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Social factors influencing cortisol modulation in dogs during a strange situation procedure

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A B S T R A C T

In human-dog relationships, positive interaction and social attention may mutually dampen stress responses. In humans, attachment representations and personality are linked to the modulation of individual stress reactions. We investigated the connections between dog attachment to the owner, owner attitudes and relationship toward the dog, and the personality of both on stress coping in dogs during the Ainsworth Strange Situation Procedure for dogs. For the first time, dog attachment patterns were assessed via the original Ainsworth attachment classification system. In addition, cortisol was measured from saliva in the context of play with the owner and 2 threat situations, once with and once without the owner present. We found that dogs classified as "securely attached" secreted less cortisol during the attachment ($P = 0.008$) and play situations ($P = 0.031$) and showed by trend a stronger cortisol reactivity during the threat situation when the owner was absent ($P = 0.086$) than dogs which were classified as "insecure." The higher the owner's self-reported insecure-ambivalent attachment toward the dog and perception of the dog as a social support, the higher was the dog's cortisol reactivity during the Ainsworth Strange Situation Procedure ($P = 0.004$ and $P = 0.018$). Furthermore, it was found that owners high in neuroticism and agreeableness had dogs with low cortisol reactivity ($P = 0.003$ and $P = 0.001$). Older dogs showed less cortisol reactivity than younger ones ($P = 0.023$). Male dogs of male owners tended to show the lowest cortisol reactivity compared to all other human gender-dog sex combinations ($P = 0.008$). In conclusion, results show that secure dog attachment to the owner, owner-dog relationship, and personality of both influence the dog's stress coping.

Introduction

Behavioral and physiological stress coping (Koolhaas et al., 1999, 2011) is an important determinant of relationship quality in humans and other animals (Aureli & de Waal, 2000). Positive social interactions may dampen stress responses and increase relaxation through oxytocin (DeVries et al., 2003; Julius et al., 2012; Uvnäs-Moberg, 1998), which has been linked to increased trust, affiliative behavior, decreased anxiety, and aggression (DeVries et al., 2003; Neumann, 2008) and to secure attachment between babies and their mothers (Feldman et al., 2011). This mechanism of emotional social support is shared with nonhuman animals (Curley & Keverne, 2005; Julius et al., 2012; Scheiber et al., 2005) and even seems to work between species (reviewed in Beetz et al., 2012; Romero et al., 2014). Because personality is generally associated with individual variation in cortisol modulation (Koolhaas et al., 1999; Korte et al., 2005), it is not surprising that human personality affects owner-dog relationships (Kotrschal et al., 2009; Reevy & Delgado, 2015) as well as dog stress coping and cortisol modulation (Schöberl et al., 2012).

The aim of our study was to examine the influence of social factors (e.g., attachment, personality) on cortisol reactivity of dogs during an attachment assessment. Attachment, a construct that is usually applied to caregiving-offspring relationships among primates, is defined as a bond between one individual and a specific other (Ainsworth, 1979). Human-infant attachment patterns are

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typically accessed via the Ainsworth Strange Situation Procedure (ASSP), in which repeated separation from the attachment figure combined with meeting a stranger in unfamiliar surroundings usually leads to stress in the infant. During the last third of this assessment the infant is left alone without any support, thus the stress load usually increases at the end of the assessment (Ainsworth et al., 1978). In general, the attachment construct (Ainsworth et al., 1978; Bowlby, 1969) seems to be applicable to the owner-dog bond (Palmer & Custance, 2008; Prato-Previde et al., 2003; Topàl et al., 1998; Voith, 1985; Zilcha-Mano et al., 2011). To assess dog attachment to humans, the ASSP has been repeatedly used (Gácsi et al., 2001; Horn et al., 2013; Palmer & Custance, 2008; Prato-Previde et al., 2003; Siniscalchi et al., 2013; Topàl et al., 1998), always in connection with behavior coding. Here, for the first time, the original Bowlby-Ainsworth-Main and Solomon attachment classification system for toddlers was adapted for dogs (Solomon et al., 2014).

We predicted that stress coping in dogs is related to owner-dog attachment and that cortisol reactivity will mainly depend on dog attachment as well as on the presence of the owner. We expected that securely attached dogs may differ in this respect from dogs which show an insecure attachment pattern; that owner attachment may influence dog cortisol patterns, given that human-dog attachment parallels human-human attachment (Siniscalchi et al., 2013; Zilcha-Mano et al., 2011) and that owner gender-dog sex combination affects dog stress coping (Mongillo et al., 2014; Pessina et al., 2009). Furthermore, previous research has demonstrated that owner personality and relationship with the dog affect dog behavior (Wedl et al., 2010), dyadic performance (Kotrschal et al., 2009), and dog morning cortisol (Schöberl et al., 2012). Thus, we predict that these factors will also influence dog stress coping during an attachment test.

Methodology

Subjects and general procedure

We collected data from 132 human-dog dyads, which participated in 2 test sessions. Of those owner-dog dyads, 59 participated in a third session which featured the ASSP attachment assessment. The first and second sessions took approximately 90 minutes and were conducted in a University of Vienna test room; the third session took about 1 hour and was conducted in another test room, which was novel to the dyads. The focus of this study is on the attachment test and hence, on the subsample of 59 dyads. Here, we present just those tests relevant for this study, which are 2 tests (play and threat) during session 2 and the attachment assessment during session 3.

Intact pet dogs (mean age ± standard deviation [SD]: 3.96 ± 1.54 years; mean weight ± SD: 30.08 ± 13.21 kg) were tested together with their primary attachment figure, the "owner" (mean age ± SD: 46.22 ± 10.22 years). All dogs were adopted as puppies (mean age ± SD: 9.80 ± 4.17 weeks). Owner gender-dog sex combination was counterbalanced, resulting in 17 female owners with female dogs, 14 female owners with male dogs, 14 male owners with female dogs, and 14 male owners with male dogs. Different dog breeds and mixed breeds participated but not more than 3 dogs of the same pure breed per owner gender-dog sex combination. The breeds were as follows: Irish red setter (2), mongrel (8), Puli (1), Bullmastiff (2), Berger Blanc Suisse (3), Australian shepherd (3), Austrian pinscher (1), Labrador retriever (4), Schapendoes (1), large Münsterländer (1), Eurasier (3), Lagotto Romagnolo (1), Kooikerhondje (1), American bulldog (1), great Dane (1), Beagle (1), English cocker spaniel (3), golden retriever (3), Newfoundland (1), Rottweiler (1), German wirehaired pointer (1), German shepherd (1), Bernese mountain dog (3), whippet (1), Briard (2), Belgian shepherd (1), Spinone Italiano (1), greyhound (1), Welsh springer spaniel (1), Epagneul Picard (2), border collie (1), and old German shepherd dog (1).

During the sessions, no individuals other than the experimenters (just one experimenter at one time point) and the participants were present. For continuous videotaping during the sessions, a camcorder (Canon Inc., Canon Austria GmbH, 1100 Vienna, Austria) with a wide-angle conversion lens (Canon Inc., Canon Austria GmbH, 1100 Vienna, Austria) was fixed on the wall of the test room. The camcorder was connected to a monitor outside the room, where an experimenter was observing the procedure during the entire testing.

Questionnaires

Owners completed questionnaires to assess human and dog personality, adult attachment representation toward humans and their dog, as well as demographics and everyday life.

- NEO-FFI: The NEO Five-Factor Inventory was developed by Costa and McCrae (1992), and translated to German by Borkenau and Ostendorf (1993). This 60-item instrument is designed to measure normal (i.e., nonclinical) adult personality via 5 domains: neuroticism, extroversion, openness, agreeableness, and conscientiousness.
- MCPQ: The Monash Canine Personality Questionnaire is a 5 dimension questionnaire developed by Ley et al. (2009). We translated the questionnaire to German in cooperation with a bilingual expert. A principal component analysis (PCA) was conducted and resulted in 5 axes (reliability revealed by Cronbach-Alpha): "active-excitable," "obedient-reliable," "insistent-goal directed," "nervous-anxious," and "cool-friendly" paralleling those found by Ley et al. (2009).
- RSQ: The Relationship Scales Questionnaire was developed by Griffin and Bartholomew (1994) and was translated to German by Mestel (1994). The RSQ captures different aspects of attachment via the scales "Separation anxiety," "Closeness anxiety," "Lack of trust," and "Wish to be independent," which are associated with adult interpersonal relationship patterns (Steffanowski et al. 2001).
- FERT: The FERT (Fragebogen zu Erfahrungen mit Tieren) is a German questionnaire on relationship with pets (Beetz & Ascione, 2004). The first part is based on the German version of the Inventory of Parent and Peer Attachment (IPPA, original questionnaire: Armsden & Greenberg, 1987, 2009; German Version: Zimmermann, 1992), and the second part on the relationship questionnaire (RSQ, Griffin & Bartholomew, 1994; Mestel, 1994). Questions were adapted to companion animals, where possible, some items were excluded. For this study, we adapted the questions by exchanging the word "pet/companion animals" with the term "dog." PCA of IPPA-based items resulted in 2 axes (reliability revealed by Cronbach-Alpha): "Dog as social supporter" and "Communication." Analysis of the RSQ-based items resulted in 2 axes (reliability revealed by Cronbach-Alpha): "Secure-caregiving" and "Insecure-ambivalent".
- MDORS: The Monash Dog Owner Relationship Scale is a validated questionnaire developed by Dwyer et al. (2006) to measure human-dog relationship. We translated the questionnaire to German in cooperation with a bilingual expert. PCA resulted in 5 axes (reliability revealed by Cronbach-Alpha): "Dog as burden," "Dog as social supporter," "Dog as cuddling partner," "Separation anxiety," and "Dog as companion". Those axes differed from the original ones by Dwyer et al. (2006).

Saliva samples

To measure salivary equivalents of systemic cortisol concentrations during the first and second session, saliva samples were taken immediately before and 15 minutes after each of the tests. During the third session, saliva samples were taken before the ASSP, immediately after, and 15 minutes after the end of the ASSP. This sampling interval was chosen because blood-cortisol levels peak approximately 20 minutes after a dog encounters a stressor (Hennessy et al. 1998). Within each dyad, all 3 sessions were held at similar times in the afternoon to control the influence of diurnal effects on cortisol. All sessions were balanced across the 4 gender categories. Control samples were taken during 1 day without testing, in the morning, at noon, and in the evening to get baseline values. All samples were taken by the owner after being instructed by the experimenter (I.S.). The owners put Salimetrics (Salimetrics Europe LTD, Suffolk, UK) oral swab under their tongue, then took the saliva sample from the dog at the same time by putting Salimetrics children swab into the dogs cheek pouch for at least 90 seconds. The dog's salivation was stimulated by smelling cheese. Although it is known that cheese does not interfere with measuring cortisol (Ligout et al., 2009), the dog received a piece of cheese only after the saliva sample was taken. Each sample was frozen at $-20°C$ (Sarstedt tubes, Sarstedt Ges.mbH, Wr. Neudorf, Austria) until analysis. An enzyme immunoassay according to Palme and Möstl (1997) was used to analyze the cortisol concentration of the owners' and the dogs' saliva samples. This enzyme immunoassay is routinely used for salivary cortisol analysis in dogs (Haubenhofer 2003; Haubenhofer & Kirchengast, 2007; Patzl 1990) and humans (Haubenhofer 2003; Haubenhofer & Kirchengast, 2007).

Tests during session 2

Test "play session"

At the beginning of the second session, the owner was asked to play with the dog for 5 minutes as he/she would like to play. Toys and treats could be used freely. Afterward, the dog was on leash for 5 minutes, and the owner was instructed to ask the dog to sit or lie down and not to move.

Test "mild threat"

This test was counterbalanced, with and without the owner present, in random order. There was a 15-minute break between the 2 threat situations. For safety reasons, the dog was tethered with a leash to the wall during both threats. When the owner was present, he/she was instructed to behave in the same way he/she would do in similar situations during their daily life. When the dog was alone during the threat, the owner could observe the situation from outside via a monitor. The experimenter observed the dog from outside during both conditions. Another person, called the "threatening person," conducted this test. The threatening person wore a long black coat with a hood and ski mask; just the eyes of the threatening person were visible.

The threatening person entered the room, knocked 2 times at the closed door from inside the room to get the attention of the dog. If the dog did not react, the threatening person knocked again 2 times. The threatening person stared at the dog's eyes or face (if the dog avoided eye contact) and moved 3 steps toward the dog; in between the steps and after the last step, the threatening person stopped walking for 3 seconds, remained still and kept her eyes directed at the dog in the same manner. At the end of the first threat, the threatening person left the room. The procedure of the second threat was exactly the same, but at the end of the second threat the situation was resolved: the threatening person moved to the corner opposite the dog. The ski mask and coat were removed, and the threatening person approached the dog while talking in a friendly manner and offering pieces of cheese. Then, the formerly threatening person left the room, and the dog was let off the leash.

Assessment of dog-owner attachment session 3

Ainsworth Strange Situation Procedure

The original ASSP protocol was developed for the assessment of attachment in human toddlers (Ainsworth et al., 1978). Some aspects of the setting were adapted to dogs while staying as close as possible to the procedures and setting of the original ASSP. Two chairs were placed in the room; 1 for the stranger and 1 for the owner. Different and diverse toys of interest to dogs were placed between the chairs and door, including socks filled with a ball, spread paper all around the inside of a box, wooden toys, as well as ropes, balls and a plastic bottle with stones inside (Figure 1).

The episodes and procedures of the ASSP were carried out in close alignment to the original Ainsworth procedure. The ASSP comprises 8 episodes, all of which are 3 minutes in length (except for the 30 second introduction to the room). Separation episodes were planned to be terminated in the case of symptoms of severe distress on side of the dog (including excessive barking, panting or hyperventilating, (self)-destructive behavior, freezing, hiding in a corner or trying to escape from the room, any kind of aggression toward the stranger). This never happened in any of our dog subjects in this study; hence, none of these sessions had to be terminated before the end of the full experiment.

The episodes consist of (1) entry to the room by dog and owner; (2) opportunity for free play with no specific instructions given to the owner; (3) entry of the "playmate," a female graduate student who is unfamiliar to the dog. The "playmate" sits silently in her chair for 1 minute and does not initiate interaction. After 1 minute, she chats in a friendly manner with the owner, and in the third minute, she initiates unstructured interaction and play with the dog; (4) the owner is signaled by the "playmate" to leave the room. He or she received no specific instructions about communicating this to the dog other than to leave as he/she might do in other similar circumstances. During this separation, the "playmate" plays with or pets the dog as seems appropriate and returns to her chair if the dog seems uninterested in or anxious about her; (5) the owner calls the dog's name and enters. No specific instructions about greeting were given; the owners were instructed to return to their chairs and interacted with their dog from there; the "playmate" leaves unobtrusively. On vibration-signal via phone the owner leaves, as mentioned previously; (6) the dog remains alone in the room; (7) the "playmate" enters the room and interacts as mentioned previously; (8) the owner calls the dog's name and enters, as mentioned previously. Owner and dog are free to interact as they choose. The owners were given instructions immediately before the session.

Figure 1. Prepared test room for the Ainsworth Strange Situation Procedure (ASSP).

Attachment classification

Dog attachment classifications were made from the videotapes of the ASSPs. Two psychologists (A.B. and J.S.) who specialize in attachment and are reliable in the Ainsworth and the Main and Solomon systems (Ainsworth et al., 1978; Main & Solomon, 1986, 1990) adapted it for this purpose. The dog-owner classification system remained very close to the originals, adjusted for species-specific behaviors of dogs. In unclear cases, a behavioral biologist (K.K.) additionally advised on dog behavior. Agreement between A.B. and J.S. on dog-owner attachment classification was 89%. Attachment classifications were defined as follows (Solomon et al., 2014):

- Secure (31 dogs): dog approaches owner promptly at reunion and follows, makes physical contact or signals for contact, seeks and is comfortable with contact. Little or no gaze aversion or proximity avoidance. Little or no resistance to contact or interaction. Unlike human infants, little independent exploration or play is usually observed at the 2nd reunion.
- Insecure avoidant (3 dogs): dog shows little tendency to approach, to seek contact, or to follow. Dog turns or looks away during reunion. Dog shows lack of response to invitations to approach or interact for 30 seconds or more. Dog explores the room and objects during preseparation and postseparation. There is little active search for owner.
- Insecure ambivalent (7 dogs): these dogs were unable to explore independently during preseparation and showed clear distress during separations. On reunion, they mixed persistent distress with efforts to maintain physical contact and/or physically intrusive behavior directed toward the owner. These dyads were characterized by a degree of conflict regarding physical contact or play activities. For example, the dog wished to maintain contact and was uncooperative with the owner's attempt to encourage play or exploration, or the owner maintained firm physical contact which the dog merely passively tolerated. (Dogs who the judges agreed seemed essentially secure but with ambivalent tendencies, were included in the secure group).
- Insecure disorganized (10 dogs): evidence of strong approach-avoidance conflict or fear on reunion, for example, circling owner, hiding from sight, rapidly dashing away on reunion, "aimless" wandering around the room, shying away from contact, or proximity. "Dissociation" may be observed, that is, staring into space without apparent cause; still or frozen posture for at least 20 seconds (in the nonresting, nonsleeping dog).
- Unclassifiable (8 dogs): dogs showed ambiguous evidence of disorganization or other disturbance, for example, "depressed"-a marked lack of enthusiasm in a dog that otherwise seemed secure or showed other behavior suggesting a neurologic or compulsive disorder. Classifiers were unable to reach consensus on group placement for dogs from this classification category. Unclassifiable dogs were excluded from further analysis on dog attachment.

Data analysis

Cortisol data were checked for outliers including the entire sample size of 132 dogs. After excluding 1 dog because of pseudopregnancy (cortisol values differed from all other dogs), we corrected for outliers. For this, the mean cortisol value of all cortisol samples from the 131 dogs were taken; just the morning samples during the resting day were excluded because morning cortisol concentrations differ from afternoon and evening samples in dogs (Dreschel et al., 2014). All values higher than 3 SDs of the mean were excluded from further analysis (Osborne & Overbay, 2004), which was the case in 1.6% of all cortisol values. The excluded values were distributed evenly over male and female dogs. For dog attachment classification, those dogs judged as unclassifiable ($n = 8$, see the previously mentioned paragraph) were excluded from further analysis. Delta cortisol (Δcort) was calculated by subtractions of the sample value before the test from the sample value after the test. Thus, Δcort values >0 indicate an increase, whereas Δcort values <0 indicate a decrease. For better understanding, we defined high Δcort values (>0) as high cortisol reactivity. Those dogs secreted more cortisol during the ASSP than dogs with Δcort values <0.

Statistical analyses

Statistical analysis was performed in SPSS 21 software (IBM Corp., Armonk, NY). The Shapiro-Wilk test was used to test for normal distribution. Data were not normally distributed; thus, Friedman, respectively, Wilcoxon were used to test for differences between cortisol samples. Mann-Whitney *U* was used to test for group differences within delta cortisol during the second session.

A generalized linear model with normal distribution and an identity link function was used to analyze dog delta cortisol (Δcort) data. Owner gender-dog sex combination and dog attachment classification toward the owner were included as factors. For dog attachment classification, we combined insecure-avoidant, -ambivalent, and -disorganized dogs in 1 group and tested them against secure ones, resulting in 2 groups: secure (31 dogs) and insecure-disorganized (20 dogs). Owner personality (NEO-FFI), dog personality (Monash), owner-to-dog relationship (Monash), owner-to-dog attachment (FERT), owner relationship to other humans (RSQ), and owner and dog age were tested as covariates. Only, terms with $P < 0.1$ remained in the final model. Excluded terms were reentered one by one into the final model to confirm that they did not explain a significant proportion of the variation (Poesel et al., 2006). Additionally, the Akaike information criterion was used to determine the best-fit model. Although all terms with $P < 0.1$ remained in the final model and therefore are presented in the results section, only terms with $P < 0.05$ were considered as having a significant influence on the dependent variable. All significances ($P < 0.05$) are given two-tailed.

Alpha correction for multiple comparisons was not considered because this generally increases the risk of type-II error at a comparatively low potential of decreasing type-I error (Nakagawa, 2004). Instead, Cohen effect size was calculated for univariate statistics, with $d = 0.2$ indicating a low effect, $d = 0.5$ a medium effect, and $d = 0.8$ a large effect (Cohen, 1988). Because nonsignificant results do not mean that there is no effect (Zakzanis, 2001) but rather could be due to a lack of statistical power to detect true differences, we also include tendencies within this article.

Results

Cortisol levels during the ASSP session

In general, cortisol decreased during the ASSP and increased again afterward (Friedman, $n = 47$, $\chi^2 = 8.043$, $df = 2$, $P = 0.018$; Wilcoxon, first vs. second sample: $n = 47$, $Z = -3.282$, $P = 0.001$, $d = 0.443$; second vs. third sample: $n = 47$, $Z = -2.554$, $P = 0.011$, $d = 0.285$; Figure 2).

When Δcort was grouped into increase and decrease, 70.6% of the dogs had a decrease and 29.4% of the dogs had an increase from the first to the second sample. A decrease was found in 37.7% and an increase in 62.3% of the dogs from the second to the third sample.

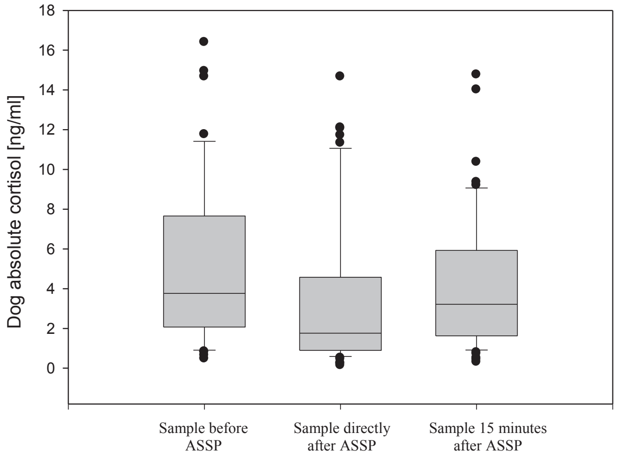

Figure 2. Absolute dog cortisol values in ng/mL during the third session (n = 47). Samples were taken directly before the ASSP, directly after and 15 minutes after the Ainsworth Strange Situation Procedure (ASSP). Median, interquartile range, and range are given.

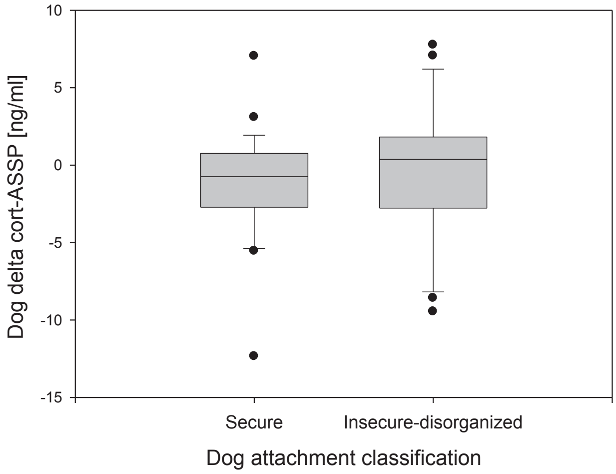

Figure 3. Dog cortisol reactivity during the Ainsworth Strange Situation Procedure given as delta cortisol (delta cort-ASSP) in ng/mL related to dog attachment classification. Median, interquartile range, and range are given. ASSP, Ainsworth Strange Situation Procedure.

From the first to the third sample, 54.2% of the dogs had a decrease and 45.8% of the dogs had an increase. This division is probably due to factors influencing cortisol modulation in dogs. Thus, for further analysis, we used Δcort from the third minus the first sample (Δcort-ASSP) as dependent variable with social and individual parameters as independent variables.

Factors influencing cortisol reactivity in dogs

Securely attached dogs showed significantly lower cortisol reactivity than dogs coded as insecure-disorganized ($P = 0.008$, Table 1, Figure 3). Cortisol decreased with increasing age of the dog and also with increasing active excitability (MCPQ PCA-axis 1) of the dog ($P = 0.023$ and $P < 0.001$, Table 1, Figure 4). As a trend, male dogs of male owners had the lowest cortisol reactivity compared to all other owner gender-dog sex combinations ($P = 0.065$, Table 1, Figure 5).

High owner neuroticism (NEOF-FFI dimension 1) and agreeableness (NEOF-FFI dimension 4) were related to cortisol decrease in dogs ($P = 0.003$ and $P = 0.001$, Table 1). The more the owner was insecure ambivalently attached to the dog (FERT PCA-axis 4) and the more the dog was considered a social supporter by the owner (FERT PCA-axis 1), the higher was the dog's cortisol reactivity ($P = 0.004$ and $P = 0.018$, Table 1). Owners who did not trust other humans had dogs with low cortisol reactivity ($P < 0.001$, Table 1).

Furthermore, during the second meeting, securely attached dogs had significantly lower cortisol reactivity during the play situation, and by trend, a higher cortisol reactivity during the threat without the owner present than dogs rated as insecure-disorganized in the ASSP. For both, Cohen's d indicates a medium effect (Mann-Whitney *U*, play: $n = 43, Z = -2.161; P = 0.031, d = 0.535$; threat without owner present: $n = 44, Z = -1.719, P = 0.086, d = 0.614$, Figure 6).

Table 1
Effects of owner and dog personality, attachment, and owner gender-dog sex combination on dog Δcort-ASSP [ng/mL] (GLM with "dog Δcort-ASSP" as dependent variable, n = 42)

Explanatory variable	Df	Wald chi-square	P
Dog attachment classification	1	7.043	0.008
Dog age	1	5.190	0.023
Owner neuroticism (NEO-FFI dimension1)	1	8.570	0.003
Owner agreeableness (NEO-FFI dimension4)	1	11.468	0.001
Dog active excitable (MCPQ PCA-axis 1)	1	26.840	<0.001
Dog as social supporter (FERT PCA-axis 1)	1	5.564	0.018
Owner insecure-ambivalent (FERT PCA-axis 4) toward dog	1	8.273	0.004
Owner lack of trust (RSQ axis3)	1	18.059	<0.001
Owner gender-dog sex combination	3	7.212	0.065

Discussion

For the first time, we show that dog attachment classification according to the Ainsworth schema is related to cortisol patterns in dogs. Securely attached dogs had lower cortisol reactivity than insecurely-disorganized attached ones, which is consistent with the predictions of attachment theory and results in human studies (Spangler & Schieche, 1998). Also, securely attached children, compared with insecure children, generally have better stress coping (Gunnar, 1998) and show a decrease in cortisol after positive affect episodes and an increase after fear episodes (Roque et al., 2012). In securely attached children, stress increases during separation from the caregiver and decreases after reunion; in disorganized children it is the opposite (Julius et al. 2012); for them the caregiver may even be the source of stress (Julius et al. 2012). Sensitive caregivers (the source of secure attachment) interact thoughtfully in accordance with the infant's needs, whereas caregivers of insecure infants are ambivalent or distanced in their interactions; caregivers of disorganized individuals may show hostile, frightened, or dissociative behavior (George & Solomon 2008; Main & Hesse, 1990). Securely attached dogs also, compared with dogs classified as insecure-disorganized, showed higher cortisol reactivity during the threat without the owner present and lower cortisol reactivity during play with the owner. Probably, owners of securely attached dogs interacted in a friendlier and more sensitive manner compared with those of insecure-disorganized dogs. This would be in agreement with the findings from Horváth et al. (2008) that friendly play is connected to a cortisol decrease in dogs, whereas intrusive play is related to an increase in dog cortisol.

The finding of a link between dog cortisol and dog attachment as predicted supports the use of the original human attachment classification system for dogs. Human-human attachment is estimated via the ASSP and observer rating, based on a schema by Ainsworth, Main and Solomon (Ainsworth et al., 1978; Main & Solomon, 1986, 1990). This rating system includes

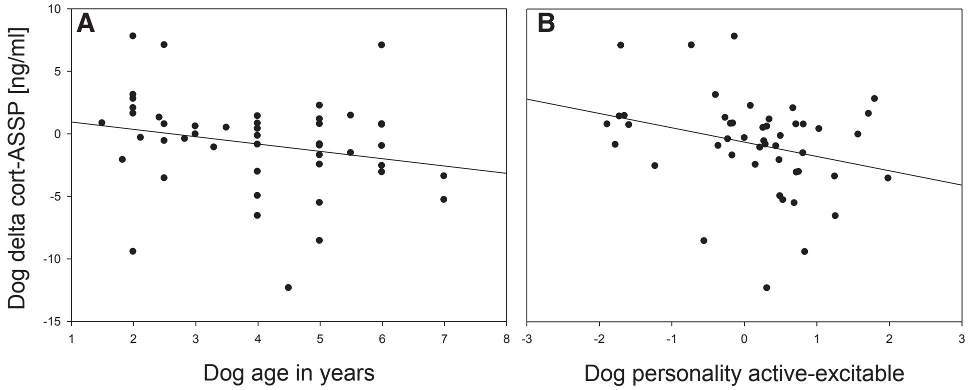

Figure 4. Dog cortisol reactivity during the Ainsworth Strange Situation Procedure given as delta cortisol (delta cort-ASSP) in ng/mL related to (A) dog age in years and (B) to dog personality PCA-axis active excitable. The fit line represents the trend of the data. The fit line is based on the least squares method. ASSP, Ainsworth Strange Situation Procedure; PCA, principal component analysis.

attachment-specific behaviors and interactions between offspring and caregiver. The raters have to gain reliability via a complex training procedure. In contrast, behavior coding (frequencies, durations) has been used in all the recent ASSP dog studies, under the assumption that this is an "objective" approach. Behavior coding does not reveal the qualitative differences in patterns of interaction that Ainsworth intended to capture in the construct of attachment security (Ainsworth et al., 1978). As yet, there are no published direct comparisons between the 2 approaches with dogs. Owner and dog behavior coding was completed as a part of this study, specifically to validate the dog attachment classification system; these results will be reported in future publications.

We found that cortisol decreased during the third session with increasing age from 1.5 up to 8 years of age, which parallels findings by Mongillo et al. (2013) that dogs between 1.5 and 7 years showed a decrease in cortisol, whereas dogs older than 7 years showed an increase after an attachment assessment. This may be because of better habituation to strangers and novel situations in adult dogs compared to younger ones. Higher cortisol levels in senior dogs found by Mongillo et al. (2013) could be explained through health issues and lower capacity to cope with stress. We tested dogs with a maximum age of 8 years; thus, we could not find this effect for senior dogs.

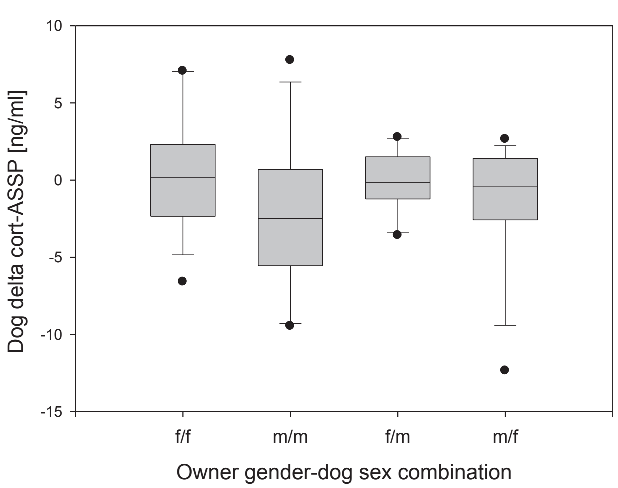

Figure 5. Dog cortisol reactivity during the Ainsworth Strange Situation Procedure given as delta cortisol (delta cort-ASSP) in ng/mL related to owner gender-dog sex combination. f/f, female owner with female dog; m/m, male owner with male dog; f/m, female owner with male dog; and m/f, male owner with female dog. Median, interquartile range, and range are given. ASSP, Ainsworth Strange Situation Procedure.

In contrast to Ottenheimer Carrier et al. (2013), we found a relationship between MCPQ-dog personality and cortisol, probably because we restructured data by PCA. More active-excitable dogs showed a lower cortisol reactivity, which may reflect a particular stress coping strategy in these active animals. The human equivalent of this would probably be "extravert"; for example, humans classified as extraverted show high satisfaction to experiments with high activity (Quattrochi-Tubin & Jason, 1983). Contrary to our results, during nonsocial stress tests extraversion correlates positively with stress reactivity (LeBlanc et al., 2004; LeBlanc & Ducharme, 2005). However, during a Trier Social Stress Test, extraversion is negatively correlated with cortisol reactivity in men (Oswald et al., 2006). Also, high novelty seeking in humans, which is related to extraversion, is associated with low cortisol response during a Trier Social Stress Test (Tyrka et al. 2007). Hence, the relationship between social stressors and cortisol reactivity is not trivial, which is also underlined by our results.

Interestingly, male dogs of male owners tended to have the lowest cortisol reactivity compared with all other combinations. There may be dog-centered or owner-centered explanations for this: male dogs indeed have higher morning basal cortisol values than female dogs (Mongillo et al., 2014), but female dogs react with stronger cortisol increase to adrenocorticotropic hormone (ACTH) injection (Pessina et al., 2009). Furthermore, male dogs of male owners are more sociable and active than male dogs of female owners (Kotrschal et al., 2009), which fits to our results that active-excitable dogs showed low cortisol reactivity. Both together may explain this specific owner gender-dog sex combination effect on dog cortisol reactivity.

We found that owner neuroticism was related to low cortisol reactivity in dogs. This is consistent with previous results that dogs of neuroticistic owners have low morning cortisol values (Schöberl et al., 2012) and also approach their owners quite often and are in proximity with them for long periods (Wedl et al., 2010). Proximity in turn is probably related to the attachment figure being available as a secure base, which leads to calming effects (Ainsworth, 1989; Bowlby, 1969) supported through oxytocin increase and cortisol decrease (De Vries et al., 2003). Owners with an insecure ambivalent representation of attachment to their dog and for whom the dog was an important social supporter had dogs with higher cortisol reactivity. This is probably because those owners may not be fully available as a reliable and calming caregiver. Owner's lack of trust in other humans seems to be an influencing factor for dog stress coping. However, the direction of our result is not clear yet. Projective instruments and behavior-based assessments may provide more insight into this aspect.

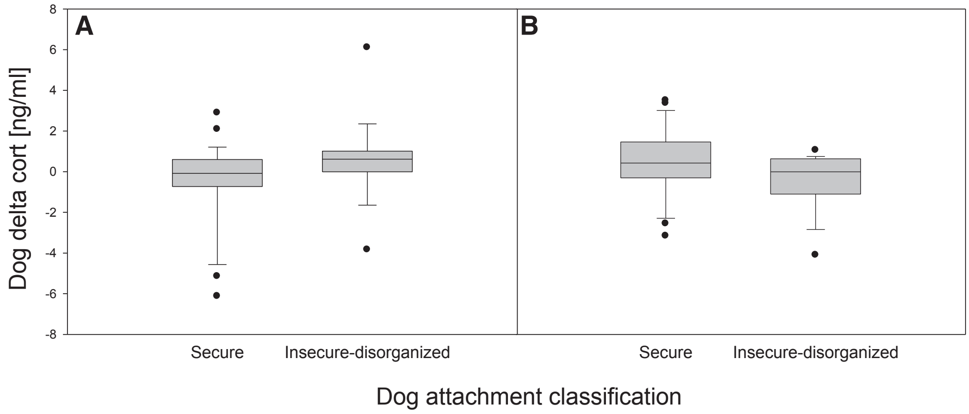

Figure 6. Dog attachment classification and dog cortisol reactivity during the second session given as delta cortisol (delta cort) in ng/mL for (A) play with the owner and (B) threat without the owner present. Median, interquartile range, and range are given.

In most of the dogs, cortisol decreased during the ASSP and increased again afterward. This is probably due to reactivity toward the novel environment even ahead of the first saliva sample. Pessina et al. (2009) found that adrenocorticotropic hormone–treated dogs showed an increase in cortisol in the first hour after injection up to 3 hours later. To optimize the evidence quality from cortisol data, in future studies samples should be taken 20 minutes before starting the test, directly before and after the test and also 20, 40, and 60 minutes after the test. With this number of samples, an area under the curve could be calculated, which gives more information on cortisol reaction over time. We may have got a decrease of cortisol during the ASSP because of the cortisol sample timing. Pretest baseline samplings are sensitive to the novelty of the setting; an adaptation to the setting may influence following samples (Nicolson 2008). However, this does not explain, why half of the dogs showed an increase and the other half a decrease over the entire meeting. Within the owner gender-dog sex groups the sample size was small, which increases type II errors. With a higher sample size, some results may be more distinct and also interactions between influencing factors could have been calculated.

We conclude that the attachment classification system as used in psychology may also be applicable for dog-owner attachment, as our study reveals similar findings concerning attachment and stress coping. In addition, we suggest that aside of dyadic attachment patterns, dog and owner characteristics affect dog stress coping. This hints at the importance of a systemic approach rather than a person-centered approach, in science as well as in practice.

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Ethical considerations

Participation in our study was voluntary; dog owners were informed that they could stop the test situation at any time and were also asked to sign 2 information and consent forms, one for the first and second meeting and one for the third meeting. Data collection was conducted according to the standards of the Code of Ethics of the World Medical Association (Declaration of Helsinki), the EU Directive 2010/63/EU for animal experiments and Uniform Requirements for manuscripts submitted to Biomedical journals and the German society of Psychology (Ethische Richtlinien der DGPs und des BDP). Ethical review for the first and second meeting was done by the Animal Welfare Committee of the Faculty of Life Sciences, University of Vienna (approval number: 2014-015). Ethical review for the third meeting was done by the German Society for Psychology (Deutsche Gesellschaft für Psychologie).

Conflict of interest

The authors declare no conflict of interest.

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