Journal of Veterinary Behavior 9 (2014) 98–106

Contents lists available at ScienceDirect

Journal of Veterinary Behavior

journal homepage: www.journalvetbehavior.com

Research

# Salivary cortisol and behavior in therapy dogs during animal-assisted interventions: A pilot study

Lisa Maria Glenk [a,b,*], Oswald David Kothgassner [c], Birgit Ursula Stetina [d], Rupert Palme [e], Berthold Kepplinger [b], Halina Baran [b]

[a] Department of Comparative Medicine, Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna, University of Vienna, Austria
[b] Department of Neurochemistry, Neuropharmacology, Neurorehabilitation and Pain Treatment, Karl Landsteiner Research Institute, Landesklinikum Mauer, Austria
[c] Department of Applied Psychology, Health, Development, Enhancement & Intervention, Working Group Clinical Psychology, University of Vienna, Austria
[d] Department of Psychology, Sigmund Freud University, Vienna, Austria
[e] Department of Biomedical Sciences, Institute of Medical Biochemistry, University of Veterinary Medicine, Vienna, Austria

ARTICLE INFO

*Article history:*
Received 16 May 2013
Received in revised form
3 February 2014
Accepted 10 February 2014
Available online 22 February 2014

*Keywords:*
animal-assisted therapy
interventions
dogs
behavior
salivary cortisol

ABSTRACT

Animal-assisted interventions (AAIs) have been associated with positive effects on human psychological and physiological health. Although the perception of quality standards in AAIs is high, only few investigations have focused on potential welfare implications for therapy dogs linked to their performance in AAIs. The standardized program “multiprofessional animal-assisted intervention (MTI)” has been carried out in adult mental health care, significantly improving patients’ prosocial behaviors. In the present study, we monitored salivary cortisol and behavioral measures in therapy dogs that participated in MTI group therapy sessions in an in-patient substance abuse treatment facility. Work-related activity (lay, sit, stand, walk, and run), behavior (lip licking, yawning, paw lifting, body shake, tail wagging, and panting), response to human action (taking food treats and obeying commands), and salivary cortisol levels were analyzed over the course of 5 subsequent MTI working sessions in experienced therapy dogs ($N = 5$), aged 5.4 ± 2.8 years (mean ± standard deviation). Salivary cortisol levels decreased from presession to postsession in sessions 1, 2, and 3. However, only in session 4 and 5, postsession cortisol levels were significantly lower than presession levels ($P = 0.043$). There was no difference between salivary cortisol levels sampled on a nonworking day at home and work-related levels sampled at the therapy site. None of the behavioral parameters varied significantly over the course of the 5 MTI sessions. Both lip licking ($P = 0.038$) and body shake ($P = 0.021$) were positively correlated with the decline in cortisol during session 5. The study results suggest that trained dogs are not being stressed by repeated participation in in-patient substance abuse therapy sessions. Further investigation into the effects of animal-assisted therapy on dogs’ physiological markers and behavior is warranted.



## Introduction

The practice of using dogs in therapy environments is constantly emerging. An extensive amount of research has attempted to link human–animal interaction during animal-assisted interventions (AAIs) with parameters important to human physical and psychological health (Friedmann et al., 2011). Using animals for human benefit, AAIs can be considered animal-assisted therapy (AAT) when they advocate the implementation of goal-directed, documented, and evaluated methodology into professional settings (Kruger and Serpell, 2006). In contrast, animal-assisted activities 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 participate in health interventions, exercise, and social interaction (Wilson and Barker, 2003).

* Address for reprint requests and correspondence: Lisa Maria Glenk, PhD, MSc, Department of Comparative Medicine, Messerli Research Institute, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria. Tel: +4366488460159.
*E-mail address:* lisa.molecular@gmail.com (L.M. Glenk).

1558-7878/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jveb.2014.02.005

Dog-assisted interventions with adult substance abuse patients improved the patient–therapist alliance, therapy motivation, and success (Wesley et al., 2009). Moreover, it has been proposed that a dog may provide more comfort and trust than a human therapist (Beetz et al., 2011). The multiprofessional animal-assisted intervention (MTI) meets all required criteria to be considered AAT (Kruger and Serpell, 2006) and is carried out by 2 human experts with a background in psychology, pedagogy, life science, and/or social science, providing wide-ranging expertise in their subject and a specially trained dog (Stetina et al., 2011). With a strong focus on positive reinforcement, respectful interaction, and appropriate dog handling, MTI has been standardized and evaluated with research outcomes that underpin the effectiveness of the intervention (Stetina et al., 2011). Pilot results by Burger et al. (2011) indicate that participation in 10 subsequent MTI sessions led to improvements in drug-addicted offenders' emotion regulation and self-control. Although the therapy animal is per definition a significant part of the AAI treatment process (Kruger and Serpell, 2006), attempts to monitor animal welfare in AAIs have been scarce (Hatch, 2007; Marinelli et al., 2009). Animal welfare has been referred to an animal's ability to adapt to and/or cope with the demands imposed by its environment (Broom and Fraser, 2007). In addition, the freedom to express natural behavior depicts an important aspect of dog welfare (Houpt et al., 2007). Behavioral concomitants of stress have been previously described in dogs (Beerda et al., 1999; Hydbring-Sandberg et al., 2004; Dreschel and Granger, 2005). Dogs subjected to social and spatial restriction showed enhanced frequencies of locomotion, yawning, paw lifting, and body shaking (Beerda et al., 2000). Bellaio et al. (2009) have identified lip licking, yawning, and body shaking as concomitants of stress in rescue dogs during training sessions. Schilder and van der Borg (2004) linked paw lifting in dogs to a state of conflict, confusion, and fear of punishment. Increased secretion of the adrenal glucocorticoid hormone cortisol has been related to cascading levels of physiological arousal (Chrousos, 2009). Although short-term effects of rising cortisol have an adaptive function in regulating an organism's bodily processes, prolonged high cortisol levels can lead to stress-related diseases and have been associated with negative effects on health (Chrousos, 2009). Over the past years, salivary cortisol in dogs has become an important marker in noninvasive stress assessment (Dreschel and Granger, 2009; Bennet and Hayssen, 2010). A combination of behavioral and physiological measurements is likely to yield reliable results in reflecting animal welfare. Behavioral studies targeting dog welfare and performance have been predominantly conducted with shelter dogs (Coppola et al., 2006; Hennessy et al., 2006), working dogs (Haverbeke et al., 2008; Horváth et al., 2008; Tomkins et al., 2011), and companion dogs (Kotrschal et al., 2009; Pastore et al., 2011). Welfare implications for therapy dogs may arise from interaction with strangers in unfamiliar environments, forced positions with no possibility to escape, and/or inappropriate training methods (Hatch, 2007; Piva et al., 2008). With regard to AAIs, animal welfare science lacks accurate studies that evaluate the effects of human–animal interaction on therapy dogs (Hatch, 2007; King et al., 2011). Preliminary investigations (Haubenhofer and Kirchengast, 2006, 2007; Marinelli et al., 2009; King et al., 2011) and anecdotal reports of case studies (Heimlich, 2001; Piva et al., 2008) have presented a conflicting picture regarding the potential welfare implications in dogs in therapy environments, but none of these studies was carried out with human adults participating in repeated AAI group sessions during in-patient substance abuse treatment. Wilson and Barker (2003) emphasized that results that were derived from experimental sampling in 1 particular facility are doubtfully generalizable to other facilities and that a clear description of an AAI needs to be given. The lack of standardized manuals, the variability in populations, working schedules, and the different contexts, in which AAIs are carried out, pose a problem for researchers (Marinelli et al., 2009; Deaton, 2005). Hence, a detailed description of the AAI program (including the duration of sessions, between session intervals, number of patients at 1 time, permanent or visiting animal), the research site, patient and dog population, from which the study samples are drawn, are crucial. The primary aim of this pilot study was to examine whether therapy dogs experience work-related stress during the MTI program, carried out with drug-addicted inpatients. Hence, we documented therapy dogs' salivary cortisol levels, activity, behaviors that have previously been related to stress, and responses to human action. To put the dogs' work-related cortisol levels into context, we compared them to samples that were collected at home on a nonworking day. Moreover, we strove to determine whether there were changes in the dogs' cortisol levels or behavioral variables linked to the effects of repeated weekly sessions.

## Materials and methods

### Animal subjects

Health care professionals who regularly work with their personal dog(s) in the MTI program were recruited via e-mail or telephone invitation. All participating dogs were privately owned and led by their handlers who had a professional background in human mental health care and also participated in the AAIs. To lessen experimenter influence, the experimenter attended 3 therapy sessions before data collection so that the dogs and patients were familiar with her presence. The 5 adult dogs (4 crossbreeds, 1 Labrador retriever) ranged in age from 3 to 10 years (mean $\pm$ standard deviation, 5.4 $\pm$ 2.8) and weighed from 20 to 35 kg (mean $\pm$ standard deviation, 27.8 $\pm$ 2.9). One dog was an intact male, 3 female dogs were spayed, and 1 female dog was intact. To be eligible for participation in the study, the dogs were required to be in good clinical health (i.e., free from pain, external and internal parasites, and immunized) and subjected to regular health screening and behavioral monitoring by a veterinarian or an ethologist. To choose a representative sample of therapy dogs, each participating dog had been awarded an AAI certificate and exhibited a minimum of 2 years of working experience. All dogs were previously trained with only positive reinforcement techniques. Moreover, only dogs that regularly (at least once a month) participated in AAIs over the past 2 years were considered.

### Study design

Sampling was carried out during 5 subsequent MTI sessions per dog, that is, 25 MTI sessions in total, with offenders in an inpatient substance abuse treatment facility in Austria. In the specialized facility, MTI was first launched in 2008 and has been established as an adjunct socialization therapy to rehabilitate offenders whose crimes have been associated with substance abuse. All the adult human participants of the MTI sessions that were analyzed over the course of this study enrolled in residential substance abuse treatment in Austria, participated voluntarily, agreed to be video-recorded for scientific purpose, underwent clinical–psychological screening, and appeared physically and mentally stable so that they posed no risk to themselves, the MTI professionals, and the therapy dog. MTI in residential substance abuse treatment aims at the training of social skills that shall ease reintegration of residents into working life and society. Each MTI session was 55-60 minutes in length and carried out once a week in groups of 8-10 participants who interacted with 1 therapy dog and 2 therapists, that is, MTI professionals, from whom 1 was owner of the respective dog (Figure 1). Therapy sessions consisted of alternating theoretical and practical parts. Participants remained the same within sessions and across the 5 different sessions. During intervention, the participants were seated in wooden chairs and only allowed to stand up, move around, and call or touch the dog in accordance with the MTI professionals' instructions. The MTI concept primarily builds on pedagogical, psychological, and biological findings and respectful human–animal interaction with a naturally behaving dog. Mediated by the MTI professionals, patients used signals to communicate with the dog. These signals were verbal or nonverbal cues including different hand signs, eye contact, mimics, words, and tone of voice. The main goal of MTI is to enhance patients' emotional and social competence through implicit learning during interaction with a therapy dog (Stetina et al., 2011). Accordingly, the participants had been instructed how to interact in an appropriate way with the therapy dog before the dog was introduced to the group. Human–animal contact was initiated by the freely moving dog, which was kept off lead. Human–animal interaction behaviors moreover included verbal contact, where people talked to the dog or spoke in a high-pitched/fluctuating voice to praise the dog. Tactile contact included softly touching, stroking, and/or grooming the dog. To play with the dog, people used dog toys and/or gently gestured with hand, arms, and fingers. For ethical reasons, dogs were never forced into positions and were able to lie down, drink water, or leave the therapy room at any time.

### *Dog behavior*

Behavioral observations were carried out via video analysis using a multicamera setup (Canon XM2, Canon MV960; Figure 1). After setting up the cameras, 50 minutes of therapeutic progress of each of the recorded sessions were coded. To guarantee the anonymity of the participants, the video material was stored in a closed facility at the Department of Clinical, Biological and Differential Psychology at the University of Vienna until analysis. Analysis of behavior was carried out using the Observer software package (Noldus Information Technology, 6702 EA Wageningen, The Netherlands).

In the prestudy phase (monitoring of 3 individual MTI sessions), we identified behavioral variables that can be reliably recognized and agreed on by different observers. Gestures and behaviors of the therapy dogs were evaluated because of their relative frequency and/or duration of occurrence during the observation period (Table 1). As described in Table 1, behavioral taxonomy was chosen in accordance with previous studies (Clark et al., 1997; Beerda et al., 1999; Ley et al., 2007; Haverbeke et al., 2008; Piva et al., 2008; Pastore et al., 2011). Intraobserver reliability calculations refer to the rating consistency in a single observer who records behavioral sequences and is usually assessed by comparing the same observer's reports from a recording of the same sequence viewed on 2 or more separate occasions (Taylor and Mills, 2006; Martin and Bateson, 2007). We assessed intraobserver reliability of the experimenter who analyzed the videos by repeated coding of independent samples of videotaped sessions and calculated the percentage of agreement, which was greater than 93%.

### *Salivary sampling*

To absorb dog saliva, we used commercial cotton rolls in tubes (Salivette®, 51.1534, Sarstedt, Wiener Neudorf, Austria). To avoid sample contamination and reduced reliability of the enzyme immunoassay (EIA), the sampling devices did not contain food-based additives that could have interfered with the EIA (Dreschel and Granger, 2009). To stimulate salivation, dogs were only allowed to sniff at the food treats in the experimenter's closed hand and not to chew on them (Bennet and Hayssen, 2010; Ligout et al., 2010). The saliva collection device was gently placed into the cheek pouch of the dog by its owner until it was saturated with saliva (approximately 40-70 seconds). Only small pieces of cheese were used as food treats to yield reliable results on cortisol (Ligout et al., 2010). In addition, we inspected each sample for visible contaminations with food or blood. Two contaminated samples (home baselines) were excluded from the analysis. Dogs were first trained for the sampling procedure at home (3 samples were taken at 0900-1000, 1300-1400, and 1800-1900 hours, respectively, on 2 nonconsecutive days). Then, before the experimental sampling protocol started, dogs were sampled twice at the therapy facility between 0930 hours and 1200 hours. Samples collected during the training phase were not included in the analysis. For ethical reasons, dogs were never restrained. After the cotton roll was soaked with saliva, the collected material was stored in an ice box before the samples were finally stored at −20°C. Before analysis, samples were thawed on ice and centrifuged at room temperature at 3000 *g* for 15 minutes to obtain clear saliva for cortisol analysis.

### *Sampling schedule*

To lessen potential circadian effects on salivary cortisol, only AAIs starting in the morning from 9.30 a.m. to 11.00 a.m. were

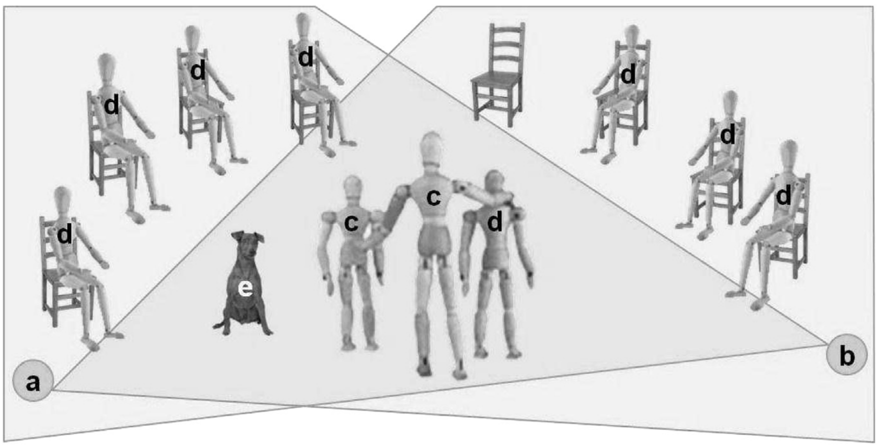


**Figure 1.** Dog-assisted group intervention (multiprofessional animal-assisted intervention [MTI]) recorded with (a, b) a multicamera setup, (c) MTI professionals, (d) participants, and (e) therapy dog.

**Table 1**
Activity, behavior, and response to human action were recorded in duration (D) or frequency (F) of occurrence

| Category | Code | D/F | Description of behavior |
|---|---|---|---|
| Activity | Lay | D | Resting position with trunk in contact with the ground |
| | Sit | D | Hindquarters and front paws only in contact with the ground |
| | Stand | D | Upright position with at least 3 paws in contact with the ground |
| | Walk | D | Taking at least 1 step, shifting body position |
| | Run | D | Any motion faster than a walk including trotting |
| Behavior | Lip licking | F | Part of the tongue is shown and moved to the upper lip |
| | Yawning | F | Mouth is open to apparent fullest extent while eyes are closed |
| | Panting | D | Mouth is open with the tongue protruding |
| | Paw lifting | F | Fore paw is lifted into a position of approximately 45° |
| | Body shake | F | Rotation of the body, starting at the head and moving caudally |
| | Tail wagging | D | Repetitive wagging movements of the tail |
| Response to human action | Takes a treat | F | Dog takes a food treat |
| | Obey to command | F | Dog responds to a human cue with a change in behavior |

considered in the analysis. Salivary samples were collected in a time frame of less than 4 minutes (Kobelt et al., 2003). As seen in Figure 2, presession sampling (T1) was carried out before each of the MTI sessions, after the dogs had approximately 30 minutes of quiet rest at the therapy site. The working sampling schedule was adjusted considering that salivary cortisol levels reflect plasma cortisol with a delay of 20-30 minutes (Vincent and Michell, 1992). After each 55-60 minute-long intervention, an additional 5 minutes were scheduled, during which the dogs received no more food treats. We used this schedule to capture postsession levels (T2, Figure 2) that correspond to the time during therapy sessions. To obtain control values for the working condition, dog owners were provided with written instructions on how to sample saliva on a nonworking day at home (3 saliva samples at 0900-1000, 1300-1400, and 1800-1900 hours). Home baselines represent the dogs' salivary cortisol levels on their daily routines without any therapy-related work. The scheduled time interval for home sampling was 3 days after a working day to avoid sampling on consecutive days. To lessen potential effects of food or exercise on home baseline cortisol levels, dog owners were advised not to feed their dogs at least 1 hour before sampling and to avoid any hard or unusual exercise on that day. Daily routines included interaction with family members and/or other dogs, quiet play, walking, and gentle obedience training.

*Sample analysis*

On average, 50 μL of clear saliva were used for the analysis. Analyses were carried out at the Institute of Medical Biochemistry at the University of Veterinary Medicine in Vienna with a highly sensitive cortisol EIA (Palme and Möstl, 1997) kit that has been previously used in dog saliva assessment (Haubenhofer and Kirchengast, 2006). Samples were assayed in duplicates (10 μL each after a 1:10 dilution with assay buffer). The average intraassay and interassay coefficients of variance were less than 10% and 15%, respectively (Palme and Möstl, 1997).

*Statistics*

Calculations were carried out using the SPSS 15.0 statistical package for Windows (SPSS, Inc., Chicago, IL). Shapiro–Wilks tests were used to examine normal distribution of each data set. Statistical analyses were the Wilcoxon signed rank test and Friedman 2-way analysis of variance (ANOVA) on the cortisol data and Friedman 2-way ANOVA to detect differences in behavioral variables across multiple testing. Spearman rank correlation was used to search for relations between behavioral variables and the difference between presession and postsession cortisol (T1-T2). All data represent group means plus standard error. We considered statistical significance at $P \leq 0.05$.

## Results

A total of 25 MTI sessions and 3 home baseline samples per dog were included in the analysis. Because data were not normally distributed and the dog sample was considerably small, we used nonparametric statistics. Each statistical unit represents an N of 5.

*Cortisol*

We looked at salivary cortisol levels that were sampled before the start of a therapy session (presession levels, T1) and postsession levels (T2) that were assessed after completion of the therapy session. As demonstrated in Figure 3, Wilcoxon signed rank test showed no significant differences in the comparison of presession and postsession levels in session 1 ($Z = -1.214$, $P = 0.225$) and 2 ($Z = -1.214$, $P = 0.225$). Session 3 was marked by a nonsignificant trend ($Z = -1.753$, $P = 0.08$), whereas in session 4 ($Z = -2.023$, $P = 0.043$) and five ($Z = -2.023$, $P = 0.043$), T1 and T2 differed significantly. Looking at the repeated sessions, there were no differences across the 5 presession ($\chi^2 = 0.320$, $P = 0.998$) or postsession levels ($\chi^2 = 3.680$, $P = 0.451$). Home baseline levels at 0900-1000, 1300-1400, and 1800-1900 hours did differ neither across the 3 time points ($\chi^2 = 0.400$, $P = 0.819$; Figure 4) nor from presession levels ($\chi^2 = 0.943$, $P = 0.988$) or postsession levels ($\chi^2 = 7.057$, $P = 2.16$) at the therapy site.

*Behavior*

As shown in Table 2, the behavioral variables lay, sit, stand, walk, and run were subsumed in the category "activity." Data were analyzed as total duration(s) of occurrence during the observation period across 5 subsequent MTI sessions. Analyses of the results using Friedman 2-way ANOVA (N = 5) did not reveal any significance in the observed parameters "lay" ($\chi^2 = 1.120$, $P = 0.891$), "sit" ($\chi^2 = 4.160$, $P = 0.385$), or "stand" ($\chi^2 = 8.960$, $P = 0.062$). Again, no

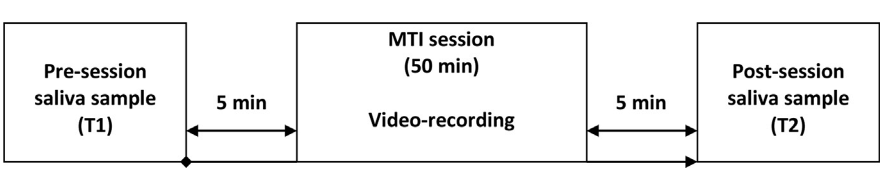


**Figure 2.** Saliva sampling and video-recording schedule.

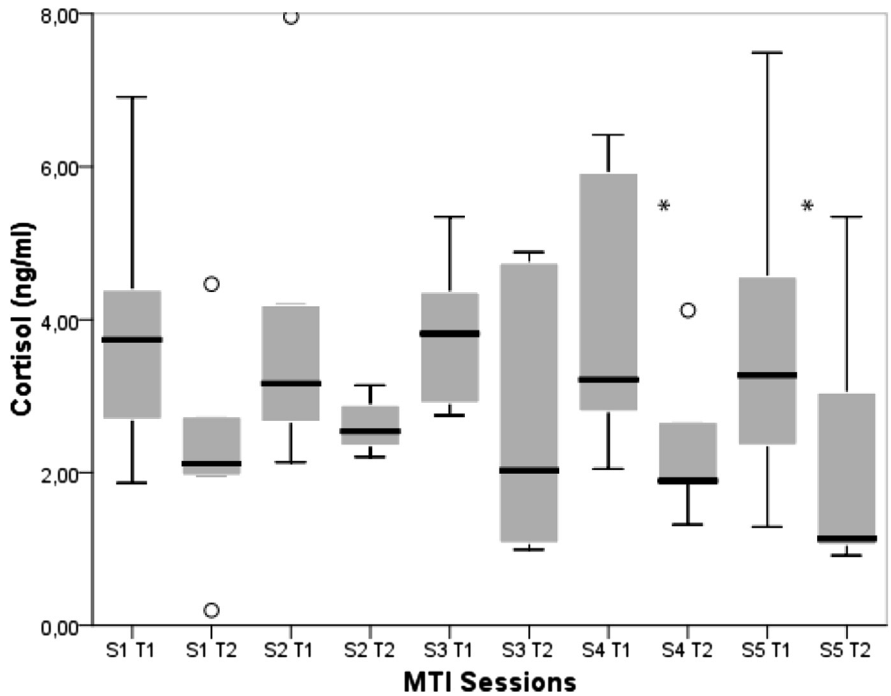


**Figure 3.** Salivary cortisol (ng/mL) levels in therapy dogs (N = 5) before (T1) and during (T2) 5 repeated MTI working sessions (S1-S5). Data are shown as box plots (circles represent outliers, and the black line represents the median). The "*" symbol indicates a significant difference with $P \leq 0.05$ (Wilcoxon test). MTI, multiprofessional animal-assisted intervention.

significant time course was found in "walk" ($\chi^2 = 4.320$, $P = 0.364$) or "run" ($\chi^2 = 2.400$, $P = 0.663$).

In addition to activity, we measured behavioral variables that have been previously linked to stress in dogs. As shown in Table 3, the results of our statistical analysis (Friedman 2-way ANOVA; N = 5) comparing behaviors (measured in frequency of occurrence) demonstrate that there was no significant effect regarding the 5 subsequent MTI sessions for behaviors "lip licking" ($\chi^2 = 4.638$, $P = 0.326$), "yawning" ($\chi^2 = 0.857$, $P = 0.931$), "paw lifting" ($\chi^2 = 1.395$, $P = 0.845$), and "body shake" ($\chi^2 = 4.790$, $P = 0.310$). Decreasing cortisol in session 5 (Figure 3) was correlated with "lip licking" ($r_s = 0.899$, $P = 0.038$) and "body shake" ($r_s = 0.931$, $P = 0.021$). Panting and tail wagging behaviors that were measured in duration of occurrence(s) revealed no significant effect over the 5 MTI sessions

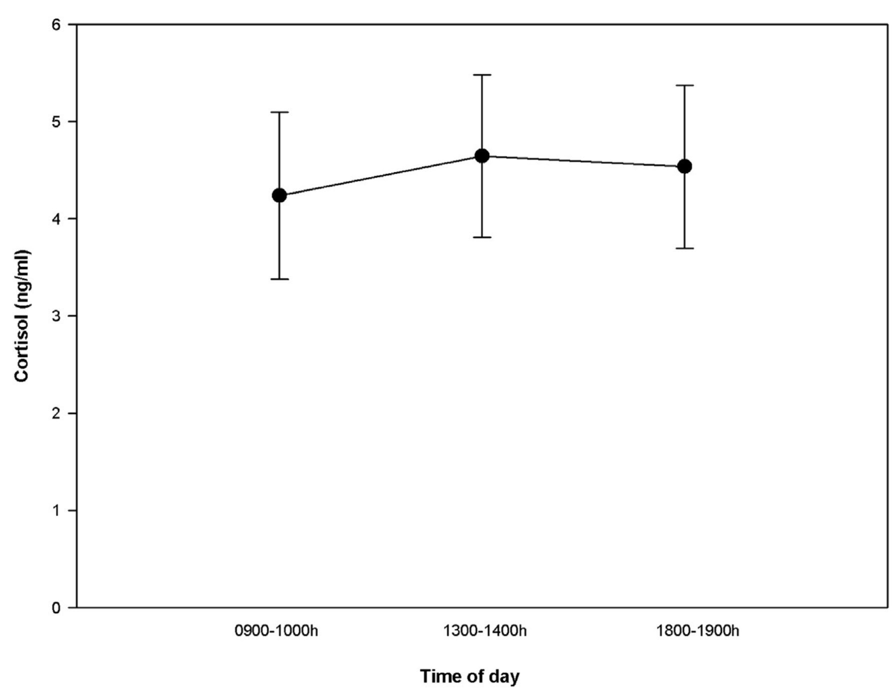


**Figure 4.** Mean (±standard error of the mean) salivary cortisol concentrations (ng/mL) in therapy dogs (N = 5) collected at 3 different time points (0900-1000, 1300-1400, and 1800-1900 hours) on a nonworking day at home.

**Table 2**
Activity in therapy dogs during 5 subsequent MTI sessions (S1-S5)

| Activity | Session 1 | | Session 2 | | Session 3 | | Session 4 | | Session 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Lay | 715.68 | 348.46 | 608.34 | 397.3 | 745.12 | 609.84 | 542.84 | 319.41 | 703.48 | 589.35 |
| Sit | 703.92 | 698.82 | 491.12 | 389.37 | 861.26 | 854.78 | 590.6 | 555.87 | 763.16 | 321.17 |
| Stand | 857.74 | 286.62 | 1110.2 | 195.03 | 638.64 | 293.06 | 942 | 446.55 | 998.64 | 453.38 |
| Walk | 511.28 | 238.65 | 544.42 | 273.17 | 556.86 | 324.09 | 416.78 | 211.94 | 464.48 | 149.89 |
| Run | 43.26 | 30.36 | 54.66 | 54.1 | 80.9 | 108.22 | 63.58 | 41.55 | 63.34 | 51.01 |

MTI, multiprofessional animal-assisted intervention; SD, standard deviation.
Behaviors were recorded in duration (D) of occurrence, given in seconds (s). Data of all variables listed were analyzed with Friedman 2-way analysis of variance (N = 5).

when analyzed with Friedman 2-way ANOVA ($\chi^2 = 0.800$, $P = 0.938$ and $\chi^2 = 1.120$, $P = 0.891$, respectively).

We also recorded responses to human action, that is, the number of food treats taken by the dog and obedience commands that caused changes in the dogs' behavior. Again, as indicated in Table 4, no significant differences over the course of the 5 sessions were found for the number of food treats ($\chi^2 = 4.404$, $P = 0.354$) and obedience commands ($\chi^2 = 2.735$, $P = 0.603$), respectively.

## Discussion

Because humans benefit from the interaction with therapy dogs, behavioral and physiological health of the therapy animal should be thoroughly reflected (Stetina and Glenk, 2011). Although dog users have a legal and moral duty of care to maximize their dogs' welfare (Rooney et al., 2009, p. 128), standardized manuals how to handle therapy dogs in AAIs do not exist. In the absence of legal regulations, some initial steps have been made and some organizations (e.g., American Veterinary Medical Association, International Association of Human Animal Interaction Organizations, International Society for Animal-Assisted Therapy, European Society for Animal Assisted Therapy) have provided minimum requirements to protect animal welfare during AAIs in closely monitoring signs of discomfort or stress of the animals. However, there is an essential need to gain a deeper understanding of which measurable variables reflect aspects of animal welfare and what standards should be achieved. The research body on animals in AAIs is limited and does not provide evidence on which standards should be issued regarding animal welfare (Beck and Katcher, 2003). In dog-assisted therapy, substantial differences exist between different programs with regard to the methods in dog training, the AAI working schedule, prearrangement of the therapy session (i.e., time span between arrival at a facility and the start of the AAI), and quality assessment and quality assurance (Stetina and Glenk, 2011). The present study is the first assessment of therapy dogs' welfare during AAIs with adult inpatients undergoing substance abuse treatment and differs from earlier studies in several aspects. Research on therapy dogs has been carried out at multiple therapy sites (hospitals, rehabilitation centers, retirement homes, and schools) by Haubenhofer and Kirchengast (2006, 2007) and Marinelli et al. (2009), hospitals (King et al., 2011), and in-patient substance abuse treatment in this study. Regarding the type of AAI, Haubenhofer and Kirchengast (2006, 2007) and Marinelli et al. (2009) included both animal-assisted activities and AAT in their analysis, whereas King et al. (2011) and we studied only interventions meeting the AAT criteria. Moreover, in our investigation, only adult participants were included and therefore, the study design differs from the other research on both adult and children participants. In contrast to King et al. (2011) who monitored only single patient AAT sessions and Marinelli et al. (2009) who included dogs in both single and group sessions, we focused on group therapy sessions only. No information about the number of participants in AAIs is given by Haubenhofer and Kirchengast (2006, 2007). The duration of therapy sessions differs markedly across and within studies from 1 to 8 hours (Haubenhofer and Kirchengast, 2006, 2007), 2 hours (King et al., 2011), 10-105 minutes (Marinelli et al., 2009), and 55-60 minutes in the present investigation. The same accounts for the between-session intervals, which were variable (9-50 sessions during 3 months) for dogs participating in the studies by Haubenhofer and Kirchengast (2006, 2007). Marinelli et al. (2009) examined daily sessions, King et al. (2011) biweekly sessions, and in the present study, weekly sessions were analyzed. Finally, dog welfare indicators ranged from salivary cortisol and emotions according to dog handlers' perception [Haubenhofer and Kirchengast (2006, 2007)] and monitoring of working activities (Marinelli et al., 2009) to salivary cortisol and behavior in the study of King et al. (2011) and this study. There is evidence that cortisol secretion in dogs is influenced by contact with humans (Coppola et al., 2006; Kotrschal et al., 2009). Positive behaviors, interactions, and quiet play with humans can decrease cortisol in dogs (Coppola et al., 2006; Hennessy et al., 2006; Horváth et al., 2008, Shiverdecker et al., 2013), whereas punitive behaviors and threats had the opposite effect (Beerda et al., 1999; Jones and Josephs, 2006; Horváth et al., 2007). Our data indicate that cortisol did not increase from presession to postsession levels. A significant decrease in cortisol was found in sessions 4 and 5.

**Table 3**
Behavior in therapy dogs over 5 subsequent MTI sessions (S1-S5)

| Activity | Session 1 | | Session 2 | | Session 3 | | Session 4 | | Session 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Lip licking (F) | 17 | 8.03 | 18 | 9.41 | 22.8 | 10.08 | 21 | 13.04 | 15.8 | 8.93 |
| Yawning (F) | 2.6 | 2.41 | 3 | 2.83 | 2.6 | 2.3 | 3 | 3.54 | 2.2 | 2.49 |
| Body shake (F) | 2 | 1 | 1.2 | 0.45 | 0.8 | 0.84 | 1.2 | 1.1 | 0.6 | 0.89 |
| Paw lifting (F) | 2.4 | 1.82 | 2.8 | 4.15 | 3.8 | 3.27 | 2.8 | 2.39 | 7.8 | 8.79 |
| Panting (D) | 848.82 | 861.87 | 908.62 | 509.96 | 809.06 | 914.92 | 848.74 | 863.78 | 802.52 | 523.34 |
| Tail wagging (D) | 796.88 | 351.98 | 939.2 | 695.13 | 867.88 | 782.71 | 941.96 | 781.34 | 1034.74 | 853.73 |

MTI, multiprofessional animal-assisted intervention; SD, standard deviation.
Behaviors were recorded in duration (D) or frequency (F) of occurrence. Duration (D) of behavior is given in seconds (s). Data of all variables listed were analyzed with Friedman 2-way analysis of variance (N = 5).

**Table 4**
Responses to human action in therapy dogs over 5 subsequent MTI sessions (S1-S5)

| Activity | Session 1 | | Session 2 | | Session 3 | | Session 4 | | Session 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Takes a treat | 47.8 | 23.44 | 48.8 | 22.26 | 56.4 | 24.89 | 45.8 | 17.04 | 52.6 | 15.27 |
| Obey to command | 30.4 | 12.42 | 27 | 10.77 | 40.6 | 19.4 | 41.2 | 23.06 | 51 | 35.04 |

MTI, multiprofessional animal-assisted intervention; SD, standard deviation.
Behaviors were recorded in frequency (F) of occurrence. Data of all variables listed were analyzed with Friedman 2-way analysis of variance (N = 5).

Thus, our study results suggest that therapy dogs, by means of salivary cortisol, were not acutely stressed by participation in the MTI program. In previous research, we showed that both in training and experienced therapy, dogs' cortisol levels did not increase during AAI sessions. However, significant decreases in cortisol were only found in experienced therapy dogs that were off lead during therapy, suggesting that the use of a lead could be an important factor (Glenk et al., 2013). It is possible that therapy dogs get accustomed to the patients over repeated sessions with the same participants, but to draw a conclusion, further study into the effects of habituation is needed. In further interpreting our cortisol data, it has to be stated that dogs could be stressed before arriving at the therapy site, for example, when they travel via public transport or are moved in or out of cars. Thus, in our study, dogs were given 30 minutes of quiet rest at the therapy site before presession data were gathered. Resulting from their previous experiences with the setting and people, the dogs may have anticipations of the sessions (e.g., play, receiving treats, being praised), which could influence their cortisol levels. The results of the present study indicate that if therapy dogs move freely, receive positive attention, and food treats during repeated 1-hour sessions in a closed group of people who are familiar to the dogs, then cortisol levels decrease. To further explore the relevance of patient familiarity, a monitoring protocol to study individual patient–dog relationships over time would be desirable. Future studies should certainly control for the effects of different working conditions and environments by manipulating the therapy sessions (e.g., food treats vs. no food treats, strangers vs. familiar people, shorter vs. longer sessions). As the present investigation was set up as a pilot field study, we could not control for these factors. In addition to manipulation of therapy conditions, it would also be important to consider activities other than the therapy condition (e.g., obedience training or quiet play with the owner, familiar and unfamiliar people) as controls. In our study, saliva samples were collected on nonworking days in home environments as controls to the therapy condition. Salivary cortisol home levels did not differ from working levels associated with performance in AAIs. No differences between the sampling locations (i.e., home, therapy facility) were found. These results are in line with the study of Wenger-Riggenbach et al. (2010), who also found that there was no difference between salivary cortisol levels sampled at home or at a veterinary clinic and furthermore corroborate our previously reported data on home- and working cortisol levels in experienced therapy dogs and therapy dogs in training (Glenk et al., 2013). However, further interpretation of home baseline cortisol levels without detailed information on the individual daily routines of the dogs is challenging. Although observation of activity can give rise to hypotheses on dog welfare, individual coping styles have to be considered (Rooney et al., 2009). Previous research has revealed that stressed dogs may either engage in motion activity (Hiby et al., 2006) or appear quiet and inactive (Rooney et al., 2009). Regarding our data from healthy dogs participating in the MTI program, no differences in activities (lay, sit, stand, walk, and run) were found over the observation period of 5 subsequent working sessions. Examining the effects of exercise on dogs' cortisol levels, no associations were found (Clark et al., 1997). Taking our findings into consideration, it is unlikely that the significant decreases in cortisol we faced during sessions 4 and 5 can be attributed to changes in activities. Apart from activity, we also recorded behaviors (lip licking, yawning, paw lifting, panting, tail wagging, and body shake) that have been controversially associated with impaired welfare. Tail wagging and panting recorded by Beerda et al. (1998, 2000) were related to chronic stress and elevation in cortisol. We found no significant temporal patterns of behaviors over the 5 sessions tested. Lip licking occurred more often than the other behavioral variables, that is, yawning, paw lifting, and body shake. In addition, lip licking was positively correlated with body shake and the significant decrease in cortisol during session 5. Lip licking and yawning have also been suspected to precede situations of social conflict in dogs (Voith and Borchelt, 1996). However, similarly to the conclusions drawn by Rehn and Keeling (2011), we suggest that lip licking and body shaking may be communicative cues in dogs that do not necessarily correspond to a stressful experience but, on the contrary, may help to manage stress. Recent data from the study of Shiverdecker et al. (2013) are in line with this assumption. In a study on behavior and cortisol associated with competition in agility dogs, Pastore et al. (2011) also proposed that body shaking may help to relieve stress-related tension. Tail wagging and panting were recorded by Beerda et al. (1998, 2000) in connection to chronic stress and cortisol elevation in dogs. However, caution needs to be taken when interpreting behavioral cues because behavior appears to be closely related to the context of a situation (McEwen and Stellar, 1993). Our behavioral data showed no further connection to cortisol, but we expect that a replication of the study with a larger dog sample may provide more insights on behaviors and their relationship to cortisol. The number of food treats taken by the dogs and number of commands that the dogs responded to with a change in behavior were analyzed. We did not observe differences in the number of food treats or obedience commands between the 5 sessions. However, other types of responses to human action that can be observed during a therapy session (e.g., physical contact, eye contact, mimics) deserve further investigation. Time frame and available resources were a limiting factor in this study. The number of sessions where sampling was possible was limited by the substance abuse treatment facility, and only a sample of 5 MTI dogs met the requirements (regular participation in the program, certification status, and working experience) to be included in the study. Nevertheless, because therapy dogs in general undergo careful selection and have to meet specific criteria to be awarded an AAI certificate (Serpell et al., 2010), the recruited sample accurately represents the target dog population. It has been suggested that the intervention context and the behaviors of humans involved influence the outcomes of AAIs (Wilson and Barker, 2003). It is likely that this assumption refers not only to the human side but also to the animal perspective. Categorization of positive, neutral, or negative human behaviors toward animals has been previously used to assess human–animal relationships (Waiblinger et al., 2004; Waiblinger et al., 2006) and may as well yield additional insights into the biobehavioral responses in therapy dogs. Accordingly, in follow-up studies, it would be interesting to monitor each patient's behavior toward the therapy dog. To interpret the quality of interactions, an analysis of patient unresponsiveness to the dog's signals and vice versa would be very interesting. To provide high quality in AAIs, it is essential to monitor and interpret physiological and behavioral parameters that are related to animal welfare. The development of a practitioner's guide on dog welfare for AAI professionals and, may be even more importantly, AAI volunteers shall be a forthcoming endeavor. AAI volunteers are often very dedicated to their work, but they also need to be well aware of subtle signs of discomfort in their dogs. Future research still needs to identify the populations or situations where contact with therapy animals may be potentially problematic or inappropriate for either the animals or the people involved (Beck and Katcher, 2003).

## Acknowledgments

The authors thank all dog owners and their therapy dogs for volunteering to participate in the study.

## Ethical considerations

The procedures of the research proposal have been approved by the Ethics Committee of the University of Veterinary Medicine Vienna. Research was based on voluntary participation and oral and/or written informed consent with the institution, patients, and animal handlers.

## Conflict of interest

The authors declare no conflict of interest. The idea for the article was conceived by L.M. Glenk, H. Baran, B.U. Stetina, and B. Kepplinger. The experiments were designed by L.M. Glenk, H. Baran, B.U. Stetina, and B. Kepplinger. The experiments were performed by L.M. Glenk. The data were analyzed by L.M. Glenk, R. Palme, and O.D. Kothgassner. The article was written by L.M. Glenk and H. Baran. The study was partially funded by a doctoral grant given to L.M. Glenk by the University of Veterinary Medicine Vienna.

## 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., Van Hoff, J.A., 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., Van Hoff, J.A., De Vries, H.W., Mol, J.A., 1999. Chronic stress in dogs subjected to social and spatial restriction. II. Hormonal and immunological responses. Physiol. Behav. 66, 243–254.
Beerda, B., Schilder, M.B., Van Hoff, J.A., De Vries, H.W., Mol, J.A., 2000. Behavioural and hormonal indicators of enduring environmental stress in dogs. Anim. Welf. 9, 49–62.
Beetz, A., Kotrschal, K., Turner, D., Hediger, K., Uvnäs-Moberg, K., Julius, H., 2011. The effect of a real dog, toy dog and friendly person on insecurely attached children in a stressful task: an exploratory study. Anthrozoös 24, 349–368.
Bellaio, E., Normando, S., Bono, G., 2009. Stress assessment in rescue dogs during routine training sessions. J. Vet. Behav.: Clin. Appl. Res. 4, 83.
Bennet, A., Hayssen, V., 2010. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences. Domest. Anim. Endocrinol. 39, 171–180.
Broom, D.M., Fraser, A.F., 2007. Domestic Animal Behaviour and Welfare, 4th ed. CABI, Wallingford, UK.
Burger, E., Stetina, B.U., Turner, K., McElheney, J., Handlos, U., 2011. Dog-assisted therapy in prison: emotional competences and emotional status of drug-addicted criminal offenders. J. Vet. Behav.: Clin. Appl. Res. 6, 79–80.
Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5, 374–381.
Clark, 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.
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.
Deaton, C., 2005. Humanizing prisons with animals: a closer look at "cell dogs" and horse programs in correctional institutions. J. Correct. Educ 56, 46–62.
Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. Appl. Anim. Behav. Sci. 95, 153–168.
Dreschel, N.A., Granger, D.A., 2009. Methods of collection for salivary cortisol measurement in dogs. Horm. Behav. 55, 163–168.
Friedmann, E., Barker, S.B., Allen, K.M., 2011. Physiological correlates of health benefits from pets. In: McCardle, P., McCune, S., Griffin, J.A., Maholmes, V. (Eds.), "How Animals Affect Us: Examining the Influences of Human–Animal Interaction on Child Development and Human Health". American Psychological Association, Washington, DC, pp. 163–187.
Glenk, L.M., Kothgassner, O.D., Stetina, B.U., Palme, R., Kepplinger, B., Baran, H., 2013. Therapy dogs' salivary cortisol levels vary during animal-assisted interventions. Anim. Welf. 22, 369–378.
Hatch, A., 2007. The view from all fours: a look at an animal-assisted activity program from the animals' perspective. Anthrozoös 20, 37–50.
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.
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.
Heimlich, K., 2001. Animal-assisted therapy and the severely disabled child: a quantitative study. J. Rehabil. 67, 48–54.
Hennessy, M., Morris, A., Linden, F., 2006. Evaluation of the effects of a socialization program in a prison on behavior and pituitary-adrenal hormone levels of shelter dogs. Appl. Anim. Behav. Sci. 99, 157–171.
Hiby, E.F., Rooney, N.J., Bradshaw, J.W.S., 2006. Behavioural and physiological responses of dogs entering re-homing kennels. Physiol. Behav. 89, 385–391.
Horváth, Z., Igyártó, B.-Z., Magyar, A., Miklósi, Á., 2007. Affiliative and disciplinary behavior of human handlers during play with their dog affects cortisol concentrations in opposite directions. Horm. Behav. 54, 107–114.
Horváth, Z., Dóka, A., Miklósi, Á., 2008. Three different coping styles in police dogs exposed to a short-term challenge. Horm. Behav. 52, 621–630.
Houpt, K.A., Goodwin, D., Uchida, Y., Baranyiova, E., Fatjo, J., Kakuma, Y., 2007. Proceedings of a workshop to identify dog welfare issues in the US, Japan, Czech Republic, Spain and the UK. Appl. Anim. Behav. Sci. 106, 221–233.
Hydbring-Sandberg, E., von Walter, L.W., Höglund, K., Svartberg, K., Swenson, L., Forkman, B., 2004. Physiological reactions to fear provocation in dogs. J Endocrinol 180, 439–448.
Jones, A.C., Josephs, R.A., 2006. Interspecies hormonal interactions between man and the domestic dog (canis familiaris). Horm. Behav. 50, 393–400.
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.
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.
Kotrschal, K., Schöberl, 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.
Kruger, K.A., Serpell, J.A., 2006. Animal-assisted interventions in mental health: definitions and theoretical foundations. In: Fine, A.H. (Ed.), Handbook on Animal-Assisted Therapy. Theoretical Foundations and Guidelines for Practice, 2nd ed. Academic Press, San Diego, CA, pp. 21–38.
Ley, J., Bennett, P.C., Coleman, G.J., 2007. Personality dimensions that emerge in companion canines. Appl. Anim. Behav. Sci. 110, 305–317.
Ligout, S., Wright, H., van Driel, K., Gladwell, F., Mills, D.S., Cooper, J.J., 2010. Reliability of salivary cortisol measures in dogs in a training context. J. Vet. Behav.: Clin. Appl. Res. 5, 49.
Marinelli, L., Normando, S., Siliprandi, C., Salvadoretti, M., Mongillo, P., 2009. Dog assisted interventions in a specialized center and potential concerns for animal welfare. Vet. Res. Commun. 33, 93–95.
Martin, P., Bateson, P., 2007. Measuring Behaviour: An Introductory Guide. Cambridge, Cambridge University Press
McEwen, B.S., Stellar, E., 1993. Stress and the individual: mechanisms leading to disease. Arch. Intern. Med. 153, 2093–2101.
Palme, R., Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int. J. Mammal. Biol. 62 (Suppl. 2), 192–197.
Pastore, C., Pirrone, F., Balzarotti, F., Faustini, M., Pierantoni, L., Albertini, M., 2011. Evaluation of physiological and behavioral stress-dependent parameters in agility dogs. J. Vet. Behav.: Clin. Appl. Res. 6, 188–194.
Piva, E., Liverani, V., Accorsi, P.A., Sarli, G., Gandini, G., 2008. Welfare in a shelter dog rehomed with Alzheimer patients. J. Vet. Behav.: Clin. Appl. Res. 3, 87–94.
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., Gaines, S., Hiby, E., 2009. A practitioner's guide to working dog welfare. J. Vet. Behav.: Clin. Appl. Res. 4, 127–134.
Schilder, M.B.H., van der Borg, J.A.M., 2004. Training dogs with help of the shock collar: short and long term behavioural effects. Appl. Anim. Behav. Sci. 85, 319–334.

Serpell, J.A., Coppinger, R., Fine, A.H., Peralta, J.M., 2010. Welfare considerations in therapy and assistance animals. In: Fine, A.H. (Ed.), Handbook on Animal-Assisted Therapy, 3rd ed. Academic Press, San Diego, CA, pp. 481–503.
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., Turner, T., Burger, E., Glenk, L.M., McElheney, J.C., Kothgassner, O.D., 2011. Learning emotion recognition from canines. Two for the road. J. Vet. Behav.: Clin. Appl. Res. 6, 108–114.
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.
Taylor, K.D., Mills, D.S., 2006. The development and assessment of temperament tests for adult companion dogs. J. Vet. Behav.: Clin. Appl. Res. 1, 94–108.
Tomkins, L.M., Thomson, P.C., McGreevy, P.D., 2011. Behavioral and physiological predictors of guide dog success. J. Vet. Behav.: Clin. Appl. Res. 6, 178–187.
Vincent, I.C., Michell, A.R., 1992. Comparison of cortisol concentrations in saliva and plasma of dogs. Res. Vet. Sci. 53, 342–345.
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. 140–152.
Waiblinger, S., Menke, C., Korff, J., Bucher, A., 2004. Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure. Appl. Anim. Behav. Sci. 85, 31–42.
Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M., Janczak, A.M., Visser, E.K., Jones, R.B., 2006. Assessing the human–animal relationship in farmed species: a critical review. Appl. Anim. Behav. Sci. 101, 185–242.
Wesley, M., Minatrea, N., Watson, J., 2009. Animal-assisted therapy in the treatment of substance dependence. Anthrozoös 22, 137–148.
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.
Wilson, C.C., Barker, S.B., 2003. Challenges in designing human-animal interaction research. Am. Behav. Sci. 47, 16–28.