Accepted Manuscript

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PII: S1558-7878(16)30148-4

DOI: 10.1016/j.jveb.2016.09.003

Reference: JVEB 997

To appear in: *Journal of Veterinary Behavior*

Received Date: 13 June 2016

Revised Date: 7 September 2016

Accepted Date: 7 September 2016

Please cite this article as: Palestrini, C., Calcaterra, V., Cannas, S., Talamonti, Z., Papotti, F., Buttram, D., Pelizzo, G., Stress level evaluation in a dog during animal-assisted therapy in pediatric surgery, *Journal of Veterinary Behavior* (2016), doi: 10.1016/j.jveb.2016.09.003.

Stress level evaluation in a dog during animal-assisted therapy in pediatric surgery

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Abstract

Animal-assisted interventions (AAIs) are associated with positive effects on human psychological and physiological health. Although quality standards in AAIs appear to be high, only few investigations have focused on potential welfare implications in therapy dogs. In the present study, we monitored behavioral measures and heart rate in a therapy dog that participated in Animal-Assisted Therapy (AAT) during post-operative awakening in a pediatric surgery ward. Work-related activity, behavior, response to human action, and heart rate were analyzed over 20 working sessions in an experienced therapy dog. No physiological or behavioral indicators of stress, fatigue, or exhaustion were present during AAT, suggesting that, with the limited generalizability of a case study, this activity did not negatively impact on the welfare of the dog. Further investigation into the effects of animal-assisted therapy on dogs' physiological markers and behavior is warranted.

Keywords: animal-assisted therapy, animal welfare, behavior, heart rate, dog, stress

Introduction

Animal-assisted interventions (AAIs) are achieving a certain level of recognition worldwide and this is accompanied by a growing body of research on the effect of these programs on human health and well-being (Bernabei et al., 2013; Marcus, 2013; Calcaterra et al., 2015). AAIs, which use animals for human benefit, can be considered animal assisted therapy (AAT) when they involve the implementation of goal-directed, documented, and evaluated methodology in professional settings. In contrast, animal-assisted

activities (AAA) are not centered on a specific goal or treatment outcome and can be carried out by nonprofessional volunteers too (Kruger and Serpell, 2006). Animals are believed to be a source of motivation to take part in health interventions, exercise, and social interaction (Wilson and Barker, 2003; Glenk et al., 2014). The widespread involvement of dogs in AAIs is grounded in the outstanding interspecific social ability of this species and in the dogs' ease in adapting to various human environments (Miklósi and Topál, 2013). Although a growing body of evidence supports the rewards and benefits of human–animal interactions for humans, only few investigations have focused on the potential welfare implications for therapy dogs as a result of their performance in AAIs. Indeed, the welfare of dogs involved in AAA and AAT has been questioned, as social interactions have been described as among the most potent stressors a dog can endure (von Holst, 1998; McEwen and Wingfield, 2003). This may be because social interactions can be unpredictable, requiring the individual to constantly adapt physiologically and behaviorally to maintain homeostasis (Karatsoreos and McEwen, 2011). Study of the physiological and behavioral effects of AAIs on registered dogs is needed to enhance our understanding of animal welfare during these interventions, to introduce evidence-based guidelines for handlers, and to establish rigorous methods for future research. Animal welfare has commonly been assessed by measuring and analyzing stress-associated behavior as well as physiological indicators of stress in dogs (i.e., heart rate) (Vincent and Michell, 1992; Beerda et al., 1999; Palestrini et al., 2005). Analysis of behavior has also long been used as a research tool to assess stress and welfare in animals. Stress-associated behavior in dogs, such as increased locomotor activity, lip-licking, yawning, and circling, have been observed to occur in response to acute stressors (Beerda et al., 1997; Palestrini, 2009). While Ferrara et al. (2004) reported the absence of stress behavior in dogs during AAA/AAT, King et al. (2011) observed multiple behavioral signs of stress (panting, yawning, whining, and lip-licking) in dogs after an AAT session. These discrepancies warrant clarification as to whether activity and therapy sessions induce stress-associated behavior.

Heart rate has a long history as a psychophysiological measure of animals' affective and cognitive responses and several studies have investigated heart rate responses of dogs to different stimuli and environmental conditions (Beerda et al., 1997). Heart rate represents an accessible, quantifiable, physiological measure underlying emotional responses in dogs and the possibility of linking physiology and observable behavior is of great importance in gaining a better understanding of the dog's reactions to environmental changes (Kostarczyk, 1992; Beerda et al., 1998; Casey, 2003). Both behavior and heart rate are considered useful indicators to evaluate stress reactions in dogs (Kostarczyk, 1992), due to the interaction between the central nervous system and the neuro-endocrine system (Henry and Ely, 1976; Beerda et al., 1998). The robustness of an animal welfare assessment is thus improved when stress-associated behavior is evaluated in conjunction with cardiac activity (Palestrini et al., 2005; Stiles et al., 2011). In this investigation, the objective was to measure and compare behavior and heart rate in an experienced therapy dog to examine whether the dog exhibited behavioral or physiological signs of stress in AAT in a pediatric surgery setting.

Materials and Methods

Participants

A carefully-screened 7 year old spayed female Golden Retriever was employed as the therapy animal. She had previous experience in AAIs and was already trained and prepared for this type of work. The dog was fully vaccinated, regularly groomed, screened for enteric pathogens, and treated for internal and external parasites on a monthly basis. The dog and handler met hospital policy for participation in AAT, including documentation of the dog's current vaccinations, controllability and temperament. Twenty immunocompetent children (15 males and 5 females), aged 3 to 17 years (mean±SD; 8.59±3.70), undergoing surgical procedures (including orchidopexy, inguinal or umbilical hernia repair, circumcision, varicocele treatment) were randomly assigned to the AAT session. In all subjects, surgery was performed between 8.30 am and 12 am under general anesthesia, at the Pediatric Surgery Unit, Fondazione IRCCS Policlinico S. Matteo, Pavia. Parental permission was obtained by means of written and oral informed consent. Written assent by the patient was also obtained in children eight years of age and older before enrollment.

Procedures and data collection

About 2 hours after surgery, during post-operative awakening, each child underwent a 20 minute session with the AAT dog. The therapy dog was specially prepared and chosen for the interactions, which were evaluated as suitable and recorded together with the handler. During the sessions, the handler monitored the dog, tended to its needs and supervised the dog-child interactions. Dog behavior was video recorded during the 20 experimental sessions. A video camera (Panasonic NV-GS330) was installed in the room (5 m × 4 m, with a constant room temperature of 22±1°C) opposite the area where the child and the dog-handler team remained during the session. Heart rate was measured using a Polar® Vantage NV system (Vincent and Leahy, 1997) in order to allow comparison between heart rate and behavior. The Polar was fixed around the chest of the animal with an elastic band. The monitor collected a reading of heart beats per minute every 5 seconds throughout each 20-minute therapy session. The results were then downloaded to a computer using Polar ProTrainer 5 software. Each session was transferred as separate file. The heart rate device was activated at the start of each session and synchronized with the video recording of behavior in order to have a perfect match between the behavioral and physiological data.

Statistical analysis

The videotaped sessions were analyzed by two trained observers, and the dog's behavior was recorded in 11 categories. Any interaction of the children with the dog, under the handler's supervision, was also recorded. Table 1 shows the list of mutually exclusive categories and their definitions. All 20 recorded AAT sessions were included in the behavior analysis. Videos were analyzed using the Solomon Coder (Version:

beta 15.11.19). Inter- and intra- observer reliability were assessed by means of independent coding of a random sample of videotaped sessions (10%) using percentage agreement: percentage agreement was always more than 92%.

A focal animal continuous recording method (Martin and Bateson, 1993) was used to describe the dog's activity. Some of the dog behaviors (exploration, passive, orientation to environment, panting, interaction with the child/handler/people, withdrawal) were recorded in terms of duration of occurrence (states), and other behaviors (licking lips, yawning, grooming) were recorded in term of frequency (events). Children's interactions with the dog were recorded in terms of duration of occurrence (states). In order to describe duration and frequency for each behavior a descriptive analysis was first performed.

Durations of states were calculated as percentage of total observation time and events were expressed as frequencies. A bivariate correlation was used to verify the relationship between child-dog interactions and variables related to the dog's stress behaviors (licking lips, yawning, grooming and withdraw). Continuous recording of heart rates was only available for 10 sessions. The remaining sessions could not be considered in the analysis because the recorded signal was either absent or incomplete. Mean (±SD) heart rate values were calculated.

Results

Behavior

Analysis of dog behavior (Figure 1) on tape showed that she spent most of her time exhibiting panting behavior (PT, 28.35% ± 18.09%) as opposed to avoiding interactions with the child or other people in the room (WT, 0.06% ± 0.15%). Most of her time she was oriented to the environment (OE, 23.22% ± 14.37%) or passive (PA, 6.58% ± 7.54%). The dog interacted more with the handler (IH, 8.61% ± 6.09%) than with the child (IC, 4.93% ± 3.80%) or other people (IP, 2.24% ± 3.03%). Exploration (EX, 3.48% ± 2.37%) was observed for very short periods especially during the early AAT sessions. GR, LL and YA were observed respectively for 1.65±1.73, 5.65±3.82 and 1.25±0.97 (Figure 2). Children behaved differently with the dog, some never sought any interaction, while others interacted with the dog for most of the time (Figure 3). No correlation was found between dog stress related behaviors (lip-licking, yawning, grooming, panting and avoidance) and children interactions.

Heart rate

Heart rate was recorded every 5 seconds for only 10 therapy sessions. The dog's heart rate levels always remained within a range of normal values (60-110 BPM) (Santilli and Perego, 2009) as reported in Table 2.

Discussion

It is well known that humans benefit from interaction with therapy dogs, therefore the behavioral and physiological health of the animal should be carefully reflected upon (Stetina and Glenk, 2011). Dedicated

research on animals in AAIs is limited and does not provide evidence on which standards can be issued regarding animal welfare (Beck and Katcher, 2003). In dog-assisted therapy there are considerable differences between different programs with regard to the procedures in dog training, the AAI working schedule, time span between arrival at a facility and the start of the AAI, and quality assessment and quality assurance (Stetina and Glenk, 2011). Consequently, it is crucial to increase knowledge of which measurable variables reflect aspects of animal welfare and provide evidence on which standards should be achieved during AAT. This study explored whether a prepared dog exhibited behavioral or physiological signs of stress during AAT in pediatric surgery setting. Of the behaviors recorded, panting was most often exhibited. In addition to being a response to heat, panting can be associated with negative stress (Godbout et al., 2007; Palestrini et al., 2010a) or with positive arousal, such as during anticipation of a desired reward (Ng et al., 2014). It must be noted, however, that the effect of room temperature on panting was probably substantial in this study, because the temperature is maintained relatively high (22±°1) during post-operative awakening and remained constant between evaluations. No prior activity or stimulation was performed that could have influenced panting in the dog. The dog did not show noticeable signs of distress: she spent most of her time oriented to the environment or being passive. She never showed any withdrawal behavior and interacted both with the child and other people present in the room. The dog explored the environment especially during the early AAT sessions. The dog did not show more lip-licking, yawning, grooming or avoidance behavior during sessions where children interacted with the dog compared to sessions without any interaction. Lip-licking, yawning and grooming have been associated with fear or anxiety (Beerda et al., 1998; Frank et al., 2007; Cannas et al., 2010; Palestrini et al., 2010b) or as a possible displacement behavior indicative of conflict (Cannas et al., 2014). Lip-licking and yawning have also been suspected to precede situations of social conflict in dogs (Voith and Borchelt, 1996). However according to Rehn and Keeling (2011), lip-licking may be communicative cues in dogs, which do not necessarily correspond to a stressful experience but, on the contrary, may help to manage stress. Recently, Shiverdecker et al. (2013) supported this assumption.

Not all dogs express stress-associated behavior in the same way because temperament and personality are influenced by many variables, including age, breed, and past experience (Hiby et al., 2006; Passalacqua et al., 2013). Different dogs often have different responses and coping strategies to the same stimulus (Rooney et al., 2007). The brain and body develop coordinated biological mechanisms in response to potent stressors to anticipate and recover from them in the future in an effort to maintain homeostasis (Karatsoreos and McEwen, 2011). Responses are also likely influenced by the type of interaction, as it has been speculated that dogs may not exhibit stress-associated behavior in the context of human–animal interactions (Kuhne et al., 2012) despite being physiologically stressed (Ng et al., 2014). Therefore, it is necessary to assess behavior in conjunction with physiological parameters such as heart rate.

In our study the 20-min AAT sessions did not result in an increase in heart rate levels during the 10 AAT sessions for which heartbeat was measured. Heart rate levels in the activity setting were no different from the normal range (60-110 BPM) (Santilli and Perego, 2009). Heart rate responses were not related to whether the dog interacted with the child during the session. This may have been because interaction during the activity was safe and predictable. The inability to predict what will happen induces significant stress in humans (Henry and Stephens, 1977) and this likely occurs in dogs as well. An AAT dog-handler team typically consists of a dog with a consistent, non-fearful and non-aggressive temperament and a handler who is trained to minimize interactions that might be perceived as threatening by the dog (Ng et al., 2014). It must be noted that the therapy dog was specially prepared and chosen for the interactions, which were evaluated as suitable and performed together with the handler. The handler monitored the dog, tended to the dog's needs and supervised each dog-child interaction.

AAT dogs are selected for this type of activity because of their temperament, and are trained to remain calm and relaxed, even in stressful situations (Viau et al., 2010). Therefore, AAT dogs may not exhibit stress-associated behaviors typically demonstrated by the rest of the canine population when physiologically aroused. This underscores the importance of measuring behavior in conjunction with heart rate. Although single AAT sessions may not induce an acute stress response, it is not known to what extent the duration or frequency of AAT sessions may induce stress, that over time may result in a disruption of homeostatic mechanisms and chronic stress (Karatsoreos and McEwen, 2011). Further studies are needed to investigate this limit. Although the dog in this study did not appear to be negatively affected by this particular AAT work, the welfare of AAT dogs should be continuously monitored. Until a gold standard measure of stress or distress is clearly established, behavioral observation remains a principal and practical method of evaluating stress and welfare in animals (Hekman, 2012). The handler must be rigorously trained on the prevention, recognition, and management of stress-associated behavior in his or her dog. It is particularly important that the handler understands normal dog behavior in the home environment in order to be able to recognize behavioral signs of stress when they occur (Ng et al., 2014).

The physical environment plays a role in dogs' stress response. An appropriately-trained handler can influence the dog's perception of the environment and minimize the stress response by facilitating controlled and predictable interactions. Continuing education of AAT dogs, monitoring for behavioral signs of stress, and intervening with mental stimulation (training obedience commands or taking a break with a short time out from patient care areas) would be helpful. An optimal work shift achieved by monitoring behavioral signs of stress is mandatory to promote healthy interactions between the dogs and the people they serve. To date, there is no single validated model to test the effect of AAT on dogs because interventions vary greatly in intensity of interaction, duration, objectives, and demographics of recipients. Our study attempted to standardize these variables in a series of 20-minute AAT sessions in pediatric

surgery. Its good feasibility and standardized data-gathering techniques suggest that the technique we used could be successfully repeated with a number of dogs simultaneously. Our study has several weaknesses, one is the limited generalizability and another is the lack of baseline heat rate measurements for the dog involved in the study. Measurement of baseline heart rates during stressful and non-stressful events would have allowed us to compare AAT-related measurements with baseline values during non-working conditions (King et al., 2011). Working dogs need outlets for good behavioral health, particularly for AAT dogs because high performance is expected in unfamiliar and unknown working areas. AAT handler reports of dog stress were a valid indicator of physiological stress. Monitoring body language in the AAT dog will help in guiding the handler to intervene earlier if the dog is showing signs of stress (King et al., 2011).

Conclusions

AAT sessions of 20 minutes for children recovering from pediatric surgery, conducted in a safe and controlled manner, did not elicit observable stress-associated behaviors or an increase in heart rate, and thus may not negatively impact the welfare of trained AAT dogs. This study may be considered as a first step towards further investigations on animal welfare in AAT. It proposes a straightforward, widely-applicable approach to data collection that allows the synchronous recording of behavioral and heart rate data, that could be used to standardize exploration of the effects of different types of AAT on animal well-being. To provide consistent high quality in AAIs, it is essential to monitor and interpret physiological and behavioral parameters that are related to animal welfare. Future studies could be aimed at ascertaining the effects of different working conditions and environments by manipulating the therapy sessions, and at validating the experimental methodology used in our study.

Ethics Statement

The study was performed according to the Declaration of Helsinki. The ethics committee of the Fondazione IRCCS Policlinico S. Matteo and Department of Internal Medicine, University of Pavia, approved the study protocol on April 11, 2013. Animal-assisted therapy was also approved on April 11, 2013 by the ethics committee of the Fondazione IRCCS Policlinico S. Matteo. No invasive intervention or drug experimentation on the dog was performed; therefore the application of D.lgs. 116/92, European Directives 86/609/EE for the protection of animals used in scientific and experimental studies and 2010-63UE was not required. The dog owner provided consent for its use in the study. Participants were recruited between September 01, 2013 and April 01, 2014. The study was registered at ClinicalTrials.gov (Identifier: NCT02284100) after enrollment was initiated, because animal assisted-therapy was considered a complementary treatment. The authors confirm that all ongoing and related trials for this drug/intervention are registered.

Conflict of interest

The authors declare no conflict of interest. All authors have approved the final article for submission.

References

Beck, A.M., Katcher, A.H., 2003. Future directions in human-animal bond research. Am. Behav. Sci. 47, 79-93.

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

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

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

Bernabei, V., De Ronchi, D., La Ferla, T., Moretti, F., Tonelli. L., Ferrari. B., Forlani, M., Atti, A.R., 2013. Animal-assisted interventions for elderly patients affected by dementia or psychiatric disorders: a review. J. Psychiatr. Res. 47:762-773.

Cannas, S., Frank, D., Minero, M., Godbout, M., Palestrini, C., 2010. Puppy behavior when left home alone: changes during the first few months after adoption. J. Vet. Behav.: Clin. Appl. Res. 5, 94-100.

Cannas, S., Frank, D., Minero, M., Aspesi, A., Benedetti, R., Palestrini, C. 2014. Video analysis of dogs suffering from anxiety when left home alone and treated with clomipramine. J. Vet. Behav.: Clin. Appl. Res., 9: 50-57.

Casey, R., 2003. Fear and stress. In: Horwitz, D., Mills, D., Heath, S. (Eds.), BSAVA Manual of Canine and Feline Behavioural Medicine. BSAVA, Gloucester, pp. 144–153.

Calcaterra, V., Veggiotti, P., Palestrini, C., De Giorgis, V., Raschetti R., Tumminelli, M., Mencherini, S., Papotti, F., Klersy, C., Albertini, R., Ostuni, S., Pellizzo, G., 2015. Post-Operative Benefits of Animal-Assisted Therapy in Pediatric Surgery : A Randomised Study. PLOS ONE, 6: e0125813.

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

Frank, D., Minero, M., Cannas, S., Palestrini, C., 2007. Puppy behaviours when left home alone: a pilot study. Appl. Anim. Behav. Sci. 104, 61-70.

Glenk, L.M., Kothgassner, O.D., Stetina, B.U., Palme, R.,Kepplinger, B., Baran, H., 2014. Salivary cortisol and behavior in therapy dogs during animal-assisted interventions: A pilot study. J. Vet. Behav.: Clin. Appl. Res. 9, 98-106.

Godbout, M., Beauchamp, G., Palestrini, C., Frank, D., 2007. Puppy behavior at the veterinary clinic: a pilot study. J. Vet. Behav.: Clin. Appl. Res. 2, 126-135.

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

Henry, J.P., Ely, D.L., 1976. Biologic correlates of psychosomatic illness. In: Grenell, R.G., Gabay, S. (Eds.), Biological Foundations of Psychiatry 2. Raven, NY.

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

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

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

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

Kostarczyk, E., 1992. The use of dog–human interaction as reward in instrumental conditioning and is impact on dogs' cardiac regulation. In: Davis, H., Balford, D. (Eds.), The Inevitable Bond: Examining Scientist–Animal Interaction. Cambridge University Press, Cambridge.

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, 33–48.

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

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

Marcus, D.A., 2013. The science behind animal-assisted therapy. Curr. Pain. Headache Rep. 17, 322.

Martin, P., Bateson, P., 1993. Measuring Behaviour: An Introductory Guide, 2nd ed. Cambridge University Press, pp. 84–85.

Miklósi, Á., Topál, J., 2013. What does it take to become 'best friend'? Evolutionary changes in canine social competence. Trends Cogn. Sci. 17, 287-294.

Ng, Z.Y., Pierce, B.J., Otto, C.M., Buechner-Maxwell, V.A., Siracusa, C., Were, S.R., 2014. The effect of dog–human interaction on cortisol and behavior in registered animal-assisted activity dogs. Appl. Anim. Behav. Sci. 159 (2014) 69–81.

Palestrini, C., Prato Previde, E., Spiezio, C., Verga, M., 2005. Heart rate and behavioural responses in dogs in the Ainsworths situation: a pilot study. Appl. Anim. Behav. Sci, 94: 75-88.

Palestrini, C., 2009. Situational sensitivities. In: Horwitz, D.F., Mills, D.S. (Eds.), BSAVA Manual of Canine and Feline Behavioural Medicine, 2nd Ed. British Small Animal Veterinary Association, Gloucester, UK, pp. 169-181.

Palestrini, C., Minero, M., Cannas, S., Rossi, E., Frank, D., 2010a. Video analysis of dogs with separation-related behaviors. Appl. Anim. Behav. Sci. 124, 61-67.

Palestrini, C., Minero, M., Cannas, S., Berteselli, G., Scaglia, E., Barbieri, S., Cavallone, E., Puricelli, M., Servida, F., Dall'Ara, P., 2010b. Efficacy of a diet containing caseinate hydrolysate on 1 stress in dogs. J. Vet. Behav.: Clin. Appl. Res. 5, 309-317.

Passalacqua, C., Marshall-Pescini, S., Merola, .I, Palestrini, C., Prato Previde, E. Different problem-solving strategies in dogs diagnosed with anxiety-related disorders and control dogs in an unsolvable task paradigm. Appl. Anim. Behav. Sci, 147, 139-148.

Rehn, T., Keeling, L.J., 2011. The effect of time left alone at home on dog welfare. Appl. Anim. Behav. Sci. 129, 129-135.

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

Santilli, R.A., Perego, M. 2009. Manuale di elettrocardiografia del cane e del gatto. Genesi ed interpretazione del ritmo cardiaco. Elsevier, pp.173.

Shiverdecker, M.D., Schiml, P.A., Hennessy, M.B., 2013. Human interaction moderates plasma cortisol and behavioral responses of dogs to shelter housing. Physiol. Behav. 109, 75-79.

Stetina, B.U., Glenk, L.M., 2011. Ethische Grundfragen der Psychologie in der Mensch-Tier-Beziehung. In: Felnhofer, A., Kothgassner, O.D., Kryspin- Exner, I. (Eds.), Ethik in der Psychologie. UTB facultas.wuv, Wien, Austria, pp. 160-180.

Stiles, E.K., Palestrini, C., Beauchamp, G., Frank, D,. 2011. Physiological and Behavioral Effects of Dextroamphetamine on Beagle Dogs. J. Vet. Behav.: Clin. Appl. Res. 6, 328-336.

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

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

Vincent, I.C., Leahy, R.A., 1997. Real-time non-invasive measurement of heart rate in working dogs: a technique with potential applications in the objective assessment of welfare problems. Vet. J. 153, 179–184.

Voith, V.L., Borchelt, P.L., 1996. Fears and phobias in companion animals. In: Voith, V.L., Borchelt, P.L. (Eds.), Readings in Companion Animal Behavior. Veterinary Learning Systems, Trenton, NJ, pp. 140e152

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

Wilson, C.C., Barker, S.B., 2003. Challenges in designing human-animal interaction research. Am. Behav. Sci. 47, 16-28.

Tables

Table 1: Behavioral categories and their definition

Behavioral category - DURATION-	Definition
Exploration - EX	Motor activity directed toward physical aspects of the environment, including sniffing, and gentle oral examination such as licking
Passive behavior - PA	Lying down with the head on ground without any obvious orientation toward the physical or social environment
Oriented to the environment - OE	Sitting, standing or lying down (the head does not rest on the ground) with obvious orientation toward the physical or social environment, including sniffing, close visual inspection, distant visual inspection (vigilance or scanning)
Interaction with the child – IC	Any behavior performed when interacting the child including active physical contact, sniffing, close visual inspection and gentle oral examination such as licking
Interaction with the handler – IH	Any behavior performed when interacting the handler including active physical contact, sniffing, close visual inspection and gentle oral examination such as licking
Interaction with the people – IP	Any behavior performed when interacting the people in the room (child's parents, hospital staff) including active physical contact, sniffing, close visual inspection and gentle oral examination such as licking
Withdrawal – WT	Avoiding interaction with the child by either moving away, very clearly turning away or looking away
Panting – PT	Rapid shallow breathing (mouth open)
Behavioral category - FREQUENCY-	**Definition**
Yawning – YA	Yawning
Lip-licking - LL	Part of tongue is shown and moved along the upper lip
Grooming – GR	The action of cleaning the body surface by licking, nibbling, picking, rubbing, scratching, etc. directed towards the animal's body (self-grooming)

366 Table 2: Mean ± SD, min and max heart rate (HR) values for each recorded session.

SESSIONS	HR MEAN±SD	MIN HR	MAX HR
1	91±15	70	121
4	140±8	88	126
6	75±3	71	138
7	93±12	78	119
11	86±8	75	134
14	100±6	94	115
15	95±18	77	132
16	85±10	72	111
19	84±10	69	127
20	104±5	95	116

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369 **Figure captions**

370 Figure 1: Proportional duration of behaviors of 20 AAT-working sessions of a therapy dog

371 Legend Figure 1: EX=exploration; PA=passive; OE=oriented to environment; IC=Interaction with the child;
372 PT=panting; IP=Interaction with the people; IH=Interaction with the handler; WT=withdrawal

373

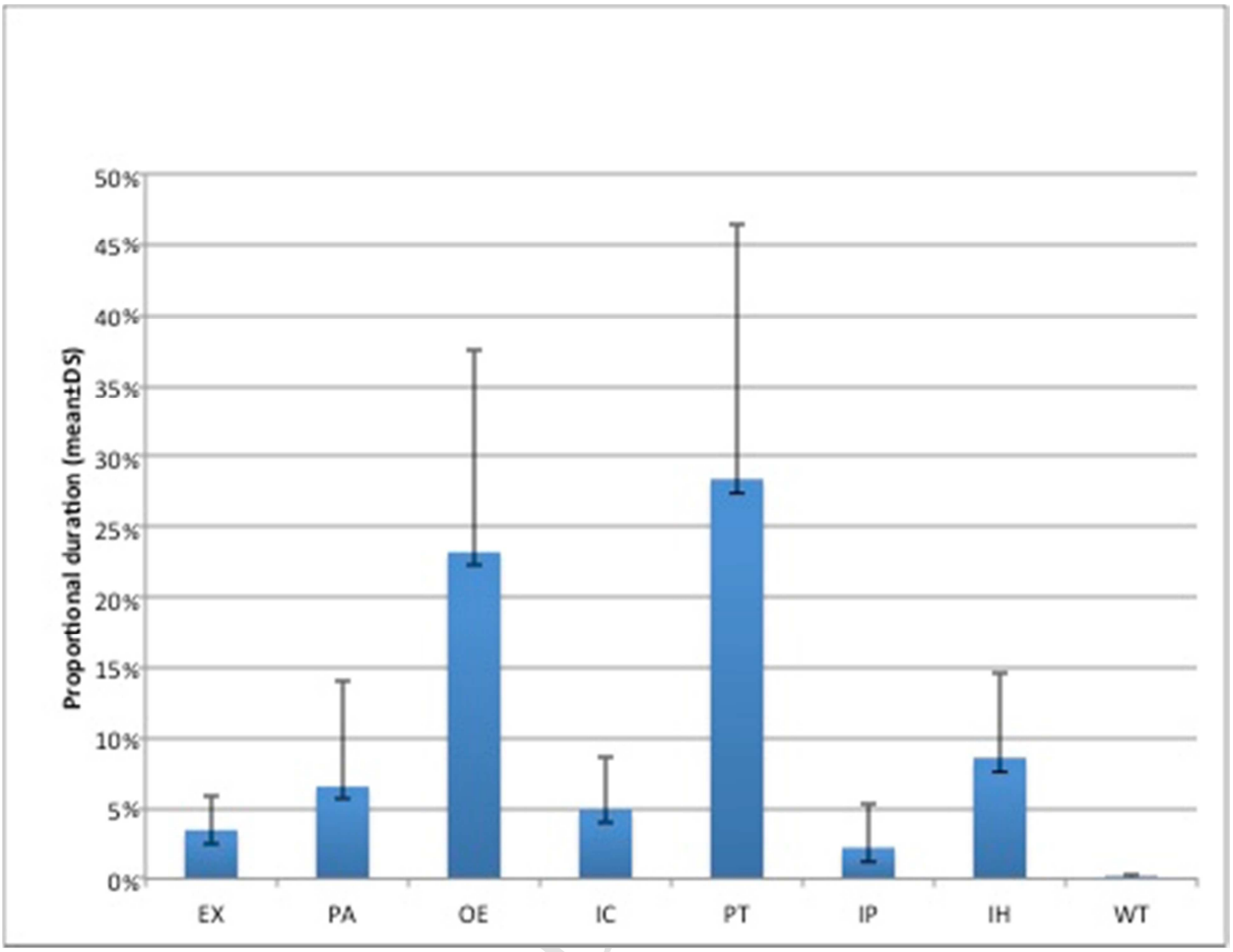
374 Figure 2: Proportional frequency of behaviors of 20 AAT-working sessions of a therapy dog

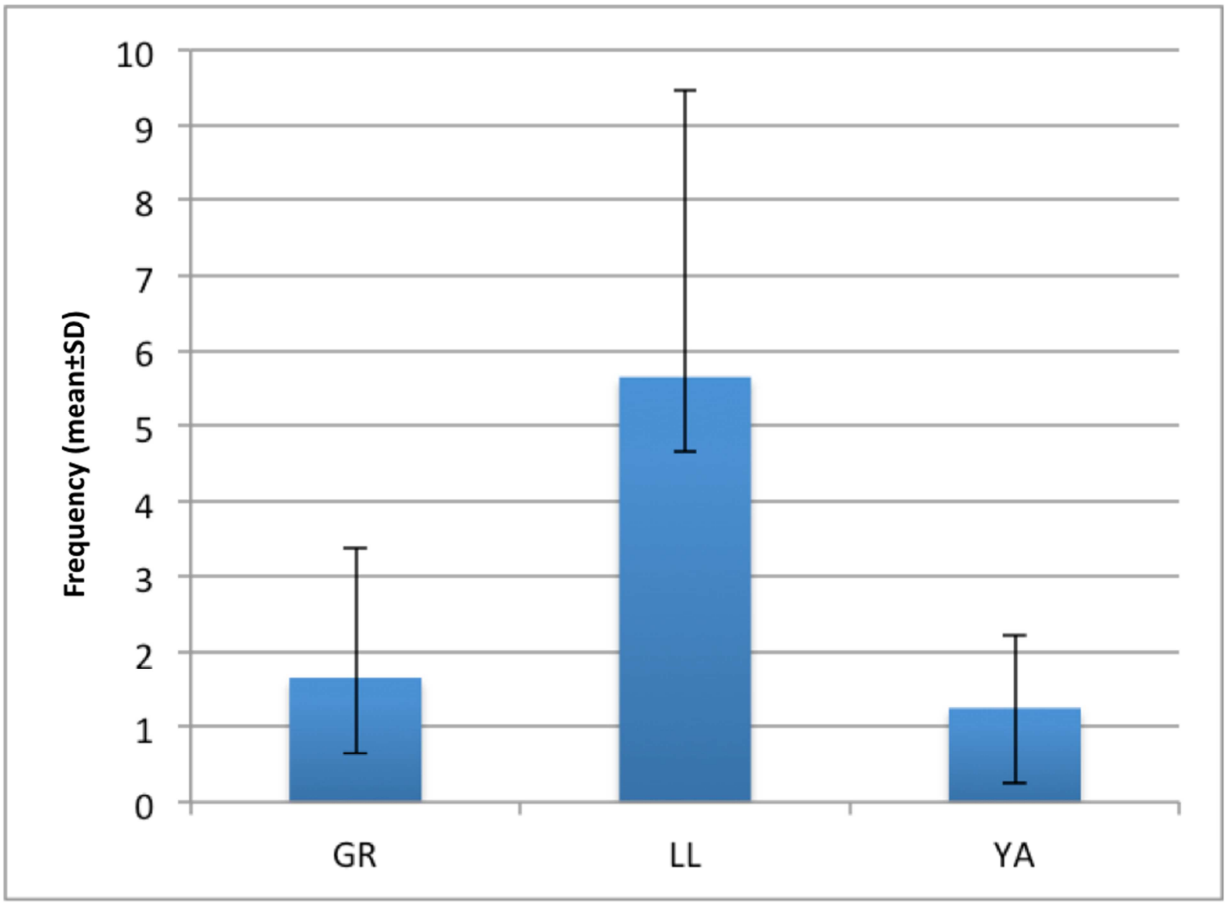
375 Legend Figure 2: GR=grooming; LL=lip-licking; YA=yawning

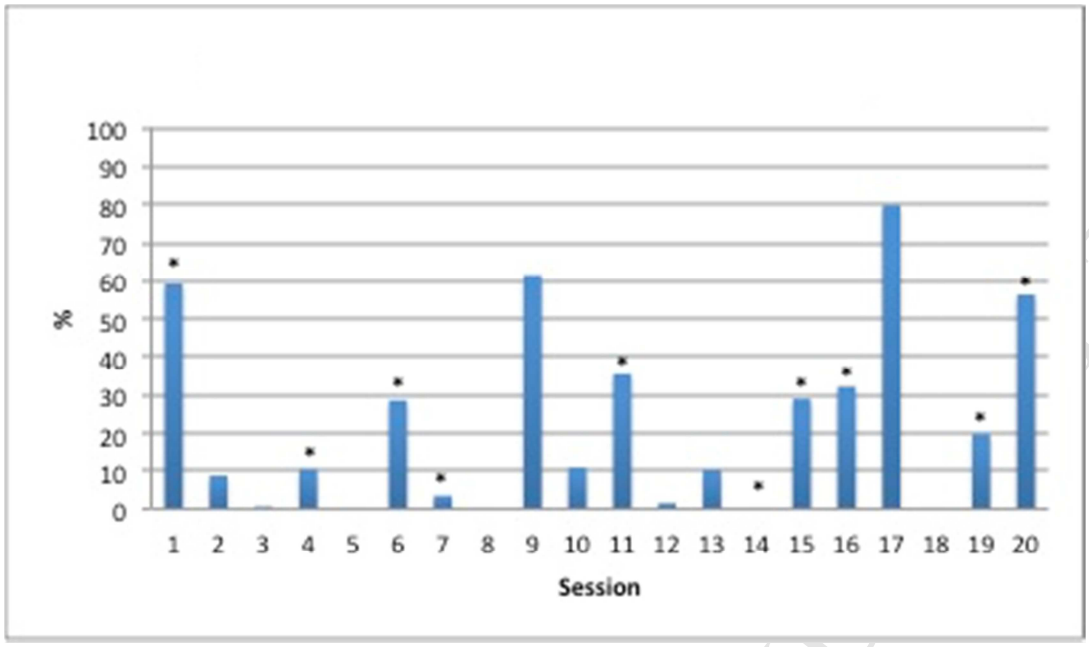
376

377 Figure 3: Proportional duration of children's interactions with the dog during 20 AAT- sessions. * refers to
378 sessions with corresponding heart rate measurements, as indicated in table 2.

379


Proportional duration (mean±DS)
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
EX
PA
OE
IC
PT
IP
IH
WT





- It is crucial to increase knowledge of which measurable variables reflect aspects of animal welfare and provide evidence on which standards should be achieved during AAT

- The robustness of an animal welfare assessment increases by comparing stress-associated behavior in conjunction with physiological parameters (i.e. cardiac activity)

- In our study no physiologic or behavioral indicators of stress, fatigue, or exhaustion were present during the AAT, suggesting that this activity did not negatively impact the welfare of the dog

- It is particularly important that the animal handler understands normal dog behavior in order to be able to recognize possible behavioral signs of stress during AAT