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Animal Welfare 2013, 22: 369-378
ISSN 0962-7286
doi: 10.7120/09627286.22.3.369

Therapy dogs' salivary cortisol levels vary during animal-assisted interventions

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Abstract

Beneficial effects of human-animal contact on human health have contributed to the wide distribution of animal-assisted interventions (AAIs). While considerable effort has been devoted to the study of human welfare during AAIs, potential effects on therapy animals have been addressed less frequently. The aim of this study was to determine baseline and work-related levels of cortisol, a glucocorticoid hormone that mediates physiological responses to arousal, in certified therapy dogs and therapy dogs in training. All dogs (n = 21) participated in weekly group-AAIs in adult mental healthcare. Saliva samples were collected over the course of AAIs and on non-working days and analysed with a cortisol enzyme immunoassay. Analysis of the results revealed that according to their cortisol responses, both therapy dogs and therapy dogs in training were not stressed by AAIs. However, cortisol levels during work in certified therapy dogs performing AAIs on- and off-lead varied significantly, suggesting that further investigation into the use of a lead or other methods of giving therapy dogs opportunities to approach or avoid human contact is needed.

Keywords: *animal-assisted interventions, animal welfare, cortisol, dogs, lead, therapy*

Introduction

The prevalence of mental health problems, including anxiety disorders, substance abuse and neurodegenerative disorders, has increased in recent years, posing a serious threat to future public health (Olesen *et al* 2012). Consequently, the need for suitable treatment and rehabilitation programmes has created socio-economic challenges for society. A considerable body of complementary therapies and interventions has emerged out of the growing need for supporting psychosocially vulnerable people (Hart 2010). The therapeutic use of animals in animal-assisted interventions (AAIs) aims to improve the psychosocial and emotional state in human patients who participate in the programme (Barker *et al* 2003). Even healthy humans can benefit from positive interactions with dogs by a decrease in cortisol along with an increase in oxytocin (Odendaal & Meintjes 2003). Investigating the effects of AAIs that use dogs' assistance to treat human patients, it has been demonstrated that animal contact can lower levels of anxiety, catecholamines, pulmonary capillary wedge and systolic pulmonary artery pressure (Cole *et al* 2007) and reduce the cortisol awakening response (Viau *et al* 2010). Moreover, acute post-operative pain, perceived physical pain and emotional distress were found to be lower in patients who had contact with a therapy dog (Sobo *et al* 2006). In psychosocial rehabilitation, AAIs for prison inmates have been developed to provide offenders social and emotional comfort through interaction with a dog (Strimple 2003; Britton & Button 2006; Hennessy *et al* 2006; Turner *et al* 2011). Despite the compelling evidence that dogs can support humans in various ways, one must not overlook that dogs have been bred primarily for assisting humans in hunting, herding and guarding; hence, they were supposed to recognise family members and be suspicious of unfamiliar individuals and/or intruders (Butler 2004). Accordingly, being approached, petted and hugged by strangers in unfamiliar environments, which is commonly featured in AAIs, may elicit comprehensible discomfort in dogs (Serpell *et al* 2010). To become an AAI working team, therapy dogs have to complete special training and a temperament screening to meet the criteria established by institutions that certify animal handlers and dogs (Haubenhofer & Kirchengast 2006b; Serpell *et al* 2010). Certification requires therapy dogs to remain calm and

relaxed in strange situations, even under stressful conditions and to be reliable with visual or vocal commands (Piva *et al* 2008; Viau *et al* 2010; King *et al* 2011). Certainly, inappropriate training methods and/or forced positions in which animals cannot avoid invasive social intrusions and do not have the opportunity to seek refuge may impair their welfare (Hatch 2007; Piva *et al* 2008; Serpell *et al* 2010; Glenk *et al* 2011). Another factor that may have been underestimated in previous investigations is the use of a lead in AAIs. Being off/on the lead has been proposed to increase arousal and affect aggressive behaviour in dogs (Roll & Unshelm 1997). Beerda *et al* (1998) demonstrated that pulling dogs on the lead can cause similar subsequent increases in cortisol as the confrontation with sudden noises or electro-shocks. In mammals, the adrenal hormone, cortisol, is regulated through the hypothalamic-pituitary-adrenal (HPA) axis and plays a major role in the response to altered internal or external stimuli (Möstl & Palme 2002). Regarding its primary function, cortisol requires modulation of bodily functions to maintain homeostasis under novel conditions (Fries *et al* 2009). However, prolonged exposure and/or excessive secretion of cortisol may lead to clinical symptoms and stress-adaptive disorders (Kooistra & Galac 2010). Salivary cortisol indicates physiological stress and is a frequently used marker for non-invasive welfare assessment in dogs (Coppola *et al* 2006; Dreschel & Granger 2009; Bergamasco *et al* 2010; King *et al* 2011). Salivary cortisol collection in dogs does not alter the activity of the HPA system itself but is a potent marker for detecting physiological responses to a stressful stimulus (Dreschel & Granger 2005). Time of day and the location of sample collection had no effect on salivary cortisol in healthy dogs (Wenger-Riggenbach *et al* 2010), nor had age or sex (Haubenhofer *et al* 2005). Preliminary investigations on therapy dogs' physiological measures have revealed that salivary cortisol levels were higher on days with AAIs and increased relative to the number of AAIs carried out in a week (Haubenhofer & Kirchengast 2007). A study by King *et al* (2011) showed that salivary cortisol levels in therapy dogs increased from before therapy sessions to after them. The experimental introduction of a short time-out session with quiet play did not cause differences when compared to the 'no time-out' control condition (King *et al* 2011). In contrast, Marinelli *et al* (2009a) examined dogs during a seven-week AAI programme in a retirement home and did not find indices of increased salivary cortisol nor behavioural signs of acute stress. Simply put, it remains difficult, if not impossible, to justify any broad conclusions from the limited number of studies that are available on therapy dogs' experiences. Marinelli *et al* (2009b) argue that studies need to be designed that focus specifically on individual aspects that may influence the welfare of dogs involved in AAIs. Most importantly, the variability in the way these interventions are conducted needs to be taken into consideration. Deaton (2005) highlights the problem by claiming that, as with many other novel disciplines, AAIs are carried out in numerous settings with different populations and lack standardised manuals. Due to these variations, animal welfare in AAIs faces difficulties. Research methods that are centered on aspects that are both characteristic and representative of individual types of AAIs are urgently needed. The present study aimed to investigate therapy dogs' salivary cortisol levels during standardised group-AAIs in adult mental healthcare. In addition, the aim was also to examine whether the dogs differ in their cortisol responses on working and non-working days and whether working experience or on-lead/off-lead working conditions affect cortisol secretion.

Materials and methods

Study animals

Animal handlers who were evaluated by an official Austrian AAI organisation and regularly work or intend to work with their personal dog(s) in dog-assisted group therapy were recruited via email or telephone or through contact with local colleagues. All participating dogs were privately owned by their animal handlers (all female), who also took part in the AAIs. Participating patients were previously informed by the facility staff members and agreed upon an experimenter's presence for the sampling procedures. There was only one experimenter (female) in this study who attended therapy sessions prior to data collection so that the animal handlers, dogs and patients were already familiar with her presence. The 21 dogs ranged in age from 1.5 to 14 years with a mean (± SD) age of 5.7 (± 4.1) years and weighed from 1.50 to 35 kg (18.7 [± 10.5]). Nine dogs (all female) were spayed and 13 were either crossbreeds or no clear breed information was given. Pedigree dogs included Australian Shepherd, Chihuahua, Flat-Coated Retriever, Golden Retriever, Labrador Retriever and Puli. To be eligible for participation in the study, the dogs were required to be in good clinical health (ie free from pain, endo- and ectoparasites and immunised). Therapy protocols stipulate in their contract that all therapy dogs have to undergo regular veterinarian screening. None of the female dogs was in oestrus or pregnant at the time of the experiments.

Therapy dogs' working schedule

All dogs and their animal handlers had to go through the same initial series of evaluations to meet the requirements by the certification programme. To be awarded an official certificate, dogs and owners undergo special training, during which animal handlers can decide whether they want to work with their dog on- or off-lead. Professional advice on dog handling is given by the staff and dog trainers of the certification programme. Thus, working conditions for certified dogs in this study were based on each dog's individual performance and differed in that one group of dogs was specifically trained to be on the lead (CTD-ON), while the other group of dogs was trained to be off the lead (CTD-OFF) during therapy sessions. CTD-ON and CTD-OFF had a minimum of one year working experience in mental healthcare facilities. The third group included dogs that were still in training to become a therapy dog (TDT-ON). Over the course of this study, these dogs attended a therapy institution for the first time, participating as assistance dogs

Table 1 Age, gender, health screening status, certification and working experience in therapy dogs (n = 21).

Factor	CTD-ON	CTD-OFF	TDT-ON
n	7	7	7
Mean (± SD) age	6.4 (± 3.9)	4.8 (± 2.9)	4.9 (± 3.9)
Gender			
Female	5	4	5
Male	2	3	2
Temperament and health screening	Yes	Yes	Yes
Certification through a recognised AAI institution	Yes	Yes	Pending
> 1 year working experience in AAIs	Yes	Yes	No

CTD-ON: Certified therapy dogs on-lead; CTD-OFF: Certified therapy dogs off-lead; TDT-ON: Therapy dogs in training on-lead.

Table 2 Interaction schedule and description of behaviours displayed by animal handlers and patients towards the CTD-ON, CTD-OFF and TDT-ON.

Human-animal interaction behaviour	CTD-ON	CTD-OFF	TDT-ON
Verbal contact: people talk softly to the dog	Yes	Yes	Yes
Praising: people speak in high-pitched/fluctuating voice	Yes	Yes	Yes
Tactile contact: people touch/stroke/groom the dog	Yes	Yes	Yes
Gesturing: people gesture with hands, arms, fingers	Yes	Yes	Yes
Treat reward: dog receives food treats	Yes	Yes	Yes
On the lead: people gently hold/pull the lead to handle the dog	Yes	No	Yes
Playing: gestures, laughing, use of dog toys	Yes	Yes	Yes
Obedience commands: dog responds to visual/verbal cues with a change in behaviour	Yes	Yes	No

CTD-ON: Certified therapy dogs on-lead; CTD-OFF: Certified therapy dogs off-lead; TDT-ON: Therapy dogs in training on-lead.

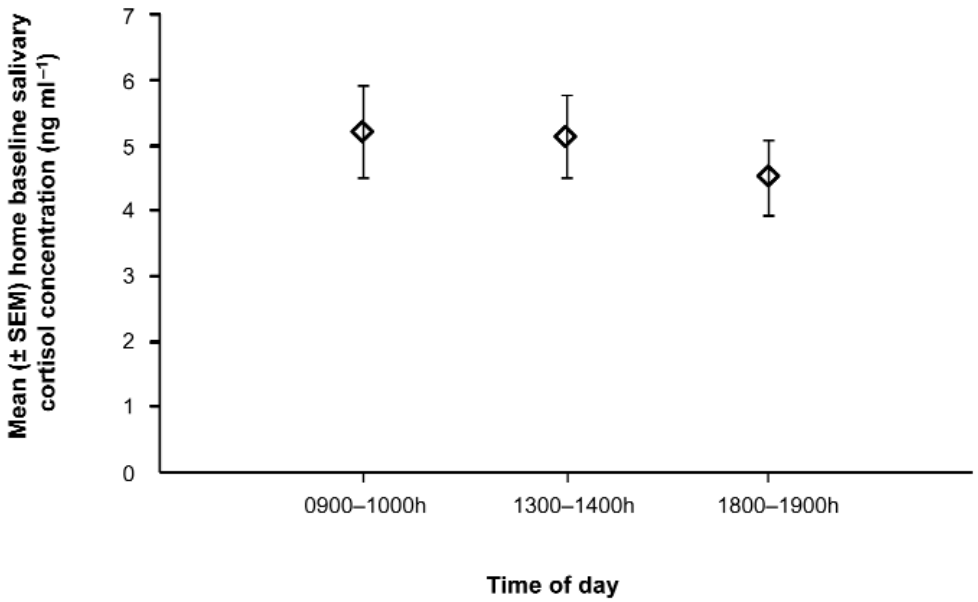
in an AAI session of CTD-ON or CTD-OFF. They were given time to quietly observe the therapy programme, explore the environment and were kept on-lead during therapy for security reasons. Towards the final evaluations and certification, animal handlers of TDT-ON decide whether they prefer to work with their dog on- or off-lead. All groups (CTD-ON, CTD-OFF and TDT-ON) included both genders of small- and large-sized dogs (see Table 1).

Study design

Sampling was carried out at three different in-patient mental healthcare facilities in Austria, which were familiar to all dogs (except TDT-ON). All therapy sessions consisted of theory parts, interpersonal communication and interaction parts with the therapy dog. Each therapy programme started with a group of ten adult patients and was run weekly for eight weeks with the same individuals (animal handler, therapy dog and patients) present. There was no patient turnover during these eight weeks. AAI programmes were supervised by each institution's staff members and participation depended on the respective physical or psychological condition of the patients. In the experimental sessions, 8–10 patients were present. The patients were informed previously how to interact in an appropriate way with the therapy dog before the dog was first introduced to the group. During therapy, the patients were seated in chairs and instructed by the animal handlers when and how to interact with the dog (ie stand up, call, touch, grab or pull the dog's lead).

In experiment one, we monitored salivary cortisol in CTD-ON and CTD-OFF at home, before and after two AAI working sessions. During each group session, one dog, one animal handler and one experimenter were present with 8–10 patients. In experiment two, we compared salivary cortisol levels of CTD-ON and CTD-OFF with TDT-ON, which participated for the first time in a therapy session. At each session, one working dog (CTD-ON or CTD-OFF), one assistance dog (TDT-ON), one animal handler and one experimenter were present with 8–10 patients. An experienced dog (CTD-ON or CTD-OFF) was regularly working, while TDT-ON were merely observing and did not interact with the working dog. Here, cortisol levels of TDT-ON were compared with cortisol levels of CTD-ON and CTD-OFF during AAI session one from the above-described experiment one. All human-animal contact in this study was guided by an experienced animal handler and based exclusively on positive reinforcement and gentle handling. Interaction schedule and behaviours towards CTD-ON, CTD-OFF and TDT-ON are categorised in Table 2.

Figure 1



Mean (± SEM) home baseline salivary cortisol concentrations of samples collected at three different time-points (0900–1000h, 1300–1400h and 1800–1900h) in therapy dogs (n = 13).

Sample collection

To absorb the saliva, we used a cotton roll (Salivette®, Sarstedt, Wiener Neudorf, Austria) in large dogs (> 15 kg) or an arrow-shaped hydrocellulose sponge attached to a plastic shaft (Sorbette, Salimetrics Europe Ltd, UK) in small dogs (< 10 kg). The saliva collection device was gently placed into the cheek pouch or under the tongue of the dog until it was saturated with saliva (approximately 40–70 s). For ethical reasons, neither of the dogs was restrained during the sampling procedure. To stimulate salivation, animal handlers presented commercial food to their dogs. In order to avoid sample contamination and, hence, reduced reliability of the enzyme immunoassay, the dogs were only allowed to sniff at the food treats in the experimenter's closed hand and not to chew on it (Bennet & Hayssen 2010; Ligout *et al* 2010). Moreover, the sampling devices contained no food-based additives that may have interfered with the enzyme immunoassay (Dreschel & Granger 2009). After the cotton roll or hydrocellulose sponge was soaked with saliva, it was replaced in the device container and closed with a plastic stopper to avoid evaporation. The collected material was stored in an ice box before samples were finally refrigerated at –20°C. Prior to analysis, samples were thawed and centrifuged at room temperature at 3,000 g for 15 min to obtain the clear saliva.

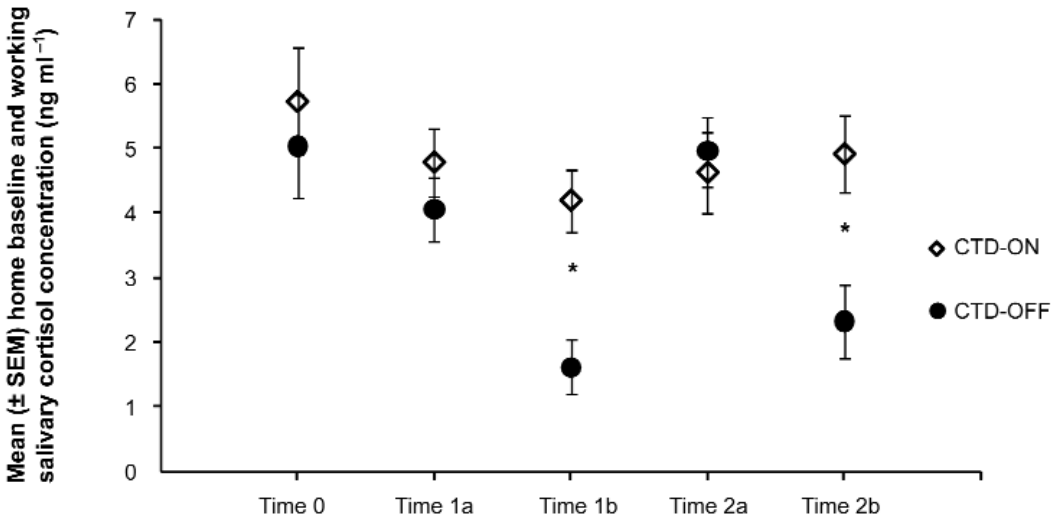
Sampling schedule

To lessen the effect of potential circadian deviation on salivary cortisol, only AAIs starting in the morning from 0930–1100h were considered in the analysis. Salivary samples were collected within 4 min (Kobelt *et al* 2003). The experimental sampling schedule was adjusted considering that salivary cortisol levels reflect plasma cortisol with a 20–30-min delay (Vincent & Michell 1992). Pre-session (Time 1a, Time 2a) sampling was carried out prior to AAIs, 15 min after arrival at the facility. After each 50–60-min intervention, an additional 5 min were scheduled where the dogs received no more food treats before the dogs were sampled to capture levels that correspond to the time during therapy work. No more than two weeks elapsed between the working sessions (Time 1 and Time 2). Animal handlers were given a demonstration how to collect a sample and were provided with written instructions to sample saliva on two non-working days at home (three saliva samples at 0900–1000h, 1300–1400h and 1800–1900h, respectively) and another sample on a therapy session day, shortly before leaving home (Time 0).

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, Austria with a highly sensitive cortisol enzyme immunoassay (Palme & Möstl 1997) that has previously been used in dogs' saliva (Haubenhofer & Kirchengast 2006a). Samples were assayed in duplicate and cortisol concentrations were assessed by double-antibody biotin-linked enzyme immunoassay (for details, see Palme & Möstl 1997). Duplicate samples with a coefficient of variance > 10% were replicated and considered in the analysis when a coefficient of variance < 10% was achieved. If the sample volume fell below the limit needed to run duplicates or ran out before reaching a coefficient of variance < 10%, the sample was dismissed from the analysis. The average intra- and inter-assay coefficients of variance were less than 10 and 15%, respectively.

Figure 2



Mean (± SEM) salivary cortisol (ng ml^{-1}) levels in CTD-ON (n = 7) and CTD-OFF (n = 7) dogs at home (Time 0), before (Time 1a, Time 2a) and during therapy (Time 1b, Time 2b). Except Time 0, data were analysed with ANOVA for repeated measure; respective groups are shown in the graph. * Indicates a significant group difference with $P < 0.05$. CTD-ON: Certified therapy dogs on-lead; CTD-OFF: Certified therapy dogs off-lead.

Statistical analysis

Calculations were carried out using the statistical package SPSS 15.0 for Windows (SPSS, Inc, Chicago, USA). We considered that $P \leq 0.05$ denotes statistical significance. Friedman two-way ANOVA was conducted to evaluate the subjects' day-time salivary cortisol levels at home. Repeated measures ANOVA with two groups were used in analyses of salivary cortisol measures, with intervention type as between-group factor and time as repeated factor in first experiment, and with animal subjects' educational status as between-group factor and time as repeated factor in the second experiment.

Ethical note

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

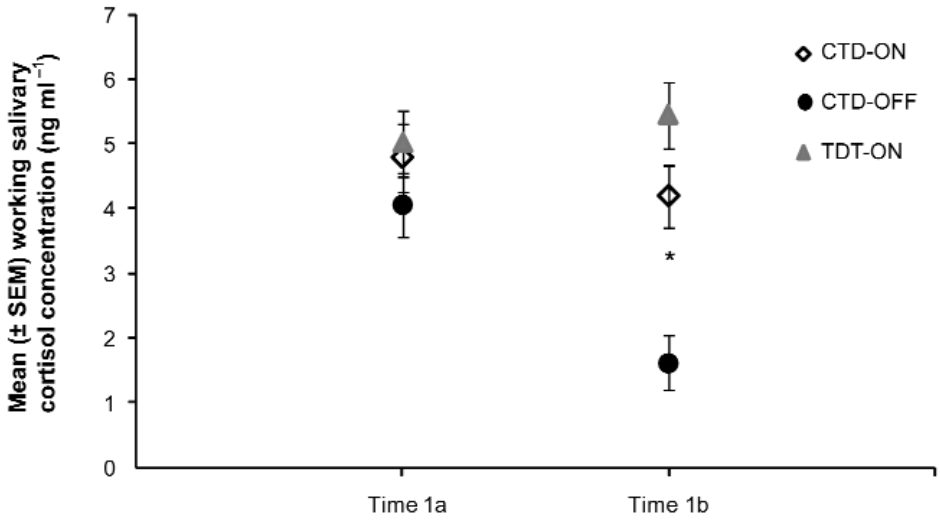
Results

A total number of 171 salivary samples were collected from seven certified therapy dogs on-lead (CTD-ON), seven certified therapy dogs off-lead (CTD-OFF) and seven therapy dogs in training (TDT-ON). No significant differences in age were found between the groups ($F_{2,20} = 0.424$, $P = 0.661$). Twenty home baseline samples (12% of all samples) were not included in the analysis because the samples yielded an insufficient volume of saliva to run duplicates. Hence, home baseline salivary sampling was carried out on a day without therapy sessions at three different time-points, resulting in 39 home baseline samples (23% of all samples) that were available in 13 of the 14 CTD-ON and CTD-OFF (Figure 1). There was no significant difference between concentrations at the different time-points ($\chi^2 = 2.92$, $P = 0.23$). Moreover, for our quasi-experimental study, we assayed salivary cortisol levels in 70 samples (41% of all samples) and 42 (24% of all samples) for experiments one and two, respectively.

Experiment 1

Repeated-measures ANOVA for two groups were used to analyse salivary cortisol measures in the first experiment. Levenes test for homogeneity was appropriate at Time 1a, the cortisol baseline of session one ($F_{1,12} = 1.48$, $P = 0.25$); Time 1b, the cortisol response during the working session one ($F_{1,12} = 2.600$, $P = 0.13$), Time 2a, the cortisol baseline of session two ($F_{1,12} = 2.77$, $P = 0.12$) and Time 2b, the cortisol response during the working session two ($F_{1,12} = 0.32$, $P = 0.58$). Mauchly's test indicated that the assumption of sphericity had not been violated ($\chi^2 = 4.286$, $P = 0.51$). Box-M-test showed no significant results ($F = 0.90$, $P = 0.53$). Mean (± SD) cortisol levels did not differ between the dogs (CTD-ON: 5.7 [± 2.4]; CTD-OFF: 5.0 [± 2.0] ng ml^{-1}) at the pre-session baseline measurements ($t = -0.59$, $P = 0.57$). ANOVA results indicate a significant main effect of time ($F_{3,10} = 5.56$, $P = 0.02$, $\eta^2 = 0.63$). In response to working sessions, CTD-OFF showed a decrease in salivary cortisol levels during both working sessions, yielding a significant group effect ($F_{1,12} = 5.06$, $P = 0.04$, $\eta^2 = 0.29$) and a significant interaction effect time by group ($F_{1,10} = 4.39$, $P = 0.03$, $\eta^2 = 0.57$). Differences between CTD-ON and CTD-OFF post-session cortisol levels emerged during both working sessions ($P < 0.01$). However, the mean absolute decreases in cortisol were at Time 1b, –2.45 ng ml^{-1} and at Time 2b, –2.34 ng ml^{-1}, respectively, in CTD-OFF. CTD-ON

Figure 3



Mean (± SEM) salivary cortisol (ng ml^{-1}) levels in CTD-ON (n = 7), CTD-OFF (n = 7) and TDT-ON (n = 7) dogs before (Time 1a) and during therapy (Time 1b). Data were analysed with ANOVA for repeated measures; respective groups are shown in the graph. * Indicates a significant group difference with $P < 0.05$. CTD-ON: Certified therapy dogs on-lead; CTD-OFF: Certified therapy dogs off-lead); TDT-ON: Therapy dogs in training on-lead. Time 1a (Session 1, baseline), Time 1b (Session 1, post-session).

showed a decrease in cortisol at Time 1b of –0.59 ng ml^{-1} and an increase in cortisol at Time 2b of +0.28 ng ml^{-1} with respect to the session's baseline (Figure 2).

Experiment 2

ANOVA for three groups with repeated measurements was used to analyse salivary cortisol data of CTD-ON, CTD-OFF and TDT-ON in the second experiment. Levenes test for homogeneity was appropriate at Time 1a (pre-session baseline: $F_{2,18} = 1.66$, $P = 0.22$); Time 1b (post-session, reflecting the cortisol response during the working session: $F_{2,18} = 1.71$, $P = 0.21$), respectively. Box-M-test showed no significant results ($F = 1.59$, $P = 0.15$). Again, mean (± SD) differences between the three groups were not seen (CTD-ON: 4.0 [± 1.8]; CTD-OFF: 4.7 [± 1.3]; TDT-ON: 5.0 [± 0.9] ng ml^{-1}) according to the Time 1b measurements ($F_{2,18} = 0.888$, $P = 0.43$). Results from the repeated measurements ANOVA point at a significant main effect of time ($F_{1,18} = 6.67$, $P = 0.019$, $\eta^2 = 0.27$) and group ($F_{2,18} = 6.316$, $P < 0.01$, $\eta^2 = 0.41$), as well as a significant interaction effect time by group ($F_{2,18} = 8.73$, $P < 0.01$, $\eta^2 = 0.492$). Bonferroni *post hoc* analysis for multiple comparisons indicated significant differences between CTD-ON and CTD-OFF ($P < 0.05$). In addition, CTD-OFF differed significantly from TDT-ON ($P < 0.01$), but there was no significant effect between CTD-ON and TDT-ON ($P = 0.67$). Experienced dogs showed decreases in their work-related cortisol responses (CTD-ON and CTD-OFF levels were taken from the first session of experiment one). However, TDT-ON exhibited a non-significant increase in cortisol (+0.43 ng ml^{-1}) during participation in their first working session (Figure 3).

Discussion

The study was designed to contribute to the limited body of research in the field of therapy animals' welfare by investigating short-term effects of human-animal interaction on salivary cortisol levels in therapy dogs. In experiment one, saliva samples were collected during two therapy group sessions with adults in mental healthcare. Pre- and post-session cortisol levels of CTD-ON and CTD-OFF were determined. Salivary cortisol samples were also collected at the dogs' homes on non-working days and before therapy. Data from CTD-ON and CTD-OFF were then compared with TDT-ON, which participated for the first time in an AAI session. *Prima facie*, our study results reveal that performance in group-AAIs in adult mental healthcare did not stimulate significant increases in salivary cortisol stress responses in CTD-ON, CTD-OFF or TDT-ON when working cortisol levels were compared to baseline levels and home levels. These are important findings, considering that in dogs, an elevation in cortisol has been associated with stressful conditions resulting from fear (Beerda *et al* 1999; Hydbring-Sandberg *et al* 2004; Dreschel & Granger 2005), controlled/authoritarian play (Horváth *et al* 2008) and human threat (Horváth *et al* 2007). Moreover, Jones and Josephs (2006) found that punitive behaviours (including pushing and yelling) towards dogs can be positively correlated with increases in dogs' cortisol concentrations. Although the role of altered cortisol in response to fluctuating environmental conditions has an adaptive function in mammals, exposition to high levels for extended periods of time may lead to harmful physiological consequences (Ebrecht *et al* 2004; Chrousos 2009). High cortisol levels have been associated with high levels of stress, thus,

cortisol is an important parameter in dog welfare research (Coppola *et al* 2006; Dreschel & Granger 2009; Bergamasco *et al* 2010). On the other hand, positive interaction with humans, quiet play and affiliate behaviours were linked to reduced cortisol levels in dogs (Coppola *et al* 2006; Horváth *et al* 2008). Our findings on the circadian pattern of home baseline salivary cortisol parallel the research results of Kobelt *et al* (2003), Hydbring-Sandberg *et al* (2004) and Haubenhofer and Kirchengast (2006a). Interpreting the cortisol results of CTD-ON, CTD-OFF and TDT-ON, the dogs in this study did not appear to be strained by participation in AAIs. Our data corroborate the findings of Piva *et al* (2008) and Marinelli *et al* (2009a) but are not in line with the reports of Haubenhofer and Kirchengast (2006a, 2007) and King *et al* (2011). Considering the controversy in these research findings, it is likely that the AAIs investigated by the different authors are not directly comparable because of their different conceptual context (eg therapy content, single patient versus group interventions, familiar versus unfamiliar patients), environment (eg therapy facility such as hospital, prison, geriatrics) and arrangement (eg frequency, intensity and duration of human-animal contact, dog on/off the lead, refuge for the dog). In the first experiment of our study, we collected saliva samples from certified therapy dogs. Two groups of dogs were distinguished by their respective working condition. While CTD-OFF were kept off the lead during the therapy sessions and, hence, able to move freely, approach or avoid human contact voluntarily or even leave the therapy setting. In contrast, CTD-ON were kept on the lead during the therapy session and had limited possibilities to move freely, approach or avoid human contact or leave the setting. Our data reveal a significant difference in salivary cortisol between CTD-ON and CTD-OFF during two therapy sessions. Being pulled on the lead can cause stress in dogs and increase cortisol levels (Beerda *et al* 1998). In our study, CTD-OFF had lower working cortisol levels than CTD-ON, thus, the use of a lead during therapy may affect physiological arousal in dogs. Positive contact with humans can decrease both heart rate (McGreevy *et al* 2005) and cortisol secretion (Coppola *et al* 2006; Bergamasco *et al* 2010) in dogs. CTD-ON could have benefited from declining cortisol levels during AAIs but additional, in-depth research is needed to clarify this hypothesis. With regard to our study design, we cannot attribute the differences in working cortisol levels solely to the use of a lead. A randomised experiment putting CTD-OFF on the lead and CTD-ON off the lead would have been necessary. Unfortunately, the certified dogs in this study were trained to work either with or without a lead and are used to their respective working condition. Randomisation of the on-lead condition in these dogs would therefore have caused a bias with potentially negative effects on the dogs. The significantly lower working cortisol levels in CTD-OFF could be related to the opportunity to approach or avoid human contact during therapy. The freedom to express behaviour not only depicts an essential aspect of dog welfare (Houpt *et al* 2007) but could be an important factor in regulating physiological responses during AAIs. To explore the relevance of freedom of choice, the study of frequency and intensity of individual behaviours as well as the context in which they appear would be necessary. There might also be a difference between animal handlers who prefer to handle their dog on- or off-lead that accounts for the differences seen in our results. Further investigation into individual relationships between therapy dogs and their handlers would therefore be desirable. Although all dogs received the same basic training and participated in the same facilities, the overall final working conditions resulting from the use of lead in CTD-ON and CTD-OFF may differ. The use of a lead may be appropriate for a therapy session or even required by facility regulations. Moreover, some dogs may feel more comfortable with a lead on. In this study, animal handlers and dog trainers of the certification programme decided what they think might be the best working condition for each individual team. Since the quality of individual human-animal relationships and the history of previous experience are important factors affecting animal welfare (Waiblinger *et al* 2006), an AAI professional should be able to distinguish the best course of action for his or her dog. Still, animal handlers with therapy dogs on the lead should be aware of subtle signals of discomfort in their dogs when they interact with patients and react accordingly (Serpell *et al* 2010). Further study on the use of a lead and other methods of giving dogs the opportunity to approach or avoid people during AAIs is definitely needed. Previous research has documented that plasma concentrations of cortisol in aggressive dogs were significantly higher than in non-aggressive dogs which, in turn, were linked to the dogs' high stress levels (Rosado *et al* 2010). According to the strong correlation between plasma and saliva cortisol levels (Kirschbaum & Hellhammer 1994), low salivary cortisol levels, along with low levels of stress and aggression, would be desirable in therapy dogs during interaction with humans. In home baseline and pre-session cortisol levels, no significant differences between CTD-ON and CTD-OFF were found. Home baseline levels appeared higher than the pre- and post-session samples that were collected after arrival at the therapy facility however, these differences were not significant. Without detailed information on the daily routine of the dogs, it is difficult to further interpret these results. King *et al* (2011) investigated the effects of age on work-related stress in therapy dogs and concluded that older and more experienced dogs exhibit less signs of stress. The authors hypothesised that dogs may undergo subsequent habituation to therapy environments and could therefore be less aroused during AAIs. Haubenhofer *et al* (2005) found no significant variations in salivary cortisol in dogs that attended a five-day training course in order to become a therapy dog. However, to finally earn a certificate in AAI, therapy dogs in training are required to get subsequently used to the therapy environments of their future workplaces. At this stage of training, they are usually supervised and accompanied by an experienced AAI-team during their regular work (Haubenhofer & Kirchengast 2006b). Objective assessment

of stress markers in therapy dogs in training has not yet been carried out during this stage of the certification process. Hence, in our second experiment, we analysed salivary cortisol in dogs that are still in training (TDT-ON), participating in a therapy session of CTD-ON or CTD-OFF. Pre-session and working levels of CTD-ON, CTD-OFF and TDT-ON were compared. Although pre-session levels did not differ among the groups, CTD-OFF had significantly lower working cortisol levels than CTD-ON and TDT-ON. Working cortisol levels in TDT-ON may have been influenced by the novel environment and the presence of the patients and the working dog. As there was no significant increase in working cortisol, we suggest that TDT-ON do not seem to be over-excited or strained by participation in AAIs. Thus, a therapy dog in training may benefit from subsequent and gentle introduction to a therapy facility and the support from a confident and experienced therapy dog. These findings corroborate the results reported by Haubenhofer *et al* (2005). All three groups of dogs included purebred dogs and crossbreeds of small and large sizes and both genders. Considering the size and heterogeneity of our dog sample, we could not attribute cortisol responses to the groups' demographics. A replication of the study with a bigger sample size would make it possible to distinguish between different groups of dogs (eg gender, breed, age, neuter status, working experience) on a larger scale. Future investigations may also benefit from looking at additional markers of arousal and behaviour. Monitoring changes in physiological markers and behaviour over time, that is, before and after completion of AAIs, and measuring the time needed for parameters to return to baseline would provide additional insight. Although it has been suggested that the collection of saliva can be carried out easily and by non-professionals (Dreschel & Granger 2009), King *et al* (2011) found that dog handlers experienced severe difficulties in dog saliva sampling, even if they were trained and instructed by scientific staff. Also, in this study, a considerable amount of home baseline samples that were collected by animal handlers did not contain sufficient saliva to run an enzyme immunoassay. To prevent data loss in forthcoming studies, dog saliva samples should be collected preferably by a previously trained experimenter.

Animal welfare implications and conclusion

Participation in group therapy sessions with mental health patients did not increase therapy dogs' working cortisol levels. Thus, our study results give rise to the conclusion that they were not acutely stressed by the workload of AAIs. This accounts for both experienced therapy dogs and dogs in training. We found different cortisol levels in dogs working on-lead and off-lead, hence, the use of leads and other methods of giving dogs the opportunity to voluntarily approach or avoid interactions require further study. To draw any broader conclusions on animal welfare, additional physiological measures and behavioural observations are needed to complement the cortisol data.

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