Physiology & Behavior xx (2007) xxx–xxx

PHYSIOLOGY & BEHAVIOR

Cortisol and behavioral responses of working dogs to environmental challenges

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Received 10 May 2006; received in revised form 25 July 2007; accepted 26 July 2007

Abstract

This paper's primary objective is to analyse the physiological (cortisol) and behavioral responses of military working dogs (MWD). Dogs (N=27) were submitted twice to environmental challenges (challenge 1 and 2, 20 days in-between) composed of social (training), visual (mobile toy car) and auditory (air blast) stimuli. Cortisol levels decreased back to the baseline after the second challenge. The behavioral observations showed that these MWD were more active, and presented less stereotypic behaviors (pacing, manipulation of the environment) during both visual challenges, whereas half low posture was observed during the first but not during the second visual challenge.

The present study shows that this group of MWD still has an adaptation capacity to an environmental challenge (return to baseline of the cortisol levels, a higher posture during the second than at the first challenge). These results are encouraging and indicate that the dogs might have a diminished welfare (i.e. stereotypic behaviors), but are not chronically stressed.

Keywords: Working dog; Cortisol; Welfare; Coping ability

1. Introduction

For several years, we have seen a growing interest concerning the welfare of dogs in various conditions (in kennels, at work, as companion animals) and the circumstances perturbing their welfare. Although there is no universal agreement about the definition or measurement of welfare [1–5] one of the most recognised ways to assess welfare is based on Broom's definition [6]: "welfare of an individual is its state as regards its attempts to cope with its environment at a physiological, behavioral and medical level". This approach has also been called "functioning-based" approach [7] or "homeostasis" approach [8]. Welfare could be graded into a continuum from very good to very poor.

There are different examples in the literature of studying both physiological and behavioral measurements for the assessment of chronic stress in laboratory dogs [9–14] and in shelter dogs [15,16]. Unfortunately, few studies treat chronic welfare problems of working dogs [17,18].

Cortisol is a good stress indicator for dogs [9,15,19]. Production of cortisol can be induced by an ACTH injection (ACTH challenge: i.e. in rodents [20,21]; in dogs [9,22]), by a challenge (i.e. sudden sound blast [9]) or by the blood sampling procedure [23–25].

Behaviors, previously associated with chronic stress, differ in dogs whether they have been challenged or not. Behaviors in challenged chronically stressed dogs (i.e. by a slamming door or by the presence of a researcher) are increased locomotor activity, circling, nosing and high levels of other behaviors (body shaking, yawning, ambivalent postures and displacement behaviors) [26]. Behaviors in unchallenged chronically stressed dogs are low posture [10], auto grooming [10,11], coprophagy [10], vocalizing [10–12], paw lifting [10,26,27], high levels of locomotor activity [11,12,26] or inactivity [28], nosing [26], urinating [26] and repetitive or stereotypic behaviors [10,12,26,28,29].

Stereotypic behaviors, defined as acts that are invariably repeated without apparent function [30] are usually considered amongst 'the most important' indicators of long-term welfare

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0031-9384/$ - see front matter
doi:10.1016/j.physbeh.2007.07.014

problems [31]. The most frequently observed repetitive or stereotypic behaviors in kennelled dogs include manipulation of environment [10,13], circling [11,28] and pacing [11,28]. A survey of Belgian military handlers reveals the presence of stereotypic behaviors among their working dogs [32].

This paper's objective is to analyse the cortisol and behavioral responses of military working dogs (MWD) after a repeated environmental challenge to confirm whether they are chronically stressed or not.

2. Material and methods

2.1. Animals

Twenty seven dogs were selected to be a representative sample of the Military canine population as regards sex (21 males, 6 females), breed (24 Belgian shepherds, 3 German shepherds) and housing conditions (15 living in a shelter at the military kennel (MK), 12 living in a shelter at the handler's home). Two additional selection criteria were added: the animals arrived in the army at least 3 months before the study (=first selection criterion) and were operational as working dogs for a maximum of 3 years (1.11 ± 0.14 years) (=second selection criterion). The first selection criterion was considered as necessary to demonstrate the adaptation of dogs in their new environment [11,12]. The second criterion was applied as these dogs will be compared to another group of dogs with new selection, training and evaluation program. Using these criteria, the dogs were between 1 and 5 years old (2.95 ± 0.23 years).

Because of the difficulty of documenting the source of many dogs acquired by the Belgian Defence, no attempt was made to distinguish dogs on basis of their provenance. All the dogs passed a clinical examination and were declared in good health and ready to participate to this study.

Using this sample, the effect of sex, breed and duty time have not been tested. The only factors that have been analyzed are age and housing.

Dogs were individually housed in typical indoor (2.9 × 1.9 × 2.5 m) — outdoor (2.9 × 4 × 2.5 m) pens. Dogs were moved to the outdoor area to record their responses to the challenges. The camera was put in front of the pen and the experimenter was out of view of the dog during filming.

2.2. Experimental design

This study has been carried out during a period of 29 days following an experimental design chosen in accordance with Hennessy et al. [15] and adapted to our own layout. All dogs were tested within 6 weeks, 5–6 dogs a day, and one dog at a time. On Days 8 and 29, dogs were exposed to the same challenges (respectively challenge 1 and challenge 2).

Both challenges were composed of social, visual and auditory stimuli. The social stimuli consisted in 8 obedience exercises (i.e. walk on leash, the recall) and 5 protection work exercises (i.e. handler defence and false run) accomplished outside in a maximum of 30 min. This social stimulus, while perhaps not stressful, may at least have been rewarding and exciting. The aim of this social stimulus is to ensure that all dogs are at the same arousal level before being kennelled. It will not be included in the analysis.

After the social stimulus the dog was placed within 5 min in the outdoor area of a pen (2.9 × 4 × 2.5 m). After being left undisturbed for two minutes, the dog was submitted subsequently to the visual and auditory stimuli. The visual stimulus consisted in the activation by a hidden assistant of a mobile and noisy toy car in the pen during 30 s. The toy car was already present in the pen at the entrance of the dog. This type of stimulus has been used by previous authors [33]: the toy car travelled in circles. The auditory stimulus (110–120 dB) was an air blast for 3 s, at a distance of 1 m from the dog. This stimulus has been chosen because it represented a loud and unknown sound, was easy to use and allowed the presentation of the same stimulus to each animal. This type of stimulus has also been used by previous authors [13].

No human disturbance was allowed during testing.

2.3. Physiological parameters

Blood samples for assessment of plasma cortisol concentrations were collected on Days 1, 3, 8, 15, 22 and 29 between 8 and 10 AM. At Day 1, the hair over the cephalic vein of the dogs was clipped to facilitate blood sample collection. The first 3 samples (Day 1, 3, 8) were taken to identify the basal levels of cortisol. On Days 8 and 29, additional blood samples (8B, 29B) were collected between 10 AM and 2 PM immediately after exposure to the challenge (Day 8: challenge 1; Day 29: challenge 2).

Samples were collected within 3 min of the beginning of the procedure. Based on data from rodents [23–25] and from dogs [19] it is known that this is rapid enough to ensure that glucocorticoid levels are not affected by the sampling procedure. All blood samples (approximately 1 ml) were obtained from the cephalic vein with a needle and syringe. One individual held the dog, and a second performed the venipuncture. The blood was rapidly transferred from the syringe to a tube containing heparin for analysis of cortisol concentration. Plasma was immediately separated in a refrigerated centrifuge during 20 min at 3800 rpm and frozen at −80 degrees centigrade until analysis. Samples were assayed in duplicate with commercially available ^{125}I radioimmunoassay kits (Spectria ®, Orion Corporation Orion Diagnostica, Espoo, Finland).

2.4. Behavioral observations

During Days 1, 3, 15 and 22, after the blood sample followed by a 10 min walk (=exercise) with the handler, dogs were filmed in the pen where the behavior was recorded (baseline of the behaviors). The aim of this exercise is to ensure that all dogs are at the same arousal level before being kennelled. During Days 8 and 29 (challenge 1 and 2), after the first blood sampling, a 10 min walk and the social stimulus presentation, dogs were filmed in the pen where the behavior was recorded. Next, the visual stimulus was presented and behaviors were recorded while the car was moving around (=mobile car: 30 s) and while the car was immobilized (=immobile car: 90 s). During the auditory stimulus, behaviors were recorded during

Please cite this article as: Haverbeke A et al. Cortisol and behavioral responses of working dogs to environmental challenges. Physiol Behav (2007), doi:10.1016/j.physbeh.2007.07.014

the stimulus presentation (3 s) and after the stimulus (=air blast: 120 s). The behaviors were recorded on videotape using a surveillance camera (Digital Video Camera Recorder, DCR-TRV27E, Sony®). The camera was placed in front of the cages. Behavioral observations were recorded 4 times during 12 min (on Days 1, 3, 15 and 22) and 2 times during 8 min (on Days 8 and 29). A total of 52 min were analysed for each dog for the entire study period as the first two minutes of each session were not analyzed, according to pilot taping sessions and data from other studies [34] that indicated that the dogs reacted most

Table 1
Behavioral taxonomy

The behaviors scored in terms of the frequency of occurrence	
Yawning	Mouth open to apparent fullest extent while eyes are closed
Oral behaviors	Includes non-directed licking (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	Fore paw lifted into a position of approximately 45°
Urinating squat	Urinating by squatting while keeping both hind limbs on cage floor
Urinating limb raised	Urinating while raising one hind limb
Defecating	Excreting the contents of the bowels
The behaviors scored in terms of the duration of occurrence	
Auto grooming	Behaviors directed towards the subjects own body like scratching, licking and biting
Nosing	Nose moved along objects and/or clear sniffing movements are exhibited
Tail wagging:	Repetitive wagging movements of the tail
Repetitive or stereotypic behavior	
Pacing	Immediately repeating a path just taken and continuing in the repetition; in circles, in a figure eight pattern or fence/wall-line running
Circling	Continuous walking in short circles, apparently chasing its tail or hind limbs
Manipulation of environment (Man Env)	Stereotypic interactions with elements from the environment; digging (=scratching the floor with the forepaws to a way that is similar to when dogs are digging holes); floor licking (=licking the floor with the tongue); rubbing legs against bars, gnawing at bars or at other material of the environment
Not seen	Unable to determine behavior of dog owing to darkness, position of dog in cage or none of these behaviors is shown
Miscellaneous oral behaviors	
Barking	Loud, rough noise
Roaring	Loud, deep sound
Growling	Low, rough sound
Whining	Long, high sound
Yelping	Sudden, short, high sound
Panting	Increased frequency of inhalation and exhalation often in combination with the opening of mouth
Teeth clapping	Making short loud noise by hitting teeth together
Not seen	Unable to determine behavior of dog owing to darkness, position of dog in cage
Locomotive states	
Prone, head down	Trunk of body on cage floor, chin or side of head in contact with cage side or floor, paws or limbs
Prone, head up	Trunk of body on cage floor, no part of head in contact with cage or paws
Sit	Hindquarters and front paws only in contact with cage floor
Stand	Upright with at least three paws in contact with cage floor
Walk	Takes at least one step, shifting body position
High active	Any motion across cage floor faster than a walk, including trotting, jumping, without coming in contact with sides of cage
Rear front	Rapidly pawing bars while rearing, with one or both front paws in contact with the front of cage
Rear back	Rapidly pawing bars while rearing, with one or both front paws in contact with the back of cage
Changing from one state of locomotion to another (Change loc)	
Not seen	Unable to determine behavior of dog owing to darkness, position of dog in cage
Postures	
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 posture shown by dogs under neutral conditions
Half low	From three features: a lowered position of the tail (compared to the neutral posture), a backward position 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	Low posture, but now the tail is curled forward between the hind legs
Not seen	Unable to determine behavior of dog owing to darkness, position of dog in cage

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intensely to the arrival of the human experimenter during the first few minutes. Videotapes were scored, using a personal computer and software designed for behavioral observation (The Observer 5.0 for Windows, Noldus Information Technology, Wageningen, The Netherlands). Intra-observer reliability exceeded 90% for all behavioral categories. Behavioral taxonomy was chosen in accordance with previous studies [12,13] and adapted to our own layout (Table 1). The behaviors included within each category (stereotypic behaviors, oral miscellaneous behaviors, locomotive states and postures) were mutually exclusive. Behavior was analysed in terms of the frequency or duration of occurrence. Environmental behaviors (manipulation environment, excluding play behavior) and mobile behaviors (circling, pacing) were considered as repetitive behaviors in accordance with Beerda et al. [10] and Hubrecht et al. [28] and were analysed in terms of duration of occurrence. Moreover Beerda et al. [10] and Hubrecht et al. [28] considered these repetitive behaviors from their first moment of occurrence. However, in the present study extreme values (below the 5th and over the 95th percentile) have been classified in separated groups in order to avoid excessive influence concerning the average and the variance of the group included between these two limits. The weakest values (below the 5th percentile) have not been analysed, as they were suspected to be irrelevant; the intermediate values were considered as robust [35] and reflecting repetitive behaviors (pacing between 2.93 s–50.21 s of duration, circling between 0.86 s–9.62 s, manipulation environment between 1.39 s–18.22 s); the superior values (over the 95th percentile) were considered as highly repetitive behaviors (pacing of more than 50.22 s duration, circling more than 9.63 s, manipulation environment more than 18.23 s).

2.5. Data analysis

Transformation (logarithmic transformation for physiological measures; square root transformation for behavioral measures) was applied for normalization of the data and for homogenisation of the variances. Data were analysed by an analysis of variance (one way ANOVA). Pair wise comparisons between groups (Tukey's studentized range test) were conducted for those parameters that showed a significant group effect as indicated by the F statistic. The level of significance was set at $p<0.05$. All the analyses were done by PROC SAS GLM [36]. For ease of presentation, all the results are expressed in raw data (mean ± SEM).

3. Results

The results presented are the average tendencies rather than individual differences. There were some individual differences among the dogs, but a detailed exposition of these is not within the scope of the present paper.

3.1. Physiological analyses

The plasma cortisol concentration was significantly higher after challenge 1 than before it (before challenge 1 (average of Day 1, 3, 8): 39.27 ± 4.91 nmol/L, after challenge 1 (Day 8B): 64.23 ± 6.19 nmol/L) ($F(7,204)=3.30$, $p<0.05$) (Fig. 1). However that was not the case after challenge 2 (before (Day 29): 43.16 ± 7.51 nmol/L, after (Day 29B): 52.39 ± 3.99 nmol/L). The plasma cortisol concentrations on Day 22 and Day 29 were significantly lower than concentration on Day 8B ($F(7,204)=5.13$, $p<0.001$).

3.2. Behavioral analyses

3.2.1. Control period

No effects of Days 1, 3, 15 and 22 have been found on the behaviors. Therefore the average of the behaviors observed on these days was calculated and considered as the control period to be compared to the responses to the challenges (challenge 1, challenge 2) (Table 2). In the control period, the most frequently observed behaviors were pacing (repetitive behavior, 31.82% ± 2.04 of the time observed with 28.58% highly repetitive pacing), panting (miscellaneous oral behavior, 33.74% ± 3.36) and the standing position (locomotion, 37.03% ± 2.05). Dogs spent 46% of the observed time in active gaits (walk, high active, rear front, rear back) and showed principally a high posture (47.71% ± 3.38).

3.2.2. Effect of challenges on behaviors

Regarding repetitive behaviors, there was significantly less pacing during the visual stimulus presentation (mobile car in challenge 1 and challenge 2) than during the control period ($F(9,259)=4.32$, $p<0.001$), but there was no significant difference of pacing in presence of the mobile car between challenge 1 and challenge 2. The challenges had no influence on circling. Whatever the challenge (1 or 2), there was less manipulation of the environment than during the control period ($F(9, 259)=29.31$, $p<0.001$). Interestingly, the highly repetitive pacing, observed during the control period, decreased into repetitive pacing during challenge 1 (visual and auditory stimuli) and challenge 2 (visual stimulus: mobile car); at the end of challenge 2 (visual (immobile car) and auditory stimuli) pacing started to be highly repetitive again. This evolution was observed neither for circling, nor for manipulation of the environment (Table 3).

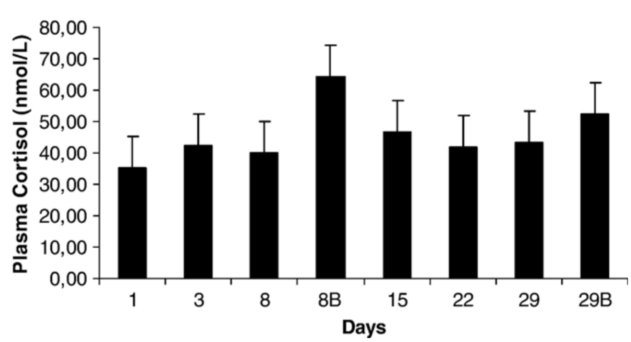

Fig. 1. Mean plasma cortisol levels of dogs sampled at Days 1, 3, 8 and 8B (=after challenge 1), 15, 22, 29 and 29B (=after challenge 2). Vertical lines represent standard errors of the means. The plasma cortisol concentration was significantly higher after challenge 1 than before it (average of Days 1, 3, 8) ($p<0.05$).

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Table 2
Behaviors of the dogs during the control period (°) and the challenges (challenge 1 and 2)

	Control period	Challenge 1			Challenge 2		
		Visual stimulus (mobile car)	Visual stimulus (immobile car)	Auditory stimulus (air blast)	Visual stimulus (mobile car)	Visual stimulus (immobile car)	Auditory stimulus (air blast)
Repetitive behavior							
Pacing	31.82±2.04	7.47±2.88	15.09±2.02	20.64±2.45	14.13±4.78	25.22±5.08	28.06±6.21
Circling	1.71±0.45	1.64±0.38	4.6±2.67	1.94±1.01	1.95±1.05	2.53±0.86	0.7±0.14
Man Env	1.02±0.3	0.0	0.0	0.05±0.05	0.0	0.2±0.2	0.33±0.30
Miscellaneous oral behaviors							
Panting	33.74±3.36	45.21±7.16	41.53±6.42	38.20±7.29	43.88±7.09	44.08±7.11	41.17±7.08
Locomotion							
Stand	37.03±2.05	17.36±4.67	41.81±5.25	42.51±5.12	22.60±6.03	40.35±5.28	47.97±5.29
Walk	24.48±1.77	15.90±5.81	13.53±3.45	8.63±2.42	6.05±2.38	13.46±3.07	10.03±4.43
High active	18.29±2.72	50.47±6.01	30.90±5.98	32.23±6.07	53.99±6.66	35.16±6.34	31.11±5.79
Rear front	3.24±0.64	1.65±1.27	2.87±1.31	4.04±1.69	3.13±2.33	3.17±2.00	2.77±1.64
Rear back	0.36±0.12	0.0	0.17±0.17	0.16±0.16	0.36±0.28	0.77±0.56	0.07±0.05
Posture							
High	47.71±3.38	49.01±8.55	41.73±8.53	55.19±8.50	65.83±7.38	67.24±7.37	56.62±8.36
Half low	0.96±0.45	22.21±6.63	4.48±3.53	5.08±3.62	7.58±3.64	7.46±4.74	6.30±4.30

Time %, means±SE.
(°) As no effects of days on the behaviors have been found ($p>0.05$), the average of the behaviors observed on Days 1,3,15 and 22 was calculated and considered as the 'control period'.

During the two visual stimulus presentations (mobile car in challenge 1 and challenge 2), dogs were more active ($F(9,259)=5.13$, $p<0.001$) and stood less ($F(9,259)=6.20$, $p<0.001$) than during the control period. There was more half low posture during challenge 1 (visual stimulus: mobile car) than during the control period ($F(9,259)=4.34$, $p<0.01$), but not during challenge 2. During the two challenges there was less tail wagging than during the control period ($F(9,259)=6.30$, $p<0.001$). There was no evidence of an increase in the number of yawning, oral behaviors (especially non-directed licking), paw lifting, urinating or defecating during the two challenges.

3.3. Effect of age and housing conditions on behaviors

The effects of age and housing conditions were subsequently examined.

In the control period and during the two challenges, 1 year old dogs spent more time pacing ($F(4,264)=9.21$, $p<0.001$)

Table 3
Repetitive behaviors of the dogs during the control period (°) and the challenges (challenge 1 and 2)

	Control period	Challenge 1			Challenge 2		
		Visual stimulus (mobile car)	Visual stimulus (immobile car)	Auditory stimulus (air blast)	Visual stimulus (mobile car)	Visual stimulus (immobile car)	Auditory stimulus (air blast)
Pacing	32.49±2.71	8.89±4.30	17.99±4.92	23.59±5.40	14.13±4.78	26.30±6.16	28.06±6.21
	0 (*)=0.67	0=1.42	0=2.90	0=2.95	0=/	0=1.08	0=/
	1=3.24	1=7.47	1=15.09	1=20.64	1=14.13	1=5.68	1=2.83
	2=28.58	2=/ (***)	2=/	2=/	2=/	2=19.54	2=25.23
Total repetitive pacing	31.82±2.04 (**)	7.47±2.88	15.09±2.02	20.64±2.45	14.13±4.78	25.22±5.08	28.06±6.21
Circling	1.71±0.45	4.84±2.91	1.94±1.01	1.99±1.09	2.53±0.86	0.95±0.39	0.83±0.31
	0=0.07	0=0.24	0=/	0=0.04	0=/	0=0.25	0=/
	1=0.40	1=0.47	1=1.94	1=0.1	1=2.53	1=0.7	1=0.83
	2=1.24	2=4.13	2=/	2=1.85	2=/	2=/	2=/
Total repetitive circling	1.64±0.38	4.6±2.67	1.94±1.01	1.95±1.05	2.53±0.86	0.7±0.14	0.83±0.31
Manipulation of environment (Man Env)	1.07	0.0	0.0	0.05	0.0	0.39	0.39
	0=0.05			0=/		0=0.19	0=0.06
	1=0.23			1=0.05		1=0.2	1=0.33
	2=0.79			2=/		2=/	2=/
Total repetitive Man Env	1.02±0.3 (**)	0.0	0.0	0.05±0.05	0.0	0.2±0.2	0.33±0.30

Time %, means±SE.
(°)As no effects of days on the behaviors have been found ($p>0.05$), the average of the behaviors observed on Days 1, 3, 15 and 22 was calculated and considered as the 'Control Period'.
(*) 0: not included as repetitive behaviors; 1: repetitive behaviors, 2: highly repetitive behaviors (see text for description of the 3 categories).
(**) One-way Anova, $p<0.001$.
(***): no behavior has been found into this category.

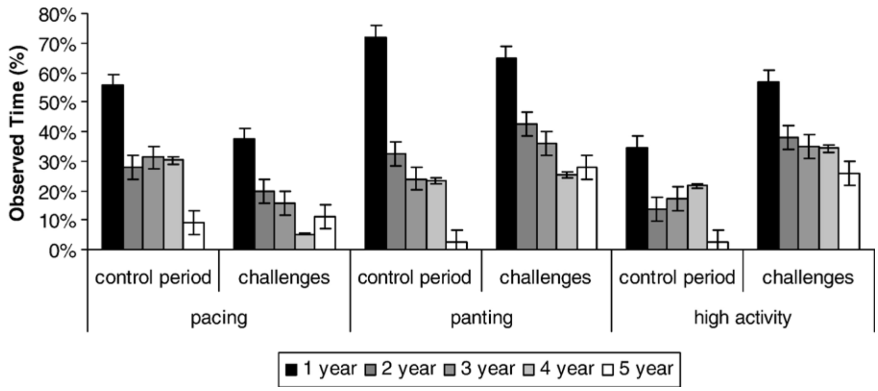

Fig. 2. Effect of age on dogs' behavior (pacing, panting, high activity) during the control period and the challenges (mean value of challenge 1 and 2). In the control period and during the challenges, 1 year old dog (black bar) spent more time pacing and panting than older dogs (the four remaining bars) ($p<0.001$).

and panting ($F(4,264)=11.00$, $p<0.001$) than older dogs (Fig. 2).

During the challenges, dogs housed at the handlers home were less active ($F(1,158)=11.62$, $p<0.001$) and exhibited more half low posture ($F(1,158)=14.97$, $p<0.001$) than dogs housed at MK. Independent of the challenges, dogs housed at the handlers home showed less pacing ($F(1,107)=8.04$, $p<0.01$), barked more ($F(1,107)=25.50$, $p<0.001$), sat more ($F(1,107)=5.36$, $p<0.05$) than dogs housed at the MK. During the challenges, in contrast to the control period ($F(1,62)=9.25$, $p<0.01$), manipulation of the environment was not influenced by housing conditions.

4. Discussion

The environmental challenges have been defined as acute stressors by previous authors [13,33]. It is interesting though to ask whether these challenges were really stressful to the dogs in the present study. Dogs might have found these challenges rather interesting and exciting compared to the barren kennel environment. The behavioral changes could all be a result of the dogs being positively stimulated or aroused rather than stressed. Henry and Stephens [37] suggest that there is only stress when there is loss of control and a reduced predictability of what will happen. So far as there is any action to obtain control with a high probability of success, there is arousal (=excitement), but no stress. In the present study, the stimuli presentations were not predictable and the interval between the challenges was long enough to prevent memorization.

4.1. Physiological responses

The importance of a challenge in the assessment of welfare has been shown in dogs. Beerda et al. [8,9] considered two groups of dogs outdoor housed under good and bad weather conditions. After having been housed as a group, dogs were individually indoor housed for 6 weeks in a novel environment. At the sixth week, a challenge was presented to determine in which group the dogs' welfare was most impoverished. When challenged, the bad weather group exhibited less behavioral and physiological responses than the good weather group. The welfare of the good weather group was considered as being more impoverished expressed by physiological (HPA hypo-responsiveness to acute stimuli) and behavioral signs (increased autogrooming, paw lifting, vocalizing, low posture, repetitive behavior, coprophagy).

Hennessy et al. [15] reported that the increase in cortisol plasma concentration in response to novel situations was almost twice as great during the second as during the first stressful battery if chronically stressed dogs had not received the program of human interaction during the intervening period and not significant if dogs had received the program of human interaction.

In the present study, the basal cortisol level was the average of the 3 first blood samples (39.27 nmol/L). We did not expect the heightened cortisol levels to reflect only a shift in Circadian rhythms of HPA activity because of the similar levels of basal cortisol concentrations found during the first 3 samples. Moreover, no Circadian rhythms have been established neither in laboratory dogs at 30 min intervals over a period of 28 h [38] nor at 20 min intervals over a period of 25 h [39] nor in working dogs exposed to defence training and trailing tasks at 90–180 min intervals over a period of 24 h [40]. The cortisol basal values in the present study do not show any significant differences from those of a similar group of military dogs undergoing an irregular environmental enrichment regimen (social+exercise) [18].

The basal cortisol level (39.27 nmol/L) remained within the normal range compared to other studies in dogs (approximately 25–55 nmol/L [15,37,41–43].

In this study, the first exposure to a challenge increased plasma cortisol level significantly. This is consistent with previous findings in dogs [9,15] and also in rodents [44,45]. After 3 weeks (Day 29), the second exposure to the same challenge no longer resulted in increased plasma cortisol levels. Between the challenges, there is complete recovery from the challenges in hormonal levels. Thus, dogs are able to cope with these challenges.

4.2. Behavioral responses

In the present study single-housed working dogs spent 46% of the observed time of the control period in active gaits (walk, high active, rear front, rear back). It was higher than previous data in working dogs (5% to 30%: [18,46]) or in laboratory dogs (5% to 15%: [11,28]). Dogs might have been aroused by the exercise (10 min walk) before being kennelled for videotaping.

According to Beerda et al. [10,13,26], active coping (e.g. restlessness: high levels of walking, nosing and changing from one state of locomotion to another…) has been observed in dogs during and after challenges. In the present study, an increase of activity has been found only during (mobile car) but not after (immobile car) the visual stimulus presentations (challenge 1 and 2). This restlessness cannot be attributed to previously defined situations in which restlessness has been observed: anticipating stimuli or the presence of humans [13], undergoing harsh training methods [27] or anticipating signalled shock avoidance trials [47]. But the restlessness could be due to the stimulus (mobile car) itself: when the stimulus moves, the dog moves and vice-versa.

Most studies emphasize that sound blasts are particularly stressful to dogs, since these stimuli are associated with a very low posture and elevated cortisol (for a review see [13]).

In the present study, no increase of activity was found during the auditory stimulus presentations. A possible explanation is that, due to their patrol work, these working dogs might often be exposed to and thus habituated to this kind of auditory stimulus.

The increase of activity during visual stimulus presentations (challenge 1 and challenge 2) was not accompanied by an increase in repetitive behaviors, as reported in previous studies [12,48]. Indeed, pacing and manipulation of environment decreased during visual stimulus presentations (challenge 1 and challenge 2). No influence on circling was found during the challenges, which is in disagreement with other authors [26], who reported an increase in circling among chronically stressed dogs during mild stimulation. But these authors [26] used different stimuli (slamming door or in the presence of a researcher) than in the present study (mobile car, sound blast). However at the end of the challenge 2 (auditory stimulus), pacing increased again and acquired the same level as during the control period. So it seems that in the present study, repetitive behaviors (namely pacing) were observed during the control period, but decreased during the challenges and came back again at the moment dogs did not feel stressed anymore (end of challenge 2). But further investigation must still be done, as this evolution was not observed for the other repetitive behaviors (circling, manipulation of environment).

One might interpret the decrease of pacing as orienting or alerting behavior that could be expected during presentation of a novel item in their otherwise barren environment. Authors cannot confirm this hypothesis, as no decrease of circling has been observed simultaneously.

While the results confirm the presence of stereotypic behaviors among MWD, their welfare is affected in a limited way. These dogs are still adapting to environmental challenges. Lefebvre et al.'s hypothesis [32], suggesting that this military working dog population suffers from an impoverished welfare, cannot be confirmed. It is true that stereotypies should always be taken seriously as a warning sign of potential suffering, but they cannot be used as the sole index of welfare [49]. Moreover, previous authors suggested that they correlate badly with other welfare parameters [50,51].

In fact, these stereotypes could be interpreted as a behavioral scar that persists long after removal from the impoverished environment. They can be considered as products of past stress, but no longer reflecting poor welfare [52]. So, it could be that these MWD have been roughly handled in the past [32]. It could also be that they have been housed in an impoverished environment or that they have been confronted with aversive stimuli.

Earlier studies on stressed dogs have reported on increased "stress-related behaviors": vocalizing, panting, paw lifting, trembling [27,47,53,54]. In accordance with Beerda et al. [13], we found no significant increase of these behaviors after challenging working dogs. There was no evidence of an increase of the number of oral behaviors (and especially non-directed licking) during challenges. Oral behaviors are typically performed in a social context. For example, snout licking or non-directed licking, may express submission in a stressful social environment [27] and can be considered as a common sign of uneasiness or anxiety in dogs [54]. It is then not surprising that our dogs showed low levels of oral behaviors during the challenges, as no stimuli involved the presence of the experimenter.

In the present work, panting was not due to high temperature.

Yawning has been associated with psychological tension or mild stress in primates [55] and in dogs [13,15,26,56] or with possible displacement behavior indicative of conflict in dogs [57]. In the present study there was no indication of increased yawning during the challenges. A lower posture has been associated with stress [58]. The present study only confirms this statement during the first visual stimulus presentation (challenge 1), though not during the second (challenge 2).

4.3. Effects of age and housing conditions on physiological and behavioral responses

Younger dogs present higher activity, more pacing and panting. Though, this behavioral observation has been confirmed by previous authors [59–62], others have failed to show any effect of age on dogs' behavior activity [63].

The results of the present study do not confirm whether dogs taken home or those living at the MK present signs of decreased welfare. Wells et al. [63] for instance, found that the activity level of shelter dogs was not correlated with the age but with the time spent at the shelter.

Indeed, no physiological differences were found between the groups of dogs. Although not supported by physiological data, dogs housed at the handler's home seemed to cope less well with their environment than dogs housed at the MK. These dogs more frequently presented a half low posture and barked more than dogs housed at the MK. The reason why the coping abilities of dogs housed at MK are better, might lie in the fact that these dogs were more familiar with the environment where the study was carried out, even if home-housed dogs were accustomed to MK.

5. Conclusion

The present study shows that this group of MWD has a good adaptation capacity to an additional challenge (return to the baseline of the cortisol levels, a higher posture at the second

Please cite this article as: Haverbeke A et al. Cortisol and behavioral responses of working dogs to environmental challenges. Physiol Behav (2007), doi:10.1016/j.physbeh.2007.07.014

than at the first challenge). These results are encouraging and indicate that the dogs might have a less diminished welfare (i.e. stereotypic behaviors) than supposed. Contrary to the original hypothesis and to Lefebvre et al. [32], MWD are still able to adapt to environmental challenges.

Acknowledgments

This study was supported by the University of Namur (FUNDP) and the Belgian Defence (research programme no. 2001_70-1233-51-20, A. Haverbeke).

The authors would like to thank all the Veterinary Service of the Belgian Defence and especially Vet LtCol Miguel Stevens and Vet Cdt Jan de Roo for their support and their help. The authors would also like to thank John Burns for proof reading the manuscript, the Laboratory of Physiology (J.L. Bister, FUNDP) for the cortisol analyses and the handlers for their participation in this research.

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Please cite this article as: Haverbeke A et al. Cortisol and behavioral responses of working dogs to environmental challenges. Physiol Behav (2007), doi:10.1016/j.physbeh.2007.07.014