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Three different coping styles in police dogs exposed to a short-term challenge

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Abstract

According to some researchers, animals show different coping styles to deal with stressful situations. In the case of social carnivores, social stress is a substantial part of the overall stress load. Previous research has established two extreme (proactive and reactive) coping styles in several animal species, but means of coping with social stress has not yet been investigated in the case of dogs. The aim of this current study was to examine whether (1) experienced working police dogs adopt different coping strategies during a short-term unexpected social challenge presented by a threatening human, (2) whether this affects post-encounter cortisol levels, and (3) whether there is an association between the cortisol response and the behavior (coping strategy) displayed during the threatening approach. Using factor analysis, we have identified three different group of dogs which were characterized by either fearfulness, aggressiveness, or ambivalence and in parallel showed specific differences in their reaction norm when threatened by an approaching stranger. This grouping also allowed to draw possible parallels between aggressiveness and the proactive behavior style and fearfulness and reactive coping style, respectively. In addition, we have revealed a third group of animals which show ambivalent behavior in a social threatening situation.

Keywords: Coping styles; Ambivalent behavior; Aggression; Police dogs; Cortisol; Threatening test; Age

Introduction

Animals adopt different behavioral strategies in order to cope with stressful events (Koolhaas et al., 1999; Korte et al., 2005). Coping strategies (or styles) have been defined as a coherent set of behavioral and physiological stress responses which are consistent over time and which are characteristic to a certain group of individuals (Koolhaas et al., 1999). Individuals that when challenged tend to adapt a fight-flight type of response have been labeled active copers or proactive. Those that adopt a conservation-withdrawal type of response have been labeled passive copers or reactive. Coping strategies have been demonstrated in fish (Øverli et al., 2004; Schjolden et al., 2005), rodents (Koolhaas et al., 1999; Rödel et al., 2006; Vegas et al., 2006), birds (Carere et al., 2003), pigs (Wechsler, 1995), and humans (Lazarus and Folkman, 1984).

Proactive coping styles are characterized by a high level of aggression, short attack latency, active attempts to counteract the stressful stimuli, low HPA-axis reactivity (Koolhaas et al., 1999), and high activation of the sympathetic-adrenomedullary system (Sgoifo et al., 1997). Reactive or passive coping involves immobility, low levels of aggression, long attack latency, higher activation of the pituitary-adrenocortical system (Bohus et al., 1987; Carere et al., 2003; Kalin, 1999; Pottinger and Carrick, 2001; Øverli et al., 2004; Von Holst, 1986), and higher parasympathetic reactivity (De Boer et al., 1990; Korte et al., 1992; Ruis et al., 2001). Proactive and reactive coping styles are typified by behavior patterns that correspond to those described for bold and shy (Coleman and Wilson, 1998; Wilson et al., 1994) or aggressive and non-aggressive individuals (Schjolden et al., 2005), respectively. Bold individuals develop routines to deal with different demands more easily while shy individuals retain more flexibility in their behavior (Verbeek et al., 1996; Wilson et al., 1994). Research on animal personality suggests that behavioral syndromes (Sih et al., 2004) are analogs of personality (Capitanio, 1999; Gosling and John, 1999; Gosling, 2001). The strategy applied to cope with stressors is influenced not only by personality factors but also by learning and rearing (Benus et al., 1991; Capitanio and Mason, 2000; Hall et al., 1997; Weiss et al., 2004).

The dogs are utilized in numerous functions within human society which exposes them to many situations involving social

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stress (environmental challenges). This is especially significant in the case of working police dogs. These dogs have been trained to deal with human aggressors and fleeing offenders. Previous studies indicated that specific personality traits contribute to increased performance in the dog during training (Svartberg, 2002; Wilsson and Sundgren, 1997). The means of coping with an acute stressor, however, has not yet been studied in detail in working dogs.

In the bulk of prior studies, dogs were exposed to various forms of non-social stress. These include experiments acute stress from events such as strong noise, electric shock, flopped bag (Beerda et al., 1998a), simulated thunderstorm (Dreschel and Granger, 2005), separation (Hennessy et al., 1997; Tuber et al., 1996), frightening objects (e.g. umbrella; Beerda et al., 1998b; King et al., 2003), and transport (Bergeron et al., 2002). After stimulation, dogs generally showed elevated levels of cortisol concentrations that correlated with characteristic behavior pattern such as a very low body posture, paw lifting, snout licking, etc.. Humans may also be the cause of stress in dogs. In some experimental situations, human behavior such as physical force to cause moderate pain was the direct cause of stress dogs (Netto and Planta, 1997; Weiss and Greenberg, 1997).

Dogs have also been exposed to a person that approached them in a threatening way (Svartberg, 2002; Vas et al., 2005). This resembles in some respect the residence intruder test developed for laboratory rodents (e.g. Ebner et al., 2005; Vegas et al., 2006). Generally, dogs showed a large variability in their behavior toward the stranger. Some dogs behaved in a 'friendly' or 'passive' manner suggesting tolerance toward the stranger. Others avoided interaction with stranger or displayed a counter attack. Vas et al. (2005) reported breed differences; Belgian shepherds displayed more threats and rarely showed friendly behavior toward the approaching stranger unlike sledge dogs and retrievers.

In the present study, we have applied a modified version of the method used by Vas et al. (2005). In the absence of their handler, police dogs were exposed to a strange human approaching threateningly. The aim of this study was to examine (1) whether experienced working police dogs adopt different behavioral coping strategies during a short-term unexpected social challenge, (2) whether such social stimulation affects post-encounter cortisol levels, and (3) whether there is an association between the cortisol response and the behavior (coping strategy) displayed during the threatening approach.

Materials and methods

Subjects

The police dogs were purchased by the Hungarian National Police Training School for Police dog Handlers (Dunakeszi, Hungary). Dogs were acquired between 1 and 3 years of age. Dogs were tested physically (i.e. for hip dysplasia) and behaviorally (i.e. reaction to gun shot, bite work). Individuals were purchased only if they did not show signs of hip dysplasia and fear of gun shot. Thereafter they participated in a 12-week-long training course together with their handlers. During this course, the dogs were trained for guarding and obedience.

Dogs that participated in the present study were purchased between 1997 and 2003, and performed patrol service with their handlers on the streets for minimum 1 year. All 60 subjects were male German Shepherds. Apart from behavioral homogeneity, the choice of a single breed was important because cortisol concentrations are known to be affected by gender, breed, and age in dogs (e.g. Hennessy et al., 1997, 2001). The dogs' age ranged from 2 to 11 years (mean age ± SD: 7.2 ± 2.18 years), and the subjects were categorized following Studzinski et al. (2006): *Adult dogs* were 2–7 years old (26 individuals; mean age ± SD: 5.19 ± 1.58 years); *Old dogs* were 8–11 years old (34 individuals; mean age ± SD: 8.77 ± 0.99 years). Fifty-seven of the handlers were men and only 3 women.

All procedures were approved by the Ethical Committee of the Eötvös Loránd University, Department of Ethology and conducted in accordance with Hungarian State Health and Medical Service (ÁNTSZ). There is a standing agreement with the Hungarian Police Force that permits testing their working dogs.

Date and premises

For the present experiment, data were collected on 6 occasions ('samples') in 2005 (April, May and September) and in 2006 (February, March and April) at the Hungarian National Police Training School for Police dog Handlers (Dunakeszi, Hungary). The tested police dogs and their handlers participated in a special 2-week training course at the time of testing. For practical reasons, the experiments in spring and summer were done in the morning hours, and in autumn and winter in the early afternoon. Many studies indicate that dogs' cortisol levels change according to a circadian rhythm, having a peak in the morning hours decreasing gradually until the evening (Beerda et al., 1996); however, some research has demonstrated just the opposite (Bergeron et al., 2002; Koyama et al., 2003). There is also some data suggesting that cortisol levels of dogs that have little exercise follow a circadian rhythm, while this could not be shown in the case of working dogs (Kolevská et al., 2003). In addition, there is considerable disagreement regarding the optimal timing for the measurement of cortisol levels in stimulation experiments (Dreschel and Granger, 2005; Jones and Josephs, 2006; Kobelt et al., 2003).

Test procedures

Behavioral tests were conducted at a separated location away from visually disturbing factors, which was familiar to the dogs, as it has been shown that introduction into a novel environment enhances HPA activity in the dog (e.g. Beerda et al., 1997). The dog, the handler, and an unfamiliar man ('decoy') took part in the observations. The behavior of the dog was recorded from about 10 m distance by a third person (cameraman).

Tests lasted a mean 6 ± 2 (SD) min and consisted of three episodes lasting 4 to 8 min that took place in a fixed order with intervals of 1 min or less.

Episode 1: dog with muzzle

The handler was asked to tie up his dog, wearing a muzzle to an isolated tree with a 2 m long chain and leave it alone (Fig. 1a). The handler hid behind a nearby tree 10 m from his/her dog, where he/she was not visible to the dog. In this first episode, the dog wore a muzzle and the decoy had a stick (40 cm) in his hand. After 1 min, the decoy, holding a 40 cm stick in his hand, appeared from behind a screen 10 m from the dog. When the dog glanced at the decoy, he started to approach the dog in normal walking speed stopping at 2 m from the dog (1 min). Next, the decoy moved forward the dog slowly, stopping several times, while looking into the dog's eyes shouting at the dog in a threatening voice (1 min). Nearing the dog within 1 m, the decoy lifted his bare hand over the head of dog, while threatened the dog with the stick (30 s) (Fig. 1b). Then the decoy went back to his hiding-place.

Episode 2: dog without muzzle

Before the start of Episode 2, the handler came back to the dog and he/she took the muzzle off the dog, and then disappeared again behind the tree. As now the dog did not wear a muzzle, the decoy had the protection-sleeve on his right hand and the stick in his left hand. The decoy approached the dog similarly as described in Episode 1. The decoy kept the protection-sleeve behind his back until he reached the dog. At about 0.5 m from the dog, the decoy brought out the protection-sleeve from behind his back. Dependent on the dog's reaction, the dog either obtained the protection-sleeve or the decoy encouraged the dog by moving the sleeve in front of the dog's head, and allowed to take it, while threatened the dog with the stick touching its front legs. The episode was terminated when the dog obtained the protection-sleeve (Fig. 1c).

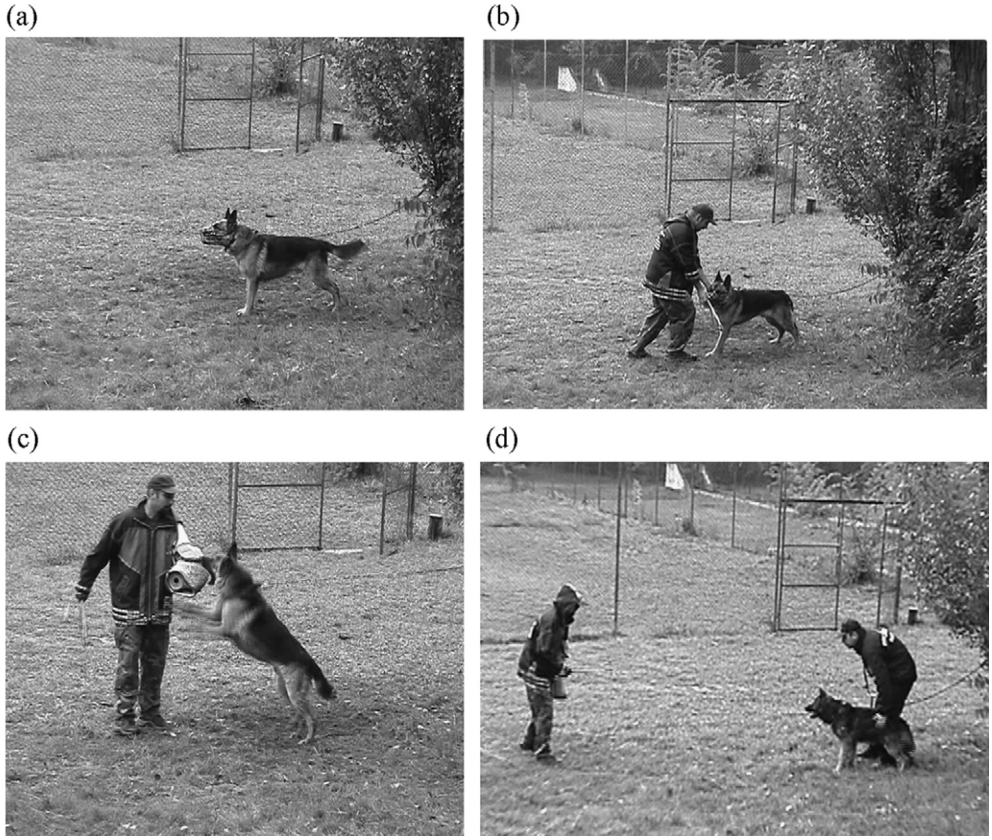


Fig. 1. Experimental arrangement: (a) the dog was tied up with a 2 m long chain to an isolated tree and left alone; (b) the dog wore a muzzle and the decoy had a stick in his hand; (c) end of Episode 2, note that the dog does not wear a muzzle and the decoy has a protection-sleeve on his right hand and a stick in his left hand; (d) beginning of Episode 3, the handler stayed near his dog and the decoy moved toward the dog waving the protection-sleeve.

Episode 3: dog with handler without muzzle

The handler came back from his/her hiding-place and stayed near his dog. The decoy started to move toward the dog waving the protection-sleeve from a distance of 1 m, and gave the sleeve to the dog when he got hold of it (Fig. 1d).

Measurement of saliva cortisol concentrations

Saliva samples were collected from dogs before and 20 min after the end of the threatening episodes (see also Beerda et al., 1998b; Dreschel and Granger, 2005; Jones and Josephs, 2006; Vincent and Michell, 1992). Substances to stimulate saliva flow were not used. The saliva was collected with cotton swabs by the handlers approximately 300 m from the site of the experiment, but in similar surroundings. While the dog was standing still, its handler reached into the mouth of the dog with the swab until it absorbed the most amount of saliva possible (lasting from 30 to 60 s). The soaked cotton swabs were temporarily stored on dry ice in numbered Eppendorf tubes. For long-term storage, the saliva samples were kept in a deep freezer (−80 °C). Just before the analysis, the tubes were warmed up to room temperature. The saliva was removed from the cotton swabs by centrifugation (3000 rpm for 15 min) using special centrifuge tubes with filters (Corning Spin-X; Sigma-Aldrich Kft., Budapest, Hungary). After separation, the saliva samples were analyzed for cortisol concentrations using a highly sensitive (from 0.003 to 3.0 μg/dl) enzyme immunoassay kit from Salimetrics (State College, PA, USA); the intra- and inter-assay coefficients of variation as provided by the manufacturer are below 10% and 15%, respectively (Salimetrics, 2005). The procedures were performed as per manufacturer's instructions.

Analysis of behavior

The behavior of dogs was video recorded and analyzed with the help of Theme-Coder software (Magnusson, 1996). From the video recordings, 18 different behaviors were scored for the duration of occurrence. The behavior of the dogs was recorded separately in the three episodes. The behavioral variables were the following:

Sitting	
Standing	
Lying	
Walking	Taking slow steps in a chain length radius
Running	Continuous movements in a chain length radius
Jumping	Jumping movements right–left, up–down, with only hind legs staying on the ground while orienting toward the decoy
Attacking	Dashing toward the decoy, jumping up
Backing up	Moving in the opposite direction from decoy, retreating, trying to hide behind the tree
Pacing	Walking back and forth, does not remain in one place
Sniffing	The nose is moved along objects and/or clear sniffing movements are exhibited
Digging	Scratching the ground with the forepaws in a way that is similar to when dogs are digging holes
Urinating	
Barking	
Whining	Soft, high pitched vocalizations
Growling	Low frequency vocalizations
Orientation toward the handler	Looking (direction of the head) at handler
Orientation toward the decoy	Looking (direction of the head) at decoy
Looking away	Looking around, but not at decoy or at handler
Attack latency	From the appearance of the decoy to the first attack (see above) of the dog (in the first episode only)

Additionally, the dogs' responses to the approaching decoy, i.e. from 2 m distance to contact during Episodes 1 and 2, were scored as (after Beerda et al., 1998a,b; Vas et al., 2005):

'Moving off'
- Score 0: the dog does not move away from the approaching decoy while gazing at him.
- Score 1: the dog moves away from the approaching decoy while gazing at him, but it does not move behind the tree.
- Score 2: the dog moves behind the tree while gazing at the decoy.

'Avert gaze'
- Score 0: the dog is continuously looking at the face of the decoy or if eye contact is interrupted, the subject re-establishes it again within 5 s.
- Score 1: the dog averts its gaze from the decoy for more than 5 s.
- Score 2: the dog averts its gaze from the decoy and does not look back even after the third 'warning noise' made by the decoy.

'Attack'
- Score 0: the dog makes sudden movements directed at the decoy associated with continuous growling or barking or attempts to bite, but is jerked back by the chain.
- Score 1: the dog initializes sudden movements toward the decoy associated with a short growling or barking response while on loose chain.
- Score 2: the dog does not make any sudden movement toward the decoy.

'Postures'
- Score 0: the breed specific posture as shown by dogs under neutral conditions, but in addition the tail is positioned higher, or the position of the head is elevated and the ears are pointed forwards, or the animal is standing extremely erect.
- Score 1: the breed specific posture shown by dogs under neutral conditions.
- Score 2: a lowered position of the tail (compared to the neutral posture), a backward positioning of the ears and bent legs.
- Score 3: the position of the tail is lowered, the ears are positioned backwards and the legs are bent.
- Score 4: a low posture with the tail curled forward between the hind legs.

'Tail wagging' (repetitive wagging movements of the tail)
- Score 0: does not occur.
- Score 1: once or twice, but not intense.
- Score 2: once or twice, but intense.
- Score 3: many times, but not intense.
- Score 4: many times and intense.

'Greeting the Handler'
- Score 0: the dog pays no attention to handler, when he/she appears.
- Score 1: the dog looks toward the handler, sometimes wagging the tail once.
- Score 2: the dog is continuously looking at the handler, wagging the tail, shifting the paws, and jumps at the approaching handler.

'Snout licking' (part of the tongue is shown and moved along the upper lip)
- Score 0: no snout licking during the test.
- Score 1: at least one snout licking during the test.
- Score 2: several times snout licking during the test.

'Paw lifting' (a fore paw is lifted into a position of approximately 45°)
- Score 0: no paw lifting during the test.
- Score 1: at least one paw lifting during the test.
- Score 2: several times paw lifting during the test.

Interobserver agreements for all of the eight scored behavior categories were assessed by means of parallel coding of the 20% of the total sample by two observers and relatively high values were calculated in all cases. Kappa coefficients are 0.92 for 'moving off', 0.83 for 'avert gaze', 0.79 for 'attack', 0.66 for 'postures', 0.63 for 'tail wagging', 1.0 for 'greeting the handler', 0.92 for 'snout licking', and 0.83 for 'paw lifting', respectively.

Statistical analysis

The normality of our variables was analyzed by using *Shapiro-Wilks tests*, and variables that failed to pas the normality test were subjected to non-parametric statistical investigations. We used *Kruskal–Wallis tests* with Dunn's post hoc test for analyzing the attack latencies, the cortisol concentrations in the 6 experimental samples which were collected at different occasions. The significance of the observed differences was calculated by using *Mann-Whitney U-test* for cortisol concentrations of adult and old dogs and morning and afternoon sessions, respectively. We used *Wilcoxon test* for comparing the baseline and 20 min post-stressor concentrations in the case of the whole group and in the case of Groups 1, 2 and 3. *Spearman's Rank correlation* was used to search for relationships between baseline and 20 min post-stressor cortisol concentrations, and to search for relationship between scores obtained on the behavioral factors ('fearfulness', 'aggressiveness, 'ambivalence') and age of dogs.

In analyzing the behavioral variables, we used multi-stage analysis.

As our experimental design yielded numerous variables, we used *factor analysis* for data reduction in order to explain the underlying variance by a smaller number of variables. The Scree test and the Kaiser Eigenvalue rule were used to determine the number of factors that could be considered, and varimax rotation was used to identify empirical groupings of variables. In order to maximize the specificity of the items ('behavioral variables') on a certain factor, we only retained an item if its loading on a factor was greater than 0.35 and its loading on any other factor was less than 0.20. Items that did not fulfil these criteria were excluded (Reise et al., 2000; Sheppard and Mills, 2002). From the measured behavioral variables (running, lying, standing, sitting, attack, backing up, pacing, walking, sniffing, urinating, barking, orientation toward handler, decoy and environment, paw lifting, snout licking, avert gaze, moving off, low posture, handler greeting, tail wagging), only 16 variables met these criteria. A further decrease in the number of variables was achieved by pooling together 6 scored variables into two derived variables. '*Acute stress*': snout licking and paw lifting (Beerda et al., 1996); '*Frightened behavior*': moving off, avert gaze, low posture, attack (Vas et al., 2005). In both cases, the values were obtained by calculating the average scores from the assigned variables.

For each factor that resulted from the factor analyses, scores were calculated for individual dogs and these were standardized used z-transformation to make the scores on different factors comparable.

Hierarchical cluster analysis was used for classification of individuals depending on their standardized individual factor scores and to separate individuals of distinct groups by maximizing their between-class separability while minimizing their within-class variability.

Discriminant analysis was used to establish if individuals were correctly classified, and to what extent the certain factors explain the variance in these groupings. The confidence radius of the group centroid was calculated by using the following formula:

$$t_{\alpha i,N_i-1}/\sqrt{N}$$

where N_i is the sample size of the ith group and where $t_{\alpha i,Ni-1}$ is the tabulated value of Student's t distribution at the α (0.01) percent for N_i-1 degrees of freedom (Colgan, 1978).

We used *one-way ANOVA* for comparing the mean scores obtained on the three behavioral factors ('fearfulness', 'aggressiveness', 'ambivalence') with Bonferroni post hoc test.

Statistical analyses were performed using SPSS (Version 13.0).

Results

The analysis of behavior

Factor analysis (FA)

Twelve variables fulfilling the criteria for inclusion (see Statistical analysis above) were entered into the main FA with varimax rotation to identify underlying background variables. Components with initial eigenvalues of greater than one were retained and subjected to varimax rotation with Kaiser Normalization. The analysis revealed three factors with the loadings of 0.47 to 0.88 (eigenvalues > 1.46; varimax rotation) that accounted for 58.53% of the total variance (Table 1).

Table 1
Results of the principal components factor analysis for each of the 12 variables on the first three varimax-rotated principal factors with Kaiser Normalization

	Factors		
	1	2	3
	Fearfulness	Aggressiveness	Ambivalence
Frightened behavior	0.783		
Handler greeting	0.730		
Oriented decoy	−0.722		
Backing up	0.690		
Attack	−0.568		
Oriented handler	0.478		
Barking		0.882	
Tail wagging		0.833	
Pacing		0.813	
Paw lifting + mouth licking			0.743
Running			−0.703
Looking away			0.593
Eigenvalues	3.51	2.04	1.46
% of variance	29.25	17.06	12.22
Cumulative %	29.25	46.31	58.53

Factor 1 included six behavioral variables related to flight or withdrawal to the approaching decoy (frightened behavior, oriented decoy, backing up, attack) and affiliation toward the returning handler (handler greeting, oriented handler) and was labeled as 'fearfulness'. Factor 2 was labeled as 'aggressiveness' because it was associated with 3 items related to a tendency to respond courageously toward the approaching decoy (barking, tail wagging, pacing). Finally, behaviors loading on Factor 3 indicated uncertainty (paw lifting and mouth licking, running, looking away, and no motion response to the decoy), thus the factor was labeled as had been 'ambivalence'.

We found weak but significant positive correlation between age and both Factor 1 ('fearfulness') and Factor 3 ('ambivalence') ($r_S = 0.270$, $p = 0.037$; $r_S = 0.295$, $p = 0.022$, respectively), suggesting that the older dogs were more fearful and/or ambivalent in their response to the approaching decoy.

Hierarchical cluster analysis (HCA)

The standardised individual factor scores ('fearfulness', 'aggressiveness', and 'ambivalence') were used to group our subjects by a HCA. On the basis of the derived dendrogram, the dogs were divided into three separate clusters (Groups 1, 2, and 3) at the rescaled distance of 22. The analysis of variance showed significant differences among the groups in the case of all three factors (Table 2). Dogs classified into Group 1 had the highest scores on factor 'fearfulness' ($F(2,83) = 8.73$; $p < 0.001$), dogs in Group 2 had the highest scores on factor 'aggressiveness' ($F(2,56) = 8.45$; $p = 0.001$), while dogs in Group 3 had the highest on factor 'ambivalence' ($F(2,38) = 20.06$; $p < 0.001$). The post hoc comparisons (Bonferroni test) showed that each group represented a distinctive pattern with regard to response to the approaching decoy.

Comparisons of the attack latency of the three groups revealed significant differences ($Chi^2 = 12.73$; $df = 2$; $p = 0.002$). The post hoc comparisons (Bonferroni test) showed that Group 2 differed both from Groups 1 and 3. Group 1 had the longest attack latency, followed by the attack latency of Group 3, while the attack latency of Group 2 was the shortest.

We found no significant differences among groups based on age, although the dogs in Group 1 were the oldest, Group 2 had the youngest ones, and in Group 3 was typified by middle-aged dogs.

Table 3
Variance explained and structure matrix for discriminant analysis on individual identity

	Functions	
	1	2
Variance explained (%)	57.9	42.1
Cumulative variance (%)	57.9	100.0
Behavioral variables		
Fearfulness	0.896	0.027
Aggressiveness	0.169	−0.316
Ambivalence	0.069	0.834

Discriminant analysis (DA)

We used DA to test the power of the scores obtained on the three behavioral factors ('fearfulness', 'aggressiveness', and 'ambivalence') to correctly classify each subject. Wilks' Lambda values relatively nearing 0 suggest strong relationships, meaning that within certain groups the homogeneity is almost complete. We found differences among groups in the case of all three independent variables ('fearfulness': Lambda = 0.424; $p < 0.001$; 'aggressiveness': Lambda = 0.853; $p = 0.011$; 'ambivalence': Lambda = 0.536; $p < 0.001$). We obtained two discriminant functions (Table 3). The variable that mostly contributed to the first function was fearfulness, followed by ambivalence, while the least critical was aggressiveness. The variable that mostly contributed to the second function was ambivalence,

Table 2
The three groups with individuals' number, age, baseline, and 20 min post cortisol concentrations, and mean of standardized individual factor scores (±SD)

	N	Age (year; mean ± SD)	Baseline cortisol concentrations (μg/dl; mean ± SD)	20 min post cortisol concentrations (μg/dl; mean ± SD)	Factor 1, fearfulness (mean ± SD)	Factor 2, aggressiveness (mean ± SD)	Factor 3, ambivalence (mean ± SD)
Group 1	28	7.71 ± 1.92	0.09 ± 0.08	0.13 ± 0.15*	0.73 ± 0.17#	0.59 ± 0.29	0.46 ± 0.25
Group 2	19	6.42 ± 2.55	0.10 ± 0.12	0.12 ± 0.13	0.22 ± 0.16	0.51 ± 0.29	0.33 ± 0.17
Group 3	13	7.23 ± 1.92	0.11 ± 0.13	0.21 ± 0.25*	0.42 ± 0.29	0.30 ± 0.24#	0.83 ± 0.11#

In Groups 1 and 3, the cortisol concentrations were significantly (*) higher after the test than before. The mean individual scores obtained on Factor 1 ('fearfulness') were significantly (#) higher in Group 1, and similarly the mean individual scores for Factor 3 ('ambivalence') were significantly higher for Group 3. Factor 2 ('aggressiveness') showed the significantly lowest mean scores in Group 3.

followed by the lack of the aggressiveness, and the least crucial was fearfulness. The two functions explain our results almost in equal proportion. The discriminant functions classified 90% of the individuals into the correct group.

Based on the confidence radius of the groups' centroids, there was no overlap among the groups (Fig. 2). Based on the position of the centroids in the two dimensional space determined by the two discriminant functions, Group 1 is characterized by fearfulness, Group 2 is typified by aggressiveness, and Group 3 can be described by ambivalent behavior.

The analysis of salivary cortisol concentrations

The effects of threatening approach on dogs' salivary cortisol concentrations

Cortisol concentrations after the test (0.14±0.31 μg/dl) were significantly higher than before the test (0.10±0.08 μg/dl) ($T- = -2.031$; $p = 0.042$; $N = 60$). A positive correlation existed between baseline and 20 min post-stressor cortisol concentrations ($r_S = 0.487$; $p < 0.001$).

The effect of age on the cortisol concentrations

Comparisons of the cortisol concentrations showed that old dogs (8–11 years; mean age±SD: 8.74±0.99 years; $N = 34$) and adult dogs (2–7 years; mean age±SD: 5.19±1.58 years; $N = 26$) did not differ significantly neither in the baseline ($Z = -0.742$; $p = 0.458$), nor in the 20 min post-stressor concentrations ($Z = -1.681$; $p = 0.093$). In the group of adult dogs, no significant differences were found between cortisol concentrations before and after the testing. On the contrary, in the group of older dogs, significant differences were found between cortisol concentrations before and after the testing ($T- = -2.49$; $p = 0.013$) (Fig. 3a).

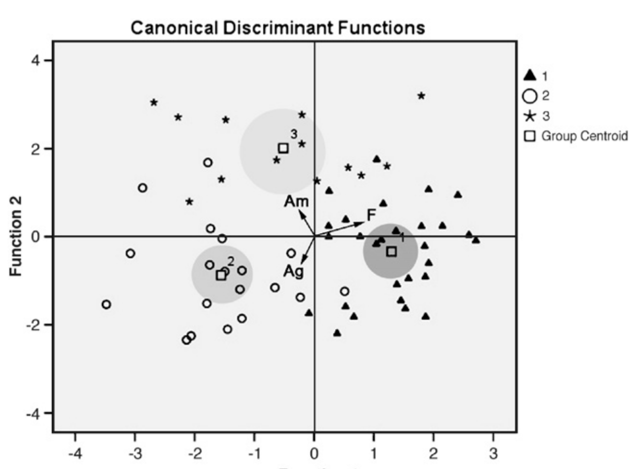


Fig. 2. Results of the discriminant analysis: the three groups (▲=Group 1; ○=Group 2; *=Group 3) were diverged along the two discriminant functions; the first group is characterized by fearfulness (F=vector of the behavioral factor ‘fearfulness’), the second group is typified by aggressiveness (Ag=vector of the behavioral factor ‘aggressiveness’), and the third group can be described by ambivalent behavior (Am=vector of the behavioral factor ‘ambivalence’); based on the confidence radius of the groups' centroids, there was no overlap among the groups.

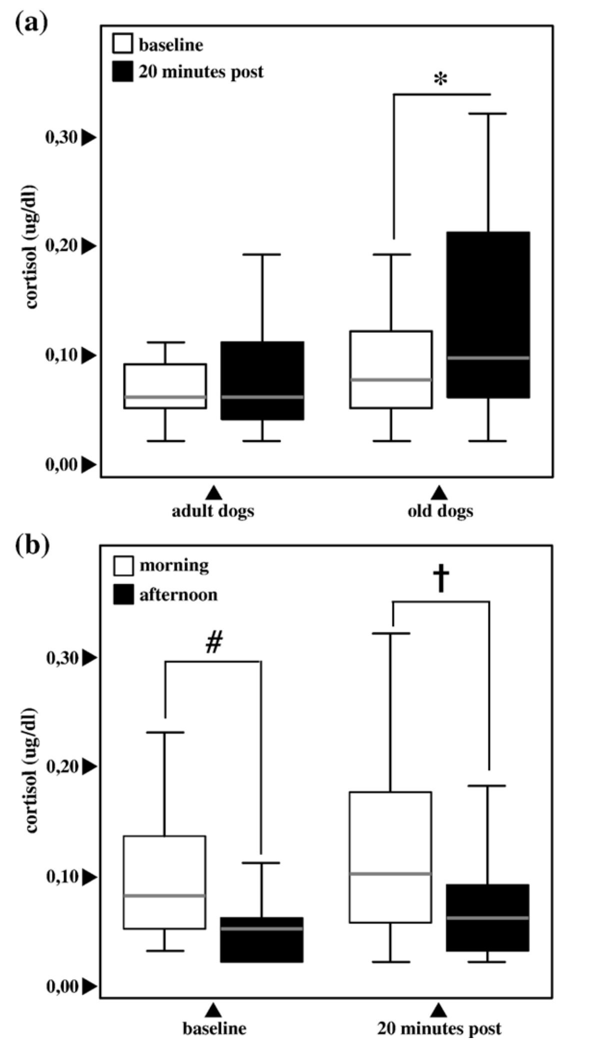


Fig. 3. The measures of cortisol concentrations by age (a) and day session (b) on the effect of threatening approach. (a) Old dogs' ($N = 34$) cortisol concentrations increased significantly (*) from baseline (□) to 20 min post-stressors (■), while this was not the case in adult dogs ($N = 26$). (b) The baseline cortisol concentrations in morning sessions (□) were significantly (#) higher than the afternoon (■) sessions, as in the case of 20 min post-stressor concentrations (†). Data are presented as median±quartiles in μg/dl, and differences are considered statistically significant if $p < 0.05$.

The effects of time of day on cortisol concentrations

Comparisons of the cortisol concentrations of morning and afternoon groups were found significantly different both before ($Z = -2.452$; $p = 0.014$) and after the stressor ($Z = -2.422$; $p = 0.015$). In the morning, the baseline was higher (0.12± 0.11 μg/dl; $N = 39$) than in the afternoon (0.07±0.07 μg/dl; $N = 21$), but in both groups a similar stressor-induced increase (0.04 μg/dl) was found (Fig. 3b).

The effect of the day of testing on the variation of cortisol concentrations

The 60 dogs were measured and tested at six different occasions (samples). Comparisons of the cortisol concentrations at these 6 occasions did not show any significant differences in the 20 min post-stressor; however, there were significant differences among the samples in the baseline ($Chi^2 = 17.299$; $df = 5$; $p = 0.004$). The Dunn's post hoc test showed that the baseline cortisol concentrations were significantly higher in Sample 6 (0.23 μg/dl; $N = 10$) than in all the others (Sample 1: 0.07 μg/dl; $N = 10$; Sample 2: 0.07 μg/dl; $N = 7$; Sample 3: 0.08 μg/dl; $N = 13$; Sample 4: 0.05 μg/dl; $N = 8$; Sample 5: 0.10 μg/dl; $N = 12$). This may have been caused by some unknown distressing event prior to our experiment. However, those individuals whose higher baseline hormonal concentrations caused the significantly higher baseline on Sample 6 level were evenly distributed over the three coping style groups, thus omitting this sample from our analysis did not influence the results of the statistical analysis.

The changes of cortisol concentrations in the three groups revealed by the cluster analysis

In Group 1, characterized by 'fearfulness' (see above), cortisol concentrations were significantly higher after the test than before testing ($T+ = -2.077$; $p = 0.038$; $N = 28$). In Group 2, with a relatively high level of 'aggressiveness', cortisol concentrations did not change significantly during the test ($T+ = -0.546$; $p = 0.58$; $N = 19$), while in Group 3, characterized by 'ambivalence', cortisol concentrations were significantly higher after the test than before the test ($T+ = -1.957$; $p = 0.05$; $N = 13$). We did not find significant differences between groups, neither in baseline nor in post-stressor cortisol concentrations (Table 2).

Discussion

In the present experiment, experienced police and patrol German shepherd dogs were exposed to a human approaching threateningly and were observed for behavioral stress responses and coping strategies. Behavioral observations were correlated with saliva cortisol concentration measurements. At the group level, the test situation proved to be stressful for the dogs, as we found a significant post stimulation increase in hormone concentrations. However, results also show that both endogenous and exogenous factors influence this phenomenon. Cortisol responses to the social stressor were modified by age with significant increases in old dogs, but not in adult dogs. This indicates a parallel with humans where a meta-analysis found greater cortisol response to a challenge in older people (Otte et al., 2005). Furthermore, baseline cortisol concentrations were higher in the morning than in the afternoon, which is in accordance with the data of Beerda et al. (1996). The time of day, however, did not affect the cortisol responses to the social stressor.

Increased saliva cortisol substantiates that the applied test was stressful and is a valid technique for investigating different behavioral response patterns or coping strategies in socially stressed dogs. The interpretation of our observations is complicated by the different approaches of modeling individual differences. In the following, we will discuss the results in the framework of the classic factorial models with reference to personality traits (e.g. Svartberg, 2002) and utilizing models on behavior or copying styles (e.g. Koolhaas et al., 1999).

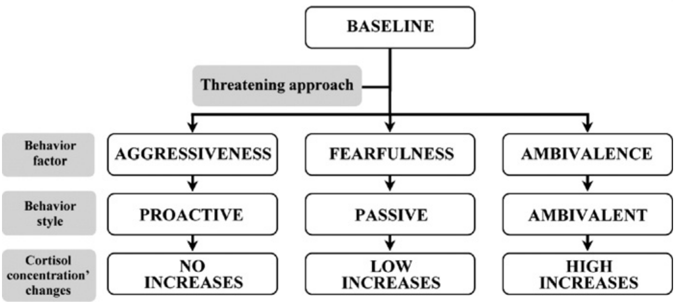
Our factor analysis identified three behavioral factors that shared many features with similar constructs described in the literature (for review see Jones and Gosling, 2005). The first factor characterized by frightened behavior, avoidance of the stranger, and increased affiliative tendencies toward the handler was labeled as '*fearfulness*' (see also 'fearfulness': Goddard and Beilharz, 1986; 'Curiosity/fearfulness': Svartberg and Forkman, 2002; opposite to 'Courage': Wilsson and Sundgren, 1997; see also Borchelt, 1983; Dreschel and Granger, 2005; Van der Borg et al., 1991). The second factor was dominated by behaviors that were associated with attacking the stranger (note that the dogs were tethered to a tree and were prohibited from performing a full attack) and not surprisingly many parallels to this factor have been published in the literature (e.g. 'Aggression-dominance': Goddard and Beilharz, 1986; 'Aggressivity': Bradshaw and Goodwin, 1999; 'Sharpness' and 'Fighting ability': Ruefenacht et al., 2002). The third factor has not been described previously but the behavioral variables loading on this factor suggest an ambivalent undecided inner state (e.g. Beerda et al., 1997).

The comparison of factor scores obtained in the three groups showed a characteristic pattern. Dogs in Group 1 were characterized by the highest values in 'fearfulness'. They were the least active, and had the longest attack latency. The social stimulation resulted in a low, but significant increase post-stressor cortisol concentrations which indicates a moderate stress reaction in these subjects. It is important to note, however, that while this group had the highest mean score in 'fearfulness', they also scored high on the 'aggressiveness' factor (see also below). This seeming contradiction may be caused by the training that they received as police dogs. It is likely that the training experience made them behave boldly in stressful situations despite their originally shy character which was only revealed by the hormonal changes during the experiment (Schjolden et al., 2005; Sluyter et al., 1996). The behavior of these dogs was similar to that reported by Vegas et al. (2006) in an 'Avoidance and Defense/Submission' and a low activity toward an aggressive opponent.

Dogs in Group 2 were characterized by high scores of 'aggressiveness' that was correlated with low scores on both other two factors. Dogs in this group behaved very actively throughout the test. They were pacing, barking (indicating excitement), and reacted to the approaching human by attacking with the shortest latency. Importantly, their hormone concentrations did not change significantly as a result of the threatening stranger which could represent an eustress state (Selye, 1975). The behavior of dogs in this group is similar to those mice that explored the environment extensively (high activity) and attacked their opponent or avoid them without showing any signs of 'Defeat or Submission' (Vegas et al., 2006).

In Group 3, the dominant behavioral factor was 'ambivalence'. Dogs in this group were even more active than dogs in Group 1. They responded ambivalently to the threatening human: while the threatening human was relatively far they were active, running, barking, but as he came closer, dogs started to show paw lifting, mouth licking, looking away, and backing down which are often interpreted as signs of acute stress (e.g. see Beerda et al., 1997, 1998a,b). The increase of cortisol levels in this group was significant and much more pronounced than in Group 1, though the difference between the post stressor levels

Table 4
Relationship between applied strategies and cortisol concentrations



Individuals in 'Proactive' group are characterized by aggressiveness and low HPA-axis reactivity (no increases); 'Passive' group consisted the fearful individuals with moderate HPA-axis reactivity (low increases), while subjects in 'Ambivalent' group showed ambivalent behavior and high HPA-axis reactivity (high increases).

in the two groups could not be supported by the statistic analysis. The subjects in Group 1 seemed the most stressed by the threatening stranger (Table 4). Their behavior parallels that of the subjects in the Vegas et al. (2006) study which responded mainly with 'Avoidance and Defense/Submission' but showed moderate activity.

It should be noted that the above analysis is also consistent with the results of the discriminant analysis which indicated that the behavior of dogs in Group 1 is mostly discriminated by their 'fearfulness', while the two other groups were specifically characterized by 'aggressiveness' or 'ambivalence', respectively (see direction of arrows in Fig. 2).

Interestingly, our observations can be also interpreted in models utilizing different coping styles (e.g. fishes: Øverli et al., 2004; Schjolden et al., 2005; rodents: Koolhaas et al., 1999; Vegas et al., 2006; mammals: Spoolder et al., 1996; Van Reenen et al., 2005; and birds: Carere et al., 2003). Dogs in Group 1 applied a passive coping strategy ('Passive' group) (Reimers et al., 2007) because these dogs were the least active. They showed long attack latency and reacted to approaching human with passivity and submission. Their HPA-axis reactivity was moderate (as in De Boer et al., 1990; Korte et al., 1992; Ruis et al., 2001). Individuals in Group 2 were characterized by low HPA-axis reactivity, high level of activity, and short attack latency with a tendency of aggression. Thus these dogs could be labeled as displaying a proactive coping strategy when facing a social challenge ('Proactive' group) (Benus et al., 1989; Koolhaas et al., 1999; Reimers et al., 2007; Schjolden et al., 2005). According to this line of modeling, subjects in Group 3 did not choose either coping styles. They were not able to deal with this exceptional social situation and, as a result, they experienced heightened stress levels indicated by ambivalent behavioral tendencies, and increased cortisol concentration ('Ambivalent' group). One could argue that both the proactive and the passive coping styles represent a natural solution to the situation for which the species is predisposed. In contrast, the dogs in the ambivalent group could reflect some problems of the individual to solve challenging social situations. One might suppose that this behavior might indicate either a characteristic behavioral malformation of the animal or it represents a state of transition when individuals switch coping styles as a result of social (or environmental) experiences and/or processes of aging (see below). This observation could also have further implications by suggesting that an inability to solve a social situation is the most dangerous for the individual in terms of experienced stress.

We have also analyzed age-distribution and found no significant difference in the mean age among the three groups. Nevertheless, it is important to note that half of the old dogs fall in the 'Passive' group while the bulk of adult dogs have been assigned to our 'Proactive' group. This could be explained by declining activity as animals grow older (mice: Lamberty and Gower, 1990; Rosenthal and Morley, 1989; rats: Dorce and Palermo-Neto, 1994; rhesus monkeys: Emborg et al., 1998; Gerhardt et al., 1995; dogs: Siwak et al., 2002), but it could also be the result of more (perhaps also negative) social experiences during their lifetime (Boccia et al., 1995). Veenema et al. (1997) found that social stress experienced over a lifetime can influence age-related changes in the brain causing decreased coping ability in complex social interactions. Thus old age can lead to differences in structure of behavior (Fitts, 1982; Spruijt, 1991). Previous research investigating consistency of coping styles over time involved a relatively short observation period (1 week in pigs, Spoolder et al., 1996; 1 year in dairy cows, Hopster, 1998). However, Liu et al. (2004) showed that the coping styles were used differentially across age. Older adolescents avoid the situation when faced with stress while younger ones tried to improve the situation. Lazarus and Folkman (1984) concluded that age may affect stress appraisal and thus choice of coping styles in humans. Our study seems to fit with this line of argument. Experiences in life may influence the expression of the behavior style (Helson and Roberts, 1994; Suomi et al., 1996) because the older dogs tended to behave passively in parallel with relatively high increases in cortisol concentration. This could be important to the welfare of police dogs since animals that show elevated stress reactions and elevated cortisol levels could be exempted from further work.

In summary, the present study identified three different groups of dogs which were characterized by either fearfulness, aggressiveness, or ambivalence and on the basis of specific differences in their reaction norm when threatened by an approaching stranger. This grouping allowed to draw possible parallels between aggressiveness and the proactive behavior style and fearfulness and passive coping style, respectively. In addition, we have revealed a third group of animals which show ambivalent behavior in a social threatening situation.

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