11-1-2015

The Physiological Consequences of Crib-Biting in Horses in Response to an ACTH Challenge Test

S. Briefer Freymond
Swiss National Stud Farm

D. Bardou
Swiss National Stud Farm

Elodie F. Briefer
Queen Mary University of London

R. Bruckmaier
University of Bern

N. Fouché
Swiss Institute of Equine Medicine

See next page for additional authors

Recommended Citation

Freymond, S. B., Bardou, D., Briefer, E. F., Bruckmaier, R., Fouche, N., Fleury, J., ... & Bachmann, I. (2015). The physiological consequences of crib-biting in horses in response to an ACTH challenge test. Physiology & behavior, 151, 121-128.

Authors

S. Briefer Freymond, D. Bardou, Elodie F. Briefer, R. Bruckmaier, N. Fouché, J. Fleury, A.-L. Maigrot, A. Ramseyer, K. Zuberbühler, and I. Bachmann

The Physiological Consequences of Crib-Biting in Horses in Response to an ACTH Challenge Test

S. Briefer Freymond[1], D. Bardou[1], E.F. Briefer[3], R. Bruckmaier[5], N. Fouché[4], J. Fleury[1], A.-L. Maigrot[1], A. Ramseyer[4], K. Zuberbühler[2,6], I. Bachmann[1]

[1] Swiss National Stud Farm
[2] University of Neuchâtel
[3] ETH Zurich
[4] Swiss Institute of Equine Medicine
[5] University of Bern
[6] University of St. Andrews

KEYWORDS

stress physiology, stereotypy, coping strategy

ABSTRACT

Stereotypies are repetitive and relatively invariant patterns of behavior, which are observed in a wide range of species in captivity. Stereotypic behavior occurs when environmental demands produce a physiological response that, if sustained for an extended period, exceeds the natural physiological regulatory capacity of the organism, particularly in situations that include unpredictability and uncontrollability. One hypothesis is that stereotypic behavior functions to cope with stressful environments, but the existing evidence is contradictory. To address the coping hypothesis of stereotypies, we triggered physiological reactions in 22 horses affected by stereotypic behavior (crib-biters) and 21 non-crib-biters (controls), using an ACTH challenge test. Following administration of an ACTH injection, we measured saliva cortisol every 30 min and heart rate (HR) continuously for a period of 3 h. We did not find any differences in HR or HR variability between the two groups, but crib-biters (Group CB) had significantly higher cortisol responses than controls (Group C; mean ±SD: CB, 5.84±2.62 ng/ml, C, 4.76±3.04 ng/ml). Moreover, crib-biters that did not perform the stereotypic behavior during the 3-hour test period (Group B) had significantly higher cortisol levels than controls, which was not the case of crib-biters showing stereotypic behavior (Group A) (B, 6.44±2.38 ng/ml A, 5.58±2.69 ng/ml). Our results suggest that crib-biting is a coping strategy that helps stereotypic individuals to reduce cortisol levels caused by stressful situations. We conclude that preventing stereotypic horses from crib-biting could be an inappropriate strategy to control this abnormal behavior, as it prevents individuals from coping with situations that they perceive as stressful.

1. Introduction

Physiological reactions are triggered during both positive, rewarding stimuli and negative, aversive stimuli [1,2]. Normal physiological reactions are thought to impact positively on individuals due to the release of energy-mobilizing glucocorticoids (GCs) and behavioral diversification [3]. Following such reactions, two systems are activated to help the individual to regain homeostasis (or steady state [3]); the hormonally based hypothalamo-pituitary-adrenocortical (HPA) axis and the neural sympatho-adreno-medullary (SAM) axis. In response to increased physical and psychological demands, the adrenocorticotropin hormone (ACTH) is released from the anterior pituitary gland, subsequently triggering a release of glucocorticoids (cortisol) from the adrenal cortex, epinephrine (adrenaline) from the adrenal medulla, and norepinephrine (noradrenaline) from the sympathetic nerves.

Chronic stress (or “stress”) occurs when environmental demands produce a physiological response that, if sustained for an extended period, exceeds the natural regulatory capacity of the organism, particularly in situations that include unpredictability and uncontrollability [2].Whether a threatening situation is perceived as a stressor, however, appears to differ between individuals due to variation in coping abilities [4]. Despite its adaptive fight-or-flight function on the short-term, long-term or chronic release of stress hormones can be detrimental. If one or both axes are persistently activated [5], individuals can be affected by cardiovascular diseases, depression or immunosuppression. In captive and domesticated animals, chronic stress can be provoked by unnatural husbandry practices, such as early weaning, social isolation, or dietary restriction, which can negatively affect the HPA-axis [5,6]. It can also trigger stereotypies and other behavioral disorders, which can be used as indicators of welfare problems, if they persist after the situation of chronic stress [7–10].

Stereotypies have been defined as repetitive, relatively invariant, patterns of behavior with no apparent goal or function [11]. They occur in various forms and contexts and have been observed in a wide range of species in captivity. In horses and other ungulates, different forms of stereotypies exist, including crib-biting, windsucking, weaving and box-walking [12]. The performance of stereotypic behavior varies between horses in terms of the percentage of time occupied by the activity, as well as the vigor and the persistence of the behavior [13]. The prevalence of crib-biting or windsucking among horses reported in Europe and Canada is 2.4–8.4% [14,15]. The causes of stereotypies are difficult to identify but have been linked to chronic stress, management factors and genetic predispositions [7,9,14].

An important problem is whether or not stereotypic behavior has an adaptive function or whether it is a functionless behavioral abnormality [11,16]. For example, stereotypies may function to cope with high levels of frustration, but the fact that stereotypic behavior often persists after the cause of frustration has been removed contradicts this hypothesis [16]. Another line of argument is that stereotypic behavior functions as a coping mechanism to reduce chronic stress or to provide animals with some form of control over their environments [7,11,17–19]. The main prediction of this argument is that the physiological response of animals should increase if they are being prevented from displaying the stereotypic behavior in response to a frustrating situation [16,20]. To our knowledge, however, there is no agreement between studies regarding the coping function of stereotypies [18,20–22].

One way to understand the nature of stereotypic behavior is to link it to coping styles. Coping styles have been defined as “a coherent set of behavioral and physiological stress responses that are consistent over time and which are characteristic of a certain group of individuals” [23,24]. The main idea is that, as soon as some “stress” threshold is reached, the coping response acts to minimize “stress” [24]. Two different coping styles have been distinguished: proactive copers try to escape or remove the stressor (“fight-or-flight” response), while reactive copers show no signs of being affected (conservation-withdrawal response) [25]. Proactive individuals tend to have a lower HPA and higher SAM axis reactivity than reactive ones [4]. One hypothesis is that stereotypic behavior reflects a proactive coping response, while depression is more typical of reactive individuals [6].

In this study, we experimentally induced a physiological stress response, in horses affected by stereotypic behavior (crib-biters) and a comparable number of non-crib-biter individuals (controls), using an ACTH challenge test, which consists of administering adrenocorticotropin [26]. To assess the relative reactivity of the HPA and SAM axes, we measured cortisol released from the adrenal cortex, as well as heartrate related measures [27]. If proactive individuals are more prone to developing stereotypies than reactive individuals, we predicted that stereotypic horses should have lower initial cortisol levels, smaller cortisol responses and higher sympathetic activity and reactivity to the ACTH challenge test than control horses [7,19,28].

2. Methods

2.1. Subjects and management conditions

The study was carried out on 22 crib-biters and 21 control horses (total = 43 horses) of various breeds, sex (mares, geldings and stallions) and ages (3 to 24 years old), housed in 19 different farms in Switzerland, between April and July 2013 (Table 1). Thirty-two horses were privately owned, and 11 horses were owned by the Swiss National Stud Farm. All the horses had been at their respective farms for at least one year. To be eligible for inclusion in the study, crib-biters were required to have demonstrated crib-biting behavior for a minimum of one year, as reported by their owners. The numbers of years that crib-biters had been observed performing the stereotypy were estimated by the horse owners to range between at least 1 and 15 years. Controls were horses that had never been observed crib-biting or performing other kinds of stereotypies (e.g. weaving or box-walking). For each crib-biting horse, we tried to find a control horse that was of similar breed, sex and age, and that was housed in the same conditions, either individually or in a group, in single box or in box with paddock, and if possible in the same farm (Table 1). Routine care was provided by the owners. The study was approved by the Federal Veterinary Office (approval number VD 26777 bis; Switzerland).

2.2. Experimental procedure

We performed an ACTH challenge test by injecting a synthetic adrenocorticotropic hormone (Synacthen® Tetracosactid 0.25 mg/l) intravenously [29]. The amount of Synacthen injection was calculated according to the weight of the subject (1 µg/kg). The cortisol secretion follows a circadian rhythm, with secretion peak occurring in the early morning. These rhythms may be influenced by exercise, copulation, learning, excitement and stressors, such as venipuncture or the removal of an animal from its familiar environment [30]. For these reasons, the injection was always carried out at 13:00 ± 10 min local time, and the subject had not been exercised in the morning on the day of the test. All the horses were housed in their usual conditions during the test.

The procedure was similar for all subjects and lasted between 3 h 20 min and 3 h 30 min. The subject's weight was estimated following the method described in Carroll and Huntington [31]. Then, a noninvasive, wireless heart-rate monitor attached to a surcingle (see below) was placed around the horse, and a camera was installed to record the behavior. After 15 min of habituation to the test conditions, the first saliva sample (sample 1) was collected to determine the subject's initial cortisol level (“Cortisol1”) before injecting the synthetic adrenocorticotropic hormone (Fig. 1). Then, a brief (15 min) clinical evaluation was performed by a veterinarian to assess body temperature, heart rate, respiratory rate and venous filling, in order to identify any potentially dangerous anomalies, such as cardiac arrhythmias or signs of febrile infectious disease that could potentially interfere with the horses' ability to respond to the ACTH challenge test. If the subject passed the health test (43 of 44 originally selected subjects), the veterinarian injected the substance intravenously. Eleven crib-biters and respective control horses housed in the same farm were tested on the same day within 10 min of each other, and 21 horses were tested individually on different days.

During the post-injection period, lasting 3 h, six further saliva samples were collected (samples 2–7) every 30 min (Fig. 1), while the ECG trace was continuously measured with the heart-rate monitor. We also monitored the behavior of the horses continuously via video recording using a Sony HandycamHDR-CX700. In total, we obtained 7 saliva samples, as well as the ECG trace and video recordings, corresponding to the 15-min habituation period (sample 1 — period 1), and 6 × 30-min periods of test post-injection (samples 2–7—periods 2–7; Fig. 1).

2.3. Response measures

2.3.1. Physiological measures

We assessed physiological measures, linked to both the hypothalamic–pituitary–adrenal (HPA) pathway and the sympathomedullary (SAM) pathway, which were likely to be affected by the ACTH challenge test [29], at least over short-time scales. Concerning the HPA axis, we collected salivary cortisol, which has been demonstrated to be affected by ACTH challenge test [29]. Saliva was collected with Salivette cotton rolls placed loosely onto the tongue of the horse for 1 min using forceps. At the end of the test, the Salivettes were centrifuged for 6 min at 5000 rpm with a Hettich EBA 20, and were then maintained at −20°C until they were sent to the laboratory for analyses (Salimetrics, USA). Concentrations of cortisol were determined with a direct enzyme immunoassay without extraction and validated for equine saliva [35]. The Salimetrics High sensitivity salivary cortisol enzyme immunoassay kit was used for the analyses.

Concerning the SAM axis, we measured heart rate (HR) and root mean square of successive inter-beat interval difference (RMSSD) [27]. Both measures were collected using a wireless, non-invasive monitor (MLE120X Bioharness Telemetry System, Zephyr), fixed to a surcingle placed around the horse's heart girth, to obtain the ECG trace, which produces more reliable HR measures compared to alternative methods [29,32]. ECG gel was applied on the electrodes before each use. The data were then transmitted and stored in real time to a laptop using LabChart software v.7.2 (ADInstrument) for later analyses. During the tests, one experimenter was entering comments in the software indicating when the ACTH challenge test started, and when each saliva sample was collected (Fig. 1). This allowed us to measure the physiological parameters precisely for each period. We analyzed HR and RMSSD from good-quality sections with clearly visible heartbeats on the ECG trace. Section durations submitted for analyses were comparable between crib-biters and controls (crib-biters, 646.62 ± 371 s; controls, 704.63 ± 406 s per horse). We ensured visually that the software tracked the heartbeats properly before extracting HR and interheartbeat (RR) intervals (ms). RR intervals were then used to calculate RMSSD (ms). If atrioventricular blocks were observed in the signal, we excluded the respective sections of the ECG trace [33,34].

Table 1

Characteristics of the horses used in the experiment. Sex (M= mare; G = gelding, S = stallion), Group (CB = crib-biters; C = non-crib-biters (controls)), age, breed, housing system (loose housing, paddock, box; alone or in group) and place (each letter refers to a given farm). Horses 1–15 (Group A) correspond to the crib-biters that did crib-bite during ACTH challenge test, and horses 16–22 to the ones that did not crib-bite (Group B).

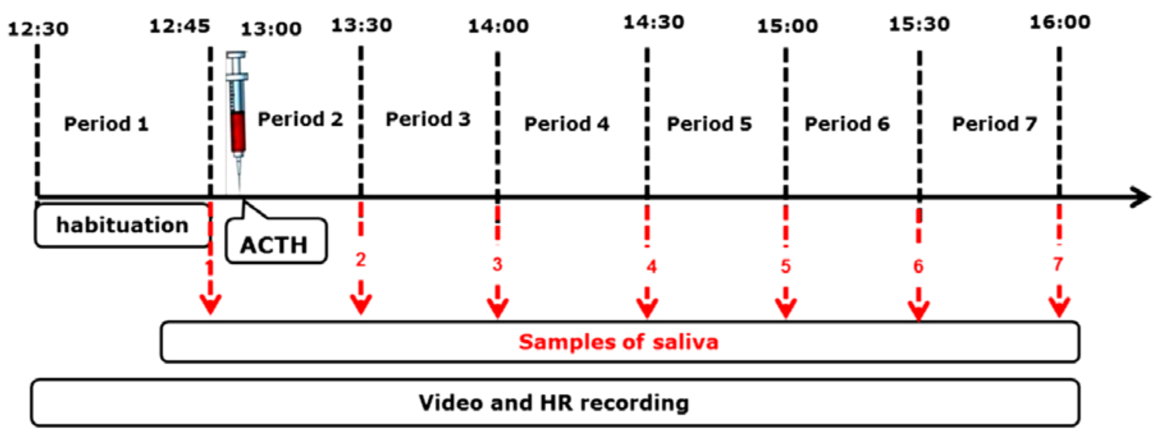
Horses	Sex	Crib-biters or controls	Age	Breed	Housing system	Alone/group	Place
1	M	CB-A	13	Shetland	Loose housing	Group	u
2	M	CB-A	6	Swiss halfbred	Box paddock	Alone	c
3	M	CB-A	22	Criollo	Box	Alone	g
4	M	CB-A	16	Franches-Montagnes	Box	Alone	y
5	M	CB-A	9	Hispano-Arabian	Box paddock	Alone	b
6	M	CB-A	5	Quarter horse	Box	Alone	s
7	M	CB-A	9	Paint horse	Box	Alone	r
8	M	CB-A	5	Paint horse	Box paddock	Alone	k
9	G	CB-A	9	Franches-Montagnes	Box	Alone	d
10	G	CB-A	11	Swiss halfbred	Box	Alone	g
11	G	CB-A	23	Franches-Montagnes	Box paddock	Group	n
12	G	CB-A	11	Franches-Montagnes	Box	Alone	bo
13	S	CB-A	9	Franches-Montagnes	Box	Alone	h
14	S	CB-A	17	Franches-Montagnes	Box	Alone	h
15	S	CB-A	15	Franches-Montagnes	Box	Alone	h
16	M	CB-B	5	Franches-Montagnes	Box paddock	Group	m
17	M	CB-B	19	Swiss halfbred	Box paddock	Alone	w
18	G	CB-B	19	Haflinger	Box paddock	Group	se
19	G	CB-B	18	Swiss halfbred	Box	Alone	a
20	G	CB-B	7	ONC	Box paddock	Alone	v
21	G	CB-B	10	English thoroughbred	Paddock	Group	d
22	S	CB-B	11	Franches-Montagnes	Box	Alone	h
23	M	C	7	Quarter horse	Box paddock	Alone	s
24	M	C	20	Friso-Arabian	Box	Alone	y
25	M	C	14	Swiss halfbred	Loose housing	Group	h
26	M	C	18	Apaloosa	Box paddock	Alone	b
27	M	C	14	Swiss halfbred	Loose housing	Group	h
28	M	C	16	Trotter	Box	Alone	h
29	M	C	18	Franches-Montagnes	Loose housing	Group	h
30	M	C	10	Swiss halfbred	Box	Alone	g
31	M	C	19	Swiss halfbred	Box paddock	Alone	w
32	G	C	4	Franches-Montagnes	Box paddock	Group	n
33	G	C	24	ONC	Box paddock	Alone	v
34	G	C	22	English thoroughbred	Paddock	Group	d
35	G	C	7	Quarter horse	Loose housing	Group	k
36	G	C	6	Franches-Montagnes	Box paddock	Alone	di
37	G	C	8	Franches-Montagnes	Box	Alone	d
38	G	C	15	Swiss halfbred	Loose housing	Group	h
39	G	C	11	Swiss halfbred	Box	Alone	h
40	G	C	12	Frison	Box paddock	Alone	se
41	S	C	3	Shetland	Loose housing	Group	u
42	S	C	17	Franches-Montagnes	Box	Alone	h
43	S	C	7	Franches-Montagnes	Box	Alone	h

2.3.2. Behavioral measures

Crib-biting events were scored directly during the tests. One experimenter was scoring as a crib-biting event the following behavior; the horse grasped a fixed object with its incisors, pulled back, contracted the neck muscles and drew air into its esophagus, emitting an audible grunt [9]. Instances when the horse performed the same behavior, but without grasping an object were also considered ("windsucking" [9]). Then, for each horse, we calculated the frequency of occurrences of crib-biting events per minute for every period (1–7; Table 2).

From the videos of the tests, we scored the physical activity (movements) of the horse, because this behavior can potentially affect physiological parameters [27,30,36]. The duration of body movements was scored continuously ("State Events") using the Observer software XT v.11 (Noldus), and considered when the horse performed more than two steps [37]. We then calculated the proportion of the total time spent performing the behavior.

Fig. 1. Experimental procedure for the ACTH challenge test. The black dotted lines indicate the time at which each period started and ended (periods 1–7). The syringe indicates when the ACTH injection took place. The red dotted lines designate when the saliva samples were collected (1–7). Video and ECG trace were recorded continuously, as indicated by the black bar.



2.4. Statistical analysis

To compare the physiological reaction of crib-biters and controls to the ACTH challenge test, we tested for group differences in cortisol increase (HPA axis), HR and RMSSD (SAM axis), using linear mixed-effects models (LMM; lme function, nlme library; [38,39]).

To calculate the cortisol increase (hereafter "Icortisol") in response to the ACTH challenge test, for each horse and each period, we subtracted the initial value, Cortisol1 (sample 1 — period 1) from its value measured at the end of each 30-min period 2–7 (i.e. after injection; samples 2–7 in Fig. 1; [40]). Cortisol1 is the value for cortisol after habituation and before Synacthen injection (Fig. 1). In order to test for group differences in HR and RMSSD while controlling for initial values before injection, we calculated HR and RMSSD ratios (hereafter "rHR" and "rRMSSD" respectively), by dividing the average HR and RMSSD values for each period (2–7) by the initial values before injection (HR1 and RMSSD1; period 1). The HR1 and RMSSD1 values are the average values of HR and RMSSD for period 1 (Fig. 1).

Table 2

Crib-biting events performed by the crib-biters for each period.

Mean ± SD of occurrences of crib-biting events for each period (period 1 (habituation)–period 7; N = 22 horses; indicated in number of events per min).

Crib-biting (nb/min)		
Period	**Mean**	**SD**
1	1.55	2.22
2	0.66	0.91
3	0.69	1.04
4	0.96	1.35
5	0.77	1.13
6	0.79	1.02
7	0.73	1.25

First, we carried out a series of models on the initial values before injection (Cortisol1, HR1 or RMSSD1). These LMMs included Cortisol1, HR1 or RMSSD1 as a response variable (3 separate models), the sex and age of the horses, the housing system (control factors) and the group (crib-biters or controls) as fixed factors. To control for differences between farms, the identity of the farms where the horses were housed was included as a random factor. Second, we carried out another series of models on the values collected after injection (Icortisol, rHR or rRMSSD). These LMMs included Icortisol, rHR, rRMSSD, or movements as a response variable (4 separate models). The sex, the age and the housing system of the horses (control factors), the period (1–7) and the group (crib-biters or controls), as well as the interaction term between period and group, were included as fixed factors. Finally, to control for repeated measurements of the same subjects and for farm differences, the identities of the horses nested within the farms where they were housed were included as random factors. As the frequency of crib-biting varies between and even within horses [13], it turned out that seven crib-biting horses did not perform the stereotypic behavior during the ACTH challenge test.We thus then reran the same LMMs including the Icortisol, rHR or rRMSSD, and movements as a response variable (4 separate models), and the same fixed and random factors as mentioned above, to compare the crib-biters that did crib-bite during the test (Group A: 15 horses, Table 1—horses 1–15 and Table 3), the crib-biters that did not crib-bite during the test (Group B: 7 horses, Table 1—horses 16–22 and Table 3) and the controls (Group C; 21 horses, Table 1—horses 23–43 and Table 3). Then, two-by-two comparisons between the three groups were carried out using LMMs including the same fixed and random factors as in the model carried out the three groups together. We applied a Tukey correction (function glht, package multcomp in R, Multiple comparisons of means) for these posthoc tests.

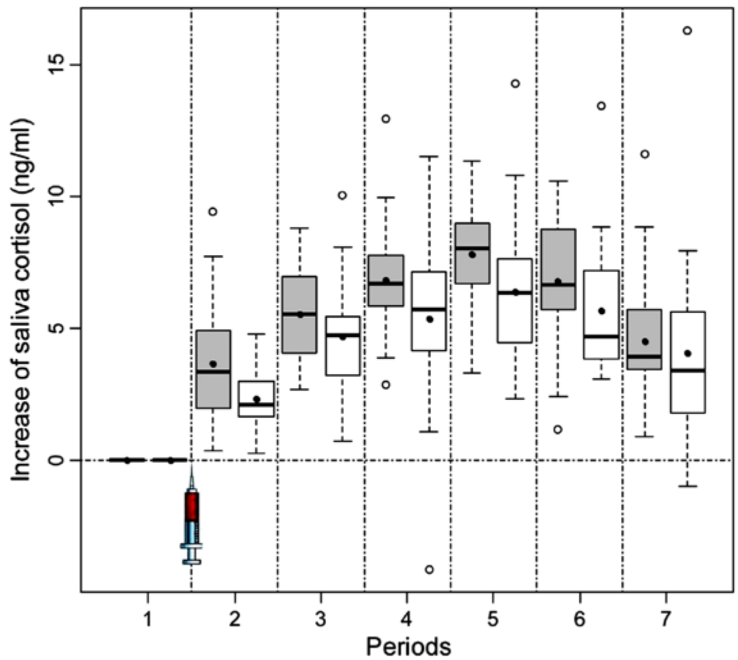
Using a standard model simplification procedure, we removed each non-significant interaction term or control factor, until the deletion did cause a reduction in goodness of fit (in this case, the term was left in the model). The residuals were checked graphically for normal distribution and homoscedasticity. To satisfy model assumptions, we used a log transformation for RMSSD1, rHR and rRMSSD. Because of a technical problem with a defective Bioharness unit, we only obtained HR and RMSSD measures on 9 crib-biters and 20 controls. Therefore, sample sizes vary between the analyses on the HPA and SAM axis responses (Table 3). Additionally, because of a technical problem with one of the videos, we scored movements on 21 crib-biters and 21 controls. The significance level of the factors was set at α = 0.05. All means are given with standard errors.

Table 3

Mean and standard deviation for SAM and HPA axis measures, and results of the models investigating the effects of various factors on the HPA and SAM axis measures. Group (CB=crib-biters, C=non-crib-biters (controls), A=crib-biters that crib-bit, B=crib-biters that did not crib-bite during the ACTH challenge test); Mean, median and standard deviation (SD) for the following measures: Cortisol1=initial value of cortisol for the period 1 (sample 1, Fig. 1), Icortisol=average cortisol increase over periods 2–7, HR1 and RMSSD1=average initial values of HR and RMSSD for the period 1, rHR and rRMSSD=average HR and RMSSD ratio; Linear mixed effects models investigating the effects of the group, the period (1 or 2–7), the sex (gelding, mare or stallion), the age (3 to 24 years old) and the housing system on the physiological measures. Only the effects of the parameters kept after the model selection procedure are shown. Significant results are shown in bold.

Group comparison	Response variables/measures	Group	N	Mean/median	SD	Fixed effect	F(df)	p value
	Cortisol1 (ng/ml)	C	21	1.03/0.56	1.60			
		CB	22	0.74/0.77	0.24	Group	0.78 (1,23)	0.40
		C	21	4.76/4.34	3.04			
	Icortisol (ng/ml)					Group	7.15 (1,21)	**0.014**
		CB	2	5.84/5.8	2.6	Sex	3.20 (2,21)	0.060
						Period	12.81 (1211)	**0.0004**
	HR1 (BPM)	C	20	40.25/39.7	4.27			
		CB	9	38.83/40.19	5.26	Group	0.71 (1,12)	0.42
CB-C	rHR	C	20	1.07/1.04	0.15			
		CB	9	1.10/1.07	0.16	Group	0.90 (1,12)	0.36
	RMSSD1 (ms)	C	20	46.63/44.20	15.46	Period	205.27 (1,144)	**<0.0001**
		CB	9	50.03/40.89	23.15	Group	1,2 (1,9)	0.32
	rRMSSD	C	20	1.06/1.00	0.34	Sex	5.00 (3,9)	**0.030**
		CB	9	1.07/0.95	0.42	Group	0.004 (1,12)	0.95
		C	21	1.03/0.56	1.60	Period	9.70 (1,144)	**0.002**
	Cortisal1 (ng/ml)	A	15	0.74/0.76	0.26			
		B	7	0.72/0.77	0.21	Group	0.40 (2,22)	0.70
		C	21	4.76/4.34	3.04			
	Icortisol (ng/ml)	A	15	5.58/5.75	2.69	Group	3.87 (2,20)	**0.038**
		B	7	6.44/6.14	2.38	Sex	3.15 (2,20)	0.065
		C	20	40.25/39.7	4.27	Period	12.82 (1,211)	**0.0004**
	HR1 (BPM)	A	6	38.64/40.72	5.57			
		B	3	39.22/39.94	5.75	Group	0.34 (2,11)	0.72
		C	20	1.07/1.04	1.15			
C-A-B	rHR	A	6	1.13/1.11	0.17	Group	2.12 (2,11)	0.17
		B	3	1.03/1.01	0.13	Period	205.27 (1,144)	**<0.0001**
		C	20	46.63/44.19	15.46	Group	0.74 (2,9)	0.51
	RMSSD1 (ms)	A	6	46.63/45.36	13.27	Sex	6.94 (2,9)	**0.020**
		B	3	56.84/34.36	39.98	Group	0.38 (2,11)	0.70
		C	20	1.06/1.00	0.34	Period	9.70 (1,144)	**0.002**
	rRMSSD	A	6	1.14/0.94	0.50			
		B	3	0.94/0.97	0.21			

Fig. 2. Cortisol response (HPA axis) to the ACTH challenge test in crib-biters and control horses. Increase in saliva cortisol between each period (2–7) and the habituation (sample1 — period 1) for crib-biters (Group CB; N=22 horses, gray) and control horses (Group C; N=21 horses, white); box-and-whiskers plot (the horizontal line shows the median, the box extends from the lower to the upper quartile, and the whiskers to 1.5* the interquartile range above the upper quartile or below the lower quartile). The black dots indicate the means. The syringe indicates when the ACTH injection took place.



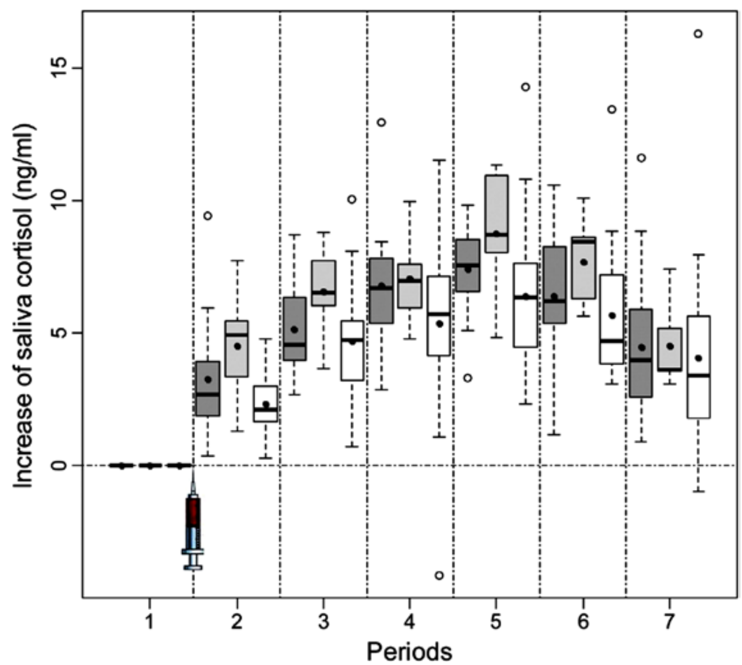
3. Results

3.1. HPA axis response

We analyzed the physiological responses of 43 horses to ACTH injections. We did not find any significant difference in Cortisol1 between groups (no effect of the group CB-C on Cortisol1, Table 3). However, there was an overall increase following ACTH injections (effect of the period on Icortisol for CB-C; Table 3; Fig. 2) Cortisol concentrations increased significantly more strongly in crib-biting horses than in controls relative to Cortisol1 (effect of the group CB-C on Icortisol; Table 3; Fig. 2; see Supplementary material 1 for raw values). Stallions tended to have lower cortisol increases than geldings and mares (effect of sex on Icortisol for CB-C; Icortisol: stallions, 4.01 ± 2.49 ng/ml; geldings, 5.63 ± 3.0 ng/ml; mares, 5.5 ± 2.87 ng/ml; Table 3)

During the experiment, only 15 of 22 crib-biters displayed stereotypic behavior (crib-biting). We thus compared cortisol levels between controls (Group C) and crib-biters that crib-bit (Group A) and did not crib-bite (Group B) during the test. The three groups did not differ in Cortisol1 (no effect of the group C-A-B on Cortisol1; Table 3), but differed significantly in their cortisol increase (effect of the group C-A-B on Icortisol; Table 3; Fig. 3; see Supplementary material 1 for raw values). Again, in the same way as for the analyses testing differences between crib-biters and controls (comparison between groups CB-C), there was a significant cortisol increase following ACTH injections (effect of the period on Icortisol for C-A-B; Table 3; Fig. 3; see Supplementary material 1 for raw values), and sex tended to affect cortisol increase (effect of the sex on Icortisol for C-A-B; Table 3). Post-hoc comparisons showed that Group B had a significantly higher cortisol increase than Group C (Table 3; Fig. 3; Multiple comparisons of means $Z = -2.44$, $N = 28$, p = 0.038). However, we did not find any difference between Group A and Group C (Table 3; Fig. 3; Multiple comparisons of means Z = −1.98, N =36, p = 0.11), nor between Groups A and B (Table 3; Fig. 3; Multiple comparisons of means Z = 0.86, p = 0.66). For the LMM carried out on Cortisol1, the age, sex and housing system were removed during model selection. For the LMM carried out on Icortisol, neither the interaction between group and period, nor the age and housing system significantly affected cortisol increase. These terms were thus removed during model selection.

Fig. 3. Cortisol response (HPA axis) to the ACTH challenge test in crib-biters that did or did not crib-bite and control horses. Increase in saliva cortisol between each period (2–7) and the habituation (sample 1 — period 1) for crib-biters that did crib-bite during the test (Group A; N = 15 horses, dark gray) crib-biters that did not crib-bite during the test (Group B; N = 7 horses, light gray) and control horses (Group C; N = 21 horses, white); box-and-whiskers plot (the horizontal line shows the median, the box extends from the lower to the upper quartile, and the whiskers to 1.5* the interquartile range above the upper quartile or below the lower quartile). The black dots indicate the means. The syringe indicates when the ACTH injection took place.



3.2. SAM axis response

We analyzed the heart-rate responses of 29 horses during the test (periods 1–7). There was no difference between the HR1 of crib-biters and controls (no effect of the group CB-C on HR1; Table 3). However, rHR (ratio between HR values at each period and the HR1 value) significantly varied between periods (effect of the period on rHR for CB-C; Table 3). When comparing rHR between controls (Group C) and crib-biters that did (Group A) or did not (Group B) show stereotypic behavior during the test, we found no significant differences between the three groups in their HR1 or rHR (no effect of the group C-A-B on HR1 or rHR; Table 3).

Finally, there was no difference in RMSSD1 between crib-biters and controls (no effect of the group CB-C on RMSSD1; Table 3). However, rRMSSD (ratio between RMSSD values at each period and the RMSSD1) significantly varied between periods (effect of the period on rRMSSD for CB-C; Table 3). Similarly as for rHR, rRMSSD did not differ between groups C, A and B (no effect of the group C-A-B on rRMSSD; Table 3). Stallions tended to have higher RMSSD1 than geldings and mares (effect of sex on RMSSD1 for CB-C and C-A-B; RMSSD1: stallions, 66.54±20.19 ms; geldings, 38.9±12.01 ms; mares, 41.52±13.65ms; Table 3). For all the LMM on the SAM axis response, neither the interaction between group and period, nor the age, sex (except for RMSSD1) and housing system, significantly affected HR1, rHR, RMSSD1 and rRMSSD. These terms were thus removed during model selection.

3.3. Behavioral measures

We analyzed the movements of 42 horses during the test (periods 1–7).We did not find any significant difference in movement between groups (CB versus C; LMM: $F_{1,36} = 2.62$, $p = 0.11$; comparison C-A-B; LMM: $F_{2,35} = 1.48$, $p = 0.24$), but we found a significant effect of the housing system on this parameter (effect of the housing system for CB-C; LMM: $F_{4,36} = 6.11$, $p = 0.0007$; for C-A-B; LMM: $F_{4,35} = 6$, $p = 0.0009$). The interaction between group and period, the age and sex of the horses did not affect significantly the movements. These terms were thus removed during model selection.

4. Discussion

Stereotypic behavior affects many domesticated species and other animals kept in captivity. The function of stereotypic behaviors is still largely unknown but it has been proposed to help affected individuals in dealing with stressful situations [18,41]. In this study, we addressed this stress-coping hypothesis in a large sample of horses, half of which showed crib-biting, one of the main stereotypies in domestic horses.

We induced stress experimentally, by injecting synthetic ACTH. Because some of the stereotypic horses did not crib-bite during the test, we could then investigate differences in the physiological responses of three groups of animals (individuals identified as crib-biters, which responded by crib-biting or not to the test, and control horses).We collected measures related to the SAM and HPA stress axes. We did not find any group difference in terms of SAM axis measures (HR and RMSSD). However, we found significant differences in the HPA axis measures, with crib-biters showing higher cortisol responses than controls. More importantly, we also found that the difference between crib-biters and controls was mainly due to the seven crib-biters that did not crib-bite during the test, whereas crib-biters that showed stereotypic behavior during the test (N=15) had cortisol levels that were indistinguishable from control animals. Our results suggest that the presence of stereotypic behavior in horses is linked to differences in HPA axis response. These differences could be either inherited, caused by chronic stress or due to the long-term performance of the stereotypic behavior. Our results also suggest that crib-biting might be an effective coping strategy that helps stereotypic individuals to gain control over stressful situations, in order to reduce their cortisol levels. We conclude that preventing stereotypic horses from crib-biting could be counterproductive, because this behavior, once established, might have some beneficial effects for the animals.

4.1. HPA axis response of crib-biters and controls

Our results show that the HPA axis stress response differs between crib-biting and non-crib-biting horses. The activation of the HPA axis is an adaptive mechanism that helps to maintain physiological stability in response to stressful stimuli. Repeated or chronic exposure to stress can induce changes in HPA axis function [33]. Because of the suggestion that crib-biters are more proactive than controls [6], we had hypothesized that these horses would have a lower HPA axis response to the ATCH challenge test (i.e. lower cortisol values) than other horses. However, contrary to our hypothesis, crib-biters had a higher cortisol increase than controls. Increased or maintained HPA responses to novel stressors are often observed in chronically stressed animals compared to control animals [33]. A higher HPA axis response could result from a “facilitation process”. This process results in an enhanced glucocorticoid (GC) response to a stressor in “acclimated” (i.e. animals that no longer respond in the same robust manner to chronic stressors) compared to “non-acclimated” animals [34]. Therefore, the high cortisol responses of crib-biters in our study could result from a “facilitation process” linked to chronic stress.

An alternative suggestion to the “facilitation process” hypothesis is that the changes that we observed in the HPA-axis response could be due to the long-term performance of the stereotypic behavior. In fact, corticosteroid hormones may have differential effects during the early and fully developed stages of a stereotypy [24]. It has been suggested that stress levels and high corticosteroids enhance the acquisition and expression of stereotypies, whereas an already-developed stereotypy may reduce corticosteroid levels [24]. It would be interesting, as suggested elsewhere [20,22,30,47], to perform a longitudinal study in order to establish whether the development of crib-biting leads to a reduction of cortisol levels from even higher original levels, and whether a transient peak in stress level occurs prior to the emergence of stereotypic behavior.

Reactive coping animals have a higher HPA axis reactivity and react with a higher cortisol response than proactive ones [4]. The higher cortisol response we observed in crib-biting horses therefore suggests that these individuals are, contrary to our hypothesis, more reactive than non-stereotypic horses. Hyperactivity of the HPA axis is also a characteristic of major depression; similar HPA axis modifications can be observed after repeated exposure to different stress procedures [42].

Finally, the observed difference in HPA axis stress response between crib-biters and controls might be related to genetic factors. Some studies have found genetic predispositions to crib-biting, which could explain why some horses but not others, develop this stereotypy after a similar period of chronic stress [15,43,44]. For instance, wind-sucking has been shown to occur more frequently in some pedigrees than others [44]. Vecchiotti and Galanti [15] reported an incidence rate of 7.4% of stereotypic behaviors in Italian thoroughbreds, and concluded that the genetic transmission of these behaviors is similar to some human mental disorders involving polygenic inheritance. Recently, Hemmann [43] found an unusually high prevalence of crib-biting in a small Finnhorse population, again suggesting that horses might inherit behavioral susceptibility to develop stereotypy. Other studies reported stress-induced alterations in the central nervous system (CNS) dopamine physiology in stereotypic animals [18,45,46]. This suggests that such alteration or sensitization in the CNSmay be the result of chronic stress in combination with a genetic predisposition. Based on the literature and our own results, we could hypothesize that this inherited behavioral susceptibility consists in a higher sensitivity to stress in crib-biting horses compared to nonstereotypic ones due to differences in HPA axis reactivity.

4.2. HPA axis response of crib-biters that did and did not crib-bite

Our results show that the differences in cortisol increase in response to the ACTH challenge test between crib-biters and controls were largely due to the crib-biters that did not perform the stereotypy during the test. Indeed, only the stereotypic horses that did not crib-bite during the test had a higher HPA axis reactivity than the controls. By contrast, there was no difference in cortisol response between the crib-biters that did crib-bite during the test and the controls. These promising results suggest that the stereotypic horses that did crib-bite during the test might have developed and installed a successful coping strategy that helped them to gain control and reduce cortisol levels during stressful situations. These results are in accordance with McBride and Cuddeford [18], who found a reduction in cortisol levels following bouts of crib-biting.

Previous studies that have investigated whether stereotypies lowered arousal and anxiety as well as corticosteroid levels, did not find consistent results [7,17–20]. If crib-biting is indeed a coping mechanism, animals should show signs of stress in situations in which they are prevented from crib-biting. McBride and Cuddeford [18] placed a collar, which prevented crib-biting, on crib-biters and controls. These authors showed differences in physiological stress responses between restricted and non-restricted horses, but could not conclude about the functionality of crib-biting, because the use of the collar also triggered a physiological stress response in the control horses. McGreevy and Nicol [20] found higher mean baseline levels of cortisol in crib-biters than in controls. However, this study did not find any significantly higher rise in cortisol levels in crib-biters transiently prevented from performing this stereotypy by removing a bar on which they could perform crib-biting, than in controls [20]. Indeed, an increase in plasma cortisol levels was found in both stereotypic and controls, when they were deprived of ad libitum hay and, for crib-biters, of the opportunity to crib-bite for 24 h.

The fact that the cortisol response in crib-biters that did show the behavior during the test was similar to the response of controls, whereas those that did not crib-bite had a higher response, suggests that preventing stereotypic horses from crib-biting, without reducing underlying motivation, could be counter-productive. Our results imply that crib-biting, once installed, has beneficial effects for the individual. Mason and Latham [8] discussed the link between welfare and stereotypies and concluded that in some cases, the performance of fully developed stereotypies could improve welfare. For example, stereotypies performed as “mantra effects”, which help an individual to calm itself through repetition, decrease arousal. We could hypothesize that crib-biting has a similar effect on some horses. However, Mason and Latham [8] also warned against generalization. Indeed, stereotypies may have different underlying causes and mechanisms and are rarely comparable among species. For example “perseveration” is another process that could underlie stereotypies and in this case, it indicates altered behavioral control [8]. It is thus important, as Mason and Latham [8] suggested, to understand the mechanisms underlying stereotypies before implying a link with good, neutral or poor welfare of the animals.

Thirty-two percent of crib-biters did not crib-bite during the ACTH challenge test. The different responses between these horses and the crib-biters that did crib-bite could be explained by the fact that cribbiters might have been at different stages of the development of their stereotypies [8]. Horses that did crib-bite could have fully-developed stereotypies, while non-crib-biting individuals might be at an early stage of development [24]. However, the owners reported that the horses that did not crib-bite in the study had been crib-biting for 8 years on average (range = 2–15 years). Alternatively, horses could have developed more or less strong stereotypies. Indeed, the performance of stereotypic behavior varies between horses in term of the daily percentage of time occupied by the activity [13]. Thus, the fact that some horses did not crib-bite during our test could be explained by a generally lower propensity to crib-bite. We suggest that it may be necessary to not only take into account how long the behavior has been present in an animal, but also at what frequency the behavior is performed. We also suggest that the crib-biters that did crib-bite during our test were at the stage when full-blown stereotypies serve their coping function of reducing stress.

4.3. SAM axis response of crib-biters and controls

We did not find any difference in HR or RMSSD ratio (i.e. ratio between HR or RMSSD values at each period and the value before injection) in response to our ACTH challenge test between crib-biters and controls. One explanation could be the small sample of crib-biters for which we were able to measure HR (N = 9). Studies investigating the effect of crib-biting on the SAM axis reactivity found similar results for HR as we found for the HPA axis. For instance, HR was shown to decrease during bouts of crib-biting [17,19]. Differences between cribbiters and controls in heart rate variability have also been found in other studies [7]. In fact, crib-biting horses seem to have a reduced reactivity range of the autonomic nervous system. Crib-biting horses may therefore not be capable to react as efficiently as other horses to an external stimulus, suggesting that crib-biters are more stress-sensitive and less flexible when coping with stress [7]. By contrast, and in accordance with our results, other studies did not find any significant difference in the mean HR or in the HR variability between crib-biters and other horses [18,30,47,48].

4.4. Behavioral activity of crib-biters and controls

We did not find any difference in the physical activity (movements) during the ACTH challenge test between crib-biters and controls. Therefore, we can suggest that the difference in the HPA axis found between crib-biters and controls is not the result of a difference in activity during the test [27].

5. Conclusion

Our results suggest that crib-biters differ from controls in their HPA axis reactivity. Further experiments need to address whether this difference is a consequence of chronic stress, or if a genetic difference could predispose horses to develop such abnormal behavior. Indeed, many studies have mentioned the importance of longitudinal studies required to investigate the HPA axis function during the development of stereotypic behavior [20,22,30,47]. In fact, it is possible that horses develop stereotypies in order to cope with stressful situations, and the stereotypy itself could be part of a coping process. If stereotypic behavior is really a coping mechanism, then any attempt to prevent stereotypic horses to crib-bite would be counter-productive. In some cases, the performance of stereotypies, once developed, could even improve welfare. These hypotheses will require further testing taking into account the possibility that there may be important individual differences in whether and how stereotypic behavior can reduce stress.

Acknowledgments

We are grateful to all the owners of the horses, who offered to participate in this study, and to Lorenz Gygax for statistical advice. EFB and ALM are funded by a Swiss National Science Foundation fellowship (grant number : PZ00P3_148200).

References

[1] A. Boissy, G. Manteuffel, M.B. Jensen, R.O. Moe, B. Spruijt, L.J. Keeling, C.Winckler, B. Forkman, I. Dimitrov, J. Langbein, M. Bakken, I. Veissier, A. Aubert, Assessment of positive emotions in animals to improve their welfare, Physiol. Behav. 92 (2007) 375–397.

[2] J.M. Koolhaas, A. Bartolomucci, B. Buwalda, S.F. de Boer, G. Flügge, S.M. Korte, P. Meerlo, R. Murison, B. Olivieri, P. Palanza, G. Richter-Levin, A. Sgoifo, T. Steimer, O. Stiedl, G. van Dijk, M. Wöhr, E. Fuchs, Stress revisited: a critical evaluation of the stress concept, Neurosci. Biobehav. Rev. 35 (2011) 1291–1301.

[3] R. Axelrod, T.D. Reisine, Stress hormones: their interaction and regulation, Science 224 (1984) 452–459.

[4] J.M. Koolhaas, S.F. De Boer, C.M. Coppens, B. Buwalda, Neuroendocrinology of coping styles: towards understanding the biology of individual variation, Front. Neuroendocrin. 31 (2010) 307–321.

[5] C. Touma, M. Bunck, L. Glasl, M. Nussbaumer, R. Palme, H. Stein, M. Wolferstätter, R. Zeh, M. Zimbelmann, F. Holsboer, R. Landgraf, Mice selected for high versus low stress reactivity: a new animal model for affective disorders, Psychoneuroendocrinology 33 (2008) 839–862.

[6] C.L. Ijichi, L.M. Collins, R.W. Elwood, Evidence for the role of personality in stereotypy predisposition, Anim. Behav. 85 (2013) 1145–1151.

[7] I. Bachmann, P. Bernasconi, R. Herrmann, M.A. Weishaupt, M. Stauffacher, Behavioural and physiological responses to an acute stressor in crib-biting and control horses, Appl. Anim. Behav. Sci. 82 (2003) 297–311.

[8] G. Mason, N. Latham, Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim. Welf. 13 (2004) 57–69.

[9] P.D. McGreevy, P.J. Cripps, N.P. French, L.E. Green, C.J. Nicol, Management factors associated with stereotypic and redirected behaviour in the thoroughbred horse, Equine Vet. J. 27 (1995) 86–91.

[10] E. Möstl, R. Palme, Hormones as indicators of stress, Domest. Anim. Endocrinol. 23 (2002) 67–74.

[11] G.J. Mason, Stereotypies and suffering, Behav. Process. 25 (1991) 103–115.

[12] G.J.Mason, J. Rushen, Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare, Second ed. CAB International, Wallingford OXON, 2006.

[13] K.A. Houpt, S.M.McDonnell, Equine stereotypies, Compend. Contin. Educ. 15 (1993) 1265–1271.

[14] U.A. Luescher, D.B. McKeown, J. Halip, A cross-sectional study on compulsive behavior (stable vices) in horses, Equine Vet. J. 27 (1991) 14–18.

[15] G.G. Vecchiotti, R. Galanti, Evidence of heredity of cribbing, weaving and stall walking in thoroughbred horses, Live. Prod. Sci. 14 (1986) 91–95.

[16] K. Nagy, G. Bodo, G. Bardos, A. Harnos, P. Kabai, The effect of a feeding stress-test on the behaviour and heart rate variability of control and crib-biting horses (with or without inhibition), Appl. Anim. Behav. Sci. 121 (2009) 140–147.

[17] D. Lebelt, A.J. Zanella, J. Unshelm, Physiological correlates associated with cribbing behaviour in horses: changes in thermal threshold, heart rate, plasma endorphin und serotonin, ß, Equine Vet. J. Suppl. 27 (1998) 21–27.

[18] S.D. McBride, D. Cuddeford, The putative welfare-reducing effects of preventing equine stereotypic behaviour, Anim. Welf. 10 (2001) 173–189.

[19] M. Minero, E. Canali, V. Ferrante, M. Verga, F.O. Odberg, Heart rate and behavioural responses of crib-biting horses to acute stressors, Vet. Rec. 145 (1999) 430–433.

[20] P.D. McGreevy, C. Nicol, Physiological and behavioral consequences associated with short-term prevention of crib-biting in horses, Physiol. Behav. 65 (1998) 15–23.

[21] A. Sarrafchi, H.J. Blokhuis, Equine stereotypic behaviors: causation, occurrence, and prevention, J. Vet. Behav. Clin. Appl. Res. 8 (2013) 386–394.

[22] C.L. Wickens, C.R. Heleski, Crib-biting behavior in horses: a review, Appl. Anim. Behav. Sci. 128 (2010) 1–9.

[23] M. Budzynska, Stress reactivity and coping in horse adaptation to environment, J. of Equine Vet. Sci. 34 (2014) 935–941.

[24] J.M. Koolhaas, S.M. Korte, S.F. De Boer, B.J. Van Der Vegt, C.G. Van Reenen, H. Hopster, I.C. De Jong,M.A.W. Ruis, H.J. Blokhuis, Coping styles in animals: current status in behavior and stress-physiology, Neurosci. Biobehav. Rev. 23 (1999) 925–935.

[25] S.C. Seaman, H.P.B. Davidson, N.K.Waran, How reliable is temperament assessment in the domestic horse (Equus caballus)? Appl. Anim. Behav. Sci. 78 (2002) 175–191.

[26] H. Hopster, R.M. Bruckmaier, J.T.N. Van der Werf, S.M. Korte, J. Macuhova, G. Korte-Bouws, C.G. Van Reenen, Stress responses during milking; comparing conventional and automatic milking in primiparous dairy cows, J. Dairy Sci. 85 (2002) 3206–3216.

[27] E. von Borel, J. Langbein, G. Després, S. Hansen, C. Leterrier, J. Marchant-Forde, R. Marchant-Forde, M. Minero, E. Mohr, A. Prunier, D. Valance, I. Veissier, Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals — a review, Physiol. Behav. 92 (2007) 293–316.

[28] P.D. McGreevy, Equine Behaviour: A Guide for Veterinarians and Equine Scientists, Saunders, London, UK, 2004.

[29] M. Peeters, S. Sulon, J.F. Beckers, D. Ledoux, M. Vandenheede, Comparison between blood serum and salivary cortisol concentrations in horses using adrenocorticotropic hormone challenge, Equine Vet. J. 43 (2011) 487–493.

[30] S.M. Pell, P.D. McGreevy, A study of cortisol and beta-endorphin levels in stereotypic and normal thoroughbreds, Appl. Anim. Behav. Sci. 64 (1999) 81–90.

[31] C.L. Carroll, P.J. Huntington, Body scoring and weight estimation of horses, Equine Vet. J. 20 (1998) 41–45.

[32] M. Parker, D. Godwin, R.A. Eager, E.S. Edhead, D.J. Arlin, Comparison of Polar© heart rate interval data with simultaneously recorded ECG signals in horses, Comp. Ex. Physiol. 6 (2010) 137–142.

[33] S. Bhatnagar, C. Vining, Facilitation of hypothalamic–pituitary–adrenal responses to novel stress following repeated social stress using the resident/intruder paradigm, Horm. Behav. 43 (2003) 158–165.

[34] M.L. Romero, Physiological stress in ecology: lessons from biomedical research, Trends Ecol. Evol. 19 (2004).

[35] A. Schmidt, E. Möstl, C. Wehnert, J. Aurich, J. Müller, C. Aurich, Cortisol release and heart rate variability in horses during road transport, Horm. Behav. 57 (2010) 209–215.

[36] A.S.T. Willener, L.G. Halsey, S. Strike, M. Enstipp, J. Georges, Y. Handrich, Reassessment of the cardio-respiratory stress response, using the king penguin as a model, Stress 0 (2014) 1–6.

[37] T. Young, E. Creighton, T. Smith, C. Hosie, A novel scale of behavioural indicators of stress for use with domestic horses, Appl. Anim. Behav. Sci. 140 (2012) 33–43.

[38] J.C. Pinheiro, D.M. Bates, Mixed-Effects Models in S and S-PLUS, Springer-Verlag, New York, USA, 2000.

[39] R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria (2013).

[40] A. Patt, L. Gygax, B. Wechsler, E. Hillmann, R. Palme, N.M. Keil, Behavioural and physiological reactions of goats confronted with an unfamiliar group either when alone or with two peers, Appl. Anim. Behav. Sci. 146 (2013) 56–65.

[41] A.K. Engel, A.N. Gross, S.H. Richter, J. Rommen, C. Touma, H. Würbel, Variation in stress reactivity affects cage-induced stereotypies in female CD-1 (ICR) mice, App. Anim. Behav. Sci. 133 (2011) 101–108.

[42] A. Raone, A. Cassanelli, S. Scheggi, R. Rauggi, B. Danielli, M.G. De Montis, Hypothalamus–pituitary–adrenal modifications consequent to chronic stress exposure in an experimental model of depression in rats, Neuroscience 146 (2007) 1734–1742.

[43] K. Hemmann, M. Raekallio, O. Vainio, J. Juga, Crib-biting and its heritability in Finnhorses, Appl. Anim. Behav. Sci. 156 (2014) 37–43.

[44] T. Hosoda, On the heritability of susceptibility to wind-sucking in horses, Jpn. J. Zootech. Sci. 22-25 (1950).

[45] H. Wurbel, Ideal homes? Housing effects on rodent brain and behaviour, Trends Neurosci. 24 (2011) 4.

[46] S.D. McBride, A. Hemmings, A neurologic perspective of equine stereotypy, J. Equine Vet. Sci. 29 (2009) 10–16.

[47] H.A. Clegg, P. Buckley, M.A. Friend, P.D. McGreevy, The ethological and physiological characteristics of cribbing and weaving horses, Appl. Anim. Behav. Sci. 109 (2008) 68–76.

[48] C. Fureix, H. Benhajali, S. Henry, A. Bruchet, A. Prunier, M. Ezzaouia, C. Coste, M. Hausberger, R. Palme, P. Jego, Plasma cortisol and fecal cortisol metabolites concentrations in stereotypic and non-stereotypic horses: do stereotypic horse cope better with poor environmental conditions? BMC Vet. Res. 9 (2013) 1746–6148.