BEHAVIOURAL AND HORMONAL INDICATORS OF ENDURING ENVIRONMENTAL STRESS IN DOGS

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Final Acceptance: 7 April 1999

Abstract

*Animal Welfare 2000, **9:*** 49-62

Four groups of dogs, which had been subjected to housing conditions of varying quality for years, were assumed to experience different levels of stress. The groups were compared for behavioural and hormonal parameters in order to identify measures that indicate chronic stress in the dog and which may help to identify poor welfare in this species. As a standard for comparison, one of the four groups was composed of privately owned dogs; we assumed that chronic stress levels were relatively low in this group (GI). The three remaining groups of dogs (GII, GIII and GIV) were kept under conditions of low to relatively high austerity, and had basal urinary ratios of cortisol to creatinine, adrenaline to creatinine and, to a lesser extent, noradrenaline to creatinine, that varied from low to high, respectively. Significant differences (P < 0.05) were found in cortisol to creatinine ratios when comparing GI to GII, GIII and GIV and when GII was compared to GIV. Statistical analyses indicated that the mean adrenaline to creatinine ratio in GI differed from that in the remaining groups and that the ratio in GII differed from that in GIII. Noradrenaline to creatinine ratios differed significantly only between GI and GIII. Dopamine to creatinine ratios and noradrenaline to adrenaline ratios did not differ significantly between groups. When dogs were not disturbed, those that were kept under the most austere conditions typically had high levels of locomotor activity, nosing, urinating and paw lifting. After mild disturbance by a slamming door or in the presence of a researcher these animals reacted actively, with increased locomotor activity, circling and nosing, and they showed high levels of behaviours that have previously been associated with acute stress: body shaking, yawning, ambivalent postures and displacement behaviours. Chronic stress in dogs may be identified by increased paw lifting when animals are not disturbed and by ample behavioural expressions of arousal when they are mildly stimulated. Since some behaviours may occur in contexts not related to stress, behavioural data are easily misinterpreted with regard to chronic stress. Interpretation will only be meaningful when physiological measures such as urinary adrenaline to creatinine ratios and, especially, urinary cortisol to creatinine ratios are also determined.

Keywords: *animal welfare, behaviour, chronic stress, dog, urinary catecholamines, urinary cortisol*

Introduction

Animal shelters, breeding stations, animal boarding houses and laboratories are examples of situations where living conditions may be austere and stressful to dogs. The assessment of welfare problems in dogs requires tools that measure poor welfare in this species. Humans who experience reduced welfare show signs of stress (Silver & Wortman 1980; Hilton 1989; Kathol *et al* 1989; Jørgensen *et al* 1990; Herbert & Cohen 1993). Following similar reasoning (for a discussion see Stafleu *et al* [1992]), it can be argued that dogs also show expressions of stress when they experience poor welfare. Thus, the measurement of stress parameters constitutes a way of identifying welfare problems in dogs. Stressful situations that endure, say for several days, may induce different stress responses, and will be more detrimental to a dog's state of welfare, than when such situations are relatively short-lasting. Since welfare is most seriously compromised in situations of enduring stress, parameters that indicate chronic stress are more useful for detecting welfare problems than parameters that change only temporarily at the onset of the stressor (ie acute stress parameters).

Previously, we have studied behavioural and physiological indicators of chronic stress in socially and spatially restricted Beagles (Beerda *et al* 1999a, b). It is unclear whether or not our findings also apply to other, non-experimental, settings. The experimental animals that we used in that study were socially and spatially restricted for only 6 weeks and they were homogenous with regards to breed, age and life history. In any non-experimental setting, the dogs under study are likely to differ from the animals that we studied. Moreover, the stressor to which they are exposed may not resemble the treatment that we applied and it may last considerably longer than 6 weeks. As a result, dogs that are exposed to stressful situations may show responses that deviate from the ones that we have reported. The present study aims to establish the practical use of behavioural and physiological measurements for the assessment of chronic stress under minimally controlled conditions.

Four groups of dogs that had been living for years under relatively enriched conditions or in conditions of three degrees of austerity were assumed to have experienced levels of stress that varied accordingly. The groups were compared with regards to behavioural parameters, urinary cortisol and urinary catecholamines. We aimed to identify behavioural and physiological characteristics that typically manifest themselves under rather austere living conditions and that indicate chronic stress in dogs. Behavioural parameters, urinary cortisol and urinary catecholamines were measured because they indicate the activity of biological systems that are known to respond in acutely stressed dogs, namely behaviour (Solomon & Wynne 1953; Corson 1971; Schwizgebel 1982; Engeland *et al* 1990), the hypothalamic-pituitary-adrenal (HPA) axis (Clower *et al* 1979; Dess *et al* 1983; Palazzolo & Quadri 1987; Assia *et al* 1989; Bueno *et al* 1989; Gue *et al* 1989; Engeland *et al* 1990) and the sympathetic-adrenal-medullary (SAM) system (Anderson & Brady 1972; Lown *et al* 1973; Gaebelein *et al* 1977; Galosy *et al* 1979; Pagani *et al* 1991). Moreover, we have previously found that behaviour and cortisol levels may also respond in chronically stressed dogs (Beerda *et al* 1999a, b).

The groups of dogs that we studied were not balanced for factors like breed, gender and age. However, intra-group comparisons were made to find out how variation in breed, gender and age may have biased the measurement of chronic stress.

In summary, we wanted to know which behavioural and hormonal parameters distinguish dogs which are kept poorly from those which are kept well, and, as such, have practical use as indicators of poor welfare. For the same reason, we investigated correlations between cortisol secretion, as an established indicator of stress, and the remainder of the parameters.

Methods

Animals

Data were obtained from 72 dogs that were categorized in accordance with the conditions under which they were maintained. Preceding the collection of data, the dogs' housing conditions had been unchanged for 1 year or longer. Group sizes and statistics with regards to gender, breed and age are summarized in Table 1. Dogs in group GI were privately owned. On working days, from 0800h to 1700h, these dogs were maintained in outdoor kennels of 4.3m^2. Between 1200h and 1400h the dogs were taken outside for a walk. GII individuals were housed individually in kennels that included an indoor section (2.1m^2) and an outdoor section (5.6m^2). GII dogs were walked outside the premises on a regular basis between 1200h and 1330h. The dogs in GIII (all female) were maintained in pairs in kennels measuring 2.4m^2. From 0800h to 1400h these dogs resided pairwise in outdoor kennels (3.6m^2). GIV individuals were maintained individually in kennels that measured 1.7m^2. From 0800h to 1400h these dogs stayed individually in outdoor kennels that were sized 3.6m^2. GIV individuals were unique in that, over the years, they had been subjected to a number of different, sometimes stressful, experiments. The animals in GII, GIII and GIV were fed once a day (brand DOKO, Trouw, Putten) between 0800h and 1100h. The privately owned GI dogs were fed at around 0800h and around 1800h. Different owners supplied different brands of food. All animals had free access to water.

Table 1 Composition of the groups and the number of animals measured.

	GI	GII	GIII	GIV
Gender	12 ♂ and 12 ♀	9 ♂ and 6 ♀	20 ♀	7 ♂ and 6 ♀
Breed	Various breeds	Various breeds	Beagles	Beagles
Mean ± SEM age	5.3 ± 0.8 years	7.2 ± 0.6 years	3.3 ± 0.5 years	6.2 ± 0.7 years
Behavioural recordings (no. of animals) when dogs were:				
- undisturbed (6 observation sessions of 10min)	8	12	18	11
- startled by a slamming door (6 observation sessions of 5min)	8	12	18	11
- approached (6min)	8	12	18	11
Hormonal measurements:				
Urinary cortisol	21	15	17	11
Urinary catecholamines	13	15	16	11

We assumed that the levels of chronic stress increased progressively from GI to GIV in accordance with increasingly austere conditions of maintenance.

Data collection

The dogs were alternating and/or limitedly available for experimental use. As a result, measurements were performed with variable sets of animals. The numbers of animals used for the different types of measurements are given in Table 1. The nature and diversity of the factors that caused animals to be unavailable for testing makes it unlikely that any sort of unintended selection of the experimental animals biased assessment of stress.

The undisturbed behaviour which the dogs displayed inside their home kennel was recorded on video, using a Panasonic M7 video recorder (van Hulskamp, Nieuwegein) for 10min on 6 different days between 1430h and 1700h. After a dog had been recorded for 10min, it was startled by slamming the entrance door of the housing facility. The dog's response to this stimulus was recorded for 5min. On one of the 6 days the dogs were observed in the presence of a female researcher. Having been in sight for 1min (t = 1), the researcher moved towards the door of a home kennel (t = 2min), from where she tried to establish physical contact with the animal (t = 3min). Next, the researcher went inside the kennel (t = 4min), tried to attract the subject (t = 5min) and, finally, retreated to the starting position (t = 6min). The female researcher was familiar to the dogs in that she had frequently visited their housing facilities. On such occasions she was in sight of the animals, sometimes handled them but did not purposely pet them.

Behaviour was analysed in terms of the frequency or duration of occurrence using the Observer software package version 3 (Noldus Information Technology, 6702 EA Wageningen, The Netherlands). Behavioural observations were conducted by one person according to the following protocol:

Behaviours scored in terms of the frequency of occurrence

Autogrooming, body shaking, changes from one posture to another, changes from one state of locomotion to another, circling, crouching, defecating, digging, drinking, eating faeces, floor licking, intentions to change from one state of locomotion to another (scored when dogs performed only the first motions of the normal repertoire that they performed when changing from, for example, a standing position to a sitting position, thus maintaining the starting, in this example standing, position), manipulating the environment, open mouth, oral behaviours, paw lifting, sighing, stretching, urinating, vocalizing and yawning (see Beerda *et al* [1998] for descriptions).

Behaviours scored in terms of the duration of occurrence

Nosing, panting, tail wagging, trembling, states of locomotion and postures were scored as described previously (Beerda *et al* 1998). Postures were only recorded when the positioning of a dog's ears, tail and legs could be readily assessed, ie when a dog stood or walked. Other behaviours that we recorded in terms of their duration of occurrence were:

Ambivalent postures: a crouched body posture accompanied by a position of the tail that is higher than the breed-specific position; or a high body posture accompanied by a position of the tail that is below normal.

Contact: dog within a radius of approximately 30cm of the researcher.

Gnawing at the rest basket.

Latency to first contact.

Raising of the hairs on the withers.

Repetitive behaviour: motions that were repeated with minimal interruptions and that were stereotyped in character.

Naturally voided urine samples were collected in the morning and stored, within 3h of being voided, at -20°C until their creatinine and cortisol concentrations could be analysed. Half of each 6ml urine sample was adjusted to pH 3–4 using methanoic acid, stored at -20°C and analysed for catecholamines.

Determinations

Urinary cortisol and creatinine concentrations were determined following the procedures described by Rijnberk *et al* (1988). Inter-assay variation was 9.5 per cent. Urinary catecholamine concentrations were measured after solid-phase extraction by HPLC followed by radiochemical detection, as validated for the dog (Beerda *et al* 1996). A commercially available kit was used for urine extraction (Pharmacia LKB, Woerden). The HPLC system consisted of a model 112 solvent delivery system (Beckmann, Mijdrecht) and a model 460 electrochemical detector (Waters, Milford, MA). The electrochemical detector data were analysed using a model 3396 integrator (Hewlett Packard, Amsterdam).

Data processing and statistical analysis

The data were normalized by means of logarithmic transformation and analysed by an analysis of variance (one-way ANOVA). Pairwise comparisons between groups were conducted for those parameters that showed a significant group effect as indicated by the F-statistic. Differences between groups were tested for significance following the Tukey method when group sizes were equal, and by the Tukey/Kramer approach when group sizes differed (for details on the Tukey method and Tukey/Kramer approach see Stoline [1981]).

Statistical tests on locomotor activity and postures were performed with ratios in order to achieve mutual independence between the different states of locomotion or postures. First, a reference state of locomotion (or posture) was selected on the basis of it being performed by all animals, under a given test condition, with relatively small differences between groups. Next, the remaining states of locomotion (or postures) were expressed as a ratio to the selected reference state of locomotion (or posture). Lying with head rested and half-low were used as the reference state of locomotion and posture, respectively, for the conditions when dogs were startled by a slamming door or not disturbed. For the condition involving approach by a female researcher, standing and neutral posture were used as references.

Per test condition, only the scores on the behaviours that significantly discriminated between any two groups are presented. However, two exceptions are made. Firstly, in the case of significant group effects in any of the locomotor states (or postures), the scores for the reference locomotor state (or reference posture) are also shown. Secondly, scores on the behaviours that significantly discriminated groups during the time that the dogs were startled by a slamming door are also presented for the undisturbed condition. Thus, the extent to which the dogs responded to the slamming door could be assessed.

Urinary levels of cortisol and catecholamines were expressed as ratios to creatinine.

Pooled Pearson's product-moment correlations (Morrison 1976) were calculated between urinary cortisol to creatinine ratios and behaviours that significantly discriminated between groups. By calculating pooled correlations, group effects, which are likely to inflate the correlations, were taken into consideration.

The effects of gender were investigated by means of setting sex as an independent variable when running ANOVAs with data from GI, GII and GIV (GIII included only females). The behaviour of 12 GII individuals (six Beagles and six dogs from different breeds) was analysed in an ANOVA for behaviours specific to Beagles. Age effects were investigated by means of calculating Pearson's product-moment correlations (not pooled) between age and behavioural or hormonal parameters. Gender, breed and age effects were only tested for the parameters that differed significantly between the groups. Results are presented as mean values ± standard errors of the means (SEM). The level of significance was set at $P < 0.05$. Our analytical techniques corrected error rates for multiple between-group comparisons, but not for the number of parameters studied.

Results

Urinary cortisol and catecholamines

The urinary cortisol and catecholamine levels for each group are presented in Table 2. Urinary cortisol to creatinine (CC) ratios were higher in GIV than in GII or GI. CC ratios in GIII were elevated in comparison to those in GI. Similarly, mean adrenaline to creatinine (AC) ratios tended to decline from GIV to GI (see Table 2). Urinary noradrenaline to creatinine (NC) ratios were significantly higher in GIII than in GI. Ratios of noradrenaline to adrenaline (NA) and dopamine to creatinine (DC) were similar for all groups.

Table 2 Mean ± SEM cortisol and catecholamine levels[1] in urine voided by dogs that were maintained under different housing conditions.

Ratio	Group			
	GI	GII	GIII	GIV
CC	4.8 ± 0.5^{c}	7.4 ± 0.6^{b}	8.7 ± 0.6^{ab}	14.4 ± 3.4^{a}
DC	9.4 ± 3.7	2.3 ± 0.6	8.3 ± 4.0	10.4 ± 3.5
NC	6.5 ± 1.1^{b}	8.7 ± 1.4^{ab}	20.5 ± 4.4^{a}	16.6 ± 4.8^{ab}
AC	1.4 ± 0.6^{c}	2.2 ± 0.6^{b}	8.5 ± 2.3^{a}	11.5 ± 4.8^{ab}
NA	15.8 ± 5.7	7.9 ± 2.0	3.6 ± 0.8	6.9 ± 3.7

[1] Urinary levels of cortisol (CC), dopamine (DC), noradrenaline (NC) and adrenaline (AC) are expressed as ratios to creatinine concentrations x 10^{-6}. Noradrenaline to adrenaline ratios are indicated by NA. Differences between lettered superscripts within a row indicate significant ($P < 0.05$) differences between groups.

Table 3 Differences in the undisturbed behaviour of dogs that were maintained under different housing conditions.

Behaviour & score	GI	GII	GIII	GIV	Unit
body shaking	0.4 ± 0.3	0.4 ± 0.2	0.8 ± 0.2	0.6 ± 0.4	instances h^{-1}
circling	0	1.3 ± 0.8	1.6 ± 0.6	3.3 ± 1.6	,,
locomotion:					
c.s.l. [1]	50.9 ± 12.3^{bc}	55.2 ± 12.3^{c}	90.0 ± 9.6^{a}	92.7 ± 16.6^{ab}	,,
lying head rested	71.3 ± 5.1	59.9 ± 7.2	50.5 ± 4.5	49.2 ± 5.1	% obs. time
sitting	1.6 ± 1.2^{b}	13.2 ± 4.4^{a}	17.3 ± 3.3^{a}	20.1 ± 5.7^{a}	,,
standing	4.1 ± 2.0^{c}	5.2 ± 1.7^{bc}	19.6 ± 3.4^{a}	12.6 ± 2.6^{ab}	,,
walking	0.9 ± 0.6^{b}	2.2 ± 0.8^{a}	5.3 ± 0.9^{a}	5.0 ± 1.8^{a}	,,
oral behaviours	21.9 ± 6.0	26.3 ± 8.2	28.5 ± 5.3	48.1 ± 9.0	instances h^{-1}
nosing	1.0 ± 0.4^{b}	2.4 ± 0.6^{b}	6.8 ± 1.0^{a}	6.3 ± 1.1^{a}	% obs. time
paw lifting	0.3 ± 0.3^{b}	0.6 ± 0.2^{ab}	2.0 ± 0.8^{ab}	3.5 ± 1.3^{a}	instances h^{-1}
posture:					
half-low	64.8 ± 13.2	64.7 ± 11.3	69.9 ± 6.7	70.6 ± 8.5	% obs. time
high	1.5 ± 1.0^{a}	1.4 ± 0.7^{ab}	2.5 ± 0.9^{ab}	0.3 ± 0.3^{b}	,,
sighing	0.9 ± 0.5^{b}	2.9 ± 0.6^{a}	0.2 ± 0.1^{b}	0.1 ± 0.1^{b}	instances h^{-1}
tail wagging	1.6 ± 1.5^{ab}	0.1 ± 0.8^{b}	1.3 ± 0.4^{a}	1.5 ± 0.7^{ab}	% obs. time
urinating	0^{b}	0^{b}	0.1 ± 0.1^{b}	0.8 ± 0.3^{a}	instances h^{-1}
yawning	1.3 ± 0.7	1.8 ± 0.4	2.4 ± 0.4	2.6 ± 0.6	,,

Mean behavioural scores (± SEM) are expressed as frequencies or percentages of the observation time. Differences between lettered superscripts within a row indicate significant ($P < 0.05$) differences between groups.

[1] changes from one state of locomotion to another. [2] changes from one posture to another.

Behaviour

Tables 3, 4 and 5 summarize the dogs' behaviour as recorded under three different conditions. Locomotor activities and levels of nosing were higher in GIV and GIII than in GII and GI. This turned out to be consistent for the situations in which the dogs were not disturbed, when they were startled by the slamming of a door and when they were approached by a familiar person. GIV individuals stood out, especially in comparison to GI individuals, in that they showed high

https://doi.org/10.1017/S0962728600022247 Published online by Cambridge University Press

levels of paw lifting and urinating when they were not disturbed (Table 3), and high levels of circling, body shaking, yawning, urinating, tail wagging and oral behaviours when they were startled (Table 4). Also, GIV individuals rarely showed a high posture. Only in GIV did the slamming of a door induce increases in circling, body shaking and oral behaviours. Increased nosing occurred in GIV and in GIII. When GIV individuals were approached by a familiar person they typically showed ambivalent postures, changed often from one posture to another and, together with GIII individuals, were the only dogs that drank (Table 5). GI individuals that were approached by a familiar person showed fewer oral behaviours and more growling than the remainder of animals. At the same time, they appeared reluctant to contact the researcher and

Table 4 Differences in the response behaviour of dogs that were maintained under different housing conditions and disturbed by slamming a door. For notes and legend see Table 3.

Behaviour & score	GI	GII	GIII	GIV	Unit
body shaking	0.1 ± 0.1[b]	0.3 ± 0.2[b]	0.8 ± 0.2[ab]	1.2 ± 0.3[a]	instances 30min^{-1}
circling	0[b]	0.4 ± 0.3[b]	0.5 ± 0.2[b]	4.9 ± 3.0[a]	,,
locomotion:					
c.s.l. [1]	27.3 ± 5.5[b]	29.2 ± 6.0[b]	51.5 ± 6.1[a]	75.2 ± 11.0[a]	,,
lying head rested	55.1 ± 9.6	57.7 ± 7.5	46.0 ± 4.9	29.9 ± 3.8	% obs. time
standing	6.1 ± 4.5[b]	5.6 ± 1.7[b]	20.4 ± 3.1[a]	24.2 ± 4.1[a]	,,
walking	1.4 ± 0.4[bc]	2.0 ± 0.6[c]	5.2 ± 0.9[ab]	7.3 ± 1.7[a]	,,
oral behaviours	6.3 ± 2.2[b]	14.5 ± 4.7[b]	12.3 ± 3.5[b]	48.4 ± 10.2[a]	instances 30min^{-1}
nosing	1.5 ± 0.5[b]	2.1 ± 0.6[b]	10.6 ± 1.2[a]	14.8 ± 2.4[a]	% obs. time
posture:					
half-low	46.4 ± 14.8	15.2 ± 13.6	75.3 ± 6.1	67.9 ± 9.0	,,
high	9.4 ± 7.1[a]	2.6 ± 1.8[a]	1.9 ± 1.0[b]	0.9 ± 0.7[b]	,,
sighing	0.5 ± 0.2[b]	1.7 ± 0.3[a]	0.2 ± 0.1[b]	0.2 ± 0.1[b]	instances 30min^{-1}
tail wagging	1.7 ± 1.1[ab]	0.2 ± 0.1[b]	2.0 ± 0.8[ab]	3.6 ± 1.1[a]	% obs. time
urinating	0[b]	0.1 ± 0.1[b]	0.1 ± 0.1[b]	0.9 ± 0.3[a]	instances 30min^{-1}
yawning	0.3 ± 0.2[ab]	0.3 ± 0.2[b]	1.3 ± 0.3[a]	2.1 ± 0.8[a]	,,

Table 5 Differences in the response behaviour of dogs that were maintained under different housing conditions and approached by a familiar person. For notes and legend see Table 3.

Behaviour & score	GI	GII	GIII	GIV	Unit
ambivalent posture	0[b]	0.2 ± 0.2[b]	0[b]	1.8 ± 0.9[a]	% obs. time
c.p. [2]	1.9 ± 1.2[bc]	1.8 ± 0.8[c]	4.7 ± 0.8[ab]	6.8 ± 2.2[a]	instances 6min^{-1}
drinking	0[b]	0[b]	0.6 ± 0.1[a]	0.2 ± 0.1[ab]	,,
growling	3.6 ± 2.7[a]	0[b]	0[b]	0[b]	,,
latency to contact	51.4 ± 11.9[a]	16.2 ± 6.4[a]	1.4 ± 0.4[b]	18.3 ± 7.4[ab]	s
locomotion:					
c.s.l. [1]	16.1 ± 3.9[b]	28.6 ± 5.3[b]	55.3 ± 3.9[a]	30.1 ± 5.4[ab]	instances 6min^{-1}
lying	29.5 ± 9.7[a]	11.2 ± 8.6[ab]	0[b]	1.3 ± 0.9[b]	% obs. time
lying head rested	27.0 ± 12.0[a]	0[b]	0[b]	2.1 ± 1.4[b]	,,
sitting	4.2 ± 2.0[ab]	26.5 ± 7.3[a]	6.7 ± 1.6[b]	22.8 ± 7.6[ab]	,,
standing	32.9 ± 14.1	24.6 ± 5.7	44.9 ± 3.4	45.5 ± 10.6	,,
standing raised	2.9 ± 2.6[ab]	26.4 ± 8.3[a]	29.1 ± 3.7[a]	13.8 ± 7.1[b]	,,
oral behaviours	5.9 ± 2.3[b]	27.8 ± 5.8[a]	16.5 ± 3.1[a]	29.1 ± 7.9[a]	instances 6min^{-1}
nosing	4.1 ± 1.9[b]	12.7 ± 2.5[a]	25.4 ± 2.5[a]	22.4 ± 4.3[a]	% obs. time
contact	26.0 ± 6.1[b]	44.7 ± 3.8[a]	49.3 ± 2.6[a]	37.8 ± 4.7[ab]	,,
tail wagging	23.7 ± 13.7[b]	45.4 ± 7.6[a]	57.3 ± 6.0[a]	37.2 ± 6.3[a]	,,

they showed relatively little tail wagging. In situations where the dogs were startled by a slamming door or left alone, GII individuals sighed relatively often and rarely wagged their tails (Tables 3 and 4).

Correlations between urinary cortisol and behavioural or urinary catecholamine parameters
We investigated correlations of urinary cortisol levels with the remainder of parameters that are listed in Tables 2 and 3–5. With regard to correlations between the basal urinary CC ratio and behavioural parameters (see Table 6), we found positive correlations with changes from one state of locomotion to another and walking when dogs were not disturbed. After being startled by a slamming door, the CC ratios correlated with these same behaviours plus circling, tail wagging and urinating. Basal urinary CC ratios and the behaviour of the dogs in the presence of a female researcher were not correlated. With regard to catecholamine measures, the urinary CC ratio was positively correlated with the basal urinary AC ratio ($r = 0.53$, n = 55, $P < 0.001$).

Table 6 Correlation coefficients between urinary CC ratios and behavioural parameters (n = 46 in all instances).

	Pearson's correlation and significance	
Behaviours		
(a) when dogs were undisturbed;		
changes from one state of locomotion to another	0.33	ns
walking	0.53	$P < 0.001$
(b) after disturbance by a slamming door;		
changes from one state of locomotion to another	0.57	$P < 0.001$
circling	0.86	$P < 0.001$
tail wagging	0.34	ns
urinating	0.38	$P < 0.014$
walking	0.67	$P < 0.001$

Pooled Pearson's correlation coefficients between basal urinary CC ratios and behavioural parameters when dogs were (a) undisturbed and (b) startled by a slamming door.

Effects of gender, breed and age
Gender effects were studied in GI, GII and GIV (GIII consisted only of females). Male dogs showed more ambivalent postures than females when they were approached by a familiar person. Ambivalent postures were shown during a (mean ± SEM) of 1.6 ± 0.8 per cent and 0 ± 0 per cent of the observation time by males and females, respectively. Undisturbed females wagged their tail during 1.5 ± 0.8 per cent of the observation time, which was longer than the 0.3 ± 0.3 per cent of the observation time that we recorded for male dogs.

With regard to stress hormone excretion, Beagles in GII did not differ from group members that were of other breeds, but they did deviate in their behaviour. GII Beagles typically showed high levels (mean ± SEM) of oral behaviours after they were startled by a slamming door: 25.7 ± 6.6 instances $30min^{-1}$ *vs* 3.3 ± 2.4 instances $30min^{-1}$ in GII individuals that were not Beagles. When not disturbed, Beagles sighed more than dogs of other breeds: 4.2 ± 0.9 instances h^{-1} *vs* 1.7 ± 0.4 instances h^{-1}.

Effects of age occurred in behavioural parameters only. In the situation that animals were not disturbed, age correlated with sighing ($r = 0.30$, n = 48, $P = 0.04$) and urinating ($r = 0.37$, n = 48, $P = 0.01$). When animals were startled by a slamming door, age correlated with urinating ($r = 0.36$, n = 48, $P = 0.01$), oral behaviours ($r = 0.42$, n = 48, $P = 0.003$) and lying ($r = 0.38$, n = 48, $P = 0.007$). Finally, when dogs were approached by a familiar person, relationships existed between age and the performance of oral behaviours (r = 0.34, n = 48, P = 0.02), drinking (r = -0.32, n = 48, P = 0.03), sitting (r = 0.41, n = 48, P = 0.004) and changes from one state of locomotion to another (r = -0.43, n = 48, P = 0.003).

Discussion

The present study aims to identify behavioural and hormonal parameters that measure chronic stress in dogs and that have practical use in the assessment of poor welfare in this species. Dogs maintained under relatively enriched (GI) or increasingly austere conditions (GII, GIII and GIV) for several years were assumed to experience higher levels of chronic stress, and were compared for behavioural and hormonal parameters. The findings with regard to urinary cortisol indicated that our experimental set-up was usable for the investigation of chronic stress parameters in dogs and that, conforming to our assumptions, chronic stress levels increased progressively from groups I to IV. There is ample evidence of increased glucocorticoid levels in acutely stressed dogs (Clower *et al* 1979; Dess *et al* 1983; Palazzolo & Quadri 1987; Assia *et al* 1989; Bueno *et al* 1989; Gue *et al* 1989; Engeland *et al* 1990). As in a number of other species (Sassenrath 1970; Craig *et al* 1986; Gamallo *et al* 1986; von Holst 1986; Roger *et al* 1989), it would appear that increased glucocorticoid secretion also indicates dogs that experience enduring stress (Beerda *et al* 1999a). This leads us to assume that elevated cortisol levels are a strong indication of chronic stress, although normal cortisol levels do not exclude chronic stress, since physiological adaptations and stressor-specific responses cannot be ruled out. Our present data show that dogs which are housed under rather austere conditions for several years show elevated CC ratios and that these values become progressively higher as living conditions worsen. The present investigations also show that adaptation of the HPA axis in chronically stressed dogs probably does not imply a normalization of cortisol levels. This makes cortisol levels a very useful measure of chronic stress.

Although the present findings on urinary cortisol indicate otherwise, we may have ranked the groups incorrectly in terms of the austerity of their housing conditions. Differences in the way GII and GIII individuals were housed suggested to us that the former were 'better off' than the latter, but the opposite may have been true. Dogs in GIII, but not GII, had the permanent companionship of a conspecific. Dogs in GII were privileged, in comparison to those in GIII, in that they were kept in a more spacious and enriched home kennel and they were regularly walked outside. The latter implies regular exposure to novel stimuli, contact with conspecifics and interactions with humans. The importance of human companionship for dogs has been highlighted by the work of Tuber *et al* (1996). Based on the way GI individuals were kept, we assumed that these dogs had the lowest chronic stress levels. It cannot be excluded that, for reasons unknown to us, some of the dogs in GI experienced chronic stress, although these privately owned animals lived in a social, spacious and enriched environment. In contrast, from all the dogs that we studied, those in GIV were subjected to the highest degree of social and spatial restriction.

An important aspect of the present study is that we measured a large number of parameters simultaneously. This complicated the statistical analysis of the results. A correction for the number of parameters that were measured would have practically ruled out the possibility of significant group effects. In our opinion, this would not have reflected the actual situation. Instead, we chose to evaluate the results less conservatively and we adopted a comparison-wise error rate of $P < 0.05$ as the level of significance. This necessitates a critical discussion of the results since there is a fair chance that some of the significant results were accidental. Only with great caution may the behavioural and hormonal differences between GIII dogs and the remainder of the animals be interpreted in terms of signs of different levels of chronic stress.

Dogs in GIII were the only ones that were kept in pairs and direct influences of a cage companion on a dog's behaviour may have obscured the effects of chronic stress. Also, these dogs were relatively young and were all female. We investigated, and discuss hereafter, if differences in the dogs' age and gender biased our assessment of stress: we cannot rule out that, at least in part, the behavioural and hormonal differences between GIII and the other groups were caused by differences in age and gender.

Hormonal measures

In accordance with our assumption that chronic stress levels increased from GI to GIV, we found that basal urinary CC ratios were highest in GIV and declined progressively to the lowest values in GI. Differences between groups were significant except when GIV was compared to GIII, or GIII to GII. Mean basal urinary CC ratios were comparable to those reported by others. Van Vonderen *et al* (1997) measured a mean ratio of 2.9×10^{-6} in morning urine samples of 88 pet dogs, which is somewhat lower than the 4.8×10^{-6} that we found for the privately owned GI dogs. A mean basal urinary CC ratio of 9.4×10^{-6} has been reported for dogs that, like our GIV dogs, were housed individually but unlike our GIV dogs were sometimes taken outside for a walk (Jones *et al* 1990). This ratio suggests that at the time that Jones *et al*'s dogs were tested these animals had stress levels that ranged between those experienced by our GIII dogs (a mean ratio of 8.7×10^{-6}) and our GIV dogs (a mean ratio of 14.4×10^{-6}). Increased catecholamine levels have been found in the plasma of acutely stressed dogs (Mekhedova & Ghadirian 1979; Engeland *et al* 1990; Parrilla *et al* 1990), but there is little evidence that increased catecholamine levels occur in the urine of chronically stressed dogs. We found that basal urinary AC ratios were highest in GIV and declined progressively to lowest values in GI. Differences were not significant between GII and GIV, or between GIII and GIV. NC ratios only discriminated GIII individuals from GI individuals, the latter having the lowest ratios.

Our findings indicate that chronically stressed dogs have increased catecholamine secretion and that this is reflected in urinary adrenaline levels and, to a lesser extent, urinary noradrenaline levels, but not in basal urinary DC or NA ratios. Hormonal measures were not significantly affected by gender, breed or age. The impression that arises when hormonal data from GIII (pair-wise housing) and GIV (solitary housing) are compared is that housing dogs pair-wise in a small kennel only moderately improves the situation for the dogs.

Behavioural measures in undisturbed dogs

The behavioural scores on undisturbed dogs confirm the findings on urinary cortisol and catecholamines, in that behavioural differences were most pronounced between GI and GIV, with GII and GIII resembling GI and GIV, respectively. The dogs that we assumed were experiencing the highest level of chronic stress, namely the GIV individuals, typically showed increased locomotor activity, nosing, paw lifting and urinating, and they rarely exhibited high postures. Past and present data indicate that increased paw lifting in dogs may indicate both chronic (Beerda *et al* 1999a) and acute stress (Beerda *et al* 1998). Measures of activity are difficult to interpret with regard to chronic stress. Both inactivity (Hubrecht *et al* 1992) and increased movement (Hetts *et al* 1992) have been connected with chronic stress induced by social isolation. In the present experiment, the relative inactivity of dogs in GI and GII may have been directly related to the fact that these animals were regularly walked out. The latter factor may also explain why GIV individuals urinated more often than dogs in the other groups. Contrary to the dogs in GI and GII, those in GIV were never walked outside and, perforce, they may have acquired a reduced inhibition to urinate inside their home kennel. Like GIV individuals, those in GIII were not walked out. However, GIII included only bitches, which urinate less often than male dogs (Beerda *et al* 1999a). We found positive correlations of age with urinating and sighing, and a negative correlation with activity. Bitches wagged their tails more often than male dogs. These effects of age and gender did not obscure the assessment of chronic stress behaviour.

High incidences of repetitive locomotor behaviour (Hubrecht *et al* 1992), manipulations of the environment, grooming, vocalizing (Hetts *et al* 1992; Beerda *et al* 1999a), bizarre movements (Hetts *et al* 1992), coprophagy and a low posture (Beerda *et al* 1999a), have previously been associated with chronic housing stress, but not in the present study. Differences in the duration of the stress period, which spanned years in the present experiment but only 3 months or 6 weeks in the studies by Hetts *et al* and Beerda *et al*, respectively (Hubrecht *et al*'s study included variable periods of restricted housing), may have been a major cause of discrepancies between the studies. Stereotyped behaviour rarely occurred in any of the groups that we studied. Mertens and Unshelm (1996) reported stereotyped behaviour in 10 per cent of 109 dogs which were housed individually in an animal shelter. Perhaps the housing conditions in the present study were not so austere that they induced stereotypies. Or perhaps we did not observe the dogs at the moments that stereotypies are typically performed. Pigs, for example, stereotype most abundantly around feeding time (Rushen 1985). It may be that dogs develop stereotyped behaviour under conditions of social and spatial restriction (Mertens & Unshelm 1996; Beerda *et al* 1999a), but that such behaviour dissipates over the years. Dynamics in abnormal behaviour have been reported by Clark *et al* (1997), who reported increasing incidences of abnormal behaviour over a 3 month period in young, poorly kept Beagles. The data that are currently available do not portray clearly how abnormal behaviour in dogs develops over time.

Behavioural measures in dogs that were startled by a slamming door or approached by a familiar person

Dogs that were mildly stimulated reacted differently according to the austerity of their living conditions. High levels of locomotor activity, changes from one posture (or state of locomotion) to another, circling, nosing, body shaking, oral behaviours, yawning, ambivalent postures and displacement behaviour may characterize the responses of chronically stressed dogs. Reactions to the sound of a slamming door typically involved a high activity (locomotor activity, circling and nosing), and the expression of behaviours that we previously associated with acute stress (body shaking, oral behaviours and yawning [Beerda *et al* 1998]), when dogs were maintained under poor conditions. It can be argued that differences in response behaviour between GI dogs and the remainder of animals merely indicate that dogs in the different groups learned different associations with the sound of a slamming door. If so, one would expect relatively high, and not low, levels of activity in GI dogs since they may have associated the sound of a slamming door with being picked up by their owner and going home. Privately owned GI individuals typically reacted minimally to an approaching female researcher. They sometimes growled, performed a few oral behaviours or spent a little time nosing and tail wagging, but spent much time lying and appeared reluctant to contact the researcher. In contrast, GIII and GIV individuals showed relatively high levels of ambivalent postures, changes from one posture (or state of locomotion) to another or drinking. The drinking of GIII and GIV individuals during the time that they were approached by a person may be interpreted as displacement behaviour. The performance of oral behaviours in stimulated dogs was positively correlated with age and appeared breed (Beagle) specific. GIV individuals were, on average, 0.9 years older than GI individuals and were all Beagles, contrary to GI individuals. This implies that in the present experiment, during mild stimulation, high levels of oral behaviours may have been falsely interpreted as an indication of chronic stress.

Correlations of urinary cortisol with behavioural and urinary catecholamine parameters

In the preceding discussion we compared dogs kept in four different housing conditions, in order to identify parameters that indicate chronic stress. Chronic stress parameters may also be identified by a positive correlation with an established sign of chronic and acute stress, namely high cortisol secretion. The present correlations of behavioural and hormonal parameters with basal urinary CC ratios suggest that chronically stressed dogs have increased basal urinary AC ratios. Also, they respond actively to mild stimulation, as indicated by positive correlations of the CC ratio with: i) changes from one state of locomotion to another and walking when dogs were not disturbed; and ii) changes from one state of locomotion to another, walking, circling, tail wagging and urinating when dogs were startled by a slamming door. This implies that the increased activity we associated with chronic stress occurred both when dogs were and were not disturbed. We do not believe that the increased activity in dogs kept under austere conditions was the predominant cause of increased cortisol excretion because the groups that were most intensely exercised (GI and GII) showed the lowest CC ratios. Also, it has been reported that exercise programmes in Beagles do not significantly affect the levels of plasma cortisol (Hughes & Campbell 1990).

In summary, the present results substantiate the measurement of urinary cortisol, adrenaline, and to a lesser extent noradrenaline, as a valid and non-invasive way to detect chronic stress in dogs. Behaviourally, chronically stressed dogs may show increased paw lifting, locomotor activity and nosing when they are not disturbed. During mild stimulation, such dogs may be identified by relatively high levels of arousal as indicated by increased locomotor activity, changes from one posture (or state of locomotion) to another, circling, nosing, body shaking, yawning, ambivalent postures and displacement behaviour. Because stress behaviour is rather variable and often nonspecific to stress, it is readily misinterpreted. In many situations, where pronounced behavioural aberrations are absent, behavioural observations may be of limited use in the assessment of chronic stress and it may only help to interpret physiological measures like urinary cortisol and adrenaline levels. The reported indications of chronic stress were associated with rather austere keeping conditions and the results must be applied with some caution when a completely different source of stress is involved.

Acknowledgements

We thank students Sandra Menting and Josefien C von Frijtag Drabbe Künzel, who helped in performing the experiments. The technical assistance of Elpetra A P Sprang and Ank van Wees (Department of Clinical Sciences of Companion Animals, Utrecht University) was highly appreciated. This work was supported by funds from the Ministry of Agriculture Nature Management and Fisheries, the Sophia Vereeniging ter Bescherming van Dieren and the Bond tot Bescherming van Honden.

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