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Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs

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Abstract

Stress parameters that can be measured noninvasively may help to identify poor welfare in dogs that live in private homes and institutions. Behavioural parameters are potentially useful to identify stress, but require further investigation to establish which behaviours are appropriate. In the present study, behaviours were recorded and analysed for signs of acute stress in dogs. Simultaneously, saliva cortisol and heart rate were measured to support the interpretation of the behavioural data with regard to stress. Ten dogs of either sex, different ages and various breeds were each subjected to six different stimuli: sound blasts, short electric shocks, a falling bag, an opening umbrella and two forms of restraint. Each type of stimulus had been selected for its assumed aversive properties and was administered intermittently for 1 min. The stimuli that could not be anticipated by the dogs, sound blasts, shocks and a falling bag, tended to induce saliva cortisol responses and a very low posture. The remainder of the stimuli, which were administered by the experimenter visibly to the dog, did not change the cortisol levels but did induce restlessness, a moderate lowering of the posture, body shaking, oral behaviours, and to a lesser extent, yawning and open mouth. Pronounced increases in the heart rate were nonspecifically induced by each type of stimulus. Heart rate levels normalized within 8 min after stressor administration had stopped. Saliva cortisol levels decreased to normal within the hour. Correlations between behavioural and physiological stress parameters were not significant. From the present results, we conclude that in dogs a very low posture may indicate intense acute stress since dogs show a very low posture concomitant with saliva cortisol responses. Dogs may typically show increased restlessness, oral behaviours, yawning, open mouth and a moderate lowering of

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the posture when they experienced moderate stress in a social setting. The nonspecific character of canine heart rate responses complicates its interpretation with regard to acute stress. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

At present, there is a growing public concern for the well-being of domesticated animals. On the one hand, housing and handling conditions of farm and laboratory animals are increasingly criticized and commended for improvement. On the other hand, such criticism of living conditions may be disputed by people whose concern is not only that of the animals' well-being, but who are also guided by, for example, economical considerations.

Discussions on issues of animal welfare will become more objective and meaningful when one can substantiate how sub-optimal living conditions are appraised by the animals that experience these. The development of methods that measure an animal's appraisal of its environment, and that may help to establish its state of welfare, is what we aim to achieve over a series of experiments. We will focus on the dog, which has received relatively little attention in the context of animal welfare.

In situations that humans perceived as undesirable, they showed behavioural and physiological indications of stress (Herbert and Cohen, 1993; Hilton, 1989; Jørgensen et al., 1990; Kathol et al., 1989a,b; Silver and Wortman, 1980). Analogous reasoning (for a discussion see Stafleu et al., 1992) leads us to assume that the same is true for dogs, and that by measuring stress one can establish if a dog perceives its environment in a negative way. For a valid assessment of stress we need to know how stress is manifested and can be measured in the dog. The present study aims to investigate the acute stress behaviour and physiology of dogs, whereas data on chronic stress will be published in separate studies.

A number of factors are known to influence stress responses and cause pronounced individual variation in stress measurements (for a review see Mason and Mendl, 1993). Especially under field conditions, where such factors are not controlled, stress measurements may vary strongly and be hard to interpret with regard to a dog's welfare status. Therefore, the assessment of welfare problems in dogs in real life situations requires stress parameters that are relatively robust and independent of those factors that cause variation between individuals. In order to identify robust parameters of acute stress, we exposed dogs to stressors without controlling those factors that are likely to cause individual variation: Ten dogs that were heterogeneous in breed, gender, age and life history, were subjected to six different types of stimuli of a more or less aversive nature.

The stimuli that were used in the present experiment included elements that have been shown to be aversive to dogs, namely restraint (Knol, 1989; Muelas et al., 1993; Rothuizen et al., 1993), loud noise (Bueno et al., 1989; Engeland et al., 1990; Gue et al., 1988, 1989a,b) electric shock (Anderson and Tosheff, 1973; Church et al., 1966; Corson, 1971; Dess et al., 1983; Koepke et al., 1984; Rothuizen et al., 1993; Solomon

and Wynne, 1953) and social punishment (Schwizgebel, 1982), and they were composed to model aversive situations occurring in the daily life of dogs.

Noninvasive sampling techniques influence the results minimally and their use is not limited to an experimental setting. Because of its non-invasive nature, measuring behavioural parameters of stress is potentially of great importance. To improve the interpretation of the behavioural data with regard to stress, we simultaneously measured saliva cortisol and heart rate. Heart rate and saliva cortisol measure the activity of two physiological systems that in dogs respond to acute stress, namely the sympathetic nervous system (Anderson and Brady, 1972; Engeland et al., 1990; Gaebelein et al., 1977; Galosy et al., 1979; Lown et al., 1973; Newton, 1969; Parrilla et al., 1990) and the hypothalamic pituitary adrenal (HPA) axis (Assia et al., 1989; Bueno et al., 1989; Clower et al., 1979; Dess et al., 1983; Engeland et al., 1990; Gue et al., 1988, 1989a,b; Knol, 1989; Palazzolo and Quadri, 1987; Rothuizen et al., 1993), respectively.

The recording of a large number of behaviours complicates the statistical validation of the present results, and gives this study an explorative character. Alternative approaches, such as using more experimental animals or investigating only a few behaviours in a series of experiments, were rejected in view of the aversive nature of the treatments.

In summary, we wanted to establish how and to what degree dogs react to aversive stimuli and to resolve to what degree stress responses in behaviour, saliva cortisol and heart rate are correlated. We also wished to find out about the speed of recovery. Our main objective was to find the most useful behavioural parameters to assess acute stress in dogs.

2. Methods

2.1. Animals

Ten dogs of both sexes, different ages and various breeds were used (Table 1). The animals were randomly selected from an existing population and had a heterogeneous

Table 1
Experimental subjects. Breed, age and gender of the experimental dogs

Name	Breed	Age (in years)	Gender
Boef	Labrador	5.2	♂
Dusty	Mongrel	5.9	♂
Hannes	Boxer	7.5	♀
Jolanda	Greyhound × Doberman	13.8	♀
Kobus	Beagle	unknown	♂
Nicky	Beagle	7.9	♂
Pat	Greyhound × Doberman	12.8	♂
Patricia	Beagle	7.9	♂
Renee	Groenendaler	7.3	♀
Wilma	Beagle	5.8	♀

history regarding experiments to which they had been subjected previously. In the year that preceded the present study none of the animals had been subjected to physiological experiments that might be expected to affect their present physiological responsiveness. The dogs were housed individually. They were allowed outside between 0700 and 1100. When outdoors, the animals were kept alone in kennels that covered approximately twice the area of their home kennels, namely 3.6 m². Food was provided at 0800 and water was available ad libitum.

2.2. Experiments

Preceding the experiments, the dogs were habituated to the experimental room for two days. During acclimatization and throughout the experiments the animals were housed inside the experimental room in kennels measuring 1.45 × 3.65 m. Treatment of the animals differed from routine in that they were kept inside continuously. During two consecutive days, the dogs were subjected to six types of short lasting stimuli. Three stimuli per day were presented intermittently during 1 min and administered at 1100, 1300 and 1500. For practical reasons electric shocks were always administered as the final stimulus: The sensitive heart rate registration apparatus was detached before applying electric shocks to the dogs, since it was liable to damage from electrical currents. By administering shocks as the final stimulus we avoided the necessity to re-install the heart rate apparatus and disturb the animals. The remainder of the stimuli were administered in a random order. Consequently, the timing and sequence of the stressor presentations added variability to the stress responses, but bias was minimal.

The following stimuli were presented.

Stimulus 1 (press): The experimenter forced the dog to the floor by pushing it down on neck and back. The dog was kept in a lying position for 20 s. The procedure was repeated after a 20 s interval.

Stimulus 2 (pull): A rope, which passed under a bar situated 10 cm above the floor, was attached to the collar of the dog and pulled for 20 s. As a result the head and fore part of the animal were forced down. After a pause of 20 s the procedure was repeated.

Stimulus 3 (umbrella): Standing inside the kennel, the experimenter pointed a folded umbrella at the dog and opened it. This procedure was performed three times with an interval of 30 s.

During the presentation of stimuli 1, 2 and 3, the experimenter was dressed in a striking outfit that consisted of a mask, hat and coat. To all of these items the same specific smell was added ('Mispoes', C&D International, Maasbree, Netherlands).

Stimulus 4 (bag): A standard sized garbage bag, filled with 600 g of paper, was released from its position on the ceiling in the center of the kennel. After subsequent lifting it was then released again after 30 and 60 s. The bag was attached to the ceiling before a dog was introduced into the experimental room and removed after the third fall. Its release was achieved through strings which were handled from behind a one-way screen.

Stimulus 5 (noise): A sound blast, at an intensity of 110–120 dB and with a duration of 1–2 s, was administered three times with intervals of 30 s. The sound blasts were

produced by a fog horn (STEBEL KDP/1 pressurized canister, 18 bar) that was handled from behind a one-way screen.

Stimulus 6 (shock): The dog was subjected to three electrical stimuli that each lasted 1–2 s. Shocks were telemetrically administered by using a shock collar (TT 100 A, Tri-tonics, Tuscon, AZ). The shock intensity was set at level 8 from the 15 possible levels. The exact intensity of the shocks could not be recovered. Technical data on the shock collar were refused by the manufacturer and the applied current alternated at such a high frequency that this prevented us from measuring it. The collar that generated the shock was worn by the animal from 15 min before until 10 min after the administration of the electrical stimuli.

Some of the above tests were derived from van der Borg et al. (1991), and from Tuber and Wright (unpublished report). The experiments were approved by the local ethical committee.

2.3. Data collection

Computer aided behavioural observations were conducted continuously from 30 min before until 30 min after the onset of each stimulus. The following behaviours were recorded on line by using the Observer software package (Noldus Information Technology, 6702 EA Wageningen, The Netherlands):

2.3.1. Behaviours scored in terms of the frequency of occurrence

Autogrooming	behaviours directed towards the subject's own body, like scratching, licking and biting-self
Body shaking	
Changes of the posture	
Changes of the state of locomotion	
Circling	continuous walking in short circles
Crouching	a rapid and pronounced lowering of the posture, sometimes in combination with movements that enlarge the distance to the eliciting stimulus
Defecating	
Digging	scratching the floor with the forepaws in a way that is similar to when dogs are digging holes
Drinking	
Floor licking	the floor is licked with the tongue
Intentions to change the state of locomotion	initial fragments of the behaviour that dogs perform in full when they actually change from one state of locomotion to another
Manipulations of the environment	playful or stereotyped interactions with elements from the environment

Open mouth	the opening of the mouth
Oral behaviours	includes tongue out: the tip of the tongue is briefly extended; snout licking: part of the tongue is shown and moved along the upper lip; swallowing; smacking
Paw lifting	a fore paw is lifted into a position of approximately 45°
Sector crossings	marks on the floor subdivided the experimental kennel into six sectors that each measured 0.88 m ² ; when a dog moved from one sector to another this was scored as one crossing
Sighing	isolated intense expiration
Stretching	stretching of the body and limbs
Urinating	
Vocalizing	barking; growling: low frequency vocalizations; whining: soft, high pitched vocalizations; yelping: loud (relative to whining), high pitched vocalizations
Yawning	

2.3.2. Behaviors scored as state and event

Nosing	the nose is moved along objects and/or clear sniffing movements are exhibited
Panting	an increased frequency of inhalation and exhalation often in combination with the opening of the mouth
Tail wagging	repetitive wagging movements of the tail
Trembling	a clear shivering of the body
<i>Locomotive states</i>	
Lying with the head rested	
Lying with the head up	
Sitting	
Walking	
Standing	
Standing up against the walls of the enclosure	
<i>Postures</i>	
High	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
Neutral	the breed specific posture shown by dogs under neutral conditions
Half low	from three features: a lowered position of the tail (com-

	pared to the neutral posture), a backward positioning of the ears and bent legs, two are exhibited
Low	the position of the tail is lowered, the ears are positioned backwards and the legs are bent
Very low	a low posture, but now the tail is curled forward between the hind legs

Depending on the type of behaviour the frequency or duration of its occurrence was recorded. All observations were made by one and the same person and conducted from behind a one-way screen.

Saliva samples were taken at 30 and 15 min before and 10, 15, 20, 30, and 60 min after the onset of a stimulus and assayed for cortisol. Prior to the taking of a sample, a few pellets of citric acid were given to the animals to stimulate their saliva flow. The saliva was collected by gently rotating two cotton buds in the dogs' cheek pouches, and recovered by using the Salivette system ('Sarstedt', 4870 AA Etten-Leur, Netherlands). Salivettes were centrifuged (3000 rpm, 10°C) for 10 min. Samples were stored at 4°C and assayed within five days of collection.

Before 0900 on the first experimental day, dogs were equipped with a heart-rate registration apparatus (Holter Monitoring System, Biosensor, Maple Grove MN 55369, 6900 Wedgewood). In order to obtain electrocardiograms, five electrodes (Medi-Trace, Graphic Controls Canada, Ontario, Canada) were attached to the animals. Data were stored in a unit that was mounted in a jacket worn by the dogs. Animals were previously accustomed to wearing this jacket during the two days of habituation to the experimental room. Throughout the first experimental day, and until 1400 on the second experimental day, heart rates were recorded continuously. One hour before electric shocks were administered, the heart rate registration equipment was detached.

2.4. Determinations

Quantitative measurements of saliva cortisol were established in a solid-phase ¹²⁵I radio immunoassay (Diagnostica Products, Los Angeles, CA). The performance deviated from standard procedures in that calibrators were diluted into the range of 1.38–138 nmol/l and 200 μl of each sample was incubated for 2 h at a temperature of 37°C. The mean intra- and inter-assay coefficients of variation were 7.4% and 11.7%, respectively. Samples collected from one individual were assayed together.

2.5. Data processing

The duration of the behaviours was expressed as a percentage of the observation time; frequency scores were analysed as such. To assess the effects of the stimulus, a subject's behaviour during the 10 min preceding a stimulus was compared to its behaviour during the first 10 min after the onset of the stimulus. Responses were expressed as the post stimulus scores minus the pre stimulus scores.

Cortisol values in saliva samples collected 30 and 15 min before stimulus administration were averaged and regarded as basal levels. The remainder of saliva samples were used to establish cortisol peak values and area under the response curves (AURC's). For

two dogs, small sample volumes and filthy saliva caused a high percentage of missing values ($\geq 15\%$). Cortisol data on these animals were omitted from further analysis.

Heart rates in beats per minute (BPM) were established by counting the number of heart beats within a 12 s time window and multiplying it by five. Over a period from 30 s before up to 300 s after the start of a stimulus, the 12-s time window was shifted repeatedly by 3 s. Heart rate responses were expressed as maximum values and AURC's. To assess the recovery of the heart rate, the mean undisturbed heart rate for each subject was added to the associated standard deviation. Heart rates were considered to be normalized from the moment that they declined below this sum value and remained there for more than 1 min. When the heart rate did not normalize before the collection of the first saliva sample (at 10 min after the onset of a stimulus), the moment of recovery was set at 600 s. Heart rates that were recorded before a bag had been dropped or noise had been administered (stimuli that could not be anticipated by the dogs) were used to establish the undisturbed heart rate.

2.6. *Statistical analysis*

Analyses of variance (ANOVA's) for repeated measurements were used to investigate if stimuli induced behavioural responses (stimulus effects), to determine if responses depended on the type of stimulus that had been administered (stimulus \times type of stimulus effects), and, when this was the case, to identify which stimuli differed with regard to the responses that they induced. Significance by chance, due to multiple testing of the various stimulus combinations, was controlled for through Bonferonni corrections. Error rates were not corrected for the number of behaviours that had been studied. A logarithmic transformation was used to normalize the behavioural data. Deviation from sphericity assumption of the data was controlled by correcting the Univariate *F*-statistic with the Huynh–Feldt epsilon adjusted degrees of freedom.

Raw data on saliva cortisol and heart rate were analysed by the statistical techniques and procedures as described above. Missing saliva cortisol values (2%) were replaced by the mean saliva cortisol concentration for the remainder of the animals.

Spearman rank correlations were calculated to identify linear relationships between responses in behaviour, saliva cortisol and heart rate. Data are presented as mean values \pm SEM

3. Results

3.1. *Behavioural responses*

Stimulated dogs showed behavioural responses that, for some behaviours, differed between the types of stimuli that had been administered. Table 2 summarizes the behaviours that the dogs typically performed after stimulation, or that they omitted. In short, upon stimulation the dogs performed more body shaking, crouching, oral behaviours, nosing and yawning. Animals were more active in that they spent less time

Table 2
Behavioural responses to different types of stimuli

	<i>P</i> -values	Stimuli					
	Stimulus/stimulus × type of stimulus	Press	Pull	Umbrella	Bag	Noise	Shock
<i>Behaviours recorded as events</i>							
body shaking	0.002/0.003	1.0 ± 0.4 ^{ab}	1.1 ± 0.3 ^a	0.3 ± 0.2 ^{ab}	0 ± 0.1 ^b	0 ^b	-0.1 ± 0.1 ^b
crouching	0.048/0.325	0	0	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
c.s.l. *	0.007/0.014	12.8 ± 5.5 ^{ab}	16.3 ± 4.4 ^a	14.8 ± 6.9 ^{ac}	2.9 ± 2.1 ^{bd}	1.5 ± 1.2 ^{cd}	0.4 ± 2.6 ^{cd}
open mouth	0.076/0.036	0.5 ± 0.3	0.4 ± 0.3	0.6 ± 0.3	-0.2 ± 1.3	0	0
oral behavior	0.013/0.019	11.5 ± 3.1 ^a	7.1 ± 3.5 ^{ab}	5.3 ± 2.7 ^{ab}	2.1 ± 2.8 ^{ab}	2.0 ± 1.4 ^b	1.0 ± 2.1 ^b
sector crossings	0.005/0.004	14.8 ± 6.5 ^{ab}	16.7 ± 5.9 ^{ab}	17.5 ± 8.3 ^a	2.1 ± 0.7 ^{abc}	-0.4 ± 0.4 ^c	1.9 ± 1.9 ^{bc}
yawning	0.009/0.056	0.6 ± 0.2	0	0.3 ± 0.2	0.1 ± 0.2	0	0
<i>Behaviours recorded as states</i>							
nosing	0.002/0.080	3.3 ± 1.6 ^{ab}	4.4 ± 1.5 ^{ab}	4.7 ± 2.9 ^{ab}	1.5 ± 0.5 ^a	0.7 ± 0.4 ^{ab}	0.1 ± 0.2 ^b
<i>Locomotive states</i>							
lying (with head rested)	0.000/0.788	-11.2 ± 17.8	-36.8 ± 11.2	-39.3 ± 13.7	-37.7 ± 9.9	-30.0 ± 15.0	-23.7 ± 15.4
standing	0.022/0.146	2.1 ± 14.5	29.1 ± 15.8	30.7 ± 14.7	21.1 ± 12.1	18.4 ± 12.6	15.2 ± 13.8
walking	0.004/0.004	8.6 ± 4.0 ^{abc}	9.7 ± 3.3 ^{ab}	12.5 ± 6.3 ^a	1.5 ± 0.4 ^{cd}	0.3 ± 0.2 ^d	1.1 ± 1.2 ^{bcd}
<i>Postures</i>							
neutral	0.000/0.185	-13.5 ± 9.2	-17.4 ± 10.9	-30.7 ± 13.2	-41.1 ± 10.6	-24.0 ± 14.1	-17.2 ± 12.7
low	0.030/0.470	1.9 ± 1.5	1.0 ± 0.8	7.5 ± 3.4	2.9 ± 2.3	9.3 ± 9.0	7.4 ± 7.5
very-low	0.120/0.042	0.5 ± 0.5	0.3 ± 0.3	0.9 ± 0.9	5.2 ± 4.2	18.9 ± 11.5	10.8 ± 9.9

Presented are the behaviours that dogs performed more or less (-) in response to acute stimuli (*P*-values < 0.05 for stimulus effects), or that changed in opposite directions after different types of stimuli (*P*-values < 0.05 for stimulus × type of stimulus effects).

In columns 3 to 8 the mean behavioural responses (± SEM, *n* = 10) are given for six different types of stimuli.

Responses are expressed as the behavioural scores in the first 10 min after a stimulus minus the scores in the 10 min preceding a stimulus.

Behaviours were recorded as states or events and expressed as frequency/10 min or percentages of the observation time, respectively.

Different stimuli induced different responses (*P* < 0.05) in the same behaviour when behavioural responses do not share a common letter in their code (superscript).

Error rates for contrasts between stimuli were Bonferonni corrected for multiple testing between stimuli, but not for the number of behaviours that were recorded.

* Changes from one state of locomotion to another.

lying with their heads rested and more time walking and standing. Concomitantly, they changed more often from one state of locomotion or sector to another. After the stimulus, the posture of the dogs tended to shift from neutral to the lower postures. Activities and performances of body shaking and oral behaviours were typically increased after the stimuli ‘pull’, ‘umbrella’ and ‘press’, which all involved the visible interference by the experimenter. Very low postures were shown by only some dogs and typically in response to loud noise and, to a lesser extent, electric shocks and a falling bag. Due to the individual variation, the mean lowering of the posture in response to loud noise was not statistically different from those that occurred after other stimuli.

3.2. Saliva cortisol responses

Saliva cortisol measurements are presented graphically in Fig. 1. In response to the stimuli ‘press’ and ‘umbrella’, saliva cortisol values remained unchanged compared to basal levels (on average 6.0 nmol/l). Significant rises in saliva cortisol were found at $t = +10, 15, 20$ and 30 min, when dogs were exposed to sound blasts. Ten minutes after being shocked or confronted with a falling bag, the dogs showed similar levels as when being exposed to loud noise, namely 13 nmol/l. However, a pronounced variation between individuals rendered these cortisol responses nonsignificant. Only when expressed as AURC’s, were noise-induced saliva cortisol responses significantly higher in comparison with responses elicited by the stimuli ‘umbrella’ and ‘bag’. Differences with cortisol responses induced by ‘press’ and ‘shock’ were nearly significant ($P = 0.06$). After the stimulus ‘pull’ (Fig. 2a), mean levels of saliva cortisol reached a maximum of

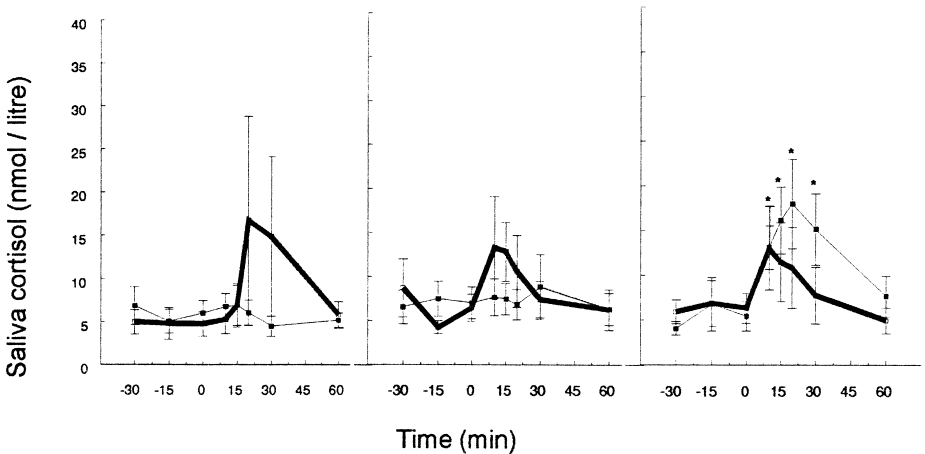


Fig. 1. Mean saliva cortisol responses in eight dogs (\pm SEM) that were restrained by being pressed down (thin line, left frame), restrained by being pulled down (thick line, left frame), confronted with an opening umbrella (thin line, middle frame), confronted with a falling bag (thick line, middle frame), exposed to sound blasts (thin line, right frame) or exposed to electric shocks (thick line, right frame). Values at $t = 0$ min represent the mean of cortisol measurements as established at $t = -30$ min and $t = -15$ min. In an intermittent way the stimuli were administered between $t = 0$ min and $t = 1$ min. * Indicates the significant differences ($P < 0.05$) between cortisol levels before and after treatments.

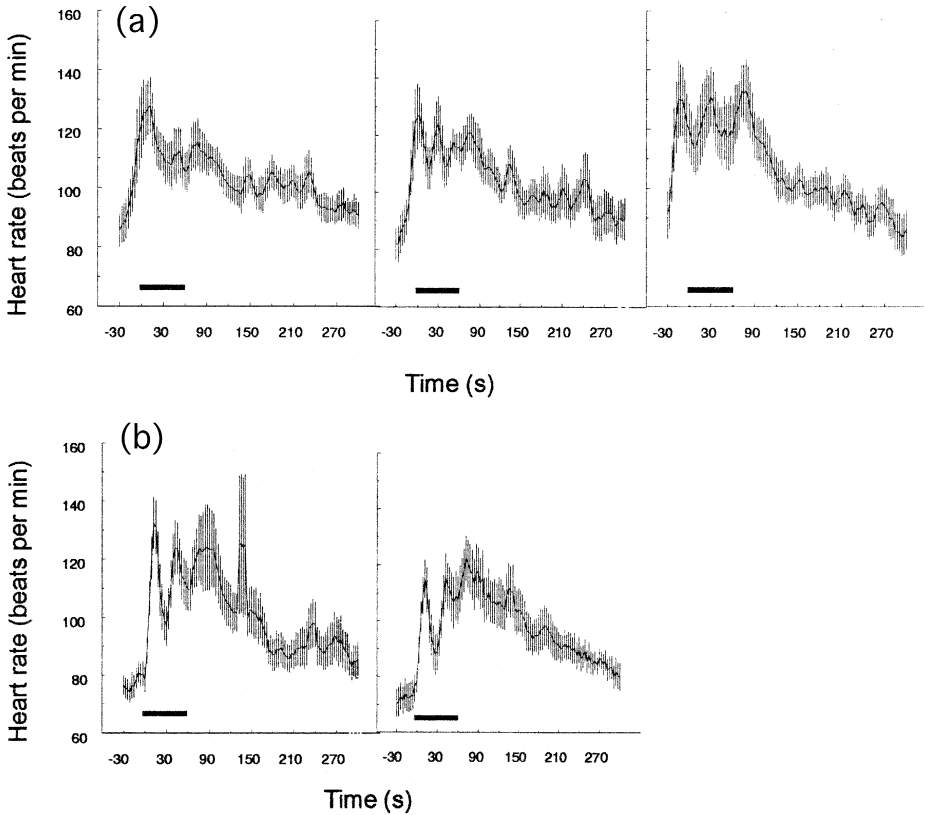


Fig. 2. (a) Mean heart rates in 10 dogs (\pm SEM) that were confronted with an opening umbrella (left frame), restrained by being pressed down (middle frame) or restrained by being pulled down (right frame). In an intermittent way the stimuli were administered between $t = 0$ s and $t = 60$ s (thick bars). (b) Mean heart rates in 10 dogs (\pm SEM) that were confronted with a falling bag (left frame) or exposed to three sound blasts (right frame). In an intermittent way the stimuli were administered between $t = 0$ s and $t = 60$ s (thick bars). Heart rate responses around $t = 140$ s in the left frame corresponded with the removal of the bag from the experimental kennel.

16.7 ± 12.1 nmol/l. This was due to one dog that showed extreme levels of 100.7 ($t = +20$ min) and 69.4 nmol/l ($t = +30$ min). Maximum saliva cortisol values were on average 20.4 ± 4.5 , 18.7 ± 6.1 and 15.5 ± 4.6 nmol/l following the presentation of loud noise, a falling bag and electric shock, respectively. In the same order of presentation, peak values were observed after a mean delay of 16.9 ± 2.3 , 16.3 ± 2.5 and 20 ± 5.8 min from the onset of the stimuli. Normalization of the cortisol levels always occurred within 60 min following the administration of a stimulus.

3.3. Heart rate responses

Heart rate recordings are presented graphically in Fig. 2a and b. The mean undisturbed heart rate frequency was 75 ± 3 BPM. Table 3 summarizes the heart rate

Table 3
 Characteristics of the dogs' heart rate responses to five acute stimuli

Stimulus	AURC	Maximum heart rate	Delay of the maximum value	Time of recovery
Press	31,593 ± 1439	156 ± 9	57 ± 19	409 ± 59
Pull	32,025 ± 1587	156 ± 7	37 ± 8	416 ± 56
Umbrella	31,451 ± 1203	157 ± 8	59 ± 27	506 ± 40
Bag	30,880 ± 5085	183 ± 20	87 ± 23	411 ± 46
Noise	29,947 ± 1599	146 ± 8	86 ± 12	372 ± 58

Data are presented as mean values ± SEM ($n = 10$), and characterize the heart rate responses as registered during 5 min after the administration of five acute stimuli.

Area under the response curves (AURC's) are expressed in beats per minute (BPM) × s. The maximum heart rates are given in BPM.

The time lags of the maximum heart rate relative to the onset of the stimuli and the time after which heart rates returned to base levels (the moment of recovery) are presented in seconds (s) from the onset of the stimuli.

parameters. Between stimuli, the heart rate responses, either expressed as AURC's or peak values, did not differ. The same was true for the delays after which the heart rate reached maximum values. The return of the heart rate to basal levels was significantly delayed after the opening of an umbrella in comparison to after a falling bag.

3.4. Correlations between behavioural, saliva cortisol and heart rate responses

Rank correlations were calculated to identify linear relationships between responses in behaviour, saliva cortisol and heart rate, but none were found that could be considered more than coincidental.

4. Discussion

4.1. Behavioural responses

This study suggests that increased performances of body shaking, crouching, oral behaviours, yawning, restlessness and a low posture constitute behavioural indications of acute stress in dogs. Acute behavioural responses followed distinctive patterns depending on the type of stimulus that had been administered. Some of the dogs tended to show a very low posture in response to a falling bag, electric shocks and, especially, to sound blasts. Restraint and an opening umbrella, stimuli that could be anticipated and involved the presence of a human, typically induced restlessness, a moderate lowering of the posture, high levels of body shaking and oral behaviours.

Shocks, sound blasts and a falling bag may have been particularly stressful to the dogs, since these stimuli were associated with a very low posture and with an established indication of stress, namely elevated cortisol (Assia et al., 1989; Bueno et al., 1989; Clower et al., 1979; Dess et al., 1983; Engeland et al., 1990; Gue et al., 1988, 1989a,b; Knol, 1989; Palazzolo and Quadri, 1987; Rothuizen et al., 1993). The absence of responses in oral behaviours, which were increased after restraint and an opening

umbrella, may be related to the intensity of the stimuli, but more likely resulted from the fact that these behaviours are typically performed in a social context. For example, snout licking, one of the oral behaviours, may express submission in a stressful social environment (Schwizgebel, 1982). With this in mind it is not surprising that our dogs typically showed high levels of oral behaviours when stimuli involved the presence of the experimenter. The foregoing could implicate that increased performances of oral behaviours represented a motivational state to perform submissive behaviours rather than that stress per se was the cause.

Lowered posture and restlessness, here indicated by high levels of walking, nosing and changing from one state of locomotion or sector to another, also occur when dogs are subjected to harsh training methods (Schwizgebel, 1982) or anticipate signalled shock avoidance trials (Solomon and Wynne, 1953), respectively. Yawning has been associated with psychological tension or mild stress in primates (Deputte, 1994).

The results on body shaking present some difficulties regarding their interpretation. The dogs showed increased body shaking in response to the stimuli that involved human interference. Possibly, the leaving of the experimenter at the end of a stimulus functioned as a safety signal, and body shaking should be interpreted as a sign of relief rather than stress. In a more direct interpretation body shaking may have functioned to rearrange the dogs' coat when this had been disturbed by human action. This assumption is supported by the relatively low performance of body shaking when dogs were subjected to stimuli not involving human interference or when human interference did not include handling (during the stimulus 'umbrella').

Earlier studies on stressed dogs have reported on increased vocalizing, panting, paw lifting and trembling (Corson, 1971; Schwizgebel, 1982; Solomon and Wynne, 1953). We observed that some dogs showed increased paw lifting and trembling upon stimulation (nonsignificant results), but found no indications that increased vocalizing or panting signals acute stress in dogs.

4.2. *Saliva cortisol responses*

In Section 4.1 we discussed how a dichotomy in the pattern of response behaviour originated from the fact that some stimuli were administered in a social context and could be anticipated, whereas others were of an opposite nature. These two characteristics also predicted if a stimulus induced cortisol responses. Saliva cortisol responses were only detectable after sudden nonsocial stimuli, namely shocks, sound blasts and a falling bag. In view of the classical experiments in stressed rats (Weiss, 1972), the predictability of the present stimuli will have been a predominant factor in modulating the saliva cortisol levels. Opposing views may rise from the earlier finding that the controllability of electric shocks, and not their predictability, significantly modulated the direct plasma cortisol responses in dogs (Dess et al., 1983). However, the latter study did report higher mean cortisol responses after unpredictable shock than after predictable shock; responses of 374% compared to 291%, respectively. Although the difference in cortisol response was not statistically significant, the results by Dess et al. (1983) do not contradict the importance of the predictability of stimuli.

The absence of saliva cortisol responses to restraint and an opening umbrella may have had other causes than only the fact that these stimuli could be anticipated. In spite of the outfit worn by the experimenter the presence of a possibly still familiar human may have exerted a stress reducing influence on the dogs (Lynch and McCarthy, 1969; Murphree et al., 1967). Also, the exit of the experimenter at the end of a stimulus presentation may have functioned as a safety signal, and may have shortened the stress period.

4.3. Heart rate responses

Contrary to the behavioural and saliva cortisol responses, heart rate responses (expressed as AURC's or peak values) occurred nonspecifically to each type of stimulus. Together with the heart rate responses that the dogs showed when they were merely approached by the experimenter, namely, before the onset of restraint or an opening umbrella and during the removal of a bag from the experimental kennel (see Fig. 2a and b), this suggests that the heart rate responds readily during arousal but cannot be used to distinguish between different types and/or levels of stress. Taking into account that similar heart rate responses have been reported in dogs that were subjected to shock avoidance tasks or food tasks (Anderson and Brady, 1972), heart rate increases should best be regarded as general responses to possibly meaningful events, irrespective of whether these are appreciated as positive or negative.

The mean heart rate increased from 75 BPM during undisturbed conditions to a maximum of 160 BPM when dogs were stimulated. Dogs that are subjected to electric shock (Anderson and Tosheff, 1973; Church et al., 1966; Gaebelien et al., 1977), hyperthermia (Hanneman et al., 1977) and loud noise (Engeland et al., 1990; Gue et al., 1987), may show heart rate responses of +20% up to +75%. However, pronounced differences in the reported control levels, which range from 80 BPM up to 170 BPM, prevent a meaningful comparison between past and present data.

In part, the present heart rate responses may have constituted a secondary effect of increased locomotor activity. Although we found no relationship between the heart rate and the state of locomotion during undisturbed conditions (data not shown), such a relationship cannot be excluded during stress when the activity of dogs tended to be increased. Moreover, the recovery of the heart rate was most delayed after the stimulus that also induced the strongest response in walking: an opening umbrella. A tendency of fast recovery was observed after the stimuli that induced negligible walking responses: a falling bag and sound blasts.

4.4. Behavioural, saliva cortisol and heart rate responses: an integrative approach

Behaviour that tended to occur concomitantly with saliva cortisol responses, i.e., a very low posture, is likely to indicate the more severe states of acute stress in dogs. Other behaviours, such as restlessness, a moderate lowering of the posture and high levels of oral behaviour, were not associated with cortisol responses and may be shown when stress is less intense and applied in a social setting. Heart rate responses occurred nonspecifically to all types of stimuli and provided no insight as to which of the behaviours were in particular indicative of stress.

Correlations among behavioural, saliva cortisol and heart rate parameters were never significant. This could be due to the pronounced individual variation in the stress responses.

4.5. The results in relation to the experimental design

Short lasting sudden stimuli induced acute behavioural, saliva cortisol and heart rate responses in 10 dogs. The assumed aversive character of some stimuli (a falling bag, sound blasts and electric shocks) was confirmed by their capacity to induce saliva cortisol responses and a very low posture. The responses to the three other stimuli suggest that these were only moderately aversive at the most. Therefore, the nature of the present experiments was not such that they were stressful beyond doubt. This leaves room for alternative explanations of our behavioural findings. Since behavioural and heart rate responses were not accompanied by increases in saliva cortisol it cannot be excluded that other factors than stress may have induced responses in body shaking, oral behaviours, yawning, restlessness, and a lowering of the posture.

On the one hand, the interpretation of these behaviours as indicators of stress seems plausible since they occurred concomitantly with saliva cortisol responses or have been associated previously with situations that are likely to be aversive to dogs (Solomon and Wynne, 1953; Schwizgebel, 1982).

On the other hand, the two forms of restraint and the unfolding of an umbrella were all administered by a person in an outfit that may have been frightening to the dogs or not. Consequently, these treatments must not be viewed as merely restraint or a sudden opening of an umbrella, but as heterogenous stimuli that included important social cues. These cues could also induce submissive behaviours. Oral behaviours as well as lowering of the posture can also be interpreted as signs of submission.

In the present study six different stimuli were administered on different times over a period of only two days. Due to this experimental design, stimuli that were administered first may have affected the dogs' responses to the stimuli occurring later on, and that, even though the stimuli were administered in random order, the time of day may have biased our results. We found no indications that this was true. The time of day and the phase of the experiment did not significantly influence basal or response measurements (data not shown). However, it cannot be totally excluded that the reported responses to electric shocks are influenced by the experimental design.

The statistical analysis of the results did not include a correction for the number of parameters that had been studied. We rejected the use of the experimentwise error rate, which would have been far more conservative than the present comparisonwise error rate, because our primary target was not to validate a select number of behaviours that were hypothesized to indicate stress. Instead we wanted to distinguish possible stress behaviours from those that are not likely to be associated with stress.

In conclusion, behavioural parameters may help to identify acute stress in the dog, but they may be misinterpreted. Behavioural responses may differ between dogs, depending on the type of stimulus and a dog's individual characteristics. Adding physiological stress measurements, and the sampling of more than one individual per stressful situation, may help to prevent the misinterpretation of behaviours. Additional

experiments have been done to investigate if the behavioural parameters that were found to be associated with acute stress are useful for measuring chronic stress and, subsequently, may help to establish welfare problems in the dog. We will report on these studies in separate publications.

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