Dr. Bess Pierce, for her support and guidance throughout my residency.

I would also like to acknowledge my friends and family, particularly my parents and grandfather, who have always provided me unconditional support throughout my endeavors.

This thesis is dedicated to Grace, who has served as the essence of the human-animal bond in my life, and all the animals and handlers who continue to make a difference in people's lives.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

HAI	Human-animal interaction
AAA	Animal-assisted activity
AAT	Animal-assisted therapy
TDI	Therapy Dogs International
AS	Animal-assisted activity session
NS	Neutral session
HS	Home session
HPA	Hypothalamic-pituitary axis
ACTH	Adrenocorticotrophic Hormone
CRH	Corticotropin releasing hormone
HAC	Hyperadrenocorticism
SCS	Salimetrics Children's swab

CHAPTER 1: LITERATURE REVIEW

A. **Human-animal interactions**

The existence of human-animal interactions or human-animal interventions (HAI) is based upon the notion that animals bring about good feelings in people. This has been founded on the principle of the human-animal bond, which is defined by the American Veterinary Medical Association as the "mutually beneficial relationship between humans and animals that is essential to the wellbeing of both" (AVMA, 1998). HAI is traditionally perceived as an activity in which an animal engages with a human to promote a wide range of physical, mental, and social benefits for the person. HAI services have been gaining significant recognition as organized programs are becoming more widespread nationally and internationally (Palley et al., 2010). The term HAI can be employed as an umbrella term to encompass animal-assisted therapy (AAT) and animal-assisted activity (AAA).

Animal-assisted therapy (AAT) is a goal-directed therapeutic intervention conducted by a human health professional, such as a social worker, psychologist, physical therapist, or nurse (DeltaSociety, 2012). This specialist utilizes an animal that meets specific criteria to reach certain goals within the scope of his or her professional expertise (Kruger and Serpell, 2010). AAT occurs in a variety of settings and is commonly implemented to assist in improving physical, social, emotional, or cognitive function in people with disabilities (DeltaSociety, 2012). Specific therapeutic endpoints are stated and progress is charted in an organized and regimented fashion. An example of AAT is a physical therapist using a dog trained to walk with and support a paraparetic individual learning to walk. The therapist designs this treatment intervention, dictates the goals of each session, frequency of sessions, and monitors the progress of each session.

Whereas AAT is an intervention guided by a human health professional to meet a specific goal, animal-assisted activity (AAA) is an intervention guided more frequently by a volunteer without professional training to broadly enhance quality of life (Kruger and Serpell, 2010). Although AAA implements a handler and approved AAA animal, it is less formal than AAT because the intervention is usually social and recreational in nature, as it is not directed to meet a specific goal (DeltaSociety, 2012). A traditional

example of an AAA is a dog and handler doing a meet and greet visit with residents and staff at a nursing home. Although general guidelines for visitations are dictated by the specific organization, the handler typically operates independently and uses his or her discretion to determine when a visit is scheduled and how long the visit lasts since it is a volunteer activity.

When discussing HAI, the conventional terminology of "therapy" dogs should be clarified to avoid confusion. An activity in which an animal interacts with a human that results in pleasurable feelings cannot simply be termed as "therapy" (Beck and Katcher, 1984). It is common to label animals that meet and greet people in various situations as "therapy" dogs when they should be more appropriately identified as AAA dogs. This misnomer likely occurs because the term "therapy dog" is universally comprehensible to the general public. There are at least 12 different terms for HAI that are used interchangeably and inconsistently in the scientific literature (Kruger and Serpell, 2010; Evans and Gray, 2012). To avoid perpetually confusing these terms, they should be distinguished and used appropriately in the scientific literature.

AAT/AAA animals are commonly required to go through a training and approval process to ensure that they obey specific commands, are friendly towards a variety of people, and do not exhibit behavioral signs of stress during the HAI. Most AAT/AAA organizations require the animal to pass a behavioral evaluation by a certified evaluator and to pass a physical exam by a licensed veterinarian (DeltaSociety, 2012). However, protocols and procedures vary between organizations because universal guidelines do not exist at this time.

B. Benefits of human-animal interaction for animals

An essential feature that characterizes the human-animal bond is that the relationship is mutually beneficial (AVMA, 1998). Much research has focused on the mental, physical, and social benefits of animals for humans (Friedmann and Son, 2009). In contrast, there is a limited amount of research to document benefits for the animal in HAI. To ensure that the relationship is beneficial to both participants, attention should be focused on the outcomes of both the human and animal (Grandgeorge and Hausberger, 2011). People working within the AAA/AAT field may have a personal and biased

perception that animals enjoy the interactions as much as the human participants do. AAA/AAT handlers in one study frequently used words such as satisfied, relaxed, and in a happy mood to describe the state of their dogs after a session (Haubenhofer and Kirchengast, 2007). However, the subjective assessment of owners is mere anthropomorphism, and objective criteria are necessary to understand the effects of HAI on animals.

Social benefits

Domestic animals may benefit from HAI because it provides opportunity for socialization, which is a necessary component of good welfare (Odendaal, 2005). Dogs, in particular, are social animals that naturally desire social contact with others of the same species (conspecifics), and have transferred this desire to humans with domestication (Wells, 2004). It has been suggested that human contact may be even more important than contact with another dog (Wolfe, 1990). Dogs have been shown to be more attentive to humans than conspecifics (Range et al., 2009). One study demonstrated that dogs preferred human contact over conspecific contact, as they solicited social interaction from and spent more time in proximity to the human rather than the kennel mate (Tuber et al., 1996).

Therefore, dogs are capable of forming close attachments to humans (Gacsi et al., 2001), which can also escalate to excessive attachments. They have repeatedly exhibited an abnormally exaggerated stress response to being separated from human attachment figures (Horvath et al., 2007; Sherman and Mills, 2008). Social isolation has been proven to be the most stressful factor in a kennel environment for dogs (Coppinger and Zuccotti, 1999). In shelter settings, where HAI programs are often implemented to provide environmental and social enrichment, human contact has been regarded as a pleasurable activity for them (Coppola et al., 2006).

Physiologic benefits

Human contact has been shown to influence multiple physiologic outcomes that can be perceived as beneficial to the animal. The interaction between human and animal consists of various forms of non-noxious sensory stimulation including touch, light pressure, warmth, and stroking as well as olfactory, auditory, and visual cues (Handlin et al., 2011). These activities have been proposed to decrease activation of the

3

hypothalamic-pituitary axis (HPA) and sympathetic and parasympathetic nervous systems (Handlin et al., 2011). For example, one study showed that a person stroking an anesthetized rat's abdomen continuously for five minutes resulted in a sedative effect that decreases in heart rate and blood pressure for several hours. These positive changes in cardiovascular parameters were implicated in increasing pain threshold and decreasing energy expenditure (Kurosawa et al., 1995; Lund et al., 1999), illustrating the indirect physiologic benefit of human contact.

Similar cardiovascular effects have been observed in dogs after human interaction. A laboratory dog's heart rate elevated significantly when a person entered an experimental room without interacting with the dog, while the same dog's heart rate decreased significantly when the person pet the dog (Gantt et al., 1966). Other studies have found that heart rate and blood pressure decreased in owned dogs after a brief interaction with either owner or stranger (Baun et al., 1984; Vormbrock and Grossberg, 1988; Odendaal and Meintjes, 2003). These studies implicate the role of human contact in generating a state of relaxation attested by decreased cardiovascular stimulation.

Human contact may also result in positive effects on endocrine function in animals. Dogs that received positive human interaction not only experienced decreases in heart rate and blood pressure, but also significant increases in β-endorphin, prolactin, phenylethylamine, and dopamine levels (Odendaal and Meintjes, 2003). Increases in these hormones have been associated with bonding, euphoria, pleasure, and happiness (Odendaal and Meintjes, 2003), suggesting positive effects of human interaction. In addition, a prominent biomarker of stress, cortisol, has been documented to decrease in response to human contact, suggesting a beneficial role in stress moderation (Coppola et al., 2006). This will be discussed in detail in later sections.

Increased oxytocin, a hormone related to bonding, affection, and pleasurable activity (Uvnäs-Moberg, 1998), may indicate a positive response of animals to HAI. Oxytocin increases in women while nursing their children, which has been associated with bonding to the child (Uvnas-Moberg et al., 1996). Higher levels of oxytocin were observed in rats after they were stroked on the abdomen by a human (Uvnas-Moberg et al., 1996). A similar increase in oxytocin was observed in dogs after human interaction (Odendaal and Meintjes, 2003). In addition, the higher the level of oxytocin in dogs, the

more likely the owner was to perceive the dog as a positive and pleasant companion in their lives (Handlin et al., 2012). Although there are limitations to the study of oxytocin, it still provides evidence to suggest that human interaction may be beneficial to animals.

Behavioral benefits

Human interaction can also result in improved behavior for the animal. Dogs that regularly interact with humans build better social habits and are easier to control and handle. Shelter dogs receiving human interaction demonstrated improved levels of sociability and diffidence compared to dogs not receiving regular human contact (Bergamasco, 2010). Another study showed significantly improved behavior scores in shelter dogs that received two, 25 minutes sessions of gentle play with a human (Menor-Campos et al., 2011). A 60-day human social enrichment program consisting of basic training, playing activity, and affective interaction for dogs in a shelter was found to improve behavioral and sociability scores (Valsecchi et al., 2007). These types of interaction programs also cause dogs to wag their tails more when introduced to people, be more active, and engage with conspecifics more frequently (Normando et al., 2009). Maintaining these interactive behaviors significantly impacts the adoptability of dogs and is important because dogs housed in kennels for long periods of time without human interaction and positive reinforcement decrease interactive greeting behaviors such as tail wagging and moving forward (Stephen and Ledger, 2005).

Dogs may also benefit from the positive mental stimulation that behavioral training provides (Zamir, 2006). Obedience training is an essential component of preparing AAA/AAT dogs, which enhances the bond and prevents a state of idleness. Although behavioral training may be tedious and perceived as work or labor, many working dogs are internally motivated to perform because they enjoy the work (Coppinger et al., 1998).

C. Animal welfare

Although there is evidence that HAI may be beneficial for animals, there is growing concern that it may have negative consequences as well. These consequences may factor into the concern for the welfare of the animal being used. When utilizing animals for any purpose, there exists an ethical obligation to ensure that animal health

and welfare needs are met, which can often be overlooked in AAA and AAT because most of the attention is focused on the human recipient of the interaction.

Animal welfare can be appropriately described as the "animal's attempt to cope with its environment at a physiological, behavioral, and medical level" (Broom, 1996). Welfare is often graded subjectively on a scale from very good to very poor (Haverbeke et al., 2008). However, grading welfare in animals can be notoriously problematic and complex because it is usually based on an observer's subjective evaluation of the animal's wellbeing (Hetts, 1992; Hiby et al., 2006). To minimize the variation in interpretation, it is important to utilize objective assessments of welfare that can be standardized across research settings (Fraser and Broom, 1997).

When utilizing animals for purposes of providing a service for humans, such as HAI, there is an ethical obligation for these animals to achieve "very good" welfare status. Good welfare and well-being can be characterized as a state in which an animal is free from distress most of the time, is in good physical health, exhibits a substantial range of the species-typical behaviors, and is able to deal effectively with environmental stimuli (Novak, 1989; Hetts, 1992).

The Farm Animal Welfare Council has proposed that five basic freedoms be assessed to determine animal welfare: Freedom from thirst, hunger and malnutrition; Freedom from discomfort; Freedom from pain, injury and disease; Freedom from fear and distress; and Freedom to express most normal behavior (FAWC, 2009). Violation of any of these freedoms can result in a poor welfare state by inciting stress.

D. Animal welfare in human-animal interactions

Although it is presumed that a mutually beneficial relationship exists for both humans and animals in HAI, few studies have investigated animal welfare aspects of HAI. As HAI programs proliferate, it is imperative that welfare be prioritized to prevent undue harm because of overlooked consequences of these activities. Harm to an animal's wellbeing as a result of HAI can falter the progress the field has worked to achieve. There are already reports in the literature commenting on the moral basis of animal-assisted therapies, suggesting that AAA and AAT exploit the animals and may be detrimental to their wellbeing (Zamir, 2006; Hatch, 2007). However, these arguments are

6

solely theories because there is minimal scientific evidence to document that AAT/AAA has truly threatened animal welfare. The following sections outline the predictions for potential threats that HAI may impose to each of the five freedoms. An underlying theme to these theories is that the handler is instrumental in preventing these risks to welfare.

Freedom from thirst, hunger, and malnutrition

This freedom requires that an animal be provided with access to fresh water and a nutritious diet appropriate for the animal's lifestyle (FAWC, 2009). An AAA/AAT animal is unlikely to suffer from thirst, hunger, or malnutrition because of the care provided by the owner. However, these basic necessities may be affected when the animal is actively engaged in an AAA/AAT session. It has been reported that handlers who are worried about dogs urinating in the facility during a session may withhold access to water, which can result in thirst and consequent dehydration (Hatch, 2007). Similarly, handlers may deny food to the animal for fear of accidental defecation in the facility, resulting in hunger. Alternatively, a handler may allow the animal to be given food during the session, which may result in the consumption of an excessive amount of treats. This excessive amount of fat and calories can lead to gastrointestinal upset and malnutrition. However, it should be noted that the handler is ultimately responsible for this aspect of animal welfare, and violations of this nature are preventable.

Freedom from discomfort

This freedom requires that an animal's environment include shelter from dangerous conditions and a comfortable resting area (FAWC, 2009). Since most AAA/AAT animals reside in the homes of handlers, this requirement is likely to be met. However, the actual AAA/AAT facilities may present certain risks that lead to discomfort. These facilities, particularly hospital environments, often subject animals to crowds of people, loud sounds, adverse smells, and unpredictable circumstances (King et al., 2011). Because the handler is in control of the animal at all times, the animal is unable to escape these conditions of its own volition (Serpell, 2010). Furthermore, standard rest and reprieve for HAI animals are not regulated and designated resting places specifically tailored for pets with water and a clean area to urinate and defecate may not be available at the facilities. Therefore, it is the handler's responsibility to

decide when and where to provide the necessary breaks for the animal before discomfort occurs.

Freedom from pain, injury, and disease

To ensure this freedom, steps should be taken to minimize the risk of disease or injury to participating animals. In addition, a plan should be in place to seek immediate medical assistance on the rare occasion that an animal becomes ill or is hurt while participating in HAI. In HAI, injury to the animal can occur due to being improperly handled by the human recipient of AAA or AAT, but these occurrences are infrequently reported (Hatch, 2007). Handlers may refrain from reporting adverse events in these situations to avoid upsetting or embarrassing the visited client, especially if the individual has special needs and the animal does not appear to have suffered severe injury. For example, one handler continued visits with an AAT dog in a study despite the dog showing signs of tentativeness towards children after it had been previously exposed to aggressive children that deliberately attempted to injure the dog (Heimlich, 2001).

AAA/AAT animals may become infected or become reservoirs for reverse zoonoses through direct or indirect contact with an infected human being. These animals commonly visit immunocompromised individuals in hospitals and nursing homes, which puts them at higher risk of acquiring multi-drug resistant staphylococci and *Clostridium dificile* (Enoch et al., 2005; Lefebvre et al., 2006; Lefebvre et al., 2009b; Gandolfi-Decristophoris et al., 2012). The handler is responsible for preventing this discomfort by avoiding visitation to individuals who are at risk of hurting animals or individuals who harbor zoonotic diseases. It is also the handler's responsibility to report and seek appropriate medical attention for the animal if any adverse event occurs.

Freedom from fear and distress

This freedom is met if the animal is free of mental suffering. Mental suffering is a difficult parameter to objectively assess, but all attempts should be made to decrease fear and stress in HAI animals. Although there are limited studies to report how well dogs tolerate this type of work, stress may be quite prevalent in AAA and AAT (Glenk et al., 2011; King et al., 2011). The presence of a human being has the potential to cause stress in animals (Jones and Josephs, 2006) and social interactions are reported to be among the most potent stressors an animal may endure (McEwen and Wingfield, 2003).

It is important to note that an animal cannot escape unwelcomed or unpleasant interactions of its own volitions because the handler commands these activities (Serpell, 2010). In addition, sleep deprivation, mental exhaustion, hectic atmosphere, inappropriate handling, and unsolicited attention, which can be prevalent in HAI, may be characteristic sources of stress for some dogs (Haubenhofer and Kirchengast, 2007). One study reported that more than 50% of handlers described therapy sessions for their dogs as straining, while over 30% of handlers described the work as stressful or physically encumbering (Haubenhofer and Kirchengast, 2006). Furthermore, handlers of dogs visiting hospice facilities reported the dogs to exhibit signs of fatigue and exhaustion after the visits (Phear, 1996).

Distress may also occur in HAI as a result of exhaustion from the work. The extensive and prolonged training involved in preparing an HAI animal has been proposed to be a violation of the animal's well-being (Zamir, 2006). This training does not guarantee that a trained animal can pass the requirements necessary to become HAI certified, and even those that do pass may still experience stress during work, especially if sessions are prolonged or environments are straining (King et al., 2011). In addition, an increase in frequency of sessions and number of clients seen was associated with a decrease in overall efficacy of the dogs (Marinelli et al., 2009). Although a single, stressful AAA or AAT session may not result in severe, long-term effects, numerous repeated sessions may.

Furthermore, the potential for animal abuse due to fatigue and burnout for HAI animals living in institutions has been described (Iannuzzi, 1991). One study also reported the diagnosis of adrenal dependent hyperadrenocorticism (HAC) in a dog after being used for AAT (Heimlich, 2001). The authors described clinical signs of exhaustion, fatigue, panting, and recurrent ear and urinary tract infections during the eight-week course of the AAT study. It was postulated that the persistent stress of AAT could have elevated stress levels, leading to elevated cortisol levels that induced a pathologic HAC (Heimlich, 2001). However, it is unlikely that HAC was due to environmental or exogenous stress since these factors have not been associated with the pathogenesis of the disease. These reports emphasize the HAI animals should be

monitored to prevent exhaustion from being overworked and consequential distress, which negatively impact animal welfare.

Freedom to express most normal behavior

This freedom requires that the animal be provided with sufficient space, proper facilities, and company of the animal's own kind (FAWC, 2009). However, this freedom is difficult to apply in HAI because the environments of AAA/AAT are unlike any environment that animal would naturally encounter. These animals did not choose to be trained for or engage in HAI out of their own volition; the owners decided this fate for them. Even if an animal achieves AAA or AAT certification, it does not necessarily mean that the animal has the desire to voluntarily participate in these activities (Serpell, 2010). HAI is unlike any other animal activity in that it requires an animal to endure intimate, unsolicited affections from a stranger for extended durations of time (Butler, 2004). Animals in these contrived circumstances must remain steady and cope with the interaction of unfamiliar people and strange settings without being able to escape of their own volition (Piva et al., 2008), which prevents expressing normal behavior.

It is also important to understand what constitutes normal behavior for the animal, which may vary between individuals. Animals may suffer if they are committed to an HAI facility that does not have an area where the animal has the freedom to exercise and play, which restricts the ability to display normal behavior. In addition, animals are not usually permitted to interact with animals of their own kind during AAA/AAT, which restricts an animal's natural desire to interact with conspecifics.

E. Use of live animals may not be necessary

There has been a continuously growing body of literature that supports the benefits of animals on physical, mental, and social health (Friedmann and Son, 2009). However, it has been argued that the use of animals in HAI may not even be necessary because of the lack of substantial evidence that AAA or AAT results in proven benefits for humans (Palley et al., 2010). Conclusions about the beneficial effects of animals on people should be interpreted with caution because of the potential problems in methodology (Wilson, 2003). Much HAI research has been qualitative and descriptive in design (Chur-Hansen et al., 2010). In addition, sample sizes are often too small for

proper statistical analyses and many of the studies utilize voluntary participation from convenience samples of a specific demographic, which introduces significant bias as compared to random sampling (Koivusilta and Ojanlatva, 2006). For example, the benefits of AAT may depend on the personality type of the individual visited or simply, whether or not the individual likes animals at all (Colby, 2002).

Other studies reveal that AAT programs may not only have no effect, but even worse, negative effects on human recipients (Wells, 2009). Human health may be at risk from interaction with animals because of infectious diseases acquired from pets as well as allergies to pets (Baxter and Leck, 1984). Allergies to pets can result in symptoms of rashes, hay fever, diarrhea and asthma, all of which can affect wellbeing (Guay, 2001). In addition, it has been reported that dogs and cats have the potential to be an environmental hazard by causing fall-related injuries in elderly people (Kurrle et al., 2004). Other traumatic incidents such as bites and scratches from animals not only result in physical harm to the person, but may also induce a fear of animals (Thompson, 1997; Voith, 2009).

If HAI is not truly effective or beneficial for humans and potential threats to animal welfare exist, some argue if there is justification for the use of animals for this purpose (McEwen, 1998; Zamir, 2006; Hatch, 2007; Marino, 2012). Perhaps there are other alternatives to AAT and AAA that achieve the same results without the use of a live animal. For example, a robotic dog used in AAA resulted in the same reduction in loneliness as a live dog used in AAA (Banks et al., 2008). Therefore, the use of non-living substitutes for these endeavors is a simple solution to ensuring that these activities present no risk or consequence to animal welfare.

F. Current human-animal interaction recommendations

The field of HAI lacks formal universal guidelines on the ethics of human-animal relationships (Antonites and Odendaal, 2004). It is presumed that most HAI organizations emphasize that animal welfare be protected and enhanced where possible (Preziosi, 1997; Santori, 2011). However, it is understood that specific guidelines are difficult to formulate because research has not identified the precise criteria necessary to guarantee welfare. Consequently, numerous AAA and AAT organizations in existence

each set their own guidelines and regulations, making standardization of the field difficult. It has been suggested that institutional animal care and use committees be developed to review the protocols for AAA and AAT (Palley et al., 2010).

Selection

The process of selecting HAI animals should be meticulous and comprehensive to ensure animals chosen are highly adaptable and predictable in all environments (Verga and Michelazzi, 2009). Most animals in AAA or AAT programs are required to pass a regimented temperament test conducted by a certified evaluator. However, exact methods of testing vary between organizations. The AAA behavior test in dogs often constitutes a variety of tasks including commands to sit, down, stay, come; walk on a loose lead; walk among a crowd; greet a stranger; and react to distractions, medical equipment, and surprising circumstances (DeltaSociety, 2012). There is no consensus as to how often these tests should be assessed, as some organizations only require one behavioral examination for the entire career, whereas others require recertification every 2 years. However, many require re-evaluation if any adverse behaviors are observed (Lefebvre et al., 2008). It is recommended for evaluators to assess if the dog demonstrates subtle stress behaviors, but it is difficult to determine how often and how stringently these behaviors are monitored. Some subtle behaviors such as panting and lip licking may go unnoticed by the evaluator. This may be important because the presence of panting and lip licking during a guide dog distraction test was positively correlated with guide dog failure (Tomkins et al., 2011).   However, there is no consensus for the selection of AAA/AAT animals based on stress-associated behavior if the animal successfully completes all other objectives of the assessment. For example, a well-trained dog may pass behavioral evaluation even though it pants and lip licks throughout the test.

Visits

A consistent guideline for animal welfare in HAI is that animals should not be forced to visit if the animal is reluctant to perform or does not enjoy sessions (Preziosi, 1997; DeltaSociety, 2012). However, this is a challenging guideline to adhere to because it is based on the subjective assessment of the handler. General recommendations warrant the animal be monitored for signs of fatigue, stress, thirst, overheating, or urges

to urinate or defecate (Lefebvre et al., 2008). More objective signs of stress including body stiffness, lowered tail, whining or increased panting, should lead to removal of the animal from this situation (Serpell, 2010). If a short and quiet break will not ease the animal's signs, then terminating the visit is warranted (Preziosi, 1997; Lefebvre et al., 2008).

It is also recommended that dogs should only visit settings that are comfortable for them, and that the session end prior to, rather than after, the dogs exhibit signs of stress (Butler, 2004). However, it is difficult for a handler to predict when the animal will demonstrate stress-associated behavior. Therefore, an AAA/AAT session should be limited to one hour or less (Iannuzzi, 1991) to reduce the risk of adverse events associated with fatigue (Lefebvre et al., 2008; Marcus, 2012). Although a duration of one-hour is given as a rough guideline, it does not account for the nature of the session, number of people visited, the environment, and activities performed. In addition, this arbitrary time standard has not been scientifically tested to guarantee prevention of stress in the animal.

Handler error

It is largely the responsibility of the handler to ensure that animal welfare be guaranteed. Many organizations require handlers to be educated in basics of HAI and the logistics of how to conduct a session. However, most handlers do not have advanced animal behavior backgrounds and may lack sufficient knowledge of stress-associated behavior, resulting in failure to read or misread specific behavioral cues (Fejsáková et al., 2009). Very few owners are able to recognize and interpret subtle behavioral cues of stress (Mariti et al., 2012). Humans often times may not recognize subtle signs of discomfort in a dog, such as yawning or lip-licking, which, if left unnoticed, may lead to consequences to the health and wellbeing of the animal (Fureix et al., 2010). Therein lies the potential for mistreatment or mishandling of the animal in HAI (Hatch, 2007).

Because it is the handler that dictates the sessions and is the one ultimately responsible for the animal participating in HAI, the handler has his or her own motivations and goals for engaging in these activities, which may not coincide with the motivation or enthusiasm of the animal for these activities. Handlers have the potential for using animals simply as tools rather than living entities with welfare needs. Some

handlers may choose to increase the duration, frequency, or intensity of AAT and AAA sessions because the human participants enjoy it, ignoring potential signs of stress in their animal partners. A handler may intentionally or unintentionally ignore signs of stress in his or her dog because the handler can work more and doesn't need a break. This underscores the need for handlers to be educated about animal welfare and stress in HAI. For appropriate education to occur, additional research is needed to define welfare, stress, and fatigue in HAI.

G. Stress

Stress can be broadly defined as the individual's characteristic behavioral and physiological response to a change in the individual's homeostatic status (Moberg, 2000; Haubenhofer and Kirchengast, 2007). Stress results from long-term physiological arousal without relief from negative stimuli (Chrousos and Gold, 1992; Beerda et al., 1997). It usually carries a negative connotation because it denotes a loss of control and a reduced predictability of what will happen (Henry and Stephens, 1977). This behavioral and physiological state is often associated with fear and anxiety; where fear is defined as an emotional response to a potentially harmful factor, while anxiety is the emotional response to something that can predict the onset of the harmful factor (Casey, 2004).

It is common for animal welfare to be described in terms of stress, with high stress levels correlating with poor welfare and low stress levels correlating with good welfare (Veissier and Boissy, 2007). Stress may occur when one of the five freedoms of animal welfare is violated.

Effects of stress

Stress can be considered a crude indicator of animal welfare because it may negatively impact the health and lifespan of dogs, resulting in a diminished quality of life (Dreschel, 2010). Stress does this by impacting multiple body systems, negatively altering the animal's physical and mental states. In humans, stress has been associated with mental health disorders, hypertension, cardiovascular disease, obesity, skin conditions, ulcerative colitis, and decreased fertility (Russell et al., 2012). Similarly, animals that experience excessive stress are thought to be more at risk of physiological dangers affecting the immune, cardiovascular, gastrointestinal, and neuroendocrine

systems (Chrousos and Gold, 1992; Beerda et al., 1997). Stress can negatively affect immune function, making the individual at increased risk of disease and illness (Clark et al., 1997; McEwen, 1998). Stress can also can result in hypertension and cardiac abnormalities (Koolhaas et al., 1997), gastrointestinal ulceration, and decreased reproductive success (Liptrap, 1993).

Stress can significantly impact mental health, the nervous system, and behavior. Protracted activation of the HPA system has certain severe detrimental consequences for brain and behavior (Sapolsky, 1996).   In humans, stress can lead to psychological suffering and be the underlying factor of major depression with melancholic features (Gold et al., 1988; Hiby et al., 2006). Stress may cause behavioral pathology resulting in stereotypies, apathy, self-mutilation, and learned helplessness as behavioral attempts to cope with stress (Broom, 1999).

Measuring stress

Objective measures of animal welfare have been attempted by measuring outcomes of various physiologic parameters and behavioral signs of stress. However, the value of using physiologic and behavioral measures as appropriate measures of welfare status is still questionable because of the inconsistencies in studies (Dawkins, 2006). Many studies have utilized a variety of tools to measure stress, such as heart rate, heart rate variability, blood pressure, cortisol, catecholamines, IgA, and behavior, but there is no consensus as to which is the single best test (Mostl and Palme, 2002). Rather, it is likely that a combination of various parameters will help to elucidate a more complete understanding of stress and welfare in animals (Hiby et al., 2006).

H. Cortisol

Cortisol is the primary glucocorticosteroid secreted in dogs and is recognized as the major physiologic indicator of response to stress in dogs (Vincent and Michell, 1992; Hennessy et al., 1997; Beerda et al., 1999b; Hennessy et al., 2002a). Cortisol plays a large role in physiologic function. In the neurologic system, it moderates learning, emotion, and memory. In the metabolic system, cortisol regulates the storage and utilization of glucose. As a participant in immune function, it regulates the duration and magnitude of inflammatory response within the body (Sapolsky et al., 2000).

Cortisol is the most commonly used hormone to detect poor welfare (Coppola et al., 2006; Verga and Michelazzi, 2009).   The initiation of cortisol release into the bloodstream begins with the secretion of corticotropin releasing hormone (CRH) from the neurons of the paraventricular nucleus of the hypothalamus. CRH then travels through the hypophyseal portal circulation to reach the anterior pituitary, which is stimulated to secrete adrenocorticotrophic hormone (ACTH) into the general circulation. When ACTH reaches the adrenal glands, it binds to the ACTH receptors of the adrenal cortex, resulting in secretion of cortisol from the zona fasciculata. When cortisol levels increase above a certain threshold, negative feedback control causes inhibition of CRH release and subsequent decrease in ACTH and cortisol secretion (Cook, 2002). This sequence of hormone secretion is commonly known as the hypothalamo-pituitary-adrenal (HPA) axis (Beerda et al., 1997).

Stimulation of cortisol

Cortisol release is activated by a variety of mental and physical stimuli. Extraordinary situations, activities, and emotions can elevate circulating cortisol concentrations (Beerda et al., 1998). For example, 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). The increase in cortisol could be the effect of a stimulating new environment or transport to the new environment (Bergeron et al., 2002). Shelter environments are sources of stress because of restricted mobility, isolation, changes in light-dark cycles, overcrowding, and unpredictable events, noises, and new surroundings (Hennessy et al., 1997). In addition, shelters often restrict dogs socially and spatially, which is stressful and significantly increases cortisol levels (Beerda et al., 1999a). Another environment which commonly incites the stress response is the veterinary hospital, as salivary cortisol levels in dogs during visits to a veterinary hospital were significantly higher compared to levels when in the home setting (van Vonderen et al., 1998). Cortisol levels also increase in response to various visual and auditory stimuli (Haverbeke et al., 2008). Specific startling and novel objects, such as an opened umbrella caused an increase in cortisol (King et al., 2003). Cortisol also increases significantly in dogs with fear response associated with exposure to loud noise, as it rises

after being exposed to the sound of a gunshot (Hydbring-Sandberg et al., 2004), sound blasts (Beerda et al., 1998), and thunder in thunderstorm-phobic dogs (Dreschel and Granger, 2005). A human approaching a dog in a threatening manner also causes the dog's cortisol level to significantly increase (Horvath et al., 2007).

Although a single stressor can cause an acute rise in cortisol, it is unclear if a persistent stressor causes cortisol levels to remain at the same level, increase, or decrease with time (Beerda et al., 1997). An isolated event, such as aggressive handling, may be frightening or stressful, but it has no long term negative impact on a healthy animal that is able to recover from this without consequence (Young, 2003). However, it has been proposed that increases in cortisol in response to acute stressors and can lead to long-term neurochemical changes (Koolhaas et al., 1997). The effect of chronic stress on cortisol levels and how long the effects last in the body are less well understood (Dreschel, 2010).

Cortisol levels are twice as high in chronically stressed dogs compared to dogs without chronic stress (Hennessy et al., 2002a). Chronic stress may induce the adrenal cortex to secrete large amounts of cortisol when challenged when compared to individuals without chronic stress (Beerda et al., 1999b). When chronically stressed animals were subjected to an acute unfamiliar stressor, they showed a stronger cortisol response to the challenge than non-stressed animals (Johnson et al., 1992; Janssens et al., 1995). This study suggested that chronic stress may increase the sensitivity of the adrenals to ACTH, which may result in a cortisol response that is higher than normal when exposed to an acute stressor. In contrast, other studies have suggested that chronic stress may decrease the sensitivity of the pituitary or adrenal gland to ACTH by increasing the negative feedback of cortisol on the HPA (Mastorakos et al., 2005). Therefore, chronic stress may lead to HPA hypoactivity rather than hyperactivity under certain conditions (Miller et al., 2007). These changes can account for the finding that cortisol levels are often reduced in humans suffering from "burnout" and other conditions involving chronic stress (Pruessner et al., 1999; Heim et al., 2000). The variability in direction of the HPA response due to chronic stress may depend on factors such as sex, species, or ontogenetic aspects (Ladewig and Smidt, 1989).

Since HPA activation is non-specific the type of change in homeostasis, it is difficult to determine whether a rise in cortisol level associated with positive and negative

emotions (Zorawski and Killcross, 2002; 2003; Boissy et al., 2007). Although cortisol is secreted in response to negative events, it can also be secreted in response to situations that are not inherently regarded as stressful, such as courtship, copulation, and hunting (Broom and Johnson, 1993; Handlin et al., 2011). Cortisol response varies because it depends on a combination of the individual's perception of the stimulus and 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).

After exposure to a stress event, researchers have reported a 15-20 minute delay in cortisol levels rising in circulation (Handlin et al., 2011). One study found that a stress event resulted in a peak level of cortisol approximately 11 minutes later (Engeland and Gann, 1989). However, this study measured cortisol at specific intervals of baseline and at 15 minutes instead of utilizing continuous sampling, so it is possible that significant changes in cortisol could have occurred earlier. In a study of 6 healthy dogs, cortisol remained significantly elevated for at least 15 minutes after a stressful exposure to a vacuum (Vincent and Michell, 1992). In a study of 10 Labrador retrievers, it took 15 to 30 minutes to observe a rise in plasma cortisol level in response to a positive three minute interaction with owners (Handlin et al., 2011). These studies demonstrate the time delay for cortisol to rise after a stressful event.

After exposure to a stress event that causes a significant rise in cortisol levels, there is a relatively slow fall of cortisol back to baseline levels. Although the half-life of cortisol is between 70 and 110 minutes (Weitzman et al., 1971), cortisol returns to baseline levels after 30 minutes (Engeland and Gann, 1989) to 40 minutes (Vincent and Michell, 1992; Dickerson and Kemeny, 2004).

Factors that influence cortisol level

Research has revealed high individual variability in baseline cortisol levels (Kirschbaum and Hellhammer, 1989). Most studies reveal no differences in basal levels of cortisol between males and females (Reimers et al., 1984; Reimers et al., 1990; Hennessy et al., 1997) and one report found no significant differences between sex in neutered dogs (Dreschel and Granger, 2005). However, in stress situations, it has been reported that intact female dogs have higher cortisol levels than intact male dogs (Garnier

et al., 1990). It has been suggested that this sex difference in humans is due to androgen-inhibited and estrogen-enhanced oxytocin effects (Taylor et al., 2000), but this explanation has yet to be investigated in dogs.

Age may also influence baseline cortisol levels, as adult dogs have higher cortisol levels than juvenile dogs (Hennessy et al., 1998). Another study found increased cortisol levels in older dogs compared to younger dogs in response to a social stressor (Horvath et al., 2007). Smaller breeds tend to have higher basal cortisol than larger breeds (Reimers et al., 1984; Reimers et al., 1990). However, other studies have found no association between age, breed, weight, or neuter status on cortisol levels (Coppola et al., 2006; Stephen and Ledger, 2006; Bennett and Hayssen, 2010; Pastore et al., 2011).

The time of day may or may not influence cortisol secretion in dogs. According to one study, cortisol peaks 1 hour after waking up and decreases to reach minimum levels in the late evening (Ojeda and Griffin, 1996). In particular, plasma cortisol levels increased slightly throughout the day, reaching maximum levels between 16:00 h and 18:00 h in a population of clinically healthy dogs (Castillo et al., 2009). Plasma cortisol was 41% higher in the morning than in the evening (Handlin et al., 2011). Although baseline cortisol levels were higher in the morning than afternoon, the time of day did not affect the cortisol response to a stressor (Horvath et al., 2007).

In contrast, other studies have found no circadian rhythm in cortisol release in dogs (Koyama et al., 2003; Wenger-Riggenbach et al., 2010). No circadian pattern was apparent in a study that measured cortisol levels every 20 minutes for 25 hours in beagle dogs (Kemppainen and Sartin, 1984). Similarly, no circadian rhythm was found in dogs for a duration of 28 hours (Takahashi et al., 1981). Another study investigating the effect of age on circadian rhythm of serum cortisol found the presence of circadian rhythm in three-year old adult beagle dogs, but absence of one in geriatric dogs and puppies of the same breed (Palazzolo and Quadri, 1987). It has been argued that ACTH secretion in the dog, which regulates cortisol production, is sporadic and does not follow a particular rhythmic pattern (Siniscalchi et al., 2012). Cortisol is secreted in periodic bursts with frequencies anywhere in the range of 3 to 90 minutes (Benton and Yates, 1990). Interestingly, there is minimal variation in levels of cortisol between different days of the week in dogs, for even changes in weather do not influence cortisol levels (Kobelt et al.,

2003).

Cortisol and human-animal interaction

Human interaction may play a role in moderating stress in animals, resulting in decreased stress and cortisol levels. Contact with humans can moderate or prevent both HPA axis activation and the autonomic response to acute stressors in different species of animals (Hennessy et al., 1998). For example, cortisol level decreased in anesthetized rats in response to gentle massage by a human (Tsuchiya et al., 1991; Lund et al., 2002; Holst et al., 2005; Handlin et al., 2011).

Numerous studies have demonstrated that dogs housed in a shelter environment maintain persistently elevated cortisol concentrations and that human contact can decrease these concentrations (Tuber et al., 1996; Hennessy et al., 1997; Hennessy et al., 1998; Horvath, 2008). For example, a single 45 minute session of human contact with dogs on the second day of admission to a shelter resulted in significantly lower cortisol levels the following day compared to dogs that did not receive human contact (Coppola et al., 2006). Similarly, 25 minutes of play and human contact in shelter dogs resulted in significantly lower salivary cortisol levels than shelter dogs that did not receive human contact (Menor-Campos et al., 2011). Regular enrichment of human contact for 20 minute intervals 3-4 times a day for 7 weeks in military working dogs reduced plasma cortisol concentration (Lefebvre et al., 2009a). In fact, human interaction lowered cortisol levels in shelter dogs for extended periods of time in a shelter with long lasting effects than previously believed (Hennessy et al., 2002b). Furthermore, human contact was found to be effective in decreasing cortisol levels in dogs with separation anxiety placed in a novel environment (Pettijohn et al., 1977; Tuber et al., 1996).

Although decreases in cortisol concentration have been consistently found in shelter dogs after human contact, findings in owned dogs have differed. Odendaal found that dogs experienced no change in plasma cortisol 5 to 23 minutes after interaction with humans (Odendaal and Meintjes, 2003). Another study found that cortisol increased in therapy dogs after a 1 hour therapy session, which was assumed to be due to stimulation and increased locomotor activity (King et al., 2011). Furthermore, cortisol levels increased in Labrador retrievers 15 and 30 minutes after a three-minute interaction of petting with their owners (Handlin et al., 2011). It was suggested that this increase was

due to an increase in locomotor activity stimulated by interaction with the owner, which was activity inducing, rather than stress inducing. However, moderate physical activity associated with training appears not to affect cortisol (Haubenhofer, 2005). In addition, Haubenhofer found that cortisol levels were higher on the days of therapy compared to days that the dogs were not working and these levels were positively correlated with the frequency of visits performed (Haubenhofer and Kirchengast, 2007). This suggested that therapy work is associated with physiologic arousal, which could be attributed to positive excitement or detrimental stress in these dogs.

The characteristics of the human, relationship between the human and dog, and context in which the interaction is conducted may also influence the change in cortisol (Bergamasco, 2010). It is interesting to note that there was a larger reduction in cortisol levels when shelter dogs were stroked by females rather than males (Hennessy et al., 1998). In addition, handler behaviors associated with control, authority, or aggression increased cortisol concentrations in police dogs while play and affiliative behaviors, such as frequent praise and petting, decreased cortisol concentrations in similar dogs (Horvath, 2008).

Measurement of cortisol

Cortisol is readily measured through plasma from a blood sample. Because blood collection can be stress inducing, the study of welfare has been shifted towards more non-invasive methods of collection. Cortisol has been validated for measurement in blood, feces, urine, saliva, and hair (Beerda et al., 1996). Feces and urine may be difficult to obtain and not reflect acute stressors, and the impact of sudden peaks in stress is minimized (Hiby et al., 2006). Therefore, saliva and hair may be more practical methods of cortisol analysis.

Salivary cortisol

Salivary cortisol represents "free" cortisol, which is a direct reflection of the biologically active portion of total cortisol level (Cook et al., 1997). Because of this representation of biologically active cortisol, it has been postulated that salivary cortisol is a more accurate indicator of adrenal function (Cook et al., 1996; Cook et al., 1997).

The saliva collection procedure is not sufficiently stressful and does not cause an HPA response in dogs if sampling does not take longer than four minutes (Beerda et al.,

1998; Kobelt et al., 2003; Dreschel and Granger, 2005). If collected within four minutes, sampling saliva produces less biased results and is more accurate in assessing HPA response compared to blood sampling (Vining et al., 1983; Beerda et al., 1996).

Vincent and Mitchell (1992) reported some evidence of a delay in the increase of cortisol in saliva compared with blood. There was a one to two minute lag of salivary cortisol to meet plasma cortisol levels (Kirschbaum and Hellhammer, 1989). This study suggested that a stress event would not result in a change in salivary cortisol until 30 minutes later. However, another study of 16 dogs did not find any delay of salivary cortisol after plasma cortisol (Beerda et al., 1996). Salivary cortisol should theoretically be reflective of plasma concentration without a lag because unbound cortisol is small and highly lipid soluble, making it able to immediately pass through cell membranes into the saliva (Beerda et al., 1996). Regardless of whether or not there is a slight delay between salivary and plasma levels of cortisol, a delay still exists for plasma levels to rise after a stress event (Handlin et al., 2011).

Research has shown high correlation between plasma cortisol and salivary cortisol levels, with concentrations in saliva being approximately 10-12% of concentrations in plasma (Vining and McGinley, 1986; Vincent and Michell, 1992; Beerda et al., 1996). High levels of salivary cortisol correlating significantly with high levels of plasma cortisol (Beerda et al., 1996; Wenger-Riggenbach et al., 2010). Published cortisol values have ranged in values from 0.02-0.3 μg/dL (Bennett and Hayssen, 2010) with a mean of 0.17 μg/dL (Dreschel and Granger, 2009) in healthy dogs. Another study determined mean baseline salivary cortisol concentration to be 0.06 μg/dL in healthy dogs and 0.37 μg/dL in dogs diagnosed with HAC (Wenger-Riggenbach et al., 2010). However, a more recent study of healthy dogs hospitalized for elective procedures reported a salivary cortisol mean of 0.48 μg/dL, which was significantly higher than previous studies (Hekman, 2012). In addition, salivary cortisol levels in dogs entering shelters ranged from as low as 0.19 μg/dL to as high as 1.09 μg/dL (Belpedio et al., 2010). The variation in values can be due to differences in laboratory error, small sample sizes, assay methodology, and individual and circumstantial differences (Hekman, 2012).

Salivary cortisol is easily obtained from dogs in non-laboratory settings and in the home environment (Dreschel and Granger, 2005). Various saliva collection techniques

have been tested to obtain the largest amount of saliva without affecting accuracy of cortisol analysis (Dreschel and Granger, 2009). A rope or swab made of cotton or hydrocellulose placed in the check pouch for one to two minutes yields an adequate amount of saliva for cortisol analysis (Dreschel and Granger, 2009). While one study reported the ease of sample collection to be "easy" (Dreschel and Granger, 2009), another study disclosed the realistic difficulty of having non-science oriented individuals collect adequate samples from therapy dogs (King et al., 2011). Another study found that 27% of saliva samples taken by veterinary students, veterinary technicians, or veterinarians in their own dogs for two minutes were deemed inadequate because they did not meet the minimum requirement of 300 μL of saliva for the electrochemiluminescense immunoassay (Wenger-Riggenbach et al., 2010). However, most other radioimmunoassays or enzyme immunoassays only need 25 μL of saliva, so this study would have likely met this requirement had they used a different assay.

Certain limitations exist in obtaining cortisol values from saliva samples. Macromolecules in the saliva, pH of the saliva, and collection material can contribute to change in salivary cortisol concentration (Vincent and Michell, 1992; Granger et al., 2007; Stevens et al., 2008). Food particles in the mouth during collection may interfere or reduce the efficacy of the ELISA assay (Ligout, 2010). Therefore, fasting may minimize contamination with particles (Restituto et al., 2008). Contamination with blood in the saliva can also pose an issue, as it can occur easily with periodontal disease and significantly increase salivary cortisol levels since there is significantly higher cortisol in blood (Vining and McGinley, 1986; Vincent and Michell, 1992). When there was gross evidence of blood in the saliva sample, the cortisol concentration was two times higher than the uncontaminated sample (Wenger-Riggenbach et al., 2010). In addition, there can be large intra and inter individual variation in salivary cortisol levels (Dreschel and Granger, 2009).

Hair cortisol

Hair cortisol has been utilized as a retrospective biomarker of major life stressors in humans and primates (Davenport et al., 2006; Karlen et al., 2011) and been associated with temperament in dogs (Siniscalchi et al., 2012). Like saliva, cortisol in hair reflects the "free" or unbound fraction of steroid in circulation, and previous research has

demonstrated a significant correlation between hair and salivary cortisol in mammals (Davenport et al., 2006). Hair cortisol has been investigated as a medium to represent basal cortisol in dogs in one study, which found that hair cortisol was positively correlated with baseline salivary cortisol samples collected from the dogs every two weeks for 12 weeks (Bennett and Hayssen, 2010). Because hair cortisol is not affected by age, breed, weight, or gender, it is a plausible measure of baseline, systemic cortisol concentration (Koren et al., 2002; Bennett and Hayssen, 2010). In addition, hair cortisol has the potential to be a better measure of responses to chronic stress compared with point sampling methods such as blood and saliva, which are more sensitive to acute stressors (Accorsi et al., 2008).

The exact mechanism of cortisol deposition into hair is still unknown, but is hypothesized to accumulate from the vascular supply of the hair follicle generating the hair shaft (Meyer and Novak, 2012). There is a high degree of chemical stability of cortisol in hair, making it an accurate method of estimating cortisol secretion over time (Stalder et al., 2012).

Since human hair grows approximately one centimeter per month, it is presumed that cortisol from the portion of the shaft closest to the skin represents the cortisol level for the first month, the second centimeter for the second month, and so on in humans (LeBeau et al., 2011). This theory has been extrapolated to dogs, but Bennett and Hayssen (2010) found that cortisol concentration was uniformly consistent between proximal and distal aspects of the shaft in healthy, unstressed dogs over a 12-week period. To the author's knowledge, there are no studies that have investigated whether stressful events result in changes in cortisol concentration along the length of the same hair shaft. Since the length of hair in dogs varies between breeds, differences in length can account for differences in cortisol concentration along the hair shaft in dogs compared to humans (Russell et al., 2012).

The timing of hair sample collection has also been reported to influence cortisol concentration. One study rated behavior in a group of 14 dogs exposed to loud noises. Two weeks later, two separate hair samples were collected on the same day: one in the morning and one in the afternoon. They found that behavioral reactivity to sounds correlated with morning samples of hair better than afternoon samples of hair (Siniscalchi

et al., 2012). The authors reasoned that afternoon cortisol may be a less reliable indicator of basal stress because it is influenced by variable activities occurring throughout the day compared to morning cortisol, which should be relatively stable since the animal is in a state of sleep (Siniscalchi et al., 2012). However, since hair cortisol represents chronic levels of stress, it is unlikely that cortisol in hair would vary between 8 hours. It is more conceivable that variability of hair cortisol concentrations exists on different areas of the body within the same individual and warrants further investigation into the effects of sampling different areas and assay methodology. Furthermore, there was no significant difference in hair cortisol within 6 to 12 weeks of sampling hair from the same area from dogs in their normal home environments and regular routines (Bennett and Hayssen, 2010).

Like salivary cortisol, the collection of hair for cortisol analysis is non-invasive. It is desirable to collect approximately 250 mg of hair for cortisol analysis, but 150 mg is sufficient (Bennett and Hayssen, 2010). Hair is collected from either right or left ischiatic region because of the hair's rapid and consistent regrowth in the area (Gunaratnam and Wilkinson, 1983; Bennett and Hayssen, 2010). This area has been utilized in previous studies, as it is unknown whether cortisol is dispersed evenly throughout the hair coat. Although testosterone was found to be consistent between hair from different regions of the body, this has not been documented for cortisol in dogs (Wheeler et al., 1998).

Hair cortisol is a relatively novel biomarker that warrants further investigation because of the inconsistencies in findings regarding stress and differences in methodology (Meyer and Novak, 2012; Russell et al., 2012). It is further challenged by variables that have been shown to reduce cortisol concentration, such as washing hair with shampoo and exposure to ultraviolet light (Hamel et al., 2011; Li et al., 2012). In addition, hair color appears to influence cortisol levels, as one study demonstrated that black (eumelananin) hair has significantly lower concentrations of cortisol than yellow (pheomelanin) hair (Bennett and Hayssen, 2010).

I. Behavior

Behavior can be considered the physical manifestation of an animal's current physical and mental health state (Broom, 1991a). Abnormal behavior can be an indicator of poor animal welfare, as different behaviors are expressed along the continuum from very good to unacceptably poor welfare (Mench and Mason, 1997). Threatening environments, conditions, interactions, and events can lead to stress, and thus negative responses in animals, which are exhibited as changes in behavior (Mason, 2004). Although animals adopt strategies to cope with stressful events in their own individual ways, many behaviors are consistently exhibited among certain groups of individuals in response to specific situations (Koolhaas et al., 1999). Therefore, specific behaviors can provide insight into the animal's welfare state. Behavioral observation is a good diagnostic measure because its non-invasive nature provides further insight into the animal's wants, needs, and internal processes without disturbing its natural state (Mason, 1991).

Several behaviors have been identified to be a normal adaptive response to situations that are perceived as stressful (Verga and Michelazzi, 2009) and therefore accurate indicators of stress in the domestic dog (Beerda et al., 1998). Behaviors associated with stress have been classified by different terminology in the literature. Displacement behavior is defined as behavior that is demonstrated out of context under normal expectations (Blackwell et al., 2010). Appeasement behavior are gestures, postures, and attitudes performed for conspecifics in situations of potential conflict (Pastore et al., 2011). Rugaas coined the term "calming signals" to describe the visual communication dogs utilize to avoid conflict with one another (Rugaas, 1997). These behaviors tend to occur in situations of psychosocial stress (Maestripieri et al., 1992). Therefore, they can be utilized as non-invasive markers of stress and welfare.

While appeasement and displacement behaviors denote mild increases in transiently stressful situations, stereotypic behaviors are often associated with prolonged, poor welfare because they are often exhibited in environments that are frustrating, threatening, or lacking in stimulation (Broom, 1991b; Haverbeke et al., 2008). Stereotypy has been characterized as repetitive movements that cannot be interrupted, serve no function or purpose, and lack reasonable explanation (Dantzer, 1986; Mason,

2004). However, it has been proposed that stereotypy is associated with the release of endorphins, resulting in an addictive-like phenomenon (Cronin et al., 1985). Examples of stereotypic behavior include manipulation of the environment, circling, and pacing (Hetts, 1992; Hubrecht et al., 1992). Displacement or appeasement behavior are more appropriate terms to describe stress-associated behavior in AAA dogs compared to stereotypy because these dogs are not expected to endure such high levels of stress that elicit stereotypic behaviors.

These stress-associated behaviors have been demonstrated by dogs in response to stressful situations such as loud gun shots, doors slamming, thunderstorm stimulation, harsh training methods, and introduction to strangers (Schwizgebel, 1982; Beerda et al., 1997). Behaviors commonly exhibited include 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; Beerda et al., 1998; Beerda, 2000).

These behaviors are demonstrated in various situations when the normal state of being is disturbed. For example, lip licking and lowering of body posture may also be interpreted as signs of submission (Beerda et al., 1998). Restlessness has been observed in dogs during and after specific challenges that result in high levels of walking, nosing, and changing locomotor states (Beerda et al., 1999b). Body shaking has been proposed to rearrange the fur after it has been disturbed, but it may be more appropriately be interpreted as the release of tension (Beerda et al., 1999b). While paw lifting can be associated with anxiety, conflict, or submission, it may also be reflective of a playful state (Hiby et al., 2006).

Oral behaviors, such as lip licking, yawning, panting, and vocalization, are typically performed in a social context. Lip licking is considered a common sign of uneasiness or anxiety in a stressful social environment (Haverbeke et al., 2008). Yawning has been found to be increased in dogs with psychological tension, mild stress, or conflict (Voith et al., 1987; Hennessy et al., 1997; Beerda et al., 1998; Hennessy et al., 1998; Beerda, 2000; Haverbeke et al., 2008). Increase in panting can be due to a rise in body temperature from physiological response to arousals (Hiby et al., 2006), or associated with exhaustion (Palestrini et al., 2010). Increased vocalization has also been

27

recognized as a response to social isolation or stress (Schwizgebel, 1982; Hetts, 1992). The specific type of vocalization can also provide insight into the emotional state of the animal. Whining may be considered a call for attention whereas barking may reflect arousal (Lund and Jørgensen, 1999).

Factors that influence behavior

Behavior is influenced by many variables, including age, breed, and past experience (Hiby et al., 2006). Variations in temperament or personality between dogs of the same age, sex, and breed result in different responses to the same stimulus (Jones and Gosling, 2005; Rooney et al., 2007). In addition, the coping strategy, or the behavioral and physiologic mechanism to master a situation, varies greatly between individuals (Rooney et al., 2007). Therefore, the expression of abnormal behavior may be dependent upon individual characteristics which differ between dogs, and which may or may not be related to welfare.

Dogs experience behavioral and cognitive change as they age (Salvin et al., 2011). Younger dogs exhibit higher activity, pacing, and panting in response to stress (Head et al., 1997; Bain et al., 2001; Haverbeke et al., 2008). For example, therapy dogs younger than six years of age demonstrated more stress associated behaviors when compared to older dogs in response to a two hour therapy session (King et al., 2011). In contrast, Beerda (2000) reported that age was positively correlated with oral behaviors in response to mild stimulation, indicating that older animals displayed more stress-associated behaviors (Beerda, 2000). Still, other studies have found no association between age and behavioral activity (Wells et al., 2002).

It has been reported that female dogs demonstrate higher levels of stress behavior than male dogs (Garnier et al., 1990). Females exhibited more paw lifting and higher locomotion, indicating increased susceptibility to stress in response to acute challenges (Beerda et al., 1999b). Even within a single breed or sex with similar rearing history, there are individual coping strategies and differences in behavioral expression of stress (Koolhaas et al., 1999). Genetics play a role in how individuals' behavioral response to stressors vary (Jones and Gosling, 2005). Individual differences in fitness, levels of hormones, and fundamental pathways in the brain influence how animal react in the face of stress and frustration (Mench and Mason, 1997).

Behavior is also dependent on the contextual cues of an environment the animal had been exposed to previously (Servatius and Beck, 2005). Previous training can play a large role in the behavioral response to stress and differences in communications (Fallani et al., 2007; Marshall-Pescini et al., 2009). Some behaviors that are typically perceived as stress-associated behaviors may actually be learned as the most effective means of gaining attention (Rooney et al., 2007). Therefore, behaviors may also be learned responses rather than true indicators of welfare (Rooney et al., 2007).

Stress is a state that occurs when there is loss of control and inability to predict what will happen (Henry and Stephens, 1977). Like cortisol, behavior can change due to being positively stimulated or aroused rather than being negatively stressed (Haverbeke et al., 2008). Change, whether it is positive or negative, is an alteration of homeostasis, which activates the stress response. It is difficult to interpret these changes in behavior, even knowing the context of the situation because it is impossible to truly know how the animal perceives a circumstance.

Behavior in human-animal interactions

Interaction with a human being certainly stimulates changes in the behavior of a dog from a solitary setting. The presence of humans, and even just the noise generated by humans can alter behavior in dogs (Lefebvre et al., 2009a). However, the behavioral response of a dog to human contact can be highly variable, as some dogs become stressed when pet by humans, while some become soothed, and others remain unaffected (Jones and Josephs, 2006). The effect of petting a dog is dependent upon the relationship between the dog and petter as well as the context of the activity (Bergamasco, 2010).

Petting from a human has generally been perceived as beneficial to the dog since it initiates contact with a human while assuming a relaxed, recumbent position when being petted (Hennessy et al., 1998). Numerous studies of human-interaction programs with dogs in shelters have shown positive changes in behavior after interaction with humans. Dogs that were subject to 25 minutes of gentle exercise, play, and human contact achieved better scores on behavioral evaluation than dogs that received no human contact (Menor-Campos et al., 2011). There was a significant improvement in shelter dogs' diffidence, temperament, and social behavior scores after engaging in an 8 week human interaction program (Bergamasco, 2010). In addition, shelter dogs that

participated in a regular human interaction program were more likely to come to the front of a kennel in a friendly manner than those that did not participate in the program (Normando et al., 2009).

However, not all human interaction may be a positive experience for a dog, as negative behavioral changes can also be observed in dogs in response to human contact. Signs of uneasiness are often observed in response to petting, especially yawning and panting (Hennessy et al., 1998). Not surprisingly, a human inflicting enough physical force to elicit pain in a dog is a cause of stress and stress associated behavior (Netto and Planta, 1997). One study reported that interaction with a stranger resulted in a significant behavioral and physiological stress response in dogs (Palestrini et al., 2005). Interestingly, it appears that dogs pet by a familiar individual demonstrated significantly more appeasement gestures and redirected behaviors than dogs pet by an unfamiliar person (Kuhne et al., 2012). These behaviors included lip licking, paw lifting, and lying down, especially when the dogs were pet on the head and shoulder, suggesting that the location on the body of where the dogs were touched may influence stress associated behavior (Kuhne et al., 2012).

Although the behavioral response is strongly dependent on the individual dog, the appearance and demeanor of the human also influences how the dog will respond. The age and sex of the human has been found to elicit different responses in dogs. Client age can influence expression of stress-related behavior, which were more evident with children under 12 rather than elderly clients (Marinelli et al., 2009). In one study, dogs being petted by a female demonstrated more yawning and remained in a relaxed, upright posture longer than when they were pet by a male human (Hennessy et al., 1998). These behavioral differences could be influenced by the past negative experience of a dog with a particular gender, and differences in size, mannerisms, and acoustics of voice between men and women (McConnell, 1990; Prato-Previde et al., 2006).

It is unlikely that appropriately selected AAA/AAT dogs would demonstrate marked behavioral signs of distress since these animals are specifically selected and trained for these purposes. However, there is a paucity of research that has evaluated the effect of AAA/AAT on behavior in dogs. The few published studies have mixed results, revealing a spectrum of change in stress behavior after an AAA session. One study

observed no signs of stress behavior in therapy dogs during an AAA/AAT session (Ferrara, 2004). In support of this, another study found that dogs participating in AAA exhibited no stress-associated behaviors and demonstrated engaging behaviors such as tail wagging, hand touching, gazing, and licking the petter, suggesting that the dogs were positively affected and relaxed by the interaction (Michelazzi et al., 2007).

However, a recent study observed the behavior of AAT dogs for 1 minute after a 2 hour AAT session, reporting the frequency of panting, pupillary dilation, yawning, whining, and air licking (King et al., 2011). Dogs that experienced increases in salivary cortisol levels tended to demonstrate more behavioral signs of stress than dogs that had no change or decreases in cortisol levels after the session (King et al., 2011). Furthermore, dogs with two or more years of therapy experience tended to exhibit less behavioral signs of stress than dogs with less experience (King et al., 2011). It is important to monitor for more subtle behavioral signs of stress in AAA/AAT animals since these sessions are not intended to be significantly stress inducing, in contrast to challenges such as being kenneled in a shelter or being exposed to loud gun-shots. The lack of knowledge of the effects of HAI on behavior in these dogs warrants further investigation.

Limitations of using behavior to assess stress

Behavior can be easily misinterpreted by an untrained observer because it is highly variable and can often be nonspecific to stress (Beerda, 2000). In addition, dogs may exhibit subtle changes in behavior that can go unnoticed, especially in stoic dogs in the presence of mild stressors (Beerda et al., 1997). Therefore, stress associated behavior should not be used as the sole index of stress or welfare (Mason, 2004). To make the best assessment of stress or welfare in animals, physiologic measures of stress such as cortisol and adrenaline levels should be monitored in conjunction with behavior to prevent the misinterpretation of behavior (Beerda et al., 1998; Beerda, 2000; Kotrschal et al., 2009). Observing an increase in stress-associated behavior that correlates with a significant increase in cortisol from basal to stress induced state provides substantial evidence that the animal is truly stressed (Hennessy et al., 2001).

Because of the wide individual variation in stress-associated behavior, it is essential to distinguish what behaviors are normal for that animal when it is not stressed.

31

A catalogue of behaviors, or ethogram, demonstrated in an unstressed situation, such as the home environment, is necessary to determine deviation from the norm when the animal is frightened, ill, or in pain (Banks, 1982).   Assessing the animal's freedom to express normal behavior warrants this ethogram to determine animal welfare. While exposure to a stressor may not result in increased stress-associated behavior, it may cause play and exploration behavior to decrease or disappear entirely (Lee, 1984). It would be valuable to validate a standardized canine ethogram for use among different observers and laboratories to progress research in canine welfare (McGreevy et al., 2012). In addition, recording behavior for future critical review and analysis minimizes the loss of subtle and quick behaviors that may be missed during real time observation. There is criticism that intraspecific variability is rarely addressed, and trends are indicated that may have been more prominent if greater numbers of dogs and more extensive checks of behavior (Hessing et al., 1993; Adams and Johnson, 1995).

J. Interaction of cortisol and behavior

It has been hypothesized that elevated cortisol levels are positively correlated with stress-associated behaviors. Some studies have found positive correlations between cortisol and behavioral indicators of stress, while many others have found less clear-cut relationships (Rooney et al., 2007).

It is important to recognize that there is a delay of increase in cortisol in response to a stress event. Therefore, stress associated behaviors are usually observed before the acute rise in cortisol. Although behavior changes appear immediately, cortisol changes in saliva take approximately 10-20 minutes to elevate. In addition, cortisol levels usually remain elevated for at least 15 minutes even though stress associated behaviors subsided and the dogs appeared relaxed (Vincent and Michell, 1992).

High arousal can lead to increased cortisol production (Hiby et al., 2006). The study of thunderstorm-phobic dogs showed a significant increase in pacing, whining, trembling, and hiding in conjunction with a substantial increase in plasma cortisol in response to thunderstorm simulation (Dreschel and Granger, 2005). In one study of shelter dogs, an inverse relationship between duration of trotting or walking and urinary cortisol levels was found, suggesting that inactivity may induce high levels of cortisol

production (Hiby et al., 2006). A similar study of chronically stressed shelter dogs found a positive correlation between urinary cortisol and intention to change state of locomotion, suggesting that the lack of stimulation and reduced locomotor activity was associated with high cortisol levels (Beerda et al., 1999a). However, a 20 minute observation of healthy dogs hospitalized in a veterinary hospital demonstrated that head resting was negatively associated with salivary cortisol, suggesting that rest is correlated with low levels of cortisol production (Hekman, 2012). The same study also found that panting and lip licking were positively associated with cortisol (Hekman, 2012). A very low posture tended to correlate with salivary cortisol in response to acute fear-provoking stimuli, but the correlation was not significant (Beerda et al., 1998). In response to a sound blast, there was an increase in frequency of lip licking, paw lifting, and body shaking, which was associated with increases in both heart rate and salivary cortisol (Beerda et al., 1997).

In contrast, most other studies have deduced that the relationship between cortisol and behavior is largely ambiguous, making appropriately assessment of welfare difficult (Hansen and Jeppesen, 2006). Increases in cortisol are not necessarily associated with increases in stress-associated behavior. A rise in cortisol may suggest an emotionally stressed state despite the concurrent display of calm and relaxed behavior (Vincent and Michell, 1992). Although there were significant rises in salivary cortisol in dogs in response to sound blasts, being electrically shocked, and subjected to a falling bag, these rises did not correlate with changes in stress associated behavior (Beerda et al., 1998). There was a similar significant rise in salivary cortisol in dogs competing in an agility competition, but no association between cortisol and behavior (Pastore et al., 2011). While urinary cortisol levels were more indicative of stress in Labrador retrievers in response to kenneling, they were not associated with any observed stress associated behaviors (Rooney et al., 2007). Although there were significant changes in cortisol levels after three minutes of play with police and guard dogs, there was no association between cortisol levels and any specific behavior changes in the dogs (Horvath, 2008).

Alternatively, increases in stress-associated behavior are not necessarily associated with changes in cortisol. In shelter dogs, significant differences in behavior factors of locomotor activity, flight, sociability, timidity, solicitation, and wariness were

not associated with differences in cortisol levels (Hennessy et al., 2001). Dogs that exhibited stress associated behaviors in response to a stressful veterinary visit did not have higher levels of urinary cortisol levels than dogs that did not exhibit stress associated behavior (van Vonderen et al., 1998). Despite an increase in paw lifting, vocalization, lowered posture, and auto-grooming in chronically stressed shelter dogs, these behaviors were not associated with a difference in urinary cortisol-creatinine ratios (Beerda et al., 1999a). Overall, there is a lack of correlation between cortisol levels and behavior, supporting the notion that there is large individual variation in physiologic and behavioral parameters of stress (Rooney et al., 2007). This area requires further study.

K. Conclusion and research justification

The main goal in the applications of human-animal interactions has largely been geared toward benefiting and ensuring the safety of the human. While this will always remain the principal objective, it must be balanced with advocating for the safety and welfare of the animal. Attention to the animal facet of human-animal interactions is essential to promoting the success and longevity of the animal's career as well as ensuring the animal's long-term health. There is evidence to suggest that human contact is beneficial for the animal while other studies imply that human contact may be stressful to the animal. The effects of HAI on animals warrants further investigation for practical applications. To assess the wellbeing of the animal, it is necessary to utilize objective measures of stress in the animal to identify any risks of these activities. Welfare parameters commonly evaluated include quantification of cortisol levels and observation of stress-associated behavior. The combination of physiologic and behavioral parameters can increase the robustness of the final assessment of animal welfare (Hiby et al., 2006).

The first objective of this study was to measure salivary cortisol levels and behavior in registered AAA dogs as a function of time in in home, neutral, and AAA settings. The second objective was to measure hair cortisol of to these dogs. The final objective was to correlate salivary cortisol, hair cortisol, and behavior in registered AAA dogs across three separate settings. These objectives tested the null hypotheses that salivary cortisol and behavior are not different between the three settings; hair cortisol do not correlate with salivary cortisol; and correlations between salivary cortisol, hair

cortisol, and behavior do not exist in registered AAA dogs. The study of physiologic and behavioral effects of AAA on registered AAA dogs can enhance our understanding of animal welfare in these interventions, introduce evidence-based applications for handlers, and establish scientific methods for future research.

CHAPTER 2: EFFECT OF DOG-HUMAN INTERACTION ON CORTISOL AND

BEHAVIOR IN REGISTERED ANIMAL-ASSISTED ACTIVITY DOGS

1. Introduction

The human-animal bond is defined by the AVMA as "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). There is a growing body of evidence to support the rewards and benefits of human-animal interactions for humans. However, there is limited evidence to document the effects of human-animal interactions on the animals themselves. This represents a significant challenge because the field is lacking in scientific based guidelines to ensure animal welfare.

An instrument of any trade requires specialized attention, care, and maintenance to enhance its efficacy, longevity, and job satisfaction. Animals utilized in human-animal interventions (HAI) are no exception to this rule and as these practices grow, the protection of the health and welfare of these animal "instruments" remains a fundamental issue to address. The welfare of dogs used in animal-assisted activities (AAA) and animal-assisted therapies (AAT) has been questioned, as social interactions are among the most potent stressors a dog can endure (von Holst, 1998; McEwen and Wingfield, 2003). Iannuzi and Rowan (Iannuzzi, 1991) commented on the potential for fatigue and burnout in therapy dogs, and Zamir (Zamir, 2006) has argued that AAT can be perceived as potentially immoral and exploitative in nature. As research supporting the benefits of animals for humans strengthens and HAI programs proliferate, it is critical that evidence based research exists to answer the question of whether the use of animals for this purpose is detrimental to animal welfare. Some have also argued that evidence has yet to prove that a live animal is necessary for a therapeutic effect (Marino, 2012). There is a need for research that demonstrates the effect of AAA/AAT on animal welfare.

The limited research involving therapy dogs have 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 one hour 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. Furthermore, it is unknown how long these cortisol levels remain elevated and whether this physiologic arousal is due to negative stress or positive excitement (Haubenhofer and Kirchengast, 2006). These uncertainties justify a need to systemically examine 1) if a significant rise and/or fall in cortisol follows a trend before, during, and after a therapy session, 2) if a significant change in cortisol is associated with stimulating interaction and/or environment, and 3) if a significant rise in cortisol can be associated with changes in other parameters of welfare to indicate negative stress or positive excitement. The design and execution of a precise protocol to effectively answer these questions can reveal important relationships between cortisol and the physiologic arousal associated with AAA/AAT sessions and can provide essential tools for future investigations aimed at better defining factors that may contribute to stress in these dogs. Although therapy dog selection requires appropriate training, veterinary health approval, and temperament testing (Davis, 1992), there is a lack of evidence to correlate the selection criteria with the actual ability of these dogs to be successful and remain unstressed as therapy animals. There is great value in defining an easily obtainable and objective measure that predicts the change in salivary cortisol during the therapy session (if one truly exists), as this measure may have the potential to be used as a screening tool to assess therapy dogs. It is conceivable that hair cortisol level may be able to predict salivary cortisol level response. In both saliva and hair, cortisol reflects the "free" or unbound fraction of steroid in circulation. A significant positive correlation between hair and salivary cortisol in mammals has been demonstrated (Davenport et al., 2006). In addition, hair cortisol has been shown to be a better measure of responses to chronic stress compared with point sampling methods (blood, saliva) that are more sensitive to acute stressors (Accorsi et al., 2008). However, the level of hair cortisol has not been previously reported to correlate with the level at which salivary cortisol rises in response to acute stress. For example, a therapy dog with significantly elevated hair cortisol may have a higher rise in salivary cortisol from

baseline in response to a therapy session than the therapy dog with lower hair cortisol. If such a correlation truly exists, hair cortisol may be a useful parameter to predict the degree of physiologic response to the therapy session.

Analysis of behavior has long been utilized 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; Beerda, 2000). These behaviors have been implicated as indicators of uncertainty, excitement, or fear (Beerda et al., 1997), suggesting their association with negative stress. The therapy session may not be perceived as a negative acute stressor by dogs, as Ferrara, Natoli, and Fantini (2004) reported the absence of observed stress behavior in dogs during AAA/AAT. However, King et al. (2011) observed multiple behavioral signs of stress (panting, papillary dilation, yawning, whining, and air licking) in dogs after a two hour AAT session. These discrepancies make it necessary to clarify whether the therapy session significantly increases stress-associated behavior. In addition, while this parameter has usually been associated with negative stress, it can also be provoked by positive excitement (Haverbeke et al., 2008). The association between stress behavior and physiologic parameters remains inconclusive (Hansen and Jeppesen, 2006), but the standard guideline for appropriate assessment of behavior requires simultaneous analysis of cortisol (Kotrschal et al., 2009). The inconsistencies that are commonly observed when comparing outcomes of behavior studies support the concept that behavior cannot be used as the sole index of welfare (Beerda, 2000). Finally, an appropriate interpretation of behavior during a stress event benefits from comparison of the animal's baseline behavior during a non-stressful event (King et al., 2011). Comparison of stress-associated behavior in conjunction with cortisol level across stimulating therapy sessions and non-stimulating settings can enhance the understanding of how HAI affects behavioral and physiologic parameters of stress in dogs, which increases the robustness of the final assessment of animal welfare (Hiby et al., 2006).

The first objective of this study was to measure salivary cortisol levels and behavior in registered AAA dogs as a function of time in in home, neutral, and AAA settings. The second objective was to measure hair cortisol of to these dogs. The final

objective was to correlate salivary cortisol, hair cortisol, and behavior in registered AAA dogs across three separate settings. These objectives tested the null hypotheses that salivary cortisol and behavior are not different between the three settings; hair cortisol do not correlate with salivary cortisol; and correlations between salivary cortisol, hair cortisol, and behavior do not exist in registered AAA dogs. The study of physiologic and behavioral effects of AAA on registered AAA dogs can enhance our understanding of animal welfare in these interventions, introduce evidence-based applications for handlers, and establish scientific methods for future research.

2. Materials and methods

2.1. Participants

Six weeks prior to the start of the study, owners of registered animal-assisted activity dogs were recruited by emails sent to current members of the University of Pennsylvania's therapy dog program, Vet Pets. Additional owners were recruited by emails sent to active and registered Therapy Dogs International (TDI) (Flanders, NJ, USA) members living within a 20-mile radius of Philadelphia, PA until 16 eligible participants were recruited. All owners served as the handlers of their dogs.

The recruitment letter briefly described the study and required that dogs were at least 1 year of age, currently active in TDI and/or Vet Pets, were up to date on rabies vaccine, had no evidence of underlying disease, and were able to attend all scheduled sessions. It also explained that dogs would be excluded if they were on non-steroidal anti-inflammatory or steroidal (topical or systemic) medications within 6 weeks, or any other medications that could affect systemic cortisol levels. Interested owners of dogs that met this criteria contacted the PI (ZN) and were sent additional details about the study with a link to complete an online survey with questions regarding age, sex, breed, medications, and availability. Eligible dog-handler dyads were invited to attend an information session at the beginning of one of the two, 2-week study periods based on availability. Owners were provided a detailed description of the study and instructed how to appropriately collect saliva samples at this information session.

After the information session, a physical examination was performed on each dog by a licensed veterinarian (ZN). Participants were excluded if the veterinary examination revealed any significant abnormalities or the owner was unable to safely obtain an appropriate saliva sample. Each dog-handler dyad was videotaped demonstrating the string of commands of sit, stay, down, and come followed by appropriate collection of saliva. A 5x5cm area of hair was taken from the left or right ischium. Handlers were provided with an instruction manual and signed the study waiver, which confirmed their attendance at each session. Handlers were compensated for their participation with a $50 gift certificate at the completion of the study. All protocols and surveys were approved by the Virginia Tech Institutional Animal Care and Use Committee (IACUC Number 11-190-CVM) and the Institutional Review Board (IRB Number 11-998).

2.2. Experimental design

2.2.1. Design

The data collection of salivary cortisol and video recording was performed across the three settings: home, neutral (novel room without human or animal interaction), and animal-assisted activity (AAA) (study break session for undergraduate students in an undergraduate residency hall) on three non-consecutive days in randomized order within a two week period. The schedule was randomized using an online randomization program (www.randomizer.org, Urbaniak, G. C., & Plous, S. (2011)) according to availability of handlers. All sessions were conducted from 15:00-20:00 in all settings. A maximum of 8 dog/handler teams were enrolled in the study during each of the two, 2 week periods, resulting in a maximum of 16 different dog/handler teams over 4 weeks from March to April 2012. Schedules were confirmed with the handlers at the information session. The dog was not given food after 12:00 (no later than 3 hours prior to collection) on any of the data collection days. The dog remained on a 6 ft leash attached to collar, harness, or gentle leader the dog was accustomed to wearing at normal visits at all times, except for indoors at the home setting. At the AAA setting, if the dog typically wore a bandana or other apparel as indication of being a therapy dog, it was placed on immediately prior to the time 0 point. Room temperature was recorded in all settings and fresh water was freely accessible within the dog's space.

2.2.2. Home setting (HS)

The home setting was the dog's residence, remaining in the room that was most frequently inhabited by the dog from 15:00-20:00. The space was at least 7 square feet. While other animals and humans may have resided in the household, interaction with the dog was discouraged.

2.2.3. Neutral setting (NS)

The neutral setting was a novel room located in a communal conference room within the school of veterinary medicine, but without exposure to the veterinary hospital. The room was 20 ft x 6 ft, and tables and chairs were arranged to demarcate a 7 square foot space for the dog to remain during the session. A maximum of 2 dogs utilized opposite sides of the room simultaneously, but dogs were prevented from interacting with one another.

2.2.4. AAA setting (AS)

The AAA setting was a communal space of the undergraduate dormitory (Rodin College House, Philadelphia, PA, USA). The room was 30 ft x 30 ft, and tables and chairs were arranged to define a 7 square foot space for the dog to remain during the session. A maximum of 8 dogs utilized separate spaces of the room simultaneously, but dogs were prevented from interacting with one another.

In the AAA setting, approximately 40-60 undergraduate students entered the room for the hour session starting at time 0 and exited at time 60. Students were recruited to attend by emails and dormitory activity announcements advertising the event at least 3 weeks prior to the event. Each student attending the session signed a consent form that stated the participant was a University of Pennsylvania student, at least 18 years old, and would be videotaped for this experiment. Participants were informed of the study protocol and asked to conduct themselves in a manner that would not interfere with data collection. No more than 8 individuals (excluding the handler and video assistant) were allowed to surround any 1 animal at any one time. Students were encouraged to use an alcohol-based hand sanitizer that was centrally accessible.

2.3. Experiment protocol

2.3.1. Experiment set-up

A trained assistant was assigned to a specific dog prior to the dog's arrival at each setting. The assistant managed a data log sheet, stopwatch (Accusplit Survivor III Magnum XL, Livermore, CA, USA), video camera (either Sony DCR-SR68, Tokyo, Japan) or Sanyo VPC-HD2000, San Diego, CA, USA), tripod, and secure digital memory card (32 gb SDHC Centon card, Aliso Viejo, CA). The assistant placed the tripod and extension cord approximately 3 ft to the side of where the handler was assigned to sit in the space. The assistant continuously focused the camera on the dog's entire body, with intent to capture the head of the dog at all times.

The assistant met the handler and dog outside of the setting. The handler was given a fanny pack (Fantasy-bag 3 zipper fanny pack, Rajaji Nagar, Banglore, India) to wear containing 5 saliva collection tubes (swab storage tube, Salimetrics, State College, PA, USA) and 5 saliva absorbent swabs (Salimetrics children's swabs (SCS), Salimetrics, State College, PA, USA) (Fig. 1). Each tube was pre-labeled with time and ID number.

The assistant continuously videotaped the dog and directed the session according to the schedule (Table 1), using the timer to instruct the handler when to pet the dog and collect samples. The assistant also recorded times and unexpected events for the two-hour session on the data log sheet. At time 0, the assistant began recording the dog with the video camera and instructed the handler to perform the command and saliva collection procedure. Following this first collection point, the handler and dog walked at a leisurely pace for approximately 10 minutes outside of the setting before walking inside. By time 25, the handler and dog were settled into the assigned space and the dog was petted for 5 minutes. Immediately following this 5-minute task, the handler performed the saliva collection procedure for the saliva to be collected at time 30. This series of petting and saliva collection was repeated at time 55 and time 85. Following the last indoor collection point, the handler and dog walked outside at a leisurely pace for approximately 10 minutes outside of the setting. At time 120, the handler performed the final saliva collection. The video camera was turned off and data collection was completed. The data log sheet was completed by the assistant. All items were returned to the PI at the completion of each session.

2.3.2. Five minute petting procedure

In the home and neutral settings, the handler served as the "petter." In the AAA setting, 8 different students that were unfamiliar to the dogs were randomly assigned to be "petters" prior to the session. Each petter was assigned to pet a different dog at each of the three 5-minute time points in consecutive order of dog ID numbers. For example, "petter number 2" pet dog 2 at time 25, dog 3 at time 55, and dog 4 at time 85. All petters were instructed to sit with the dog and gently stroke, pat, massage, scratch the dog anywhere on the body with at least 1 hand remaining on the dog at all times.

2.4. Data collection and analysis

2.4.1. Hair cortisol and analysis

Hair was collected from the dog at the information session. A 5 cm square area was removed from a randomly assigned right or left ischiatic region using a size 40 clipper blade on commercially available pet grooming clippers. Hair samples were stored in aluminum foil and kept in a -20°C freezer until analysis.

The samples were transported to the Endocrine Technology Services Core Laboratory of the Oregon National Primate Research Center. Cortisol levels from each sample were obtained using previously validated methods (Davenport et al., 2006; Accorsi et al., 2008). The samples were washed in isopropanol and allowed to air dry to remove any surface contaminants. Cleaned hair samples were minced into 1-3 mm length segments by a custom-made cutting device. For duplication, each trimmed hair sample was weighed out twice at 120 mg each for duplicate-1 and -2. Each duplicated sample was extracted and assayed as individual samples. Extraction of cortisol was performed via incubation in methanol at room temperature with mild agitation for 24 hours. Following extraction, samples were centrifuged and the extract (liquid phase) was transferred to a new tube and dried down under a stream of air. The dried extracts were reconstituted using buffer prior to assay for cortisol. Radioactive cortisol was used for determination of hot recovery, and the methanol extraction efficiency was between 75-85%. Cortisol assay was performed using commercial cortisol kits (Cat. # 1-3002, Salimetrics, State College, PA, USA). The inter-assay variation was less than 15%, the intra-assay and duplication variation were less than 12%.

2.4.2. Saliva collection and analysis

For each saliva collection, the handler cued the appropriately time labeled tube and Salimetrics children swab (SCS). Following the command procedure, the handler placed the SCS in the dog's mouth continuously for 90 seconds, which was timed by the assistant. The swab was placed in both cheek pouches and between the teeth to encourage the dog to chew on the swab. After 90 seconds, the swab was removed and folded into the Salimetrics tube. The handler gave the tube to the assistant to place into a styrofoam cooler with ice packs. All saliva samples were frozen in laboratory freezer at -20°Celcius after each session until analysis.

The frozen samples were delivered to Salimetrics. All samples were centrifuged at 3000 rpm for 15 minutes. All samples were assayed for salivary cortisol using a highly sensitive enzyme 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.

2.4.3. Behavioral observations

All sessions were downloaded in .mpg format. Each 2 hour session was split into three separate 5 minute video clips to contain only the 5 minute petting procedure (30, 60, and 90 minutes) using a video splicing software (OJOSoft Minnetonka, MN, USA).

An ethogram (Table 2) was developed with the assistance of a veterinary behaviorist (CS) from previously recorded sessions in a pilot study. Using the Observer XT data recording system (Noldus Information Technology, Wageningen, the Netherlands), the video clips were coded by trained users (ZN and DJI) using continuous sampling. Behaviors were coded and analyzed according to frequency and duration. Inter-observer reliability exceeded 90% for all behavioral categories.

2.5. Statistical analysis

Descriptive statistical analysis was 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.

Exact Kruskal-Wallis test with Dunn's procedure for multiple comparisons was used to assess associations between hair cortisol and sex, therapy dog organization, duration of ownership, therapy visits per month, and length of therapeutic session per day. Scatterplot and Spearman correlation coefficient were used to assess correlations between hair cortisol and number of animals in the house, age, weight, number of years of therapy, salivary cortisol at all time points in all locations, change in salivary cortisol at all points in all locations, and behavior.

Friedman's Chi-square 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 minutes after the behavior was observed in each setting (eg., body shaking at time 30 in AS was correlated with salivary cortisol at time 60 in AS). Statistical significance was set at $p<0.05$. All analyses were performed using SAS version 9.3 (Cary, NC, USA).

3. Results

3.1. Demographics

Sixteen participants meeting the criteria were recruited and enrolled online. Of the 16 participants, one was excluded because the handler was unable to safely collect an adequate saliva sample at the research orientation meeting. Fifteen dog-handler teams completed the study. The demographics of the participants are shown in Table 3.

There were seven spayed female dogs and eight neutered male dogs. There were eight mixed breed dogs and one of each of the following breeds: Akita, American Staffordshire terrier, Cavalier King Charles spaniel, golden retriever, miniature schnauzer, Rhodesian ridgeback, and Pembroke welsh corgi. Two dogs were considered non-shedding breeds. The mean weight was 9.14 kg (median 17.3 kg, (6.4-44.5 kg)). The mean age was 4.6 years (median 4 years, (2-10 years)). The mean years of therapy certification was 2 years (median 2 years, (1-8 years)).

Ten dogs were members of TDI only; 3 dogs were members of Vet Pets only; and 2 were members of both. Two dogs participated in AAA one day per month; 6 dogs participated 2-3 days per month; 5 dogs participated 4-6 days per month; 1 dog participated 7-10 days per month, and 1 dog participated >10 days per month. Seven dogs participated in AAA 30 minutes to 1 hour; 6 dogs usually participated 1-1.5 hours; 2 dogs participated >2 hours. Nine dogs had never visited the AAA setting; 5 dogs had visited the AAA setting one to two times; 1 dog had visited the AAA setting 2-5 times.

Eight dog/handler teams were present in the AAA room at the same time in the first study period; 7 dog/handler teams were present in the AAA room at the same time in the second study period. Three out of the 15 dog/handler teams were present in the neutral room without another team in the room; 12 dog/handler teams were present in the neutral room with another team across the room. There were 13 female handlers and 2 male handlers. The room temperature in all indoor settings ranged between 20.6 to 23.9°C. The temperature in all outdoor settings ranged between 11.1 to 21.1°C. No adverse events occurred during the study period.

3.2. Salivary cortisol

Two-hundred twenty-four salivary samples were collected from 15 dogs. Of these samples, 218 samples yielded sufficient saliva for cortisol analysis. One dog (dog

number 14, a five year old female spayed Rhodesian ridgeback) was identified as a persistent outlier in all settings. Dog number 14's salivary cortisol levels at 0, 30, 60, 90, and 120 were 1.918, 2.668, 1.750, 1.372, 1.341 µg/dl respectively in the AS; 2.853, 2.957, 4.615, 2.081, 3.059 µg/dl respectively in the HS; and 15.291, >30, >30, 14.911, 12.810 µg/dl respectively in the NS. Because all salivary cortisol levels were persistently elevated and less likely to be due to laboratory error, it was determined that there was either contamination of the samples from an abnormality persistent in the oral cavity or there was something unique about this dog's cortisol physiology that was different from the rest of the population. Therefore, the salivary cortisol values from this dog were excluded from the data set.

The means and medians of 203 salivary cortisol samples from 14 dogs separated by time or location are shown in Table 4. Box plots of salivary cortisol at each time point in HS, NS, and AS are shown in Fig. 2-4, respectively. The mean (median) salivary cortisol was 0.305 (0.236) in the AS; 0.277 (0.232) in HS; and 0.554 (0.304) in NS.

The salivary cortisol geometric means of each setting are compared against one another across time in Fig. 5. Salivary cortisol in the NS was higher than HS at time 0 (NS mean of 0.423, median 0.325 vs HS mean of 0.213, median 0.191, p=0.0002); and time 30 (NS mean of 0.397, median 0.332 vs HS mean of 0.255, median 0.245, p=0.0149). Salivary cortisol in the NS was higher than AS at time 30 (NS mean of 0.397, median 0.332, AS mean of 0.257, median 0.291, p=0.0255), time 60 (NS mean of 0.371, median 0.276; AS mean of 0.246, 0.29, p=0.0251), and time 90 (NS mean of 0.351, median 0.3065, AS mean of 0.229, median 0.197, p=0.0518).

The geometric mean percentage change of salivary cortisol from Time 0 is shown in Fig. 6. There was no significant effect of time on salivary cortisol in each setting (p=0.3370). However, there was a significant difference in percentage change of salivary cortisol over time from time 0 between HS and AS in addition to HS and NS. The percentage change cortisol from time 0 to time 60 was significantly higher in HS (mean 30.6%, median 36.36%) compared to AS (mean -14.92%, median -23.07%) (p=0.009). The percentage change cortisol from time 0 to time 120 was significantly higher in HS (mean 17.35%, median 2.01%) compared to NS (mean -23.31%, median -23.51%) (p=0.0194).

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

3.3. Hair cortisol

Fifteen hair samples from 15 dogs were analyzed for cortisol. Although dog 14's hair cortisol level at 58.9 pg/mg was higher than the median value, it was not the highest outlier in this population. However, because it was deemed that there was something unique about this dog's cortisol physiology based on salivary cortisol levels, hair cortisol from this dog was also excluded from statistical analysis.

A box plot of hair cortisol from the 14 dogs is shown in Fig. 7. The median of hair cortisol was 17.7 pg/mg (mean 25.41 pg/mg). The outlier at 94.42 pg/mg was dog 6, a 3 year old female spayed mixed breed dog.

There were no significant correlations between hair cortisol and salivary cortisol at any point in any setting. Analysis of demographic factors revealed no significant correlations between hair cortisol on therapy dog organization, duration of ownership, therapy visits per month, length of therapeutic session per day, number of animals in the house, age, and number of years of therapy. However, there were significant correlations between hair cortisol and sex and weight. Females had significantly higher levels of hair cortisol (mean 35.13, median 23.02) than males (mean 18.12, median 10.65), p=0.028, (Fig. 8). In addition, there was a negative correlation between weight and hair cortisol, as the higher the weight, the lower the hair cortisol (p=0.0068) (Fig. 9).

3.4. Behavior

Five minute video clips at time 30, 60, and 90 were coded and analyzed in the HS, NS, and AS from 15 dogs, 15 dogs, and 14 dogs respectively. The behavior from dog 1 was not recorded in the AS. The frequency of observed behaviors is listed in Table 5.

There were significant differences between settings in percentage of observed behaviors of standing, ambulating, and recumbency. The percentage standing was higher in the AS compared to HS at time 30 (AS median 58.99, HS median 0) (p=0.0075), time 60 (AS median 20.21, HS median 0) (p=0.0126), and time 90 (AS median 22.43, HS median 0) (p=0.0067). The percentage standing was higher in NS compared to HS at time 90 (NS median 20.59, HS median 0) (p=0.0067) (Fig. 10). The percentage

ambulating was higher in AS compared to HS at time 30 (AS median 5.6%, HS median 0%) (p=0.0013), time 60 (AS median 1.98%, HS median 0%) (p=0.0114), and time 90 (AS median 4.04% vs HS median 0%) (p=0.0016). The percentage ambulating was higher in AS compared to NS at time 30 (AS median 5.6, NS median 0.477) (p=0.0209) (Fig. 11). The percentage recumbent was higher in HS compared to NS at time 30 (HS median 99.18%, NS median 0%) (p=0.0325) and time 90 (HS median 100%, NS 33.57%) (p=0.0075). The percentage recumbent was higher in HS compared to AS at time 30 (HS median 99.18%, AS median 2.67%)(p=0.0325) and time 90 (HS median 100%, AS median 11.53%) (p=0.0075) (Fig. 12).

There were no significant differences between settings in observed behaviors of exploring, scratching, stretching, self-grooming, licking object, licking person, vocalizing, paw lifting, or jumping, mouth neutral, panting, alert, resting, lip licking, mouth opening, and body shaking. There were no significant effects of environment on percent changes in any observed behavior from time 0.

3.5. Relationship between behavior and salivary cortisol

Salivary cortisol was significantly associated with sitting, standing, panting, and neutral mouth at specific time points in various settings. Salivary cortisol was positively correlated with percentage sitting at time 90 in NS (p=0.0388) (Fig. 13). Salivary cortisol was positively correlated with percentage sitting at time 30 in AS (p=0.0400) (Fig. 14). Salivary cortisol was negatively correlated with percentage standing at time 30 in AS (p=0.0219) (Fig. 15). Salivary cortisol was positively correlated with percentage the mouth neutral at time 60 in NS (p=0.0019) (Fig. 16). Salivary cortisol was negatively correlated with percentage panting at time 60 in NS (p=0.0284) (Fig. 17). There were no significant correlations between any other observed behavior and salivary cortisol.

4. Discussion

Since there was no difference in salivary cortisol levels or observed behaviors between the home and AAA settings, it was concluded that a 60-minute AAA for college students in a dormitory setting does not induce significant physiologic or behavioral stress in registered AAA dogs. The data suggests that the welfare of these dogs is not compromised by the AAA in which they participated.

There is reason to believe that AAA may induce an elevation in cortisol level because of the stimulation from meeting and interacting with a variety of unfamiliar people. It is reasonable to assume that the act of interacting with an unfamiliar human may be stimulating to a dog, as they may demonstrate behaviors that could be perceived as excitement, such as jumping, vocalizing, or play bowing. This physiologic response does not necessarily denote a negative or positive response, but is rather the result of stimulation from a change in daily activity. However, this was not found in the current study. For example, King reported significant elevations in salivary cortisol levels in 21 dogs from baseline to after one hour of AAT in a hospital environment (King et al., 2011). Haubenhofer and Kirchengast (2006) found that cortisol levels in 18 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 did not control for factors such as time of therapeutic work, frequency, type (AAA vs AAT), and location of therapeutic work. The current study attempted to standardize the variation in time of day, location, intensity of work, and sample collection, which revealed no difference between the day at home and day of AAA. Since the AAA was not a high intensity or physically demanding activity to result in physiologic stimulation, cortisol values were not significantly different in this setting. In addition, the dogs were likely not stressed because AAA was a familiar and predictable activity since they were trained and selected for this interaction.

However, a significant change in behavior was not observed in the AAA setting, suggesting that these dogs are trained to behave appropriately in the AAA setting and typically demonstrate impulse control. Although these dogs may exhibit behavioral self-control, cortisol elevation is an autonomic response and can still be stimulated. For example, King reported significant elevations in salivary cortisol levels from baseline to

after one hour of AAT in a hospital environment (King et al., 2011). This significant rise could reflect a true difference in physiologic stimulation between AAT in a hospital environment and AAA in a college dormitory environment. Perhaps a hospital or medical environment is, in fact, physiologically stimulating. Salivary cortisol levels were found to be significantly elevated in the veterinary hospital compared to the home setting (van Vonderen et al., 1998). In the current study, the neutral setting yielded significantly higher cortisol values than the AAA or home settings. Although the neutral setting was not located directly within the veterinary hospital, the dogs could have perceived subtle cues of a hospital, inducing higher levels of cortisol.

It has been hypothesized that cortisol levels in dogs may decrease in response to human interaction, suggesting that AAA can decrease stress in dogs. In contrast, numerous studies have demonstrated that dogs housed in a shelter, which have persistently elevated cortisol due to chronic stress, have decreased cortisol levels after human contact (Tuber et al., 1996; Hennessy et al., 1997; Hennessy et al., 1998; Horvath, 2008). For example, 25 minutes of play and human contact in shelter dogs resulted in significantly lower salivary cortisol levels than shelter dogs that did not receive human contact (Menor-Campos et al., 2011). However, most of these studies evaluated cortisol over an extended period time rather than from a single session. In addition, the dogs are unable to serve as their own controls in a non-stressful environment since they are limited to the shelter setting. Regardless, a shelter dog lives a much different lifestyle from an AAA dog, so comparisons should be made cautiously. Although not statistically significant, there was an overall decrease in cortisol from beginning to end of the AAA session in the current study. Haubenhofer also showed that, while not statistically significant, cortisol levels tended to be lower immediately after the session if samples were taken after 2:00 pm (Haubenhofer and Kirchengast, 2007). A study of 18 pet dogs that showed no significant change in plasma cortisol before and after a short, positive interaction with a human (Odendaal and Meintjes, 2003). Regardless, a significant decrease in cortisol in response to human interaction in AAA dogs has not been documented.

Interestingly though, cortisol levels were significantly higher in the NS than AS or HS at certain time points. It is plausible that the NS could be perceived as a stressful

environment, just as the kennel could be perceived as stressful to a shelter dog (Hennessy et al., 1997), and stranger interaction in a stressful environment can reduce cortisol levels. The AS was novel and unfamiliar to most dogs, as only four of them had visited the location prior to the study. Thus, it is interesting that cortisol levels did not rise in the AS. This significant difference between AS and NS would have been more provocative had the NS been the same location as the AS, only without human interaction. Although this study planned to achieve this, the same location was unable to be utilized due to practical reasons.

The finding that salivary cortisol levels in the NS were higher than HS at time 0 and 30 and AS at time 30, 60, and 90 was unexpected because the NS was not intended to be stimulating. This location was supposed to be an environment outside of the home that was novel, but not arousing. However, the novelty of the NS did end up being physiologically rousing, which is consistent with another study of eight dogs that demonstrated significantly higher cortisol levels in the novel environment compared to the home environment when alone (Tuber et al., 1996). For example, 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). However, the significant increase in cortisol levels in the NS was important in this study because it demonstrated that physiologic stimulation was possible in these dogs. It makes the finding that cortisol levels were not higher the AAA more meaningful.

The neutral setting could have had the highest cortisol values because the room was located in the veterinary school. One study found that cortisol levels rose in some dogs in response to procedures performed in a veterinary hospital, but not in others (van Vonderen et al., 1998). Another study found no difference in salivary cortisol between the veterinary clinic and home settings (Wenger-Riggenbach et al., 2010). Although the dogs were not introduced to or walked through the lobby of the veterinary school, smells and other cues of hospitalization could have been apparent to the dog. Since past experience can modify the degree of stress elicited in a particular individual (Rooney et al., 2007), a dog that had previously had a stressful experience at the veterinary hospital could possibly exhibit a rise in cortisol in response to the environment. Approximately

half of the participating dogs had been previous patients at the veterinary hospital. All dogs entered the veterinary school's lecture hall for the introductory research seminar at the beginning of the study period, where no invasive procedures were performed. Therefore, these dogs were familiar with the general location of the hospital, but were not exposed to the novel room or the veterinary hospital. However an increase in cortisol could still have been apparent.

Another rationalization for rise in cortisol in the NS was that these dogs remained in the NS with their handlers without an obvious purpose. The dogs were expected to stay on leash in the assigned area of the novel environment, without human interaction except for the five minutes of petting by the handler and subsequent saliva collection every 30 minutes. Therefore, these dogs were waiting in anticipation of what would happen next, making the circumstances unpredictable. The inability to predict what will happen induces significant stress in humans (Henry and Stephens, 1977). They could have also been uneasy and anxious because they were not allowed to interact with another human or dog that passed by the room. However, the unpredictable nature of a situation can also be stress inducing to a dog, as it may feel insecure due to inability to control its environment. If the dog perceived a distinct purpose for being in a new environment such as interacting with a stranger or engaging in a specific activity with the handler, perhaps cortisol levels could have returned to normal at a faster rate. In addition, the handler may have also inadvertently influenced the setting. There has been evidence to show that changes in humans' hormones and behavior can significantly influence hormonal and behavioral changes in other animals (Jones and Josephs, 2006). Since the handler and dog relationship is so closely bonded in AAT and AAA, it is possible that increases in stress in the handler may cause increases in stress in the dog. Since the handlers were also waiting without a specific purpose, it could be speculated that their attitudes may have influenced the cortisol levels to be high. It has been shown that stress in handlers can induce stress in their working dogs based on increases in the dog's heart rate, temperature, and subjective ratings of stress (Lit et al., 2010). Interestingly, there was no difference in salivary cortisol levels between the environments at time 120, suggesting that the environment was initially stimulating, but the dog adapted with time. This was demonstrated in another study of Labrador retrievers that took at least 30

minutes to adjust to an unfamiliar setting (Handlin et al., 2011).

Although the significant elevation of cortisol in the NS was not accompanied by significant increases in stress-associated behavior, it still emphasizes the important role the environment plays in the physiologic stress response in these dogs. This implies that any cortisol elevation during AAA is probably not strictly due to the interaction itself, but rather a combination of the environment and nature of the situation. Future studies attempting to utilize a specific setting as a control variable should utilize careful consideration when selecting a "neutral" environment that is truly perceived as neutral to the dog.

The human interaction protocol of five minutes petting was standardized across all settings with the exception that the petter was the familiar handler in the HS and NS, while it was a stranger in the AS. A previous study showed that when these dogs were placed in the novel environment in the presence of a human, cortisol levels significantly decreased compared to being alone in the novel environment (Tuber et al., 1996). Although the salivary cortisol of the dogs was not different in the AAA with strangers from the HS, it is interesting that cortisol levels were significantly higher in the presence of the handler in the NS. It could be presumed that the individual with the greatest influence in mediating the stress response of a dog in a novel environment would be a familiar individual. It would have been interesting to investigate if a stranger in the NS, thus turning the setting into an AAA in this environment, would have influenced cortisol levels. If cortisol levels did change, it could be suggested that human interaction with a stranger can moderate the stress response in novel environments. It could be supportive of the theory that human interaction may be a method of mitigating the HPA response and autonomic response of dogs in aversive situations, such as visits to the veterinary clinics or shelters (Hennessy et al., 1998).

Adequate saliva collection from dogs can be challenging, regardless of the methodology (Dreschel and Granger, 2009). The procedure required the dog to remain still as the handler controlled its head and inserted the swab in its mouth for 90 seconds. Although the procedure needs only a minimal amount of technical expertise on the part of the handler, it requires a significant amount of compliance on the part of the dog. Even trained veterinarians had difficulty in obtaining adequate samples from their dogs

(Wenger-Riggenbach et al., 2010). It was anticipated that collection from trained AAA dogs would be easier than a normal population of dogs because of their calm temperaments. However, significant challenges were encountered in collecting saliva from AAA dogs using sorbettes in one study because handlers had inadvertently forgotten some significant aspect of the collection protocol (King et al., 2011). This finding highlighted that it is unrealistic to expect perfect compliance from non-scientifically trained people (King et al., 2011). An adequate amount of saliva for analysis was obtained in 97% of the samples in the current study. This may be attributed to the fact that a trained research assistant was present to instruct the handler when and how to collect each sample. In addition, this study utilized Salimetrics Children's Swab, which was effective in yielding an adequate amount of saliva in most samples.

One study reported that more than half of dogs with HAC were excluded from the investigation because they displayed signs of fearfulness or aggression (Wenger-Riggenbach et al., 2010). In this study, one registered AAA dog was excluded upon enrollment because of the handler's inability to safely obtain a saliva sample. This two-year old male neutered mixed breed dog exhibited fear and avoidance behavior when it was approached with the collection swab, disqualifying it from the study. Although unexpected, this demonstrates that even a trained AAA dog may not tolerate this unique type of sampling. Safe collection of saliva is paramount in conducting an appropriate research study.

The current study also showed that saliva collection was not perceived as a stressful event to potentially confound interpretations of elevations in cortisol. As long as the saliva collection technique does not exceed four minutes, it should not affect salivary cortisol values (Kobelt et al., 2003). In addition, there was no significant change in serial salivary cortisol levels in any setting and even a tendency for values to decrease with time in each setting, illustrating that saliva collection was unlikely to be physiologically stimulating in these dogs. If saliva collection was physiologically stimulating, this could potentially pose an issue in obtaining an adequate sample because stress can increase sympathetic tone, resulting in vasoconstriction and subsequent decrease in rate of salivary flow (Ganong and Ganong, 2005). However, most samples in this study were adequate, revealing that either these dogs were not stressed by the situation or that, similar to

another study, there was no effect of stress on the volume of saliva collected (Dreschel and Granger, 2009).

It could be reasoned that the duration of activity could be associated with the degree of stress response because the dog may become more tired, restless, or agitated the longer it is engaged in activity. However, a negative correlation between salivary cortisol and duration of AAA/AAT was identified in one study, revealing that longer sessions actually resulted in lower salivary cortisol levels (Haubenhofer and Kirchengast, 2006). It was reasoned that longer sessions were often low in intensity and included frequent breaks, whereas shorter sessions were higher in intensity without breaks (Haubenhofer and Kirchengast, 2006). In the current study, salivary cortisol levels did not change significantly with time for the two-hour testing period. To our knowledge, this is the first study in therapy dogs that has examined a serial change in cortisol as frequently as every 30 minutes in three different settings. One study found that owners petting their Labrador retrievers for three minutes resulted in significant increases in plasma cortisol 15 and 30 minutes later (Handlin et al., 2011). After exposure to a stress event, researchers have reported a 15-20 minute delay in cortisol levels rising in circulation (Vincent and Michell, 1992) and a relatively slow fall of cortisol back to baseline levels. The half-life of cortisol is between 70 and 110 minutes (Weitzman et al., 1971). One study reported that cortisol returns to baseline levels after 30 minutes (Engeland and Gann, 1989), while others have reported a return to baseline within 40 minutes (Vincent and Michell, 1992; Dickerson and Kemeny, 2004). Therefore, it was reasonable to select this interval of time for serial measurements of cortisol. Because levels did not change, this may denote that future studies do not need to collect saliva samples as frequently. A baseline pre-sample and post-sample may be sufficient to reveal a change in this type of AAA.

Despite the lack of change in cortisol within each setting, there was a significant difference in change in cortisol between the settings. Percentage change in salivary cortisol change tended to increase across time in the home setting and tended to decrease across time in the neutral and AAA settings. In the HS, the cortisol level at time 0 was reflective of the dog's resting state at home prior to the challenge. The following tendency for increase in percentage change of cortisol from time 0 in the HS may have

been due to physiologic stimulation from the research assistant entering the home, and the protocol itself. In contrast, the cortisol level at time 0 in the NS and AS was likely reflective of the physiologic stimulation from the anticipation or excitement of traveling to these locations. People have a tendency to worry about upcoming work-related events (Schlotz et al., 2004), and it is suggested that dogs can experience a similar type of anxiety. Haubenhofer reported that owners of AAA dogs recalled that their dogs appeared more anxious before the therapeutic work (Haubenhofer and Kirchengast, 2007). Therefore, travel or anticipation could have explained the slightly higher cortisol values in the AS and NS at time 0. Furthermore, the tendency for decrease in percentage change of cortisol following time 0 in both NS and AS may have represented the dogs' acclimation to the environment. It takes approximately 30 minutes for dogs to adjust to an unfamiliar setting (Handlin et al., 2011). In addition, the only time point where there was no difference in cortisol levels between settings was at time 120, suggesting that collecting salivary cortisol 120 minutes after arriving to a novel setting may be the most representative of baseline cortisol level.

Although normal ranges for salivary cortisol in dogs have not yet been established, the levels in this study were higher than reported values from many previous studies. Mean basal cortisol level in the home setting in the current study was 0.27 μg/dL. Mean basal levels of cortisol in normal, healthy pet dogs in the home setting have ranged from 0.06 μg/dL (Wenger-Riggenbach et al., 2010) to 0.1 μg/dL (Dreschel and Granger, 2005) to 0.156 μg/dL (Bennett and Hayssen, 2010). Therapy dogs had median cortisol values of 0.06 μg/dL on days at home (Haubenhofer and Kirchengast, 2007). Interestingly, mean levels of cortisol in the NS, a physiologically arousing state, was yielded a mean of 0.55 μg/dL. Other studies have reported cortisol levels in response to stress to be as low as 0.1 μg/dL (Bergamasco, 2010) to 0.19 μg/dL (Menor-Campos et al., 2011) in shelter dogs and 0.2 μg/dL in thunderstorm-phobic dogs (Dreschel and Granger, 2005). However, another study of stress in shelter dogs reported a mean salivary cortisol level of 0.409 μg/dL (Coppola et al., 2006), while another study of the effect of air transport on dogs reported a mean salivary cortisol of 0.58 μg/dL (Bergeron et al., 2002). In addition, a more recent study of healthy, hospitalized dogs reported a mean of 0.48 μg/dL (Hekman, 2012). These values are closer to the results of the current study, but

still higher.

One possible reason for this discrepancy can be attributed to inherent laboratory variation in assay or sample processing. Since the assay may be laboratory specific, cortisol concentrations should only be referenced to the same laboratory (Briegel et al., 2009). In addition, the type of material used to collect the saliva can impact the level of cortisol (Dreschel and Granger, 2009). Although the Salimetrics Children's swab has not been validated for use in dogs previously, this instrument was selected at the recommendation of Nancy Dreschel via personal communication. However, the effect of this swab is not expected to result in significant change. Food particles in the saliva sample have also been implicated in assay interference and resulted in increased cortisol levels (Dreschel and Granger, 2009). Since the dogs were fasted and food was not given during the session, food contamination would be unlikely. In addition, blood contamination could have played a role since contamination with blood can elevate levels (Wenger-Riggenbach et al., 2010), but this is unlikely as the dogs enrolled in this study were evaluated to have low-grade periodontal disease and no macroscopic evidence of blood on the samples.

An unusual finding from the current study was that the cortisol values of dog number 14, an eight-year old female spayed Rhodesian ridgeback, were consistent outliers in all settings. The dog was assessed as clinically healthy with no clinical signs of HAC. A few of the salivary cortisol values from this dog in the NS were almost 60 fold higher than published values of salivary cortisol for dogs with HAC (Wenger-Riggenbach et al., 2010), making it physiologically improbable that a dog could have circulating cortisol levels this high. Interestingly, higher levels of cortisol in the NS could have been justified in this dog because the handler reported that the dog was anxious in the car prior to the session because they were delayed in traffic. However, behavior in the NS was not different from behavior in HS or AS. One study excluded any salivary cortisol value greater than 3 μg/dL because it was believed these samples were contaminated (King et al., 2011). Because cortisol values were high in all settings, it was presumed that there was contamination of the sample with an abnormality that persisted in the oral cavity, such as particles of food, blood, or oral lesion. However, the dog was fasted for four hours prior to sampling, there was no evidence of blood on the

swab, and physical exam did not reveal any apparent oral abnormalities. Alternatively, there could have been something unique about this dog's HPA physiology that resulted in persistently high circulating cortisol. Although hair cortisol for this dog was higher than the mean for the population, it was not the highest. The explanation for the cortisol levels in this dog is inconclusive; nevertheless, the cortisol values from this dog were excluded from the data set.

Elevated cortisol levels can be suggestive of HAC. One case report describes a therapy dog that continued working for weeks with clinical signs before it was diagnosed with HAC (Heimlich, 2001). In a study of dogs with HAC, salivary cortisol levels yielded a mean of 0.37 μg/dL (Wenger-Riggenbach et al., 2010). One study even excluded outliers >0.318 μg/dL to achieve normal distribution (Bennett and Hayssen, 2010). Although the values of many of the dogs where higher than this value, the dogs were unlikely to have this condition because they were in good health based on history and physical exams and lacked clinical signs of disease. In addition, the results from the HAC study measured salivary cortisol using a competitive electrochemiluminescence immunoassay (ECLIA) that required a minimum saliva volume of 300 μL using a cotton pad (Wenger-Riggenbach et al., 2010), which has been reported to have 38% lower cortisol than polyester pads (Hansen et al., 2008). Therefore, this study could have underestimated the cut-off value of dogs with HAC if compared to other laboratories with different analysis or sampling techniques.

An alternative theory for increased salivary cortisol levels in these dogs is that AAA dogs are truly physiologically different from the normal population of dogs in regard to the HPA. The fact that hair cortisol, which was analyzed in a different laboratory from salivary cortisol, was also higher than previous reports lessens the possibility of laboratory variation and also supports this theory. Perhaps dogs with higher cortisol levels are more likely to have a friendly and engaging temperament, predisposing them to be candidates as AAA dogs. For example, a higher baseline cortisol was found in working dogs that were more motivated to play (Horvath, 2008). It is possible that cortisol levels could be higher in dogs that are more motivated to interact with unfamiliar humans while being well-trained. Alternatively, the nature of AAA work could cause cortisol levels to be chronically elevated. The lifestyle of these AAA dogs could be

inherently stressful due to the unpredictability of day-to-day life, which is different from the normal daily routine of a household pet dog.

Despite being higher than previous reports, these levels of cortisol were not detrimental to health or wellbeing of these AAA dogs. The clinical relevance of this finding supports that if a baseline cortisol is found to be elevated in a dog, it should be interpreted in light of whether or not it is used for AAA. In the absence of clinical signs of HAC, a higher baseline cortisol level may be considered normal in an AAA dog. It is important to measure multiple cortisol levels across multiple settings to gain an accurate assessment of circulating cortisol level in an individual dog.

Although both salivary and hair cortisol levels in the current study were higher than previous reports, there was no correlation between the two parameters. This is in contrast to another study reporting that hair cortisol was positively correlated with baseline salivary cortisol samples collected over the 6 to 12 week hair regrowth period in healthy, unstressed dogs (Bennett and Hayssen, 2010). In addition, the study was comprised only of Labrador retrievers and German shepherd dogs (Bennett and Hayssen, 2010), in comparison to the current study which was comprised of dogs of various breeds, which could have accounted for a lack of significant correlation between hair and salivary cortisol.

In addition, there was also no correlation between hair cortisol and any specific behavior at any time point in any setting. A recent study reported a significant positive correlation between behaviors of hiding, running away, attention seeking, panting, and lowering of body posture in response to thunderstorm stimulation to hair cortisol collected two weeks after the challenge (Siniscalchi et al., 2012). However, the same study did not find a correlation between behavior and hair collected in the afternoon of the same day. Despite the positive morning correlation, it is unlikely that behavior from an isolated incident could influence hair cortisol, a measure of chronic stress, two weeks later, only in the morning. It is more reasonable to consider hair cortisol as a variable that may be representative of the way in which a dog will react in response to stressful events. One study reported that low plasma cortisol values in puppies adopted from a shelter was associated with an increase in undesirable behavior problems six months later, which proposes that a simple endocrine measure could be useful in predicting

longer term behavior (Hennessy et al., 2001). A significant correlation between hair cortisol and behavior could have provided the potential for hair to be utilized as a screening tool in AAA dogs. However, since the settings were not stressful to the dogs, there was no significant change in behavior. It is plausible that hair cortisol could be associated with stress-associated behavior in highly stressful circumstances. Additional demographic factors could have also contributed to the lack of association.

One of the demographic factors that impacted hair cortisol levels was gender. Females had significantly higher hair cortisol values than males, which is consistent with the results of a study that found female dogs to have significantly higher serum cortisol levels at baseline as well as in response to environmental stress than male dogs (Garnier et al., 1990). It has been suggested that this sex difference in humans is due to androgen-inhibited and estrogen-enhanced oxytocin effects (Taylor et al., 2000). However, all the dogs used in this study were neutered, so hormones are less likely to play such a role. In contrast, most other studies reveal no differences in basal levels of cortisol between intact sexes (Reimers et al., 1984; Reimers et al., 1990; Hennessy et al., 1997). In addition, another study reported no significant differences in cortisol levels between sex in neutered dogs (Dreschel and Granger, 2005). Therefore, this finding should be interpreted cautiously since the significance of this sex-associated difference is unknown.

In addition, a negative correlation was found between weight and hair cortisol. The more the dog weighed, the less the concentration of hair cortisol. Although other studies did not find a correlation between weight and hair cortisol (Bennett and Hayssen, 2010), there wasn't much variation in weight because the study consisted of large breed dogs such as Labrador retrievers or German shepherds (Accorsi et al., 2008; Bennett and Hayssen, 2010). The current study included a wide variety of sizes of dogs, ranging from 6.8 kg to 45 kg. This finding could suggest that smaller dogs experience higher levels of chronic stress than larger dogs or that cortisol sequestration into hair is dependent upon body surface area. Although there is no current evidence to suggest breed differences in hair cortisol levels, this finding may insinuate that smaller breed dogs have higher cortisol levels than larger breed dogs. However, breed can influence coat color, which does affect hair cortisol levels (Bennett and Hayssen, 2010). Additionally, breed can influence characteristics of hair type, such as texture, length, and shedding cycle, which

have not yet been investigated to affect hair cortisol levels. There were only two dogs in this study that were considered to be non-shedding, which was not enough to investigate a difference in hair cortisol between shedding and non-shedding dogs. Other studies investigating the effect of size on plasma cortisol levels have found that smaller breed dogs tend to have higher basal cortisol levels than large breed dogs (Reimers et al., 1984; Reimers et al., 1990). Further investigation into the association between hair cortisol and weight is warranted.

Like salivary cortisol, although there are no established reference values for hair cortisol, the levels in this study were found to be higher than previous reports. The current study reports a mean of 17.7 pg/mg and median of 25.41 pg/mg. Hair cortisol in 23 Labrador retrievers and 25 German shepherd dogs reported a mean of 10.9 to 12.6 pg/mg (Bennett and Hayssen, 2010). Another study of 29 domestic dogs reported a mean of 2.10 pg/mg (Accorsi et al., 2008). The difference in hair cortisol has been attributed to technique of preparation for analysis, as there was a 3.5 fold increase in concentration when hair was powdered compared to chopping method (Davenport et al., 2006). However, the current study utilized a chopping method of hair preparation, so hair cortisol would have been expected to be lower according to this theory. A more recent study utilizing the chopping method from 11 dogs reported mean hair cortisol values between 65.08 and 95.36 pg/mg (Siniscalchi et al., 2012), which are higher than the values in the current study. This discrepancy underscores the importance of factoring laboratory methodology, such as the use of enzyme immunoassay versus radioimmunoassay, in interpretation of results (Bennett and Hayssen, 2010). Furthermore, variables such as washing hair with shampoo and exposure to ultraviolet light, which were not controlled for, can also alter cortisol concentrations (Hamel et al., 2011; Li et al., 2012). In addition, demographic factors of age, sex, and weight may still be influential in cortisol levels, despite the current lack of evidence at this time (Bennett and Hayssen, 2010). If hair cortisol values in AAA dogs are, in fact, truly higher than the normal population, these chronically high cortisol values could be the cause of, or effect of AAA, as discussed regarding salivary cortisol.

The observed behaviors that differed significantly between settings at specific time points were in regard to position and locomotor state. The dogs stood significantly

more in the AS than HS at all time points and in the NS than HS at time 90. In addition, dogs ambulated significantly more in the AS than HS at all time points and in the AS than NS at time 30. Finally, the dogs were recumbent significantly more in the HS than both AS and NS at time 30 and 90. These positional differences can be attributed to the varying degree of stimulation in each setting. Dogs rested more in the home setting compared to environments outside the home, as they were more comfortable and relaxed. Consequently, the dogs likely stood less in the home because they were more frequently recumbent.

Dogs stood and ambulated more in the NS compared to HS because they were stimulated by the novelty and unpredictability in the NS. Increased ambulation may be construed as a sign of stress if the dog was anxious and could not get settled. However, this activity was also likely more frequently observed in the NS because of the dog's hypervigilance and response to outside sounds and people passing by outside the room since there was nothing else that maintained its attention other than the presence of the other dog/handler team across the room. All but three dogs were tested in the NS with another dog/handler team in the room. Statistics could not be run to test the effect of the presence of another team in the NS on behavior and cortisol since there were only three teams that were alone in the room.

Dogs stood and ambulated more in the AS compared to HS because they were stimulated by interaction with strangers. In the absence of other signs of stress, the increase in ambulation during the AS was not interpreted to be an indicator of stress, especially since it did not correlate with a rise in salivary cortisol. However, it is important to consider that it is more desirable for an AAA dog to remain in a stationary position to allow a person to interact and engage with the dog through gentle petting. Ambulating while a person was attempting to interact with the dog could have possibly indicated that the dog was disinterested, preoccupied, or anxious. The direction of ambulation, whether it be towards or away from the petter or handler, was not coded. This level of detail could have added information to denote if the dog was attention seeking or attempting to leave the circumstance. Regardless, increased ambulation and standing during an AAA compared to a home setting could be considered normal for an AAA dog.

Interestingly, there were no significant changes in other stress-associated behaviors between settings. It would have been conceivable to observe a significant change of behavior in the AS because of the nature of the stimulating activity of interacting with a stranger, which is a much different circumstance than resting at home. It is likely that the AAA was not exceedingly stressful enough to cause changes in behavior. It has been proposed that dogs may not exhibit stress-associated behavior due to the mild restraint in the context of human-animal interactions (Kuhne et al., 2012). Furthermore, there were no significant changes in stress-associated behaviors with time in each setting. Of particular interest is the fact that these dogs did not show signs of stress, anxiety, or indicators of fatigue or exhaustion towards the end of the session in the AS, suggesting that one hour duration of AAA is not stressful to these dogs.

In addition, because cortisol levels were significantly higher in the NS than AS and NS, it would have been reasonable to observe stress-associated behaviors in conjunction with the rise in cortisol. However, stress behaviors were not observed to be different from the home or neutral setting. Again, this was likely due to the fact that these settings were not perceived to be stressful to these AAA dogs. There could be a threshold of what each dog considers to be distressing enough to elicit significant behavioral change, but neither a novel environment nor an AAA reached this threshold. It is important to remember that these AAA dogs were trained and selected specifically for this type of activity because they respond to stress in a very specific way. The temperament of these dogs is typically calm with low reactivity, which could be a reason why it may be difficult to observe stress-associated behavior compared to the normal population.

It is also intriguing that stress-associated behavior was not different between being petted by an unfamiliar stranger compared to being petted by the familiar handler. Additionally, there has been evidence to show that changes in humans' hormones and behavior can significantly influence hormonal and behavioral changes in other animals (Jones and Josephs, 2006). One study showed that dogs demonstrated significantly increased redirected behaviors, appeasement gestures, and social approach behavior when they were petted by a familiar person versus an unfamiliar person (Kuhne et al., 2012). Redirected behaviors included sniffing/licking the floor, digging, drinking, visual

scanning, and excessive activity; appeasement gestures included blinking, sitting, laying down, lip licking, and paw lifting; and social approach behavior included seeking out human contact, gazing at a human, and sitting/lying next to a human with body contact (Overall, 1997; Casey, 2002; Kuhne et al., 2012). However, this study grouped multiple behaviors within each category, which may have accounted for an absence of significant difference that might have been apparent had behaviors been analyzed individually. Still, it would have been plausible to find significantly fewer stress-associated behaviors in the presence of the handler, as the dog would be more likely to be comfortable and relaxed with a familiar person. Although it was an unfamiliar stranger who petted the dog in the AAA, the dog was still always in the presence of the handler. A difference may have been seen if the dog interacted with the stranger alone, without the handler. In addition, the location where the dog was petted, which was not standardized in this study, may have had an influence on the dog's behavioral response (Fatjó et al., 2007; Kuhne et al., 2012).

There were few significant correlations between observed behavior and salivary cortisol. Sitting behavior was positively correlated with salivary cortisol in AS and NS settings at time 30 and time 90, respectively. This is further supported by the fact that standing was negatively correlated with salivary cortisol in AS at time 30. This was an unexpected finding because it is reasonable to believe that lower cortisol levels would be associated with higher percentage of sitting because the dog would be more relaxed and feel secure in its environment enough to sit. However, the opposite was found since the more time the dog spent sitting, the higher cortisol levels were. It is suspected that the handler influenced the sitting and standing of the dogs in these settings. In these environments, the handlers had a tendency to command the dog to sit to be petted. If the dog was more inclined or more comfortable with standing or walking, being instructed to sit could have been restricting or frustrating for the dogs, and thus be more physiologically stress inducing under these circumstances. Since the dogs likely stood out of their own volition without being commanded to do so by the handler, it may have been less restricting and frustrating for the dog, resulting in a decrease in cortisol. Furthermore, it has been proposed that dogs may be more reactive or frustrated if freedom of movement is restricted (Haug, 2008). Movement was limited in the AS and

NS because the dogs were kept on leash and instructed to sit within the confines of the assigned 7 foot by 7 foot space.

In addition, salivary cortisol was positively correlated with the percentage of time the mouth was closed in a neutral position and negatively correlated with the percentage of time panting in the NS at time 60. This finding contradicts other studies which have associated panting with stress and anxiety (Beerda et al., 1997; Dreschel and Granger, 2005). However, few studies have documented a true relationship between elevated cortisol levels and increased panting (Hekman, 2012). King observed panting in AAT dogs with elevated, decreased, and no changes in levels of salivary cortisol from baseline to completion of a two hour therapy session (King et al., 2011). It is possible that panting may not always be an indication of stress, especially in the absence of other signs of stress. Increase in panting can be due to a rise in body temperature from physiological response to arousals (Hiby et al., 2006) or associated with exhaustion (Palestrini et al., 2010). Although panting can be an effect of thermoregulation, the temperature in the room was regulated and there was no correlation between panting and cortisol at other time points in the NS, so increased body heat was unlikely to be a factor in the association. The intensity of panting may play a role in association with stress. While dogs may pant heavily or have labored respiration when extremely distressed, they may also pant when in a relaxed state. These dogs were not observed to exhibit difficulty breathing. Furthermore, absence of panting may be associated with a relaxed state, but may also be associated with a degree of anxiety, as dogs can freeze when exposed to a fearful situation (Ogata et al., 2006).

Similar to other studies, the widely accepted stress-associated behaviors such as lip licking, yawning, paw lifting, etc. were not correlated with cortisol (Rooney et al., 2007). Even though the NS revealed significantly higher cortisol levels, they were not associated with increased stress associated behaviors. One study in Labrador retrievers revealed that although being pet by their owners resulted in a significant increase in plasma cortisol, all dogs displayed normal behaviors for the one hour observation (Handlin et al., 2011). It may be difficult to discern a relationship between cortisol and stress-associated behavior in response to human interaction. Despite the higher levels in the NS, it is likely that these settings were not perceived to be stressful enough to induce

a significant physiologic or behavioral stress response. Correlations between cortisol and behavior may be uncovered at high levels, but not mild or moderate levels of stress.

The few correlations found between cortisol and behavior should be interpreted cautiously because these did not persist through all settings or time points. This merely illustrates the challenge in correlating physiologic and behavioral parameters (Hansen and Jeppesen, 2006). It should be emphasized that dogs likely do not express stress-associated behavior in a standard way since there is high variability in how an animal perceives stress and how it responds to a particular stressor. Behavior is influenced by many variables, including age, breed, and past experience (Hiby et al., 2006). Variations in temperament or personality between dogs of the same demographic result in different responses to the same stimulus (Jones and Gosling, 2005; Rooney et al., 2007). In addition, the coping strategy, or the behavioral and physiologic mechanism to cope with a situation, varies greatly between individuals (Rooney et al., 2007). Furthermore, individual dogs may not express stress-associated behavior consistently at all times since behavior is not likely to be strictly associated with the animal's emotional state.

Behavior was correlated with cortisol levels 30 minutes after the behavior was observed. This was analyzed because it takes approximately 15-20 minutes for cortisol levels to increase in response to a stress event (Vincent and Michell, 1992), and an additional 4 minutes for salivary cortisol levels to equilibrate with plasma levels (Kobelt et al., 2003). Therefore, it was reasonable to assume that the cortisol level at the time behavior was observed would be more accurately depicted by salivary cortisol 30 minutes later rather than salivary cortisol taken at the same behavioral observation time point. Using this 30 minute delay, it is possible that rate at which cortisol levels increases varies between individual dogs. This variation in cortisol physiology, combined with variation in individual behavior complicates the correlation between the two variables. However, this method of correlation is more detailed and precise than other studies that correlate a single salivary cortisol value with subjective ratings of behavior (Menor-Campos et al., 2011), with behavior observed at an arbitrary time in relation to cortisol (Hekman, 2012), or with very few specific behaviors within a small time frame (Diederich and Giffroy, 2006).

This study provides practical evidence that one hour of AAA with college

students does not induce significant stress and is not likely to be detrimental to animal welfare, validating the widely accepted recommendation of limiting visits to one hour in duration (Iannuzzi, 1991). Although the current study analyzed this particular type of working dog in a specific setting, the applications can be broadly applied to other working dogs. The research application of this study proposes an original method of measuring cortisol and behavior across different environments. This standardized approach may serve as the preliminary construct of a much needed stress research tool in animal welfare (Hekman, 2012). This investigation also reports a reference range for salivary and hair cortisol in healthy AAA dogs, although variation between laboratories should be considered before direct comparison is made.

Although a few individual behaviors correlated with cortisol levels at specific time points, there was insufficient evidence to justify them as true indicators of stress because the correlations were not consistent among multiple settings and none of the settings were deemed to be sufficiently stress inducing. Until a gold standard measure of stress or distress is clearly established, behavioral observation still remains a principal method of evaluating stress in animals (Hekman, 2012), especially for the handler in a real-life setting. An individual AAA session cannot realistically rely on the overseeing organization or facility to monitor and enforce protocols, which leaves the handler with the responsibility of attending to the animal's needs. Therefore, it is imperative to the welfare of the animal that the handler be properly educated on prevention, recognition, and management of stress associated behavior in his or her dog (Mariti et al., 2012).

Since humans can influence the stress response in animals, it is recommended that handlers implement methods to prevent and minimize stress in their AAA dogs. Affiliative interactions such as positive playing or petting initiated by the handler has been shown to reduce stress levels in the dog (Jones and Gosling, 2005). The handler is key to the success of the dog because dogs demonstrate the best cognitive performance and responsiveness in the presence of the most familiar human in a familiar setting (Topal, 1997). In the current study, being petted by the handler did not appear to influence cortisol levels since they remained elevated in the NS. However, we believe that this elevation could have been due to the unpredictability of the setting. Predictable or purposeful activity, such as human interaction with a stranger in a busy and

stimulating environment, was less physiologically stimulating than an unpredictable activity in a quiet environment. The handler may be able to foster a more predictable environment by initiating familiar activities such as positive play to distract the dog from the environment and focus the attention on the positive interaction.

Another practical research application of this study is that the physical location and environment of the session may influence physiologic and behavioral indicators of stress based on the finding that the NS was not necessarily "neutral" to the dog. The environment in which the AAA occurs should be considered before attributing significant changes exclusively to the human interaction.

Published protocols detailing how to conduct an AAA session are lacking. This protocol for an AAA session involving multiple dogs in a college "study break" setting was deemed safe and appropriate because no adverse events occurred and student participants subjectively enjoyed the session. Most importantly, the welfare of the dogs was not compromised since neither cortisol levels nor behavior changed significantly over time and was not different from a comparable protocol at home. The essential component of the protocol was effectively orienting all student participants on the appropriate rules of conduct in an AAA prior to the session and displaying written guidelines in the room. The specific guidelines directed participants how to safely greet the dogs, to not crowd a dog, and to comply with instruction given by the handler. In addition, alcohol based hand sanitizer was also made readily available in the setting, which participants utilized frequently between dogs. The dogs were kept on leash and evenly spaced within the room with fresh water available, which was consumed by most dogs within the hour. All participants were compliant with the regulations of the session and respectful of the dogs.

There were a number of limitations to this study. Not only was the study size small with significant variation in demographic factors of breed, age, and weight, but the participants were not randomly selected. The handlers were recruited as a convenience sample for the first available teams that met criteria and were able to adhere to the time-intensive requirements of the protocol, which restricted many people from participating. This method of recruitment likely selected for owners that were highly motivated and more bonded to their animals than the average pet owner (Dreschel and Granger, 2005).

It would have been more appropriate to randomly select dog-handler teams from an approved AAA organization's registry. In addition, the level of familiarity with the this particular type of AAA varied between dogs, as some had participated in this college break setting previously while it was a novel situation for others. This could have had an impact on outcomes had a uniform population of dogs that perceived this particular AAA to be novel or familiar.

Although the AAA dogs in this study had higher cortisol levels than what has been previously reported, it is unknown whether this elevation was due to laboratory variation, sampling techniques, or represented true values. It would have been ideal to compare these cortisol values to those collected from a randomized population of inexperienced, non-working dog population utilized for HAI. However, randomly selecting dogs from a population could pose a safety risk to strangers interacting with the dogs. Some dogs may be aggressive towards humans or be truly stressed by and avoid human interaction (Donaldson, 2005). AAA dogs are selected because of their ability to interact with humans without showing fear, aggression, or stress. Therefore, it would have been impractical to gather this information since serious precautions need to be considered before attempting to challenge a non-trained, inexperienced dog in the context of an AAA.

The behavioral analysis was dependent on the single perspective of one camera, which was operated by multiple research assistants. Therefore, the angle of the video camera varied for the dogs in this study. Some behaviors were likely missed because of the particular angle or focus of the camera. Additional inherent limitations of videotaping included the dog moving out of the frame and inadvertent obstruction of view by participants or objects. Although other behaviors may have been observed with live observation (Hekman 2012), many other subtle behaviors could have been missed. Furthermore, only three, five-minute standard intervals of observation were analyzed in each session. While analysis of the entire duration (120 minutes) of each session would have been ideal, it would have been exceedingly labor intensive and impractical. Using this method permitted more frequent, effective, and objective assessment of behavior. However, any extraordinary events during the session were recorded by the assistant and accounted for in the analysis.

This study provides the platform for future investigations of animal welfare in AAA. Future work should randomly select dogs while controlling for demographic factors such as age, sex, and breed by recruiting dogs randomly from a service dog organization. In addition, it is important to control for and compare certain aspects of AAA, such as the demographic of the population served (children versus geriatrics). The effect of the location of AAA, whether it be hospital, long-term assisted living center, or school, on physiologic and behavioral variables will be valuable. Prospective studies should also attempt to determine the maximum duration at which cortisol level or behavioral signs warrant termination of the session. The influence of frequency and intervals of AAA in addition to breaks within AAA on animal welfare is important as well. Finally, research should also compare the response of AAA dogs in the AAA to their responses to a known stressor, such as kenneling, exposure to loud sounds, or procedures at the veterinary hospital.

To strengthen the assessment of animal welfare, cortisol and behavior should be interpreted with other physiologic parameters influenced by stress such as heart rate variability (Vaisanen et al., 2005; Bergamasco, 2010), salivary IgA (Skandakumar et al., 1995; Kikkawa et al., 2003), and neutrophil to lymphocyte ratios (Beerda et al., 1999a). Although hair cortisol was not determined to be a measure of salivary cortisol, this method warrants further investigation.

5. Conclusions

A one-hour AAA session for college students does not elicit significant physiologic or behavioral stress, and thus is not likely to negatively impact welfare of registered AAA dogs. Hair cortisol is not an appropriate substitute for assessing salivary cortisol in AAA dogs. Although stress-associated behavior did not correlate with cortisol, it is still important that the handler closely monitor the behavior of the dog in these situations and consider that the environment plays a role in the stress response in dogs. Further investigation should explore the effect of various kinds of AAA on additional parameters of stress and welfare.

REFERENCES

Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., Seren, E., 2008. Cortisol determination in hair and faeces from domestic cats and dogs. Gen Comp Endocrinol 155, 398-402.

Adams, G.J., Johnson, K.G., 1995. Guard dogs: sleep, work and the behavioural responses to people and other stimuli. Applied Animal Behaviour Science 46, 103-115.

Antonites, A., Odendaal, J., 2004. Ethics in human-animal relationships. Acta Veterinaria Brno 73, 539-None.

AVMA, 1998. Human-animal bond issues. J Am Vet Med Assoc 212, 1675.

Bain, M.J., Hart, B.L., Cliff, K.D., Ruehl, W.W., 2001. Predicting behavioral changes associated with age-related cognitive impairment in dogs. J Am Vet Med Assoc 218, 1792-1795.

Banks, E.M., 1982. Behavioral research to answer questions about animal welfare. J Anim Sci 54, 434-446.

Banks, M.R., Willoughby, L.M., Banks, W.A., 2008. Animal-assisted therapy and loneliness in nursing homes: use of robotic versus living dogs. J Am Med Dir Assoc 9, 173-177.

Baun, M.M., Bergstrom, N., Langston, N.F., Thoma, L., 1984. Physiological effects of human/companion animal bonding. Nursing Research; Nursing Research.

Baxter, D.N., Leck, I., 1984. The deleterious effects of dogs on human health: 2. Canine zoonoses. Community medicine 6, 185-197.

Beck, A.M., Katcher, A.H., 1984. A new look at pet-facilitated therapy. J Am Vet Med Assoc 184, 414-421.

Beerda, B., Schilder, M.B., Bernadina, W., van Hooff, J.A., de Vries, H.W., Mol, J.A., 1999a. 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., Janssen, N.S., Mol, J.A., 1996. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. Horm Behav 30, 272-279.

Beerda, B., Schilder, M.B., van Hooff, J.A., de Vries, H.W., Mol, J.A., 1999b. Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. Physiol Behav 66, 233-242.

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. Applied Animal Behaviour Science 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. Applied Animal Behaviour Science 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. Animal Welfare 9, 49-62.

Belpedio, C., Buffington, L., Clusman, C., Prete, F., Sadler, A., Whittemore, L., Mungre, S., 2010. Effect of multidog play groups on cortisol levels and behavior of dogs (Canis lupus familiaris) housed in a humane society. . J. Appl. Compan. Anim. Behav. 4, 15–27.

Bennett, A., Hayssen, V., 2010. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences. Domest Anim Endocrinol 39, 171-180.

Benton, L.A., Yates, F.E., 1990. Ultradian adrenocortical and circulatory oscillations in conscious dogs. Am J Physiol 258, R578-590.

Bergamasco, L., Osella, MC, Savarino, P, Larosa, G, Ozella, L, Manassero, M, Badino, P, Odore, R, Barbero, R, Re, G, 2010. Heart rate variability and saliva cortisol assessment in shelter dog: Human-animal interaction effects. Applied Animal Behaviour Science 125, 56-68.

Bergeron, R., Scott, S.L., Emond, J.P., Mercier, F., Cook, N.J., Schaefer, A.L., 2002. Physiology and behavior of dogs during air transport. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 66, 211-216.

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. Applied Animal Behaviour Science 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. Physiology & Behavior 92, 375-397.

Briegel, J., Sprung, C.L., Annane, D., Singer, M., Keh, D., Moreno, R., Mohnle, P., Weiss, Y., Avidan, A., Brunkhorst, F.M., Fiedler, F., Vogeser, M., 2009. Multicenter comparison of cortisol as measured by different methods in samples of patients with septic shock. Intensive Care Med 35, 2151-2156.

Broom, D.M., 1991a. Animal welfare: concepts and measurement. J Anim Sci 69, 4167-4175.

Broom, D.M., 1991b. Assessing welfare and suffering. Behavioural Processes 25, 117.

Broom, D.M., 1996. Animal welfare defined in terms of attempts to cope with the environment. Acta Agriculturae Scandinavica Section A Animal Science, Supplement 27, 22-28.

Broom, D.M., 1999. Animal welfare: the concept of the issues, in: Dolins, F. (Ed.), Attitudes to Animals, Cambridge University Press, Cambridge, pp. 129-142.

Broom, D.M., Johnson, K.G., 1993. Stress and animal welfare. Springer, London.

Butler, K., 2004. Therapy Dogs Today: Their Gifts, Our Obligation. Funpuddle Publishing, Norman, OK.

Casey, R., 2002. Fear and stress, in: Horwitz, D.F., Mills, D.S., Heath, S. (Eds.), BSAVA Manual of Canine and Feline Behavioural Medicine, British Small Animal Veterinary Association, Dorset, UK, pp. 144-153.

Casey, R.A., 2004. Mechanisms and consequences of fear and stress in dogs and cats, 10th Eur. Congr. on Companion Animal Behav- ioural Medicine, Cremona, Italy, pp. 70-73.

Castillo, V.A., Cabrera Blatter, M.F., Gomez, N.V., Sinatra, V., Gallelli, M.F., Ghersevich, M.C., 2009. Diurnal ACTH and plasma cortisol variations in healthy dogs and in those with pituitary-dependent Cushing's syndrome before and after treatment with retinoic acid. Res Vet Sci 86, 223-229.

Chrousos, G.P., Gold, P.W., 1992. The Concepts of Stress and Stress System Disorders. JAMA: The Journal of the American Medical Association 267, 1244-1252.

Chur-Hansen, A., Stern, C., Winefield, H., 2010. Gaps in the evidence about companion animals and human health: some suggestions for progress. International journal of evidence-based healthcare 8, 140-146.

Clark, J.D., Rager, D.R., Crowell-Davis, S., Evans, D.L., 1997. Housing and exercise of dogs: effects on behavior, immune function, and cortisol concentration. Lab Anim Sci 47, 500-510.

Colby, P., Sherman, A., 2002. Attachment styles impact on pet visitation effectiveness. Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals 15, 150-165.

Cook, C.J., 2002. Glucocorticoid feedback increases the sensitivity of the limbic system to stress. Physiol Behav 75, 455-464.

Cook, N., Schaefer, A., Lepage, P., Jones, S.M., 1996. Salivary vs. serum cortisol for the assessment of adrenal activity in swine. Canadian Journal of Animal Science 76, 329-335.

Cook, N.J., Schaefer, A.L., Lepage, P., Jones, S.D.M., 1997. Radioimmunoassay for cortisol in pig saliva and serum. Journal of agricultural and food chemistry 45, 395-399.

Coppinger, R., Coppinger, L., Skillings, E., 1998. Observations on assistance dog training and use. J Appl Anim Welf Sci 1, 133-144.

Coppinger, R., Zuccotti, J., 1999. Kennel Enrichment: Exercise and Socialization of Dogs. Journal of Applied Animal Welfare Science 2, 281-296.

Coppola, C.L., Grandin, T., Enns, R.M., 2006. Human interaction and cortisol: can human contact reduce stress for shelter dogs? Physiol Behav 87, 537-541.

Cronin, G.M., Wiepkema, P.R., van Ree, J.M., 1985. Endogenous opioids are involved in abnormal stereotyped behaviours of tethered sows. Neuropeptides 6, 527-530.

Dantzer, R., 1986. Behavioral, physiological and functional aspects of stereotyped behavior: a review and a re-interpretation. J Anim Sci 62, 1776-1786.

Davenport, M.D., Tiefenbacher, S., Lutz, C.K., Novak, M.A., Meyer, J.S., 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. General and Comparative Endocrinology 147, 255-261.

Davis, K.D., 1992. Therapy Dog: Training Your Dog to Reach Others. Macmillan, New York, NY.

Dawkins, M.S., 2006. A user's guide to animal welfare science. Trends in Ecology & Evolution 21, 77-82.

DeltaSociety, 2012. Student Guide Pet Partners Handler Course. Delta Society, Bellvue, WA.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol Bull 130, 355-391.

Diederich, C., Giffroy, J.-M., 2006. Behavioural testing in dogs: a review of methodology in search for standardisation. Applied Animal Behaviour Science 97, 51-72.

Donaldson, J., 2005. The culture clash. Dogwise Publishing, US.

Dreschel, N.A., 2010. The effects of fear and anxiety on health and lifespan in pet dogs. Applied Animal Behaviour Science 125, 157-162.

Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. Applied Animal Behaviour Science 95, 153-168.

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

Engeland, W.C., Gann, D.S., 1989. Adrenal medullary and adrenal cortical secretory responses to haemorrhage and to acoustic stimuli in awake dogs, in: Van Loom, G.R.,

Kvetnansky, R., McCarty, R., Axelrod, J. (Eds.), Stress: Neurochemical and Humoral Mechanisms, Gordon & Breach, New York, pp. 613-625.

Enoch, D., Karas, J., Slater, J., Emery, M., Kearns, A., Farrington, M., 2005. MRSA carriage in a pet therapy dog. The Journal of hospital infection 60, 186-188.

Evans, N., Gray, C., 2012. The Practice and Ethics of Animal-Assisted Therapy with Children and Young People: Is It Enough that We Don't Eat Our Co-Workers? British Journal of Social Work 42, 600-617.

Fallani, G., Prato Previde, E., Valsecchi, P., 2007. Behavioral and physiological responses of guide dogs to a situation of emotional distress. Physiology & Behavior 90, 648-655.

Fatjó, J., Feddersen-Petersen, D., Ruiz de la Torre, J.L., Amat, M., Mets, M., Braus, B., Manteca, X., 2007. Ambivalent signals during agonistic interactions in a captive wolf pack. Applied Animal Behaviour Science 105, 274-283.

FAWC, 2009. Farm Animal Welfare Council - 5 Freedoms.

Fejsáková, M., Kottferová, J., Mareková, J., Jakuba, T., Ondrašovičová, O., Ondrašovič, M., 2009. Ethical aspects related to involvement of animals in animal assisted therapy, 52nd Student Scientific Conference, Kosice, Slovakia, 28 April 2009., University of Veterinary Medicine, pp. 62-64.

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

Fraser, A.F., Broom, D.M., 1997. Farm animal behaviour and welfare. CAB International, Wallingford, OX10 8DE.

Friedmann, E., Son, H., 2009. The human-companion animal bond: how humans benefit. Vet Clin North Am Small Anim Pract 39, 293-326.

Fureix, C., Menguy, H., Hausberger, M., 2010. Partners with bad temper: reject or cure? A study of chronic pain and aggression in horses. PLoS One 5, e12434.

Gacsi, M., Topal, J., Miklosi, A., Doka, A., Csanyi, V., 2001. Attachment behavior of adult dogs (Canis familiaris) living at rescue centers: forming new bonds. J Comp Psychol 115, 423-431.

Gandolfi-Decristophoris, P., De Benedetti, A., Petignat, C., Attinger, M., Guillaume, J., Fiebig, L., Hattendorf, J., Cernela, N., Regula, G., Petrini, O., 2012. Evaluation of pet contact as a risk factor for carriage of multidrug-resistant staphylococci in nursing home residents. American Journal of Infection Control 40, 128-133.

Ganong, W.F., Ganong, W., 2005. Review of medical physiology. McGraw-Hill Medical ^ eNew York New York.

Gantt, W.H., Newton, J.E., Royer, F.L., Stephens, J.H., 1966. Effect of person. Integr Physiol Behav Sci 26, 145-160.

Garnier, F., Benoit, E., Virat, M., Ochoa, R., Delatour, P., 1990. Adrenal cortical response in clinically normal dogs before and after adaptation to a housing environment. Lab Anim 24, 40-43.

Glenk, L.M., Stetina, B.U., Kepplinger, B., Baran, H., 2011. Salivary cortisol, heart rate variability and behavioral assessment in dogs during animal-assisted interventions (AAI) in neuropsychiatry. J Vet Behav 6, 81-82.

Gold, P.W., Goodwin, F.K., Chrousos, G.P., 1988. Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress (2). N Engl J Med 319, 413-420.

Grandgeorge, M., Hausberger, M., 2011. Human-animal relationships: from daily life to animal-assisted therapies. Ann Ist Super Sanita 47, 397-408.

Granger, D.A., Cicchetti, D., Rogosch, F.A., Hibel, L.C., Teisl, M., Flores, E., 2007. Blood contamination in children's saliva: prevalence, stability, and impact on the measurement of salivary cortisol, testosterone, and dehydroepiandrosterone. Psychoneuroendocrinology 32, 724-733.

Guay, D.R.P., 2001. Pet-assisted therapy in the nursing home setting: potential for zoonosis. American Journal of Infection Control 29, 178-186.

Gunaratnam, P., Wilkinson, G., 1983. A study of normal hair growth in the dog. Journal of Small Animal Practice 24, 445-453.

Hamel, A.F., Meyer, J.S., Henchey, E., Dettmer, A.M., Suomi, S.J., Novak, M.A., 2011. Effects of shampoo and water washing on hair cortisol concentrations. Clin Chim Acta 412, 382-385.

Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdeb, ck, M., Jansson, A., Uvn, s-Moberg, K., 2011. Short-Term Interaction between Dogs and Their Owners: Effects on Oxytocin, Cortisol, Insulin and Heart RateAn Exploratory Study. Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals 24, 301-315.

Handlin, L., Nilsson, A., Ejdeback, M., Hydbring-Sandberg, E., Uvnas-Moberg, K., 2012. Associations between the Psychological Characteristics of the Human-Dog Relationship and Oxytocin and Cortisol Levels. Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals 25, 215-228.

Hansen, A.M., Garde, A.H., Persson, R., 2008. Measurement of salivary cortisol--effects of replacing polyester with cotton and switching antibody. Scandinavian journal of clinical and laboratory investigation 68, 826-829.

Hansen, S.W., Jeppesen, L.L., 2006. Temperament, stereotypies and anticipatory behaviour as measures of welfare in mink. Applied Animal Behaviour Science 99, 172-182.

Hatch, A., 2007. The View from All Fours: A Look at an Animal-Assisted Activity Program from the Animals' Perspective. Anthrozoos 20, 37-50.

Haubenhofer, D., Mostl, E., Kirchengast, S., 2005. Cortisol concentrations in saliva of humans and their dogs during intensive training courses in animal-assisted therapy. Wiener Tierarztliche Monatsschrift 92, 66-73.

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, vi.

Haverbeke, A., Diederich, C., Depiereux, E., Giffroy, J.M., 2008. Cortisol and behavioral responses of working dogs to environmental challenges. Physiol Behav 93, 59-67.

Head, E., Callahan, H., Cummings, B.J., Cotman, C.W., Ruehl, W.W., Muggenberg, B.A., Milgram, N.W., 1997. Open Field Activity and Human Interaction as a Function of Age and Breed in Dogs. Physiology & Behavior 62, 963-971.

Heim, C., Ehlert, U., Hellhammer, D.H., 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25, 1-35.

Heimlich, K., 2001. Animal-assisted therapy and the severely disabled child: a quantitative study. Journal of Rehabilitation 67, 48-54.

Hekman, J.P., 2012. Salivary cortisol concentrations and behavior in a population of healthy dogs hospitalized for elective procedures. Applied Animal Behaviour Science 141, 149-157.

Hennessy, M.B., Davis, H.N., Williams, M.T., Mellott, C., Douglas, C.W., 1997. Plasma cortisol levels of dogs at a county animal shelter. Physiol Behav 62, 485-490.

Hennessy, M.B., T. Williams, M., Miller, D.D., Douglas, C.W., Voith, V.L., 1998. Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public animal shelter? Applied Animal Behaviour Science 61, 63-77.

Hennessy, M.B., Voith, V.L., Hawke, J.L., Young, T.L., Centrone, J., McDowell, A.L., Linden, F., Davenport, G.M., 2002a. 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.

Hennessy, M.B., Voith, V.L., Mazzei, S.J., Buttram, J., Miller, D.D., Linden, F., 2001. Behavior and cortisol levels of dogs in a public animal shelter, and an exploration of the ability of these measures to predict problem behavior after adoption. Appl Anim Behav Sci 73, 217-233.

Hennessy, M.B., Voith, V.L., Young, T.L., Hawke, J.L., Centrone, J., McDowell, A.L., Linden, F., Davenport, G.M., 2002b. Exploring human interaction and diet effects on the behavior of dogs in a public animal shelter. J Appl Anim Welf Sci 5, 253-273.

Henry, J., Stephens, P., 1977. Stress, Health, And The Social Environment: A Sociobiologic Approach To Medicine (Topics In Environmental Physiology And.

Hessing, M.J.C., Hagels¯, A.M., van Beek, J.A.M., Wiepkema, R.P., Schouten, W.G.P., Krukow, R., 1993. Individual behavioural characteristics in pigs. Applied Animal Behaviour Science 37, 285-295.

Hetts, S., Clark, J. D., Arnold, C. E., Mateo, J. M., 1992. Influence of housing conditions on beagle behaviour. Appl Anim Behav Sci 34, 137-155.

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.

Holst, S., Lund, I., Petersson, M., Uvnas-Moberg, K., 2005. Massage-like stroking influences plasma levels of gastrointestinal hormones, including insulin, and increases weight gain in male rats. Autonomic neuroscience : basic & clinical 120, 73-79.

Horvath, Z., Doka, A, Miklosi, A, 2008. Affiliative and disciplinary behavior of human handlers during play with their dog affects cortisol concentrations in opposite directions. Hormones and Behavior 54, 107-114.

Horvath, Z., Igyarto, B.Z., Magyar, A., Miklosi, A., 2007. Three different coping styles in police dogs exposed to a short-term challenge. Horm Behav 52, 621-630.

Hubrecht, R.C., Serpell, J.A., Poole, T.B., 1992. Correlates of pen size and housing conditions on the behaviour of kennelled dogs. Applied Animal Behaviour Science 34, 365-383.

Hydbring-Sandberg, E., von Walter, L.W., Hoglund, K., Svartberg, K., Swenson, L., Forkman, B., 2004. Physiological reactions to fear provocation in dogs. J Endocrinol 180, 439-448.

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

Janssens, C.J., Helmond, F.A., Loyens, L.W., Schouten, W.G., Wiegant, V.M., 1995. Chronic stress increases the opioid-mediated inhibition of the pituitary-adrenocortical response to acute stress in pigs. Endocrinology 136, 1468-1473.

Johnson, E.O., Kamilaris, T.C., Chrousos, G.P., Gold, P.W., 1992. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. Neurosci Biobehav Rev 16, 115-130.

Jones, A.C., Gosling, S.D., 2005. Temperament and personality in dogs (Canis familiaris): A review and evaluation of past research. Applied Animal Behaviour Science 95, 1-53.

Jones, A.C., Josephs, R.A., 2006. Interspecies hormonal interactions between man and the domestic dog (Canis familiaris). Horm Behav 50, 393-400.

Karlen, J., Ludvigsson, J., Frostell, A., Theodorsson, E., Faresjo, T., 2011. Cortisol in hair measured in young adults - a biomarker of major life stressors? BMC clinical pathology 11, 12.

Kemppainen, R.J., Sartin, J.L., 1984. Evidence for episodic but not circadian activity in plasma concentrations of adrenocorticotrophin, cortisol and thyroxine in dogs. J Endocrinol 103, 219-226.

Kikkawa, A., Uchida, Y., Nakade, T., Taguchi, K., 2003. Salivary secretory IgA concentrations in beagle dogs. J Vet Med Sci 65, 689-693.

King, C., Watters, J., Mungre, S., 2011. Effect of a time-out session with working animal-assisted therapy dogs. Journal of Veterinary Behavior: Clinical Applications and Research 6, 232-238.

King, T., Hemsworth, P., Coleman, G., 2003. Fear of novel and startling stimuli in domestic dogs. Applied Animal Behaviour Science 82, 45-64.

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

Kobelt, A.J., Hemsworth, P.H., Barnett, J.L., Butler, K.L., 2003. Sources of sampling variation in saliva cortisol in dogs. Res Vet Sci 75, 157-161.

Koivusilta, L.K., Ojanlatva, A., 2006. To Have or Not To Have a Pet for Better Health? PLoS One 1, e109.

Koolhaas, J., Meerlo, P., De Boer, S., Strubbe, J., Bohus, B., 1997. The temporal dynamics of the stress response. Neuroscience & Biobehavioral Reviews 21, 775-782.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 23, 925-935.

Koren, L., Mokady, O., Karaskov, T., Klein, J., Koren, G., Geffen, E., 2002. A novel method using hair for determining hormonal levels in wildlife. Animal Behaviour 63, 403.

Kotrschal, K., Schoberl, I., Bauer, B., Thibeaut, A.M., Wedl, M., 2009. Dyadic relationships and operational performance of male and female owners and their male dogs. Behav Processes 81, 383-391.

Koyama, T., Omata, Y., Saito, A., 2003. Changes in salivary cortisol concentrations during a 24-hour period in dogs. Horm Metab Res 35, 355-357.

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. Applied Animal Behaviour Science 142, 176-181.

Kurosawa, M., Lundeberg, T., Agren, G., Lund, I., Uvnas-Moberg, K., 1995. Massage-like stroking of the abdomen lowers blood pressure in anesthetized rats: influence of oxytocin. J Auton Nerv Syst 56, 26-30.

Kurrle, S.E., Day, R., Cameron, I.D., 2004. The perils of pet ownership: a new fall-injury risk factor. Med J Aust 181, 682-683.

Ladewig, J., Smidt, D., 1989. Behavior, episodic secretion of cortisol, and adrenocortical reactivity in bulls subjected to tethering. Horm Behav 23, 344-360.

LeBeau, M.A., Montgomery, M.A., Brewer, J.D., 2011. The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair. Forensic science international 210, 110-116.

Lee, P., 1984. Ecological constraints on the social development of vervet monkeys. Behaviour, 245-262.

Lefebvre, D., Giffroy, J.M., Diederich, C., 2009a. Cortisol and behavioral responses to enrichment in military working dogs. Journal of ethology 27, 255-265.

Lefebvre, S.L., Golab, G.C., Christensen, E.L., Castrodale, L., Aureden, K., Bialachowski, A., Gumley, N., Robinson, J., Peregrine, A., Benoit, M., Card, M.L., Van Horne, L., Weese, J.S., 2008. Guidelines for animal-assisted interventions in health care facilities. American Journal of Infection Control 36, 78-85.

Lefebvre, S.L., Reid-Smith, R.J., Waltner-Toews, D., Weese, J.S., 2009b. Incidence of acquisition of methicillin-resistant Staphylococcus aureus, Clostridium difficile, and other health-care-associated pathogens by dogs that participate in animal-assisted interventions. J Am Vet Med Assoc 234, 1404-1417.

Lefebvre, S.L., Waltner-Toews, D., Peregrine, A.S., Reid-Smith, R., Hodge, L., Arroyo, L.G., Weese, J.S., 2006. Prevalence of zoonotic agents in dogs visiting hospitalized people in Ontario: implications for infection control. Journal of Hospital Infection 62, 458-466.

Li, J., Xie, Q., Gao, W., Xu, Y., Wang, S., Deng, H., Lu, Z., 2012. Time course of cortisol loss in hair segments under immersion in hot water. Clin Chim Acta 413, 434-440.

Ligout, S., 2010. Reliability of salivary cortisol measures in dogs in training context. Journal of veterinary behavior 5, 49.

Liptrap, R.M., 1993. Stress and reproduction in domestic animals. Ann N Y Acad Sci 697, 275-284.

Lit, L., Boehm, D., Marzke, S., Schweitzer, J., Oberbauer, A.M., 2010. Certification testing as an acute naturalistic stressor for disaster dog handlers. Stress 13, 392-401.

Lund, I., Ge, Y., Yu, L.C., Uvnas-Moberg, K., Wang, J., Yu, C., Kurosawa, M., Agren, G., Rosen, A., Lekman, M., Lundeberg, T., 2002. Repeated massage-like stimulation induces long-term effects on nociception: contribution of oxytocinergic mechanisms. Eur J Neurosci 16, 330-338.

Lund, I., Lundeberg, T., Kurosawa, M., Uvnäs-Moberg, K., 1999. Sensory stimulation (massage) reduces blood pressure in unanaesthetized rats. Journal of the autonomic nervous system 78, 30-37.

Lund, J.D., Jørgensen, M.C., 1999. Behaviour patterns and time course of activity in dogs with separation problems. Applied Animal Behaviour Science 63, 219-236.

Maestripieri, D., Schino, G., Aureli, F., Troisi, A., 1992. A modest proposal: displacement activities as an indicator of emotions in primates. Animal Behaviour 44, 967-979.

Marcus, D.A., 2012. Adding Therapy Dogs to Your Cancer Treatment Team, Therapy Dogs in Cancer Care, Springer, pp. 77-101.

Marinelli, L., Normando, S., Siliprandi, C., Salvadoretti, M., Mongillo, P., 2009. Dog assisted interventions in a specialized centre and potential concerns for animal welfare. Vet Res Commun 33 Suppl 1, 93-95.

Marino, L., 2012. Construct Validity of AnimalAssisted Therapy and Activities: How Important Is the Animal in AAT? Anthrozoos: A Multidisciplinary Journal of The Interactions of People & Animals 25, 139-151.

Mariti, C., Gazzano, A., Moore, J.L., Baragli, P., Chelli, L., Sighieri, C., 2012. Perception of dogs' stress by their owners. Journal of Veterinary Behavior: Clinical Applications and Research.

Marshall-Pescini, S., Passalacqua, C., Barnard, S., Valsecchi, P., Prato-Previde, E., 2009. Agility and search and rescue training differently affects pet dogs' behaviour in socio-cognitive tasks. Behavioural Processes 81, 416-422.

Mason, G.J., 1991. Stereotypies: a critical review. Animal Behaviour 41, 1015-1037.

Mason, G.J., Latham, N. R., 2004. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Animal Welfare 13, 57-69.

Mastorakos, G., Pavlatou, M., Diamanti-Kandarakis, E., Chrousos, G.P., 2005. Exercise and the stress system. Hormones (Athens) 4, 73-89.

McConnell, P.B., 1990. Acoustic structure and receiver response in domestic dogs,< i> Canis familiaris</i>. Animal Behaviour 39, 897-904.

McEwen, B.S., 1998. Protective and damaging effects of stress mediators. N Engl J Med 338, 171-179.

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

McGreevy, P.D., Starling, M., Branson, N.J., Cobb, M.L., Calnon, D., 2012. An overview of the dog–human dyad and ethograms within it. Journal of Veterinary Behavior: Clinical Applications and Research 7, 103-117.

Mench, J.A., Mason, G.J., 1997. Behaviour, in: Appleby, M.C., Hughes, B.O. (Eds.), Animal Welfare, CAB International, Oxon.

Menor-Campos, D.J., Molleda-Carbonell, J.M., Lopez-Rodriguez, R., 2011. Effects of exercise and human contact on animal welfare in a dog shelter. Vet Rec 169, 388.

Meyer, J.S., Novak, M.A., 2012. Minireview: Hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. Endocrinology 153, 4120-4127.

Michelazzi, M., Besana, F., Santarato, D., Giudici, P., Verga, M., 2007. AAA and AAT projects in a geriatric institute: effects on the patients welfare, 6th Int. Veter. Behav. Meet., Riccione, Italy., pp. 92-93.

Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychological bulletin 133, 25.

Moberg, G., 2000. Biological response to stress: implications for animal welfare, in: Moberg, G.P., Mench, J.A. (Eds.), The Biology of Animal Stress, CAB International, Oxford, UK, pp. 1-21.

Mostl, E., Palme, R., 2002. Hormones as indicators of stress. Domest Anim Endocrinol 23, 67-74.

Netto, W.J., Planta, D.J.U., 1997. Behavioural testing for aggression in the domestic dog. Applied Animal Behaviour Science 52, 243-263.

Normando, S., Corain, L., Salvadoretti, M., Meers, L., Valsecchi, P., 2009. Effects of an Enhanced Human Interaction Program on shelter dogs' behaviour analysed using a novel nonparametric test. Applied Animal Behaviour Science 116, 211-219.

Novak, M.A., Drewsen, K. H., 1989. Enriching the lives of captive primates: issues and problems, in: Segal, E.F. (Ed.), Housing, Care, and Psychological Wellbeing of Captive and Laboratory Primates, Noyes, Park Ridge, NJ, USA, pp. 161-185.

Odendaal, J., 2005. Science-based assessment of animal welfare: companion animals. Revue scientifique et technique-Office international des épizooties 24, 493.

Odendaal, J.S., Meintjes, R.A., 2003. Neurophysiological correlates of affiliative behaviour between humans and dogs. Vet J 165, 296-301.

Ogata, N., Kikusui, T., Takeuchi, Y., Mori, Y., 2006. Objective measurement of fear-associated learning in dogs. Journal of Veterinary Behavior: Clinical Applications and Research 1, 55-61.

Ojeda, S.R., Griffin, J.E., 1996. Organization of the endocrine system. Textbook of Endocrine Physiology 1, 3-16.

Overall, K.L., 1997. Normal canine behavior, in: Overall, K.L. (Ed.), Clinical Behavioral Medicine for Small Animals, Mosby, St. Louis, pp. 9-44.

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.

Palazzolo, D.L., Quadri, S.K., 1987. The effects of aging on the circadian rhythm of serum cortisol in the dog. Exp Gerontol 22, 379-387.

Palestrini, C., Minero, M., Cannas, S., Rossi, E., Frank, D., 2010. Video analysis of dogs with separation-related behaviors. Applied Animal Behaviour Science 124, 61-67.

Palestrini, C., Riva, J., Verga, M., 2005. Evaluation of the owner's influence on dogs' behavioural and physiological reactions during the clinical examination, Current Issues in Veterinary Behavioural Medicine Proceedings of the 5th International Veterinary Behavioural Meeting, Purdue University Press.

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

Pastore, C., Pirrone, F., Balzarotti, F., Faustini, M., Pierantoni, L., Albertini, M., 2011. Evaluation of physiological and behavioral stress-dependent parameters in agility dogs. Journal of Veterinary Behavior: Clinical Applications and Research 6, 188-194.

Pettijohn, T.F., Wong, T., Ebert, P., Scott, J., 1977. Alleviation of separation distress in 3 breeds of young dogs. Developmental psychobiology 10, 373-381.

Phear, D.N., 1996. A study of animal companionship in a day hospice. Palliative medicine 10, 336-338.

Piva, E., Liverani, V., Accorsi, P.A., Sarli, G., Gandini, G., 2008. Welfare in a shelter dog rehomed with Alzheimer patients. Journal of Veterinary Behavior: Clinical Applications and Research 3, 87-94.

Prato-Previde, E., Fallani, G., Valsecchi, P., 2006. Gender Differences in Owners Interacting with Pet Dogs: An Observational Study. Ethology 112, 64-73.

Preziosi, R., 1997. For your consideration: A pet-assisted therapist facilitator code of ethics. The Latham Letter, 5-6.

Pruessner, J.C., Hellhammer, D.H., Kirschbaum, C., 1999. Burnout, perceived stress, and cortisol responses to awakening. Psychosom Med 61, 197-204.

Range, F., Horn, L., Bugnyar, T., Gajdon, G.K., Huber, L., 2009. Social attention in keas, dogs, and human children. Anim Cogn 12, 181-192.

Reimers, T.J., Lawler, D.F., Sutaria, P.M., Correa, M.T., Erb, H.N., 1990. Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res 51, 454-457.

Reimers, T.J., Mummery, L.K., McCann, J.P., Cowan, R.G., Concannon, P.W., 1984. Effects of reproductive state on concentrations of thyroxine, 3,5,3'-triiodothyronine and cortisol in serum of dogs. Biol Reprod 31, 148-154.

Restituto, P., Galofre, J.C., Gil, M.J., Mugueta, C., Santos, S., Monreal, J.I., Varo, N., 2008. Advantage of salivary cortisol measurements in the diagnosis of glucocorticoid related disorders. Clinical biochemistry 41, 688-692.

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.

Rugaas, T., 1997. On talking terms with dogs: calming signals. Dogwise Publishing, Wenatchee, WA.

Russell, E., Koren, G., Rieder, M., Van Uum, S., 2012. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology 37, 589-601.

Salvin, H.E., McGreevy, P.D., Sachdev, P.S., Valenzuela, M.J., 2011. Growing old gracefully—Behavioral changes associated with "successful aging" in the dog,< i> Canis familiaris</i>. Journal of Veterinary Behavior: Clinical Applications and Research 6, 313-320.

Santori, P., 2011. Problems related to the use of animals for therapeutic and care purposes. The Document of the National Committee for Bioethics. Ann Ist Super Sanita 47, 349-352.

Sapolsky, R.M., 1996. Why stress is bad for your brain. Science 273, 749-750.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21, 55-89.

Schlotz, W., Hellhammer, J., Schulz, P., Stone, A.A., 2004. Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response. Psychosom Med 66, 207-214.

Schwizgebel, D., 1982. Zusammenhänge zwischen dem Verhalten des deutschen Schäferhundes im Hinblick auf tiergerechte Ausbildung. Aktuel. Arbeit. Artgemass. Tierh, 138-148.

Serpell, J.A., Coppinger, R., Fine, A. H., Peralta, J. M., 2010. Welfare considerations in therapy and assistance animals, in: Fine, A. (Ed.), Handbook on Animal-Assisted Therapy: Theoretical Foundations and Guidelines for Practice, Associated Press, San Diego, CA, pp. 481-503.

Servatius, R.J., Beck, K.D., 2005. Mild interoceptive stressors affect learning and reactivity to contextual cues: toward understanding the development of unexplained illnesses. Neuropsychopharmacology 30, 1483-1491.

Sherman, B.L., Mills, D.S., 2008. Canine anxieties and phobias: an update on separation anxiety and noise aversions. Vet Clin North Am Small Anim Pract 38, 1081-1106, vii.

Siniscalchi, M., McFarlane, J.R., Kauter, K.G., Quaranta, A., Rogers, L.J., 2012. Cortisol levels in hair reflect behavioural reactivity of dogs to acoustic stimuli. Res Vet Sci.

Skandakumar, S., Stodulski, G., Hau, J., 1995. Salivary IgA: a possible stress marker in dogs. Animal Welfare 4, 339-350.

Stalder, T., Steudte, S., Miller, R., Skoluda, N., Dettenborn, L., Kirschbaum, C., 2012. Intraindividual stability of hair cortisol concentrations. Psychoneuroendocrinology 37, 602-610.

Stephen, J.M., Ledger, R.A., 2005. An audit of behavioral indicators of poor welfare in kenneled dogs in the United Kingdom. J Appl Anim Welf Sci 8, 79-96.

Stephen, J.M., Ledger, R.A., 2006. A longitudinal evaluation of urinary cortisol in kennelled dogs, Canis familiaris. Physiol Behav 87, 911-916.

Stevens, R.C., Soelberg, S.D., Near, S., Furlong, C.E., 2008. Detection of cortisol in saliva with a flow-filtered, portable surface plasmon resonance biosensor system. Anal Chem 80, 6747-6751.

Takahashi, Y., Ebihara, S., Nakamura, Y., Takahashi, K., 1981. A model of human sleep-related growth hormone secretion in dogs: effects of 3, 6, and 12 hours of forced wakefulness on plasma growth hormone, cortisol, and sleep stages. Endocrinology 109, 262-272.

Taylor, S.E., Klein, L.C., Lewis, B.P., Gruenewald, T.L., Gurung, R.A., Updegraff, J.A., 2000. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. Psychol Rev 107, 411-429.

Thompson, P.G., 1997. The public health impact of dog attacks in a major Australian city. Medical Journal of Australia 167, 129-132.

Tomkins, L.M., Thomson, P.C., McGreevy, P.D., 2011. Behavioral and physiological predictors of guide dog success. Journal of Veterinary Behavior: Clinical Applications and Research 6, 178-187.

Topal, J., Miklosi, A., Csanyi, V., 1997. Dog-human relationship affects problem solving behavior in the dog. Anthrozoos 10, 214-224.

Tsuchiya, T., Nakayama, Y., Sato, A., 1991. Somatic afferent regulation of plasma corticosterone in anesthetized rats. The Japanese journal of physiology 41, 169-176.

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.

Uvnäs-Moberg, K., 1998. Oxytocin may mediate the benefits of positive social interaction and emotions. Psychoneuroendocrinology 23, 819-835.

Uvnas-Moberg, K., Alster, P., Lund, I., Lundeberg, T., Kurosawa, M., Ahlenius, S., 1996. Stroking of the abdomen causes decreased locomotor activity in conscious male rats. Physiol Behav 60, 1409-1411.

Vaisanen, M.A., Valros, A.E., Hakaoja, E., Raekallio, M.R., Vainio, O.M., 2005. Pre-operative stress in dogs - a preliminary investigation of behavior and heart rate variability in healthy hospitalized dogs. Vet Anaesth Analg 32, 158-167.

Valsecchi, P., Pattacini, O., Beretta, V., Bertozzi, J., Zannoni, S., Viggiani, R., Accorsi, P., 2007. Effects of a human social enrichment program on behavior and welfare of sheltered dogs. Journal of Veterinary Behavior: Clinical Applications and Research 2, 88-89.

van Vonderen, I.K., Kooistra, H.S., Rijnberk, A., 1998. Influence of veterinary care on the urinary corticoid: creatinine ratio in dogs. Journal of Veterinary Internal Medicine 12, 431-435.

Veissier, I., Boissy, A., 2007. Stress and welfare: two complementary concepts that are intrinsically related to the animal's point of view. Physiol Behav 92, 429-433.

Verga, M., Michelazzi, M., 2009. Companion animal welfare and possible implications on the human-pet relationship. Italian Journal of Animal Science 8, 231-240.

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.

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

Vining, R.F., McGinley, R.A., 1986. Hormones in saliva. Critical reviews in clinical laboratory sciences 23, 95-146.

Vining, R.F., McGinley, R.A., Maksvytis, J.J., Ho, K.Y., 1983. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem 20 (Pt 6), 329-335.

Voith, V., McGrave, E., Marder, A., 1987. Yawning "licking," and sleep behaviors in dogs in relationship to conflict, anxiety, and fear, Annual meeting of the Animal Behavior Society, Williamsburg, MA.

Voith, V.L., 2009. The impact of companion animal problems on society and the role of veterinarians. Vet Clin North Am Small Anim Pract 39, 327-345.

von Holst, D., 1998. The Concept of Stress and Its Relevance for Animal Behavior, in: Anders Pape M̄ller, M.M., Peter, J.B.S. (Eds.), Advances in the Study of Behavior, Academic Press, pp. 1-131.

Vormbrock, J.K., Grossberg, J.M., 1988. Cardiovascular effects of human-pet dog interactions. J Behav Med 11, 509-517.

Weitzman, E.D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T., Hellman, L., 1971. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. Journal of Clinical Endocrinology & Metabolism 33, 14-22.

Wells, D., Graham, L., Hepper, P., 2002. The influence of length of time in a rescue shelter on the behaviour of kennelled dogs. Animal Welfare 11, 317-325.

Wells, D.L., 2004. A review of environmental enrichment for kennelled dogs, *Canis familiaris* Applied Animal Behaviour Science 85, 307-317.

Wells, D.L., 2009. The Effects of Animals on Human Health and Well-Being. Journal of Social Issues 65, 523-543.

Wenger-Riggenbach, B., Boretti, F.S., Quante, S., Schellenberg, S., Reusch, C.E., Sieber-Ruckstuhl, N.S., 2010. Salivary cortisol concentrations in healthy dogs and dogs with hypercortisolism. J Vet Intern Med 24, 551-556.

Wheeler, M.J., Zhong, Y.B., Kicman, A.T., Coutts, S.B., 1998. The measurement of testosterone in hair. J Endocrinol 159, R5-8.

Wilson, C.C., 2003. Challenges in Designing Human-Animal Interaction Research. The American behavioral scientist (Beverly Hills) 47, 16-28.

Wolfe, T.L., 1990. Policy, program and people: the three P's to well-being., Scientists center for animal welfare. , Canine Research Environment, Bethesda, MD, pp. 41-47.

Young, R.J., 2003. Environmental enrichment for captive animals. Blackwell Publishing, Oxford.

Zamir, T., 2006. The Moral Basis of Animal-Assisted Therapy. Soc Anim 14, 179-199.

Zorawski, M., Killcross, S., 2002. Posttraining glucocorticoid receptor agonist enhances memory in appetitive and aversive Pavlovian discrete-cue conditioning paradigms. Neurobiology of learning and memory 78, 458-464.

Zorawski, M., Killcross, S., 2003. Glucocorticoid receptor agonist enhances pavlovian appetitive conditioning but disrupts outcome-specific associations. Behavioral neuroscience 117, 1453.

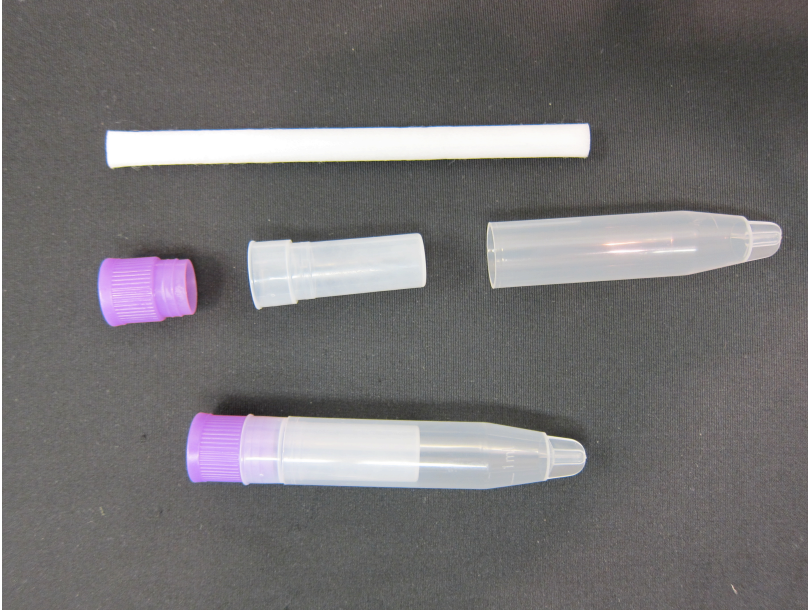Fig. 1. Salimetrics children's swab and tube

Fig. 2. Salivary cortisol box plot across time in home setting. N=66. The outlier at time 60 (0.727 ug/dL) and 90 (0.671 ug/dL) was dog 7, a 2 year old female spayed Staffordshire terrier.
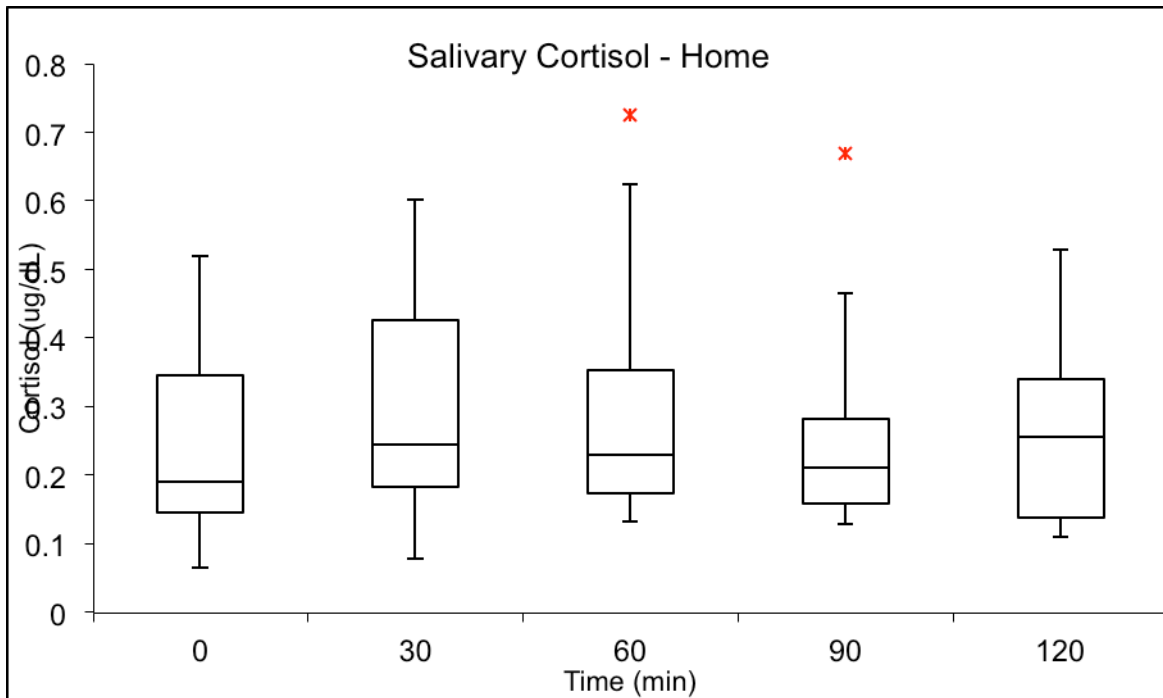
Fig. 3. Salivary cortisol box plot across time in neutral setting. N=68. The outlier at time 0 (3.438 ug/dL) was dog 7 (a 2 year old female spayed Staffordshire terrier). The outlier at time 30 (1.933 ug/dL), 60 (2.719 ug/dL), 90 (2.346 ug/dL) , and 120 (1.271 ug/dL) was dog 4, a 10 year old male neutered mixed breed dog.
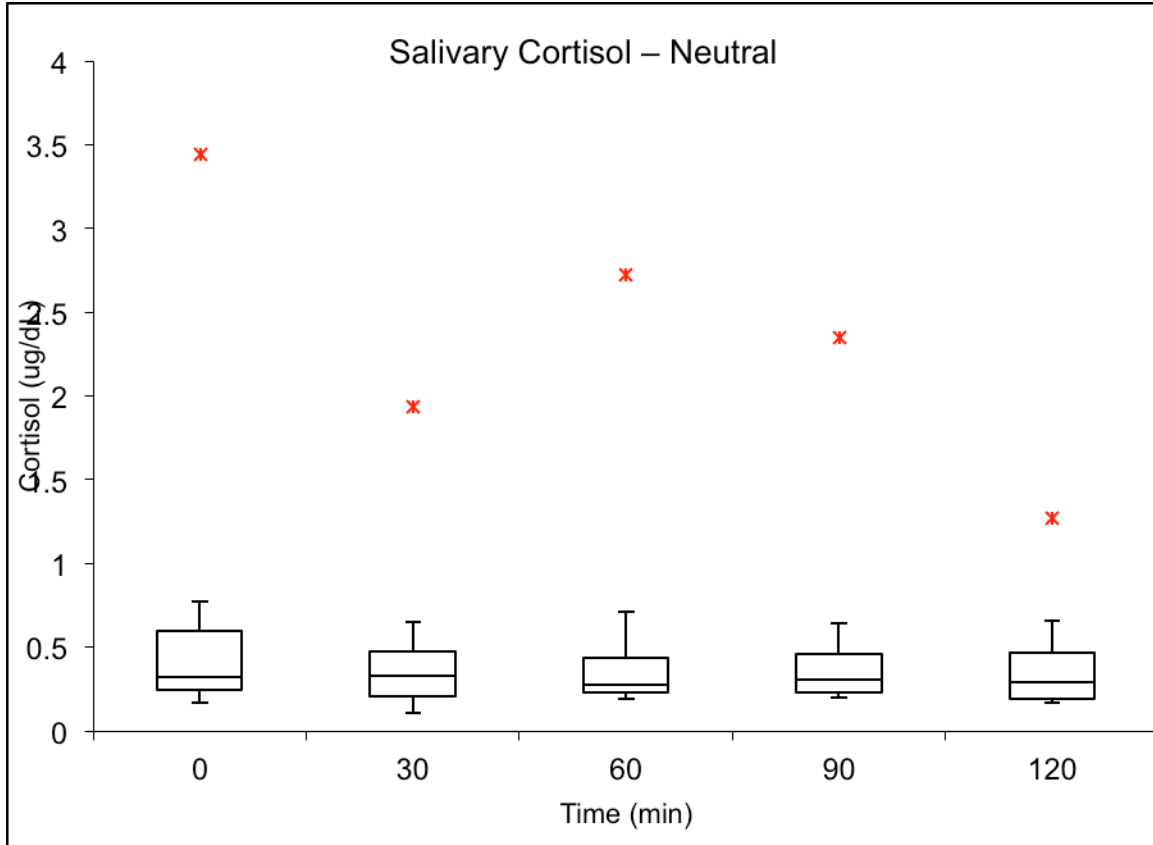
Fig. 4. Salivary cortisol box plot across time in animal-assisted activity setting. N=69. The outlier at time 30 (1.0 ug/dL), 90 (0.849 ug/dL), and 120 (1.315 ug/dL) was dog 1, a 4 year old female spayed mixed breed dog.
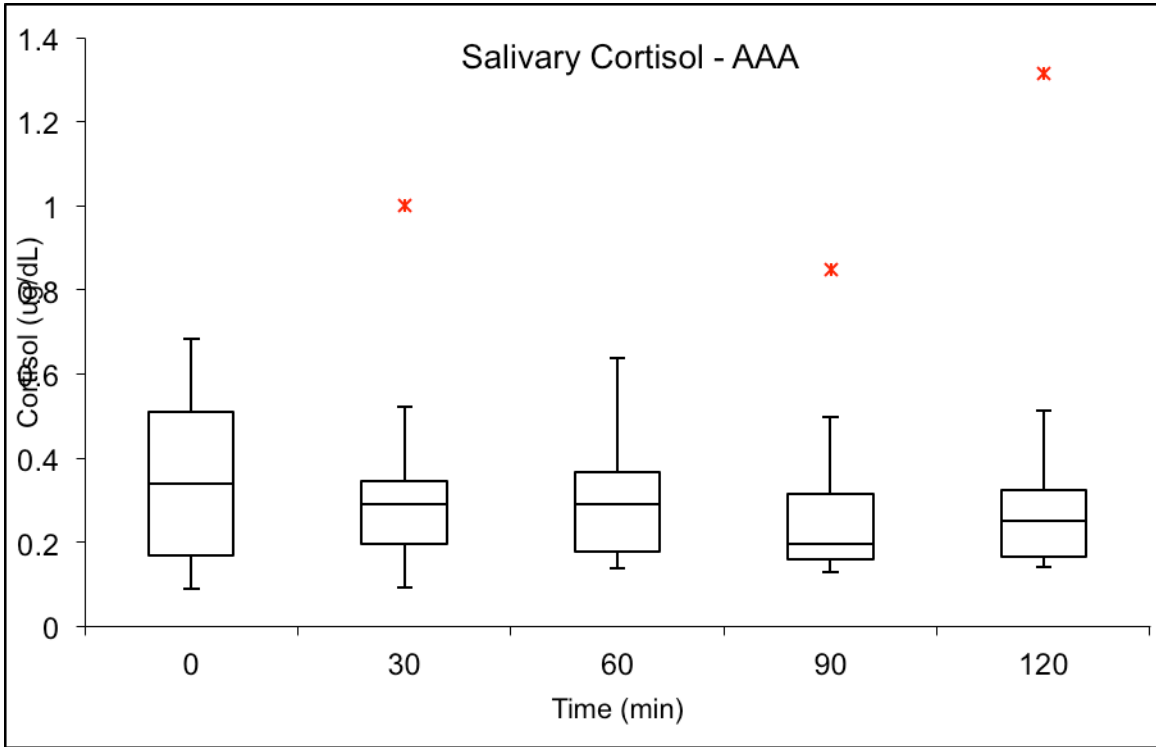
Fig. 5. Salivary cortisol geometric mean of each setting across time. Upper 95%
confidence levels are displayed for the NS and lower 95% confidence levels are
displayed for HS and AS. "a" represents a significant difference between NS and HS.
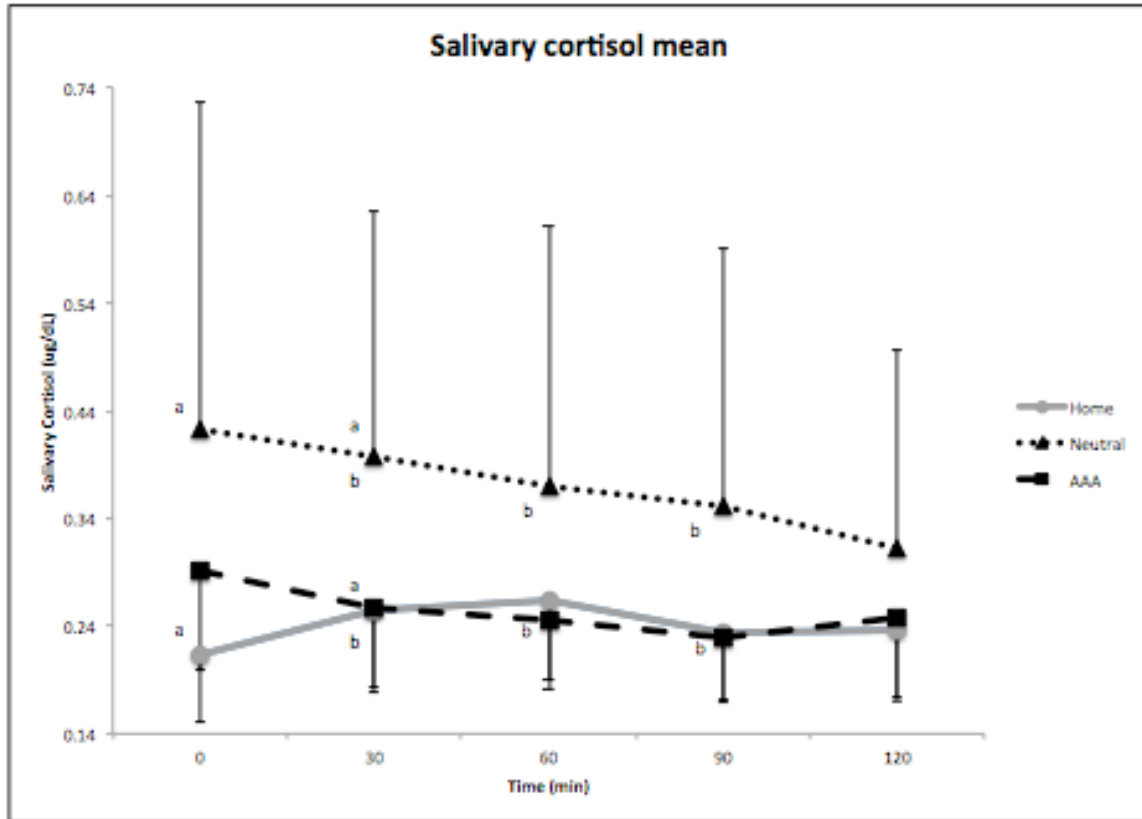"b" represents a significant difference between NS and AS.

Fig. 6. Geometric mean percentage change of salivary cortisol from time 0. The upper standard error of the means are displayed for the HS and lower standard error of the means are displayed for the NS and AS. "a" represents a significant difference between HS and AS. "b" represents a significant difference between HS and NS.
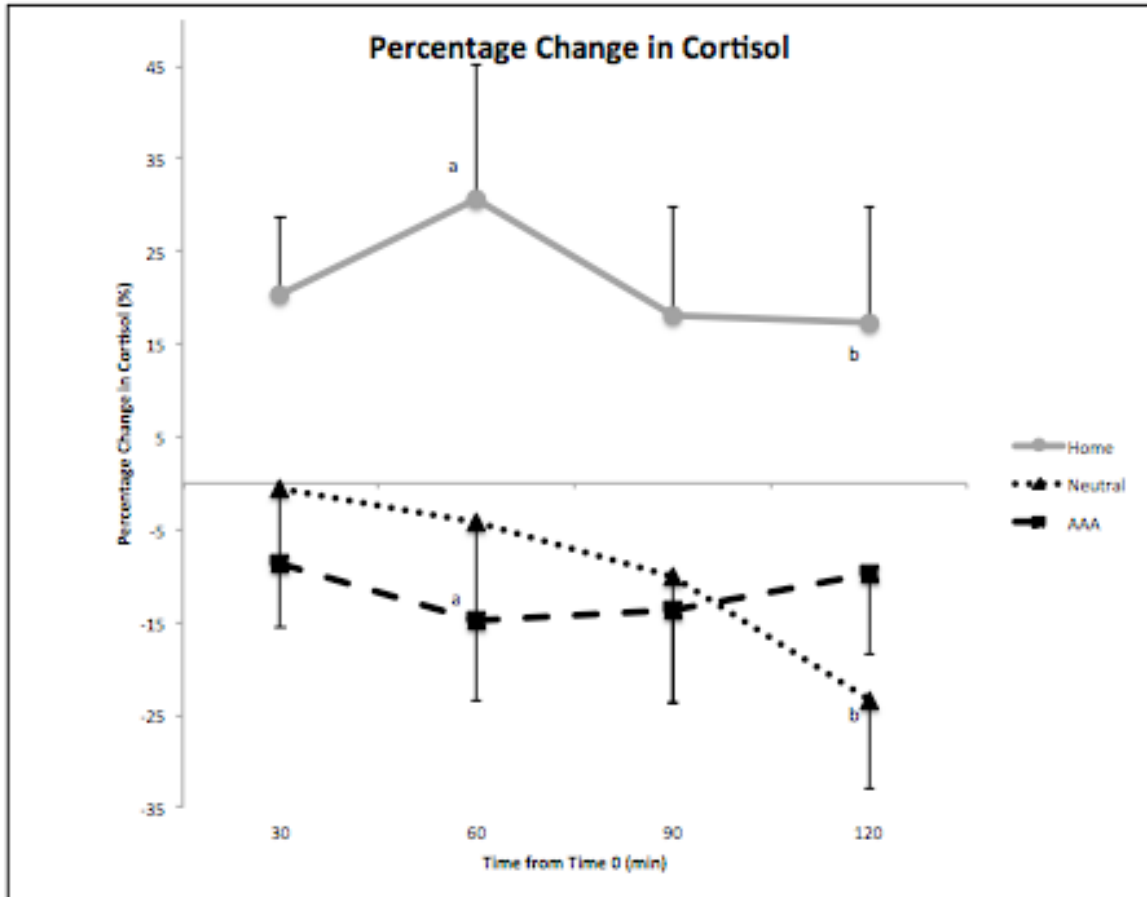
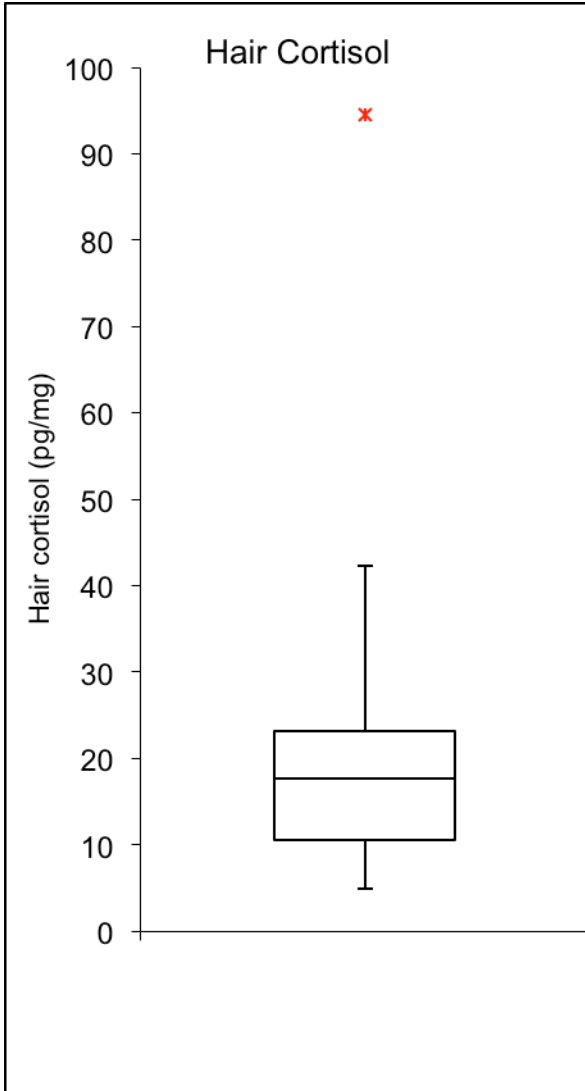Fig. 7: Hair cortisol. N=14. The outlier at 94.42 pg/mg was Dog 6, a 3 year old female spayed mixed breed dog.

Fig. 8. Sex difference in hair cortisol. N=14, (p=0.028). The female outlier at 94.42 pg/mg was dog 6, a 3 year old female spayed mixed breed dog, while the male outlier at 70.33 pg/mg was dog 4, a 10 year old male neutered mixed breed dog.
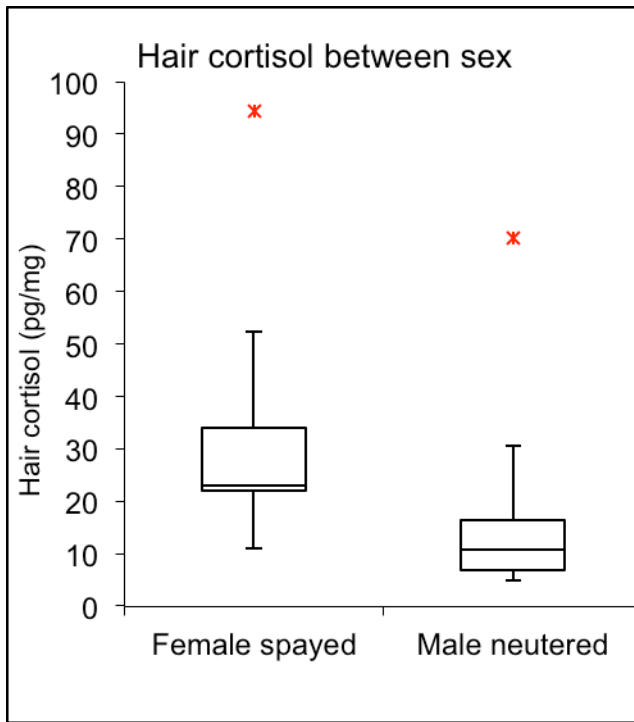
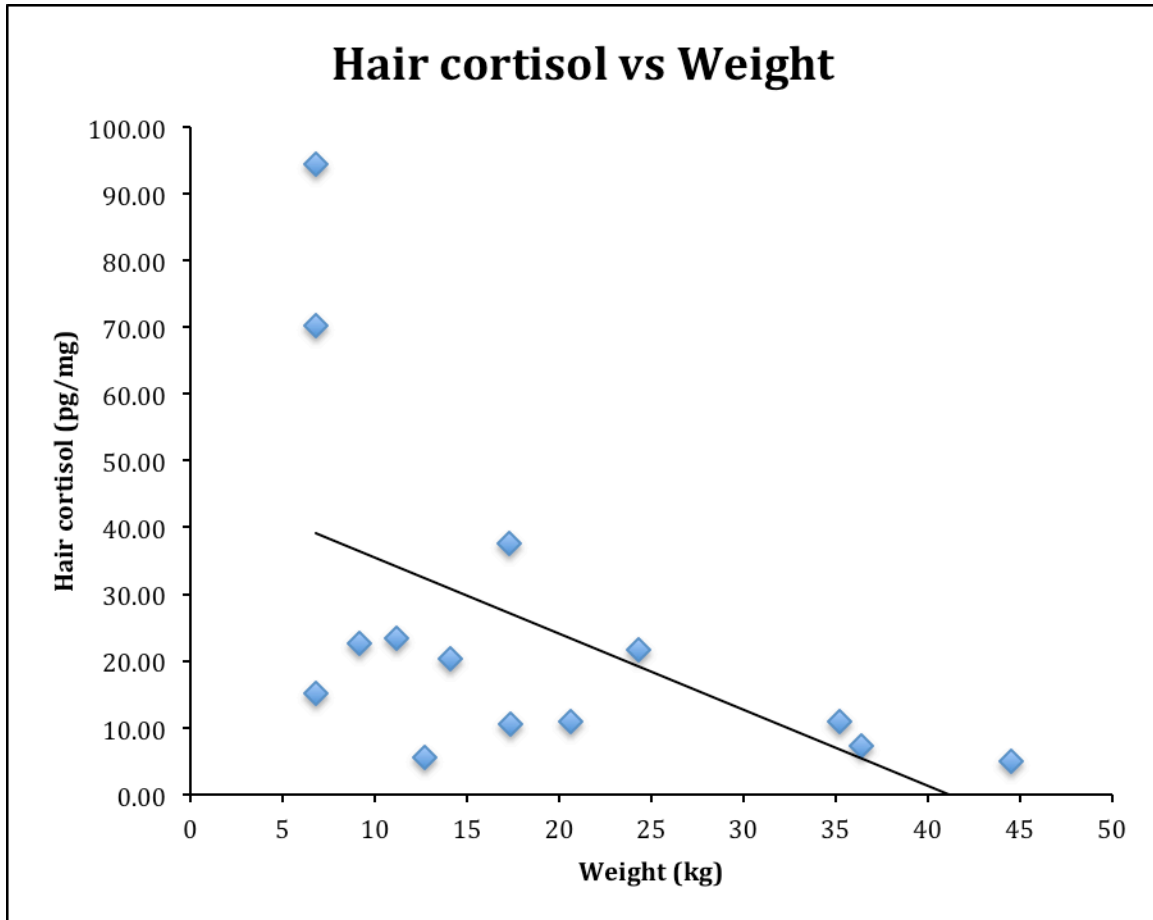Fig. 9. Hair cortisol compared to weight. N=14, (p=0.0068).

Fig. 10. Median percentage standing in each setting over time. The error bars represent the standard error of the means. "a" represents a significant difference between AS and HS. "b" represents a significant difference between NS and HS.
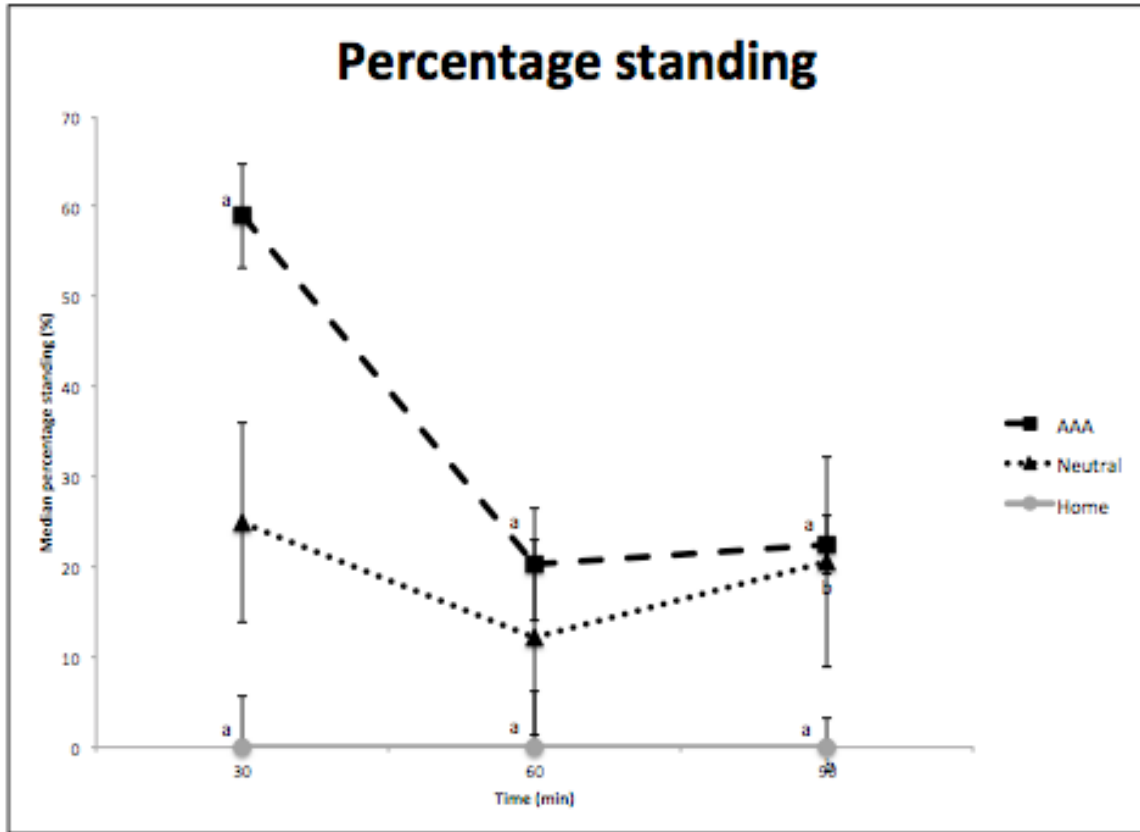
Fig. 11. Median percentage ambulating in each setting over time. "a" represents a significant difference between AS and HS. "b" represents a significant difference between AS and NS.

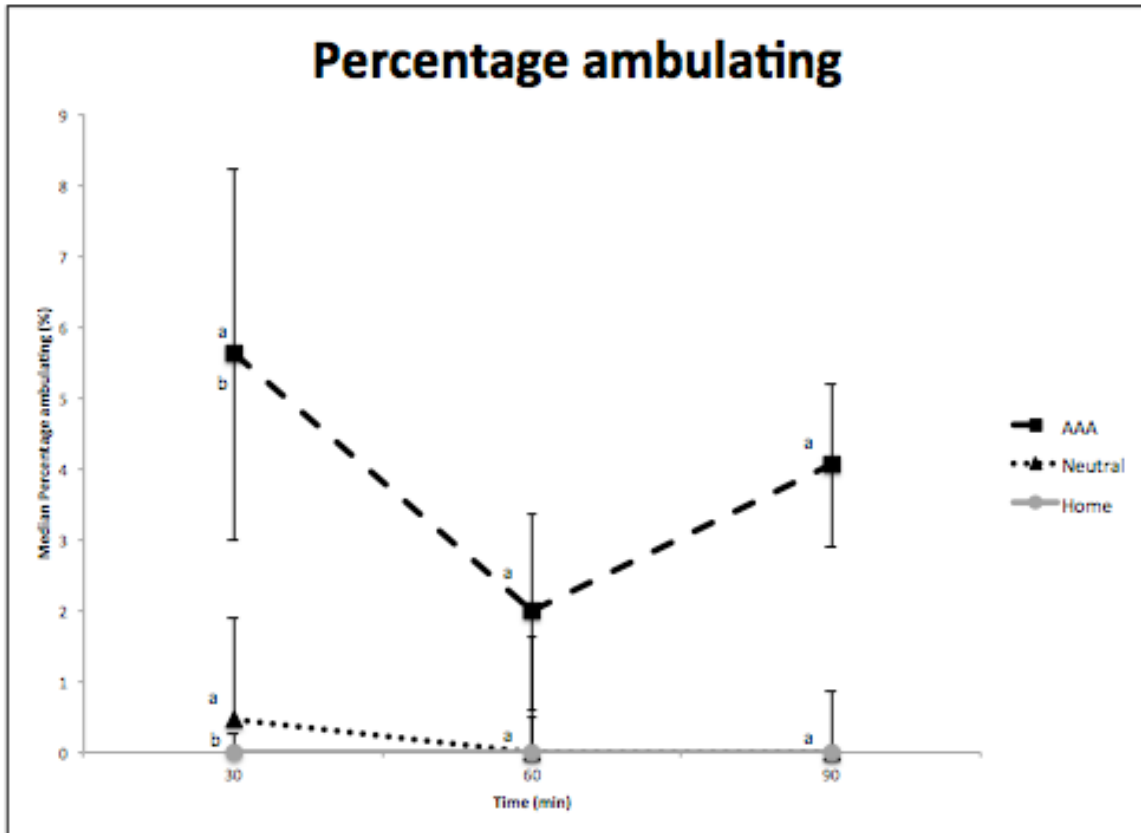Fig. 12. Median percentage recumbent in each setting over time. The error bars represent the standard error of the means. "a" represents a significant difference between HS and AS. "b" represents a significant difference between HS and NS.
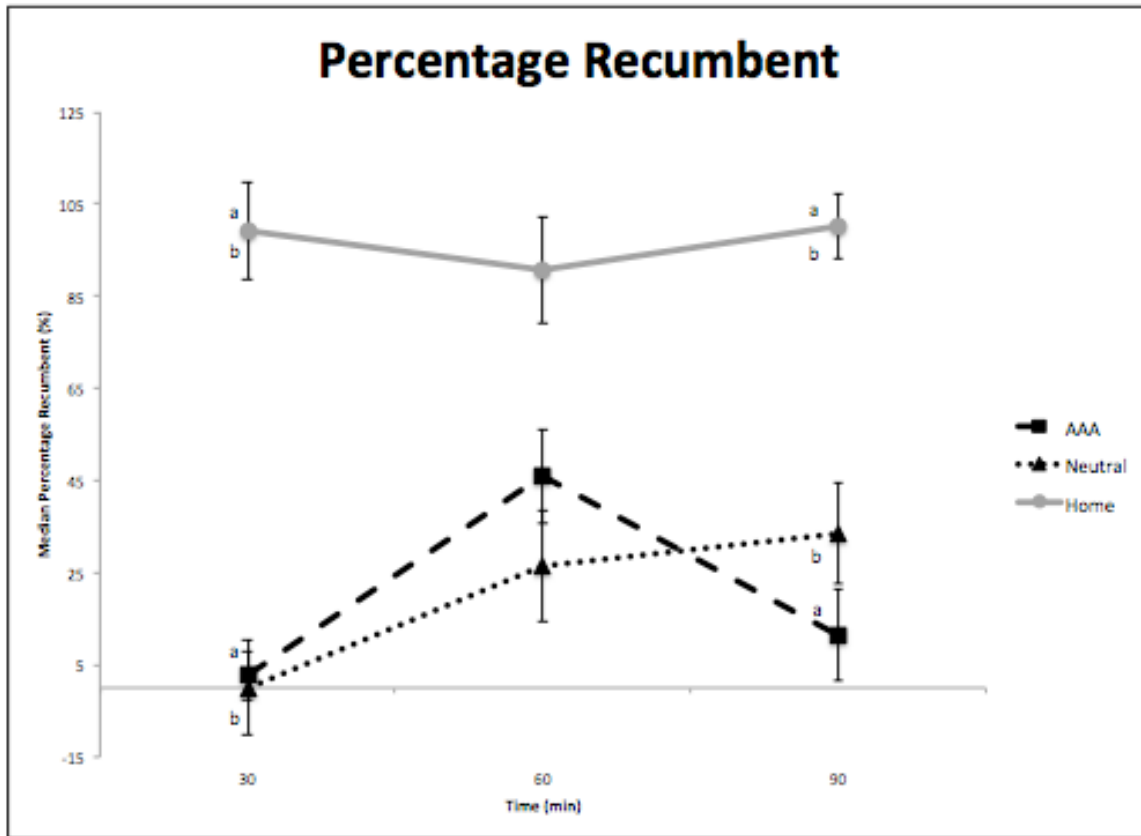
Fig. 13. Salivary cortisol vs percentage sitting at time 90 in NS. N=15, (p=0.0388).

98
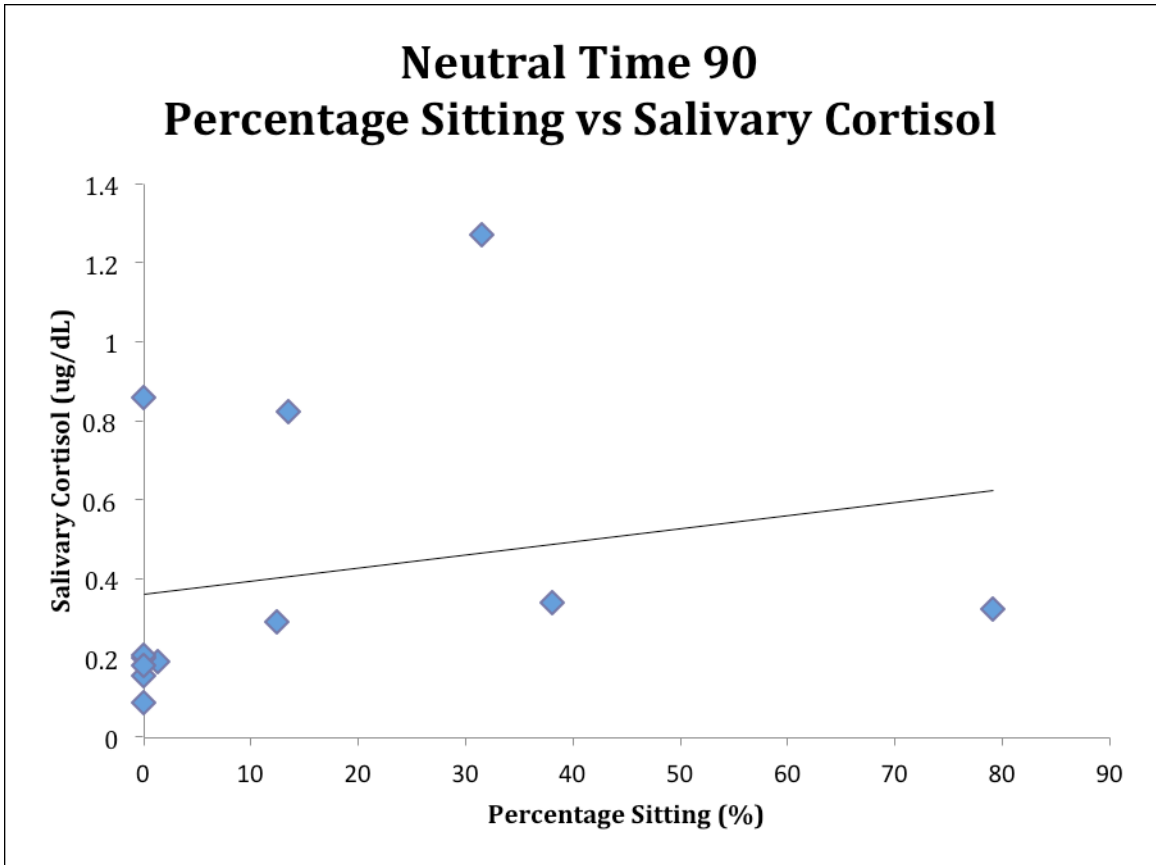
Fig. 14. Salivary cortisol vs percentage sitting at time 30 in AS. N=14, (p=0.04).
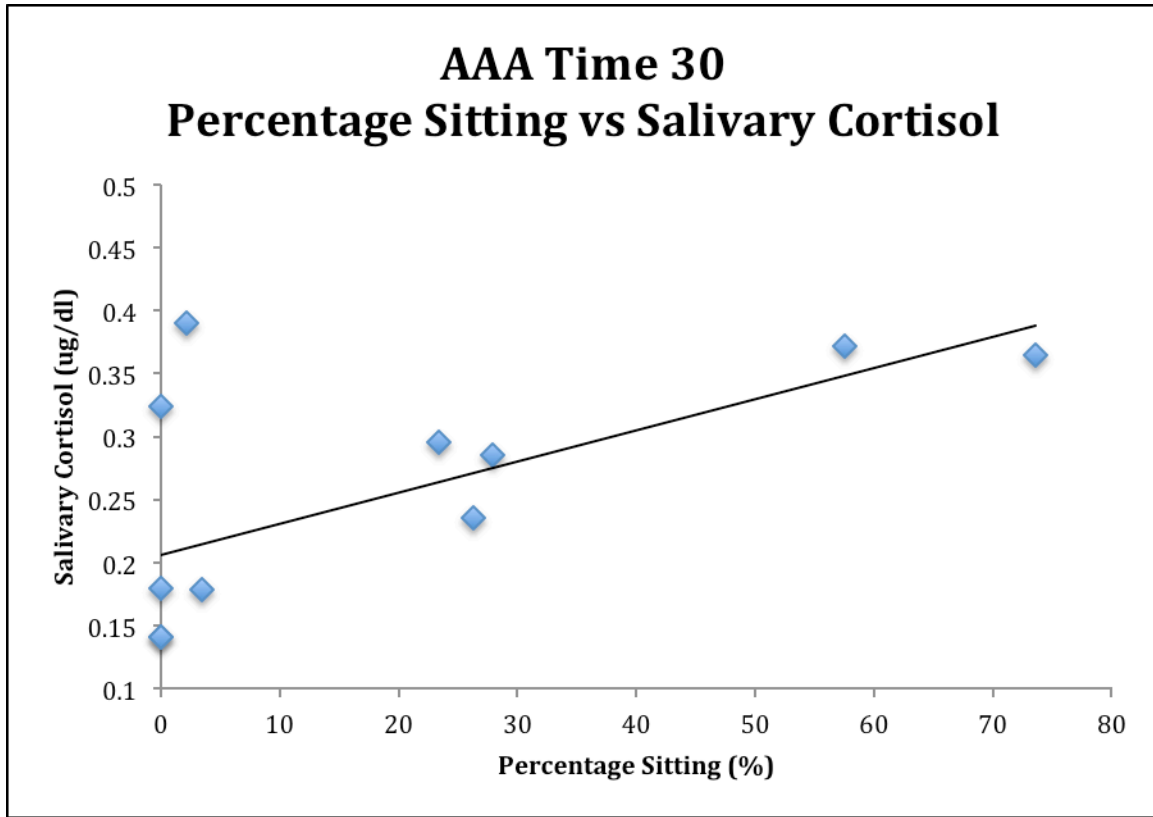
Fig. 15. Salivary cortisol vs percentage standing at time 30 in AS. N=14, (p=0.0219).

AAA Time 30
Percentage Standing vs Salivary Cortisol
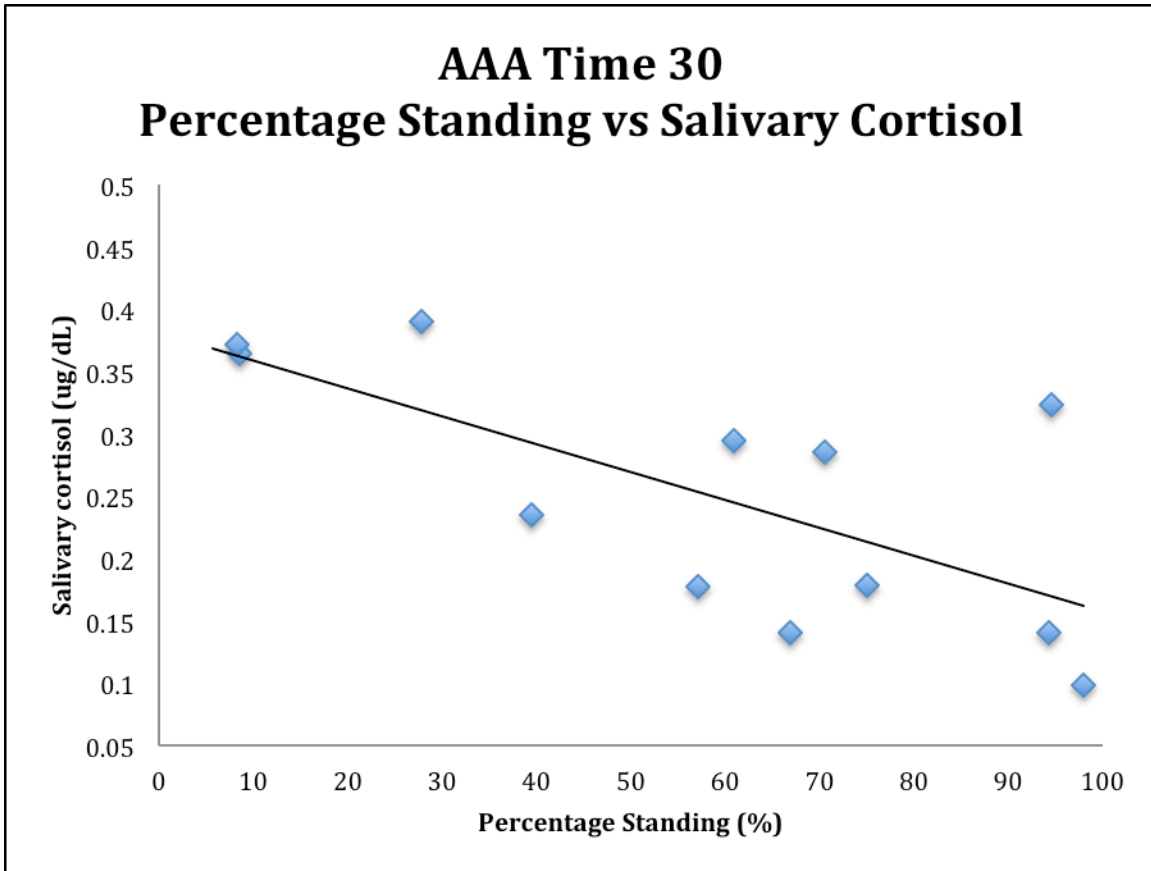
Fig. 16. Salivary cortisol vs percentage of neutral mouth at time 60 in NS. N=15, (p=0.0019).
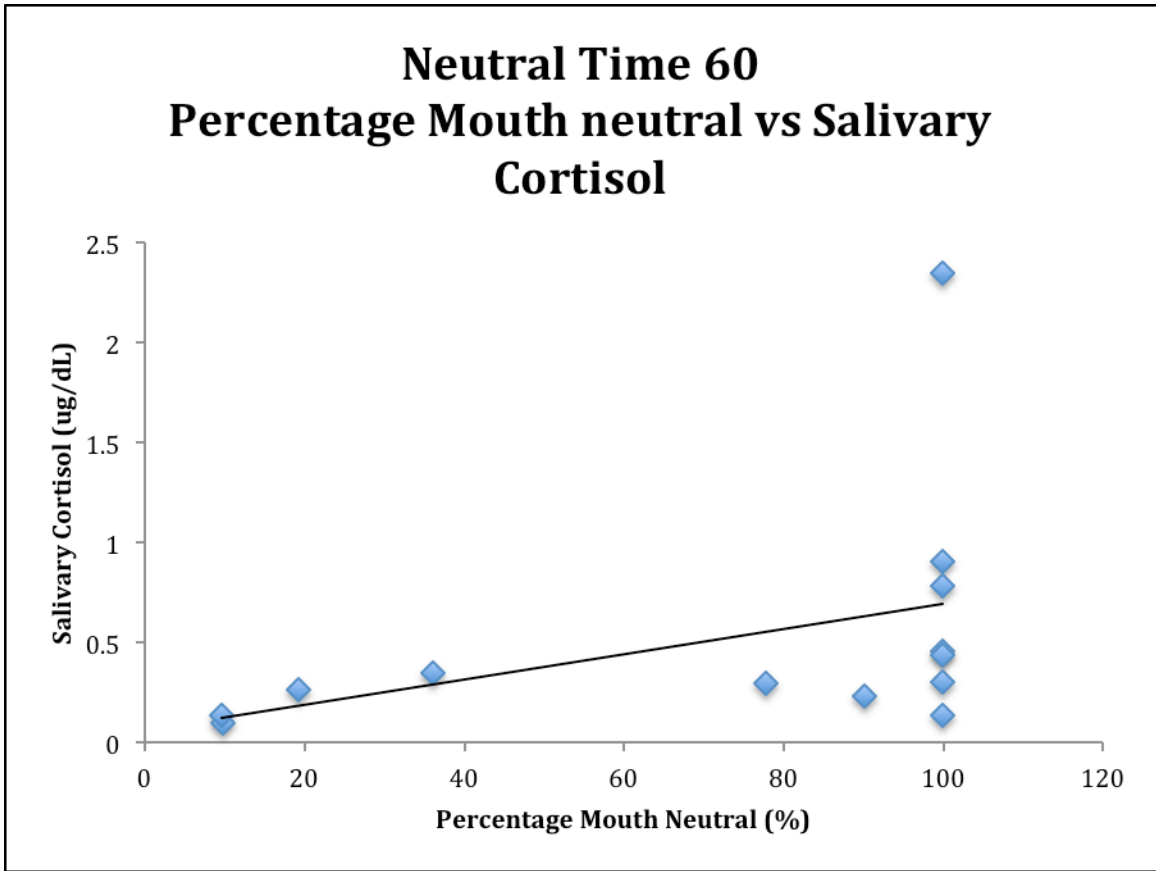
Fig. 17. Salivary cortisol vs percentage panting at time 60 in NS. N=15, (p=0.0284).

Neutral Time 60
Percentage panting vs Salivary cortisol
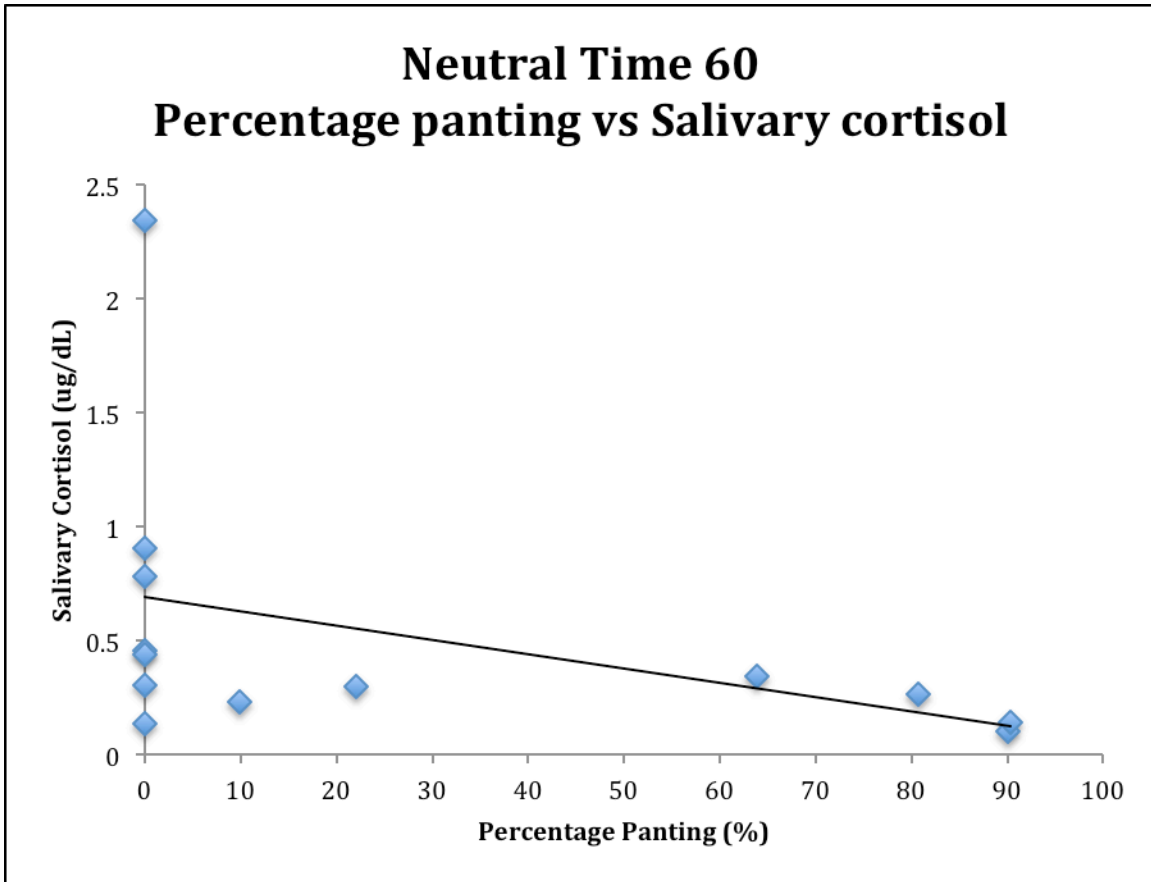
APPENDIX B: TABLES

Table 1. Timeline summary

Time (min)	Location	Action *Video continuous recording
0	Outdoors	Command, Saliva collection
25	Indoors	5 minute petting
30	Indoors	Command, Saliva collection
55	Indoors	5 minute petting
60	Indoors	Command, Saliva collection
85	Indoors	5 minute petting
90	Indoors	Command, Saliva collection
120	Outdoors	Command, Saliva collection

Table 2. Ethogram

Behavior	Description
Position (mutually exclusive, exhaustive)	
Sitting	Sitting on the ground with the pads of the front paws in contact with the floor and forelimbs straight
Standing	Positioned with just 4 paws in contact with the ground or 2 in contact with the ground and 2 in contact with a wall
Recumbent	Fully positioned, lying with 1 side in complete contact with the ground
Ambulating	Movement from 1 point to another, with no clear effort to explore
Exploring	Moving slowly, sniffing, and investigating the environment
Crouching	Rapid and pronounced lowering of the posture, sometimes in combination with movements that enlarge the distance to the eliciting stimulus; posture shows lowered position of the tail, backward positioning of the ears, and legs bent
Alertness (Mutually exclusive, exhaustive)	
Alert	Eyes kept open
Rest/Sleep	Eyes closed, dog inactive >10 seconds
Oral behavior (Mutually exclusive, exhaustive)	
Neutral	Mouth closed
Panting	Increased frequency of inhalation and exhalation often in combination with the opening of the mouth
Oral behavior (point event)	
Self grooming	Oral behaviors directed towards the dog's own body (licking and chewing the skin and coat)
Lip licking	Includes tongue out: tip of the tongue is briefly extended; snout licking: part of the tongue is shown and moved along the upper lip; swallowing; smacking
Mouth opening	Opening and closing the mouth with rapid movements without extending the tongue; possibly yawning
Licking person	Extending the tongue to touch a person's body
Licking object	Extending the tongue to touch an inanimate object or floor
Miscellaneous behavior (point event)	
Paw lifting	Raising a forepaw into a position of approximately 45 degrees
Vocalizing	Any form of vocalization, including barking, growling, whining, yelping
Scratching	Purposeful movement of limb to scratch any part of the body
Body shaking	Purposeful shaking of the full body
Trembling	Body shaking with small, high-frequency movements, clear shivering of the body
Jumping	Springing into the air, either to make contact with an object or a person or for no apparent reason
Repetitively moving head	Changing head position continuously >3 seconds
Stretching	stretching of the body and limbs

Table 3: Descriptive demographics of study population.

ID number	Age (years)	Sex	Weight (kg)	Breed	Number of other animals in household	Number of humans in household	Years of therapy certification	Years owned	Number of days dog participates in AAA visits/month:	Length of therapeutic session/day:	Number of times visited AS location	Sex of handler	Hair cortisol pg/mg
1	4	Female spayed	20.64	Mixed breed	0	5	2	2-4	7-10	>2 hour	0	F	10.99
2	3	Female spayed	24.27	Mixed breed	0	2	1	2-4	4-6	30 min-1 hour	0	M	21.69
3	2	Male neutered	44.55	Mixed breed	0	1	1	2-4	2-3	30 min-1 hour	1-2	F	4.97
4	10	Male neutered	6.82	Mixed breed	0	2	8	>6	2-3	30 min-1 hour	2-5	F	70.33
5	3	Male neutered	6.82	Mixed breed	1	1	1	2-4	2-3	30 min-1 hour	1-2	F	15.08
6	3	Female spayed	6.82	Mixed breed	1	1	2	2-4	4-6	30 min-1 hour	0	F	94.42
7	2	Female spayed	17.27	American Staffordshire Terrier	2	5	1	1-2	1	1-1.5 hour	1-2	F	37.62
8	3	Male neutered	14.09	Mixed breed	0	1	2	1-2	4-6	1-1.5 hour	1-2	F	20.31
9	4	Female spayed	9.18	Cavalier King Charles Spaniel	3	2	2	4-6	1	>2 hour	0	F	22.64
10	10	Male neutered	35.18	Golden Retriever	2	3	6	>6	>10	30 min-1 hour	0	M	10.87
11	4	Male neutered	36.36	Akita	1	1	2	2-4	4-6	1-1.5 hour	0	F	7.34
12	5	Female spayed	11.18	pembroke welsh corgi	0	1	3	2-4	2-3	30 min-1 hour	1-2	F	23.41
13	5	Female spayed	39.55	Rhodesian Ridgeback	0	1	3	4-6	2-3	1-1.5 hour	0	F	58.88
14	3	Male neutered	12.73	Mixed breed	0	1	1	1-2	2-3	1-1.5 hour	0	F	5.61
15	8	Male neutered	17.36	Mixed breed	0	1	2	>6	4-6	1-1.5 hour	0	F	10.44

Table 4. Medians and means of salivary cortisol separated by time or location

Location or Time	SampleSize	Means	Medians	SD	SEM	Minimum	Maximum
0	42	0.43285	0.255	0.55661	0.08589	0.065	3.438
30	41	0.39038	0.2895	0.38325	0.05985	0.079	1.933
60	40	0.38853	0.27025	0.45144	0.07138	0.0985	2.719
90	39	0.3509	0.2305	0.38447	0.06156	0.101	2.3455
120	41	0.33104	0.252	0.27882	0.04354	0.0865	1.3145
HS	66	0.2773	0.2325	0.15553	0.01914	0.065	0.7265
NS	68	0.5538	0.3035	0.64006	0.07762	0.0865	3.438
AS	69	0.30469	0.236	0.22184	0.02671	0.0935	1.3145

Table 5. Frequency of observed behaviors

Behavior	SampleSize	Means	Medians	SD	SEM
State events					
Position					
Percentage sitting	15	4.4	4	2.47271	0.63845
Percentage standing	15	5.53333	5	1.80739	0.46667
Percentage recumbency	15	6.06667	6	2.08624	0.53866
Percentage ambulating	15	3.73333	3	1.83095	0.47275
Percentage exploring	9	2.11111	2	1.2693	0.4231
Percentage crouching	1	1	1.	.	
Alertness					
Percentage alert	15	8.53333	9	0.91548	0.23637
Percentage rest/ sleep	11	2.90909	2	1.7581	0.53009
Oral behavior					
Percentage mouth neutral	15	8.06667	9	1.43759	0.37118
Percentage panting	9	5.11111	5	3.37062	1.12354
Point events					
Oral behavior					
Number mouth opening	13	4.84615	5	2.26738	0.62886
Number lip licking	15	6.93333	7	2.1202	0.54743
Number self-grooming	9	1.33333	1	0.70711	0.2357
Number licking object	4	1.75	1.5	0.95743	0.47871
Number licking person	8	2.75	2.5	1.58114	0.55902
Misc events					
Number paw lifting	8	2.25	2	1.28174	0.45316
Number body shaking	13	2.30769	2	1.54837	0.42944
Number stretching	5	1.6	1	1.34164	0.6
Number scratching	5	1.6	1	0.89443	0.4
Number jumping	6	1.33333	1	0.8165	0.33333
Number repetitively moving head	2	1	1	0	0
Number trembling	0	0	0	0	0
Number vocalizing	4	2	1.5	1.41421	0.70711