Behavioural and physiological responses to an acute stressor in crib-biting and control horses

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Abstract

The responses of eleven pairs of crib-biting and non-crib-biting horses (controls) to an arousal-inducing stimulus were studied. Video-observation of the horses revealed that crib-biting horses spent between 10.4 and 64.7% of their stabling time performing the stereotypy. During the first 2 days of an experimental period, the horses were conditioned to receive food from a special bucket. On the third day the food bucket was presented, but the horses were not allowed to feed. Arousal behaviour and crib-biting intensity as well as plasma cortisol concentration, heart rate (HR) and heart rate variability (HRV) were recorded at rest, and during and after presentation of the food stimulus. The stimulus induced a significant increase of HR and arousal behaviour in crib-biters and in controls, whereas the crib-biting frequency decreased. Power spectral analysis of the HRV revealed significant differences between crib-biters and controls at rest: crib-biters had a lower vagal tone (high frequency component, HF) and a higher sympathetic tone (low frequency component, LF) than controls. The lower basal parasympathetic activity might be an indication why crib-biting horses, in contrast to the controls, showed neither a significant decrease of the HF component during presentation of the food stimulus nor an increase of the HF component after presentation. Thus, there might be differences in the tuning of the autonomous nervous system and of the stress reactivity in crib-biting and in control horses. The results suggest that the crib-biting horses are more stress sensitive and physiologically and psychologically less flexible than the control horses.

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Keywords: Horse welfare; Stereotypies; Crib-biting; Pituitary-adrenocortical axis; Sympatho-adrenomedullar axis; Stress response; Stress sensibility

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1. Introduction

By definition, stereotypical behaviour is repetitive, invariant in form and has no obvious goal or function (Mason, 1991). Yet, the absence of a proximate function is often difficult to determine. It has been suggested that stereotypies may have beneficial consequences, which reinforce their performance (Cronin et al., 1985; Wiepkema et al., 1987; Dantzer, 1991; Mason, 1991). Without relieving an animal from actual suffering, such a coping effect would reduce the stress to keep it within optimal physiological and psychological limits (Wiepkema et al., 1987; Ödberg, 1989; Fraser and Broom, 1990). However, the results from studies on stereotypic behaviour and stress are controversial (Wechsler, 1995). Experiments preventing animals from performing stereotypic behaviour showed no evidence that stereotypies lead to reduced stress (Schouten et al., 1991; Terlouw et al., 1991; Würbel and Stauffacher, 1996). These inconsistencies could be interpreted in a way that stereotypies may differ greatly in their causation, in their aetiology, and in their potential prevention, regarding the process of development from a source behavioural pattern to an established stereotypy (Mason, 1991). Therefore, potential “coping” mechanisms underlying the performance of stereotypies should be studied separately with respect to species and to stereotyped behaviour pattern.

With a prevalence of 2.1%, crib-biting is among the most frequently observed stereotypies in the Swiss horse population (Bachmann and Stauffacher, 2002). It has been suggested that crib-biting might help to reduce stress and quicken adaptation to a stressor (Dodman et al., 1987; Lebelt et al., 1998; Minero et al., 1999). Regularly occurring stress as well as arousal could be a predisposing factor for the development of stereotypies, or trigger their frequency (Gillham et al., 1994; Bachmann et al., 2003). Another function suggested for crib-biting was a partial substitute for food intake (McGreevy and Nicol, 1998). Crib-biting seems to facilitate the release of β-endorphin (Lebelt et al., 1998), which again reinforces the performance of the stereotypic behaviour pattern due to self-stimulation. Dodman et al. (1987) successfully treated crib-biting horses with opioid antagonists. The successful therapy of crib-biting with opioid antagonists is the result of an unspecific blocking of the opioid receptors, and thereby reducing the effect of endogenous opioids (Dodman et al., 1987).

In contrast to pelleted alfalfa hay, feeding a grain diet caused a significant increase in the crib-biting frequency (Gillham et al., 1994). The authors suggested that the palatability of grain triggers the release of endogenous opioids.

Epidemiological studies investigating the association between behavioural disorders in horses and environmental (e.g. housing systems, feeding management, utilisation) or horse specific factors (e.g. breed, sex, age) revealed that stress sensitive horses, such as thoroughbreds, had an increased incidence of stereotypies (Lüscher et al., 1998; Bachmann et al., 2003). Furthermore, housing and management factors associated with chronic stress or with increased anticipation behaviour were found to have a strong influence on the prevalence of stereotypies (McGreevy et al., 1995a; Bachmann et al., 2003).

So far, physiological correlates associated with crib-biting behaviour are inconsistent and sometimes contradictory. Crib-biting seems to be associated with a decrease in heart rate compared to samples taken during other activities (Lebelt et al., 1998; Minero et al., 1999), as well as with a decrease of the nociceptive (thermal) threshold (Lebelt et al., 1998). Gillham et al. (1994) found a lower baseline plasma β-endorphin level in five crib-biting horses compared to six controls. They suggested that crib-biting horses might have an impaired
release of β-endorphin resulting in an up-regulation of the opioid receptor sensitivity and therefore enhanced response to external stimuli. In contrast to these results, Lebelt et al. (1998) measured three times higher basal plasma β-endorphin levels in crib-bitters than in controls, whereas McGreevy and Nicol (1998) and Pell and McGreevy (1999) did not find any differences at all. Either increased (McGreevy and Nicol, 1998) or similar (Lebelt et al., 1998; Pell and McGreevy, 1999) levels of plasma cortisol between crib-bitters and controls have been reported.

Controversial results of stress-related blood parameters may be related to methodology as, for example, the blood sampling procedure itself may cause significant stress to an animal. Furthermore, the basal level of physiological parameters may differ between individuals depending on breed, age, sex, management and previous experience. Often, the inter-individual variation of such parameters is higher than the intra-individual difference between basal and experimental levels. This makes inter-individual comparisons difficult and complicates the interpretation of results (Dechamps et al., 1989; von Borell and Ladewig, 1989).

If stereotypies would significantly contribute to reduce chronic stress, basal levels of physiological correlates should show the same values in established crib-bitters as in reference (non-crib-biting) horses. Differences to reference horses would only be expected due to specific situations (e.g. when crib-biting is prevented). It might be promising to compare the baseline values but also the responses of crib-bitters and controls to a standardised stimulus under controlled conditions. The aim of our study was to evaluate the response of crib-biting and control horses to a food stimulus. Under the assumption that stereotypies are “stress reducing” mechanisms, crib-biting frequency would increase during the confrontation with the food stimulus. The behavioural and physiological responses would be expected to be lower than those of the controls.

The type of stimulus is decisive. Stimuli causing fear (rapid inflation of a balloon: Minero et al., 1999), pain (lip twitch: Minero et al., 1999), and release of endogenous opioids (palatable food: Gillham et al., 1994), or measures to prevent crib-biting and/or food intake (McGreevy and Nicol, 1998) revealed different results. Therefore, in this experiment an aversive, but neither painful nor frightening stimulus was used; waiting for being fed is a common “stress” situation known to any stabled horse.

Several physiological systems are involved in an animal’s response to stress (Henry and Stephens, 1977). Thus, parameters of the two main stress axes were measured: the concentration of plasma cortisol, a widely recognised parameter of the hypothalamo-pituitary-adrenocortical axis activated in response to arousing and aversive stimuli, and the heart rate and the heart rate variability (HRV), both representing the sympatho-adrenomedullary axis. HRV is an established parameter to quantify the state of the autonomic nervous system (Cerutti et al., 1995) reflecting the sympatho-vagal balance (Tiller et al., 1996). Finally, arousal-indicating behaviours and the crib-biting frequency were recorded.

2. Materials and methods

2.1. Animals and housing

Eleven crib-biting horses (4 mares and 7 geldings) and 11 control horses (3 mares and 8 geldings) of different breeds (Warmblood, thoroughbred, Swiss Franches-Montagnes)
and age (6–20 years; mean ± S.E.M.: 13.8 ± 1.0) were selected (Table 1). In this field study all horses belonged to different private owners. The owners answered to particular advertisement in popular Swiss horse journals, reporting about the study, and agreed their horses to be part of it. According to the horse owners, all crib-bites had practised this stereotypy for 2 years, at least. The crib-biting and control horses were stabled pairwise at different locations and were housed solitary in conventional loose boxes with visual contact. All crib-bits and control horses were housed adjacent for several years, except two pairs which were together since 4 months. Because of the limited number of horses at disposal, it was not possible to particularly match crib-bites and control horses for sex, breed and age.

2.2. Experimental design

Eight months to 1 week before the start of the experiment the behaviour of the 11 crib-biting horses was continuously video-taped in their loose boxes during 1–3 consecutive days (Table 1). The owners were advised not to change the normal management and utilisation habits during this time.

To estimate the total duration of crib-biting (based on the analysis of the mentioned video tapes), each minute with at least one crib-biting movement was registered. This approach was chosen as according to McGreevy et al. (1995b) the oesophageal distension, during a crib-biting bout, seems to be the source of gratification to the horse, not the grasping of an object or the passage of air as far as to the stomach. McGreevy et al. (1995b) were not able to accurately quantify the duration of this distension using videofluoroscopic examination, since the retained streak of air was regularly supplemented by subsequent crib-bites. Thus, crib-biting is a repeated action occurring usually several times a minute, and oesophageal distension lasts longer than the visible contraction of the neck muscles or the emission of the characteristic grunt.

During the experiment lasting for 4 consecutive days, horse owners were advised not to exercise their horses too intensively, and not to pasture them without surveillance. Roughage should be fed at 7:30 a.m. During experimental treatments the owners should not enter the stable.

On day 0 of the experiment, crib-bites and controls were physically examined by a veterinarian, and a blood sample was taken for a haemogram. Following local anaesthesia (Lidocain Vet. 2%, G. Streuli & Co. AG, Uznach, CH) an indwelling intravenous catheter (Secalon® (∅ 2.0 mm, length 160 mm), Ohmeda, Swindon, UK) was placed in the jugular vein and prolonged with a 15 cm extension tube (Discofix®, B. Braun Melsungen AG, Melsungen, D). A three-way stopcock (Discofix®, B. Braun Melsungen AG, Melsungen, D) was added allowing easy blood sampling without disturbing the horse. Catheters were flushed with heparinised saline solution to maintain patency. The catheter remained in the vein for 4 days until the end of the experiment. For electrocardiogram recordings (ECG) a Polar R-R Recorder® system (Polar Electro Oy, Kempele, FI) was used. Two electrodes were placed on the left chest wall under a stable girth and connected to the receiver unit fixed onto the girth. The ECG recording system (girth) was carried only during the experimental trials.

On days 1 and 2, horses were habituated to the experimental procedure, and reference data for ECG and blood cortisol were collected. ECG was recorded continuously from
7:50 to 10:15 a.m., and blood samples were taken at 8:00 (pre1), 8:50 (pre2), 9:05 (stress), 9:15 (post1), 9:30 (post2) and 10:00 a.m. (post3), first from the crib-biter and immediately afterwards from the control horse. For each blood sampling procedure, horses were loosely tied up in their boxes. The average duration of this procedure was 98 ± 4.3 s (mean ± S.E.M.), and aversive reactions of the horse were rarely observed. At 10:15 a.m., horses were conditioned to receive a ration of palatable food out of a specific bucket.

On day 3 (Fig. 1), ECG recording and blood sampling followed the same protocol as described for day 1 and 2. In addition, the behaviour of both horses was recorded simultaneously and continuously by direct observation from 8:00 to 10:00 a.m. At 9:00 a.m., food was presented in exactly the same manner as on the 2 preceding days, but was placed in front of the boxes so that the horses could see but not reach it. Food buckets were removed after about 13 min (12 min 53 s ± 1 min 39 s; mean ± S.E.M.).

2.3. Behaviour

Behaviour was recorded continuously using the software Behavior 3.6 (Hammerschmidt, K., et al., Scientific Software Products, Berlin, D) and transcribed to Microsoft Excel 97 (Microsoft, Redmont, Washington, USA). The total duration of behaviour indicating arousal (i.e. motor activity, oral manipulation, agonistic behaviour, vocalisation, snorting, kicking, pawing, head-shaking, head-tossing, head-circling, nodding) and the total frequency of
crib-biting were calculated for five 10-min intervals across the experimental period (*pre1*, *pre2*, *stress*, *post1*, and *post2*; Fig. 2). The behaviours indicating arousal were chosen after literature study (e.g. Kiley-Worthington, 1987; Weeks and Beck, 1996) and pilot observations. Some of them are direct indicators of arousal (motor activity, vocalisation, snorting, kicking, pawing, head-shaking, head-tossing, head-circling), others can be regarded as displacement or redirected activities, indicating a conflict and therefore a potentially arousing situation for the horse (oral manipulation, nodding, agonistic behaviour).

2.4. Heart rate and heart rate variability

Heart rate (HR) and heart rate variability (HRV) were analysed using ProBeat® (ProBeat, Bellinzona, CH) and ProFit® (Quantum Soft, Zurich, CH) software. The mean HR was calculated for five predetermined 5-min intervals (*pre1*, *pre2*, *stress*, *post1*, and *post2*; Fig. 2), and the HRV for three intervals (*pre*, *stress*, and *post*). Within each of these intervals 170.3 ± 21.9 beats corresponding to 269.9 ± 35.9 s (mean ± S.E.M.) were selected for further analysis. The frequency components of the HRV were calculated using power spectral analysis (PSA; Bernasconi et al., 1998). The low frequency components (LF) of the signal are assigned to the tone of the sympathetic system, the high frequency components (HF) to the tone of the vagal system, and their ratio (LF/HF), the sympatho-vagal balance, represents a measure for the stress level.

2.5. Blood sampling and cortisol assay

After discarding the first 10, 9 ml blood were collected in heparinised polypropylene tubes (Greiner Labortecnik, Kremsmünster, A). Samples were kept in crushed ice until centrifugation at 3000 rpm for a maximum of 15 min at 4 °C. Aliquots of plasma were immediately placed on dry ice and stored at −20 °C for further processing. Plasma cortisol
concentrations were measured using a commercial $^{125}$I radioimmunoassay kit (EURO/DCP (Cat No. KCOD1), Llanberis, UK). The intra-assay variation was 4.5%, and the inter-assay variation was 3.4%. Mean recovery was 100%. The mean binding percentage of the tracer to the antibody was 45%, while mean non-specific binding was 2.6%.

2.6. Statistical analysis

Differences between time intervals were tested non-parametrically by the Friedman two-way analysis of variance, and in cases of significance, pairwise comparisons were done using the Wilcoxon signed rank test (Siegel and Castellan, 1988). Differences between crib-biters and control horses were tested by Wilcoxon signed rank test. Because of multiple comparisons of dependent groups or conditions, the alpha-level was corrected using the Bonferroni adjustment (Snedecor and Cochran, 1989). If not indicated otherwise, significance level was set at $P < 0.05$. Statistical analyses were carried out using STATA 5.0 (Stata Corporation, College Station, TX, USA) and SYSTAT 97 (Systat Inc., Evanston, IL, USA).

3. Results

3.1. Crib-biting intensity and veterinary health control

The total of minutes including one or more crib-biting events was found at 26.5% in median of the time the horses spent in their boxes, with a minimum at 10.44% and a maximum at 64.7% (Table 1).

At day 0, physical and haematological examinations of all animals revealed no disease which would interact with the experiment. Six horses showed mild signs of chronic obstructive pulmonary disease; three of them with intermittent cough. One horse showed a slight lameness at the trot. None of the horses had clinical signs of cardiac disease. According to the owners, six of the 11 crib-biters suffered regularly from recurrent colic episodes. However, this was never observed during the experiments.

3.2. Behaviour during the stress experiment

For both crib-biters and control horses, total duration of arousal behaviour was lower during $pre_2$ compared to $stress$ ($P < 0.05$), and higher during $stress$ compared to $post_1$ ($P < 0.05$). Neither for crib-biters nor for controls, any significant differences were found between $pre_1$ and $pre_2$, and between $post_1$ and $post_2$. The behaviours recorded also did not differ between the two groups of horses (Table 2).

3.3. Crib-biting frequency

Crib-biting frequency was significantly higher during $pre_2$ compared to $stress$, and lower during $stress$ compared to $post_1$. No differences were found between $pre_1$ and $pre_2$ and between $post_1$ and $post_2$, respectively (Table 3).
### Table 1
Individual data of crib-biting and control horses (n = 11, each), and crib-biting “duration”

<table>
<thead>
<tr>
<th>Horse pair</th>
<th>Crib-biting horses</th>
<th>Control horses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex (G: gelding/ M: mare), age (years), breed</td>
<td>Sex (G: gelding/ M: mare), age (years), breed</td>
</tr>
<tr>
<td>1</td>
<td>G, 16, FM</td>
<td>G, 20, FM</td>
</tr>
<tr>
<td>2</td>
<td>G, 15, FM</td>
<td>G, 9, FM</td>
</tr>
<tr>
<td>3</td>
<td>G, 17, WB</td>
<td>G, 9, FM</td>
</tr>
<tr>
<td>4</td>
<td>M, 6, WB</td>
<td>M, 19, FM</td>
</tr>
<tr>
<td>5</td>
<td>G, 12, WB</td>
<td>G, 14, WB</td>
</tr>
<tr>
<td>6</td>
<td>G, 10, WB</td>
<td>M, 19, FM</td>
</tr>
<tr>
<td>7</td>
<td>G, 12, WB</td>
<td>M, 18, WB</td>
</tr>
<tr>
<td>8</td>
<td>G, 12, THB</td>
<td>G, 19, WB</td>
</tr>
<tr>
<td>9</td>
<td>M, 15, WB</td>
<td>G, 6, WB</td>
</tr>
<tr>
<td>10</td>
<td>M, 17, FM</td>
<td>G, 6, FM</td>
</tr>
<tr>
<td>11</td>
<td>M, 19, WB</td>
<td>G, 14, WB</td>
</tr>
<tr>
<td>Median</td>
<td>26.5</td>
<td></td>
</tr>
</tbody>
</table>

FM: Swiss Franches-Montagnes horse; WB: warmblood; THB: thoroughbred.

### Table 2
Duration (s/10 min) of arousal-indicating behaviour (locomotor activity, oral manipulation, agonistic behaviour, vocalisation, snorting, kicking, pawing, head-shaking, head-tossing, head-circling, nodding)

<table>
<thead>
<tr>
<th>Observation interval</th>
<th>Crib-biters (s/10 min)</th>
<th>Control horses (s/10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre1</td>
<td>29.4 ± 8.9</td>
<td>39.6 ± 9.1</td>
</tr>
<tr>
<td>pre2</td>
<td>27.6 ± 11.9</td>
<td>58.4 ± 17.1</td>
</tr>
<tr>
<td>stress</td>
<td>338.7 ± 41.7</td>
<td>361.9 ± 32.8</td>
</tr>
<tr>
<td>post1</td>
<td>26.7 ± 6.1</td>
<td>50.2 ± 16.0</td>
</tr>
<tr>
<td>post2</td>
<td>38.9 ± 12.2</td>
<td>49.5 ± 12.9</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. for 11 crib-biters and 11 control horses (Wilcoxon signed rank test with alpha error adjustment).

* Significant difference (P < 0.05) between pre2- and stress-interval.

b Significant difference (P < 0.05) between stress- and post1-interval.

### Table 3
Crib-biting frequency (n = 11 crib-biters; Wilcoxon signed rank test with alpha error adjustment)

<table>
<thead>
<tr>
<th>Observation interval</th>
<th>Crib-biting frequency (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre1</td>
<td>28.5 ± 7.2</td>
</tr>
<tr>
<td>pre2</td>
<td>25.6 ± 6.1</td>
</tr>
<tr>
<td>stress</td>
<td>7.7 ± 2.9</td>
</tr>
<tr>
<td>post1</td>
<td>28.7 ± 8.8</td>
</tr>
<tr>
<td>post2</td>
<td>21.5 ±6.9</td>
</tr>
</tbody>
</table>

* Significant difference (P < 0.05) between pre2- and stress-interval.

b Significant difference (P < 0.05) between stress- and post1-interval.
3.4. Plasma cortisol concentration

Days 1 and 2: There were no significant changes in cortisol levels over the six samples taken between 8:00 and 10:00 a.m. (Fig. 3), except for the overall plasma cortisol level, which was significantly higher in crib-bites than in control horses on day 1 (Wilcoxon signed rank test with alpha error adjustment, \( P < 0.001 \); Table 4).
Table 4
Overall plasma cortisol levels (nmol/l)

<table>
<thead>
<tr>
<th></th>
<th>Crib-bite</th>
<th>Control horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 1</td>
<td>87.2 ± 4.0(^a)</td>
<td>65.6 ± 5.7(^a)</td>
</tr>
<tr>
<td>day 2</td>
<td>69.3 ± 2.7</td>
<td>62.8 ± 5.7</td>
</tr>
<tr>
<td>day 3</td>
<td>69.0 ± 3.6</td>
<td>64.7 ± 2.4</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. for 11 crib-bite and 11 control horses (Wilcoxon signed rank test with alpha error adjustment).

\(^a\) Significant difference (\(P < 0.001\)) between crib-bite and control horses.

Day 3 (stressor experiment): For both crib-bite and control horses, plasma cortisol concentrations were highest at the beginning of the experiment (\(preL\): mean ± S.E.M.: 85.7 ± 6.0 nmol/l and 72.3 ± 6.2 nmol/l, respectively), were decreased at \(pre2\) (64.0 ± 7.4 nmol/l and 61.2 ± 3.8 nmol/l, respectively), and showed a slight increase after presentation of the food stimulus (\(post1\): 70.5 ± 7.5 nmol/l and 69.5 ± 6.0 nmol/l, respectively; Fig. 3). Plasma cortisol concentrations were declining after \(post1\) until the end of the experiment at \(post3\) (61.7 ± 6.3 nmol/l and 55.8 ± 4.9 nmol/l, respectively). However, differences did not reach significance. There were also no significant differences in the plasma cortisol concentrations between crib-bite and control horses.

Fig. 4. Heart rate (mean ± S.E.M.) on day 3 (\(N = 9\) crib-bite and 9 control horses; Wilcoxon signed rank test with alpha error adjustment). Presentation of the food stimulus from 09:00 to 09:10 a.m.
Table 5
Heart rate variability (mean ± S.E.M.) for nine crib-biters and nine control horses (Wilcoxon signed rank test with alpha error adjustment).

<table>
<thead>
<tr>
<th></th>
<th>Crib-biters</th>
<th>Control horses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>pre</td>
<td>31.6 ± 4.41a</td>
<td>68.4 ± 4.41a</td>
</tr>
<tr>
<td>stress</td>
<td>25.8 ± 4.51</td>
<td>73.7 ± 4.46</td>
</tr>
<tr>
<td>post</td>
<td>30.0 ± 3.95</td>
<td>70.0 ± 3.95</td>
</tr>
</tbody>
</table>

HF: tone of the vagal system; LF: tone of the sympathetic system; LF/HF: stress level.

a Significant difference (P < 0.05) between crib-biters and controls.
b Significant difference (P < 0.05) between pre- and stress-interval.
c Significant difference (P < 0.05) between stress- and post-interval.

3.5. Heart rate

In two pairs of horses ECG recordings were incomplete due to technical difficulties with the telemetric system. These pairs were excluded from analyses. For both crib-biters and controls (n = 9, each), HR values were significantly lower before (pre2, mean ± S.E.M.: 35.0 ± 1.3 and 35.5 ± 1.8 bpm, respectively) and after (post1, 36.5 ± 2.1 and 35.6 ± 1.5 bpm, respectively) the stimulus presentation (stress: 47.5 ± 2.4 and 55.5 ± 4.7 bpm, respectively; Fig. 4). No significant differences were found between crib-biters and controls (pre1: P = 0.67; pre2: P = 0.37; stress: P = 0.17; post1: P = 0.59; post2: P = 0.86, Wilcoxon signed rank test).

3.6. Sympatho-vagal balance

LF, HF and LF/HF differed significantly between pre and stress, and stress and post for control horses, but not for crib-biters. During the pre-interval, LF, HF and LF/HF differed significantly between crib-biting and control horses. However, during the stress- and post-intervals, no difference was detected between crib-biters and controls (Table 5).

4. Discussion

Presentation of the food stimulus on day 3 induced a significant reaction in heart rate and arousal behaviour in crib-biting and control horses. However, cortisol plasma concentrations did not differ significantly. The food stimulus may not have been stressful enough, or the exposure time to the stimulus too short to trigger a response from the hypothalamo-pituitary-adrenocortical axis. Furthermore, Schrader and Laedewig (1999) described a process of adaptation to a repeated stressor in hormones of the pituitary–adrenocortical axis in domestic pigs, whereas no adaptation was found in adrenalin concentrations, in heart rate and in arousal behaviour. A food stimulus (i.e. waiting for food) is a well known situation to any stabled horse. Thus, the absence of a reaction of the pituitary–adrenocortical axis could be due to adaptation to the food stimulus and appears to be in complete accordance with Schrader and Laedewig (1999).
Highest values for plasma cortisol concentration were found at the beginning of each day during the experimental week. The first entry of a person taking blood samples might have an influence on these values. On the other hand the high plasma cortisol concentrations may be due to the circadian rhythm of horses. In fact, peak cortisol concentrations between 06:00 and 09:00 a.m. have previously been described (Irvine and Alexander, 1994; Toutain et al., 1988). According to Bradbury et al. (1991) stress induces greater release of corticotrophin releasing factor during the circadian trough. Therefore, it might have been more favourable to carry out the experiments in the evening, where low cortisol levels have been reported (Irvine and Alexander, 1994; Toutain et al., 1988). However, this was not possible due to management practices of the private horse operations (leisure activities after work-time).

No significant differences between crib-biting and control horses were found in plasma cortisol concentration, heart rate and arousal-indicating behaviour, neither in baseline values nor as reaction to the food stimulus. This is in complete accordance to other studies reported earlier (Lebelt et al., 1998; Pell and McGreevy, 1999). However, McGreevy and Nicol (1998) reported higher mean baseline levels of cortisol concentrations in crib-biting horses compared to controls using various test situations. In the same study, baseline HR and HR during treatment (preventing crib-biting, food intake, crib-biting and food intake), were not found different between crib-biters and control horses. Minero et al. (1999) reported the overall mean heart rate to be higher in crib-biters when horses were restrained with a lip twitch or suddenly exposed to the rapid inflation of a balloon. Furthermore, crib-biting horses reacted less to the lip twitch than to the inflation of the balloon.

Our results do not allow to demonstrate a stress-coping function of crib-biting. Crib-biting was not triggered by our food stimulus. Quite the reverse, crib-biting mostly disappeared in favour of arousal behaviour (e.g. pawing, snorting or vocalisation). Therefore, crib-biting seems to have no coping function in relation to a short term activation of the sympatho-adrenomedullar-axis. However, no significant increase of plasma cortisol concentration was measured. Thus, the hypothesis that crib-biting behaviour reduces the stress reaction of the hypothalamo-pituitary-adrenocortical axis, cannot completely be rejected. It is worth noting that all observed crib-biting horses have performed this stereotypic behaviour for a long time. McGreevy and Nicol (1998) suggested that long term crib-biters have a reduced basal cortisol level due to adaptation to the condition.

In human medicine, the power spectral analysis (PSA) of the HRV signal is an established method to quantify the activity of the autonomic nervous system (Bernasconi et al., 1998; Hansen, 1999). The HF component relates to the vagal tone, whereas the LF component is primarily a general indicator of the sympathetic activity but also a feedback influenced by the vagal activity (Bernasconi et al., 1998; Hansen, 1999; Kuwahara et al., 1996). Consequently, changes in the LF/HF ratio are an indicator of alterations in the sympathetic-vagal balance. The normal state of rest in horses is characterised by a predominant parasympathetic nervous activity (Kuwahara et al., 1996). In stressful situations, the parasympathetic nervous activity will decrease in favour of a higher sympathetic activity, enabling the animal to react appropriately in a biological manner, for example with flight. There is evidence, that the vagal tone is a very useful tool to assess stress and stress sensitivity in animals (Porges, 1995). At rest, the baseline values of the HRV components (LF and HF) as well as their ratio (LF/HF) were significantly different between crib-biters and controls in our study. Compared to crib-biters control horses had a higher HF component (tone of the vagal system).
and a lower LF component (tone of the sympathetic system), resulting in a lower LF/HF ratio (stress level). During presentation of the food stimulus, control horses reacted with a significant increase of the LF and a significant decrease of the HF component, resulting in a significant higher LF/HF ratio. In crib-biting horses, this reaction was not significant.

According to these results, crib-biting horses seem to have a significantly lower basal parasympathetic activity than the controls. This indicates that crib-biting horses were not in a normal state of rest during the pre-interval of our experiments. It is known, that crib-biters frequently suffer from indigestion. However, there is still little proof for a causal association (McGreevy and Nicol, 1998; Lebelt et al., 1998; Lane, 1998). Recent studies indicate that links between crib-biting and indigestion are more important than previously anticipated (Hillyer et al., 2002; Nicol et al., 2001; Waters et al., 2002). Visceral function is controlled by the parasympathetic activity (Porges, 1995) and might therefore be reduced in crib-biting horses because of their lower basal parasympathetic activity found in this study.

Due to the lower basal parasympathetic and higher sympathetic activity, the reactivity range of the autonomic nervous system of crib-biting horses may not be capable to react as competent to an external stimulus as in normal horses. According to Friedmann and Thayer (1998) the vagal tone controls physiological and psychological flexibility. Thus, horses with low basal vagal tone may be regarded as very stress sensitive. Therefore, it may be concluded that crib-biting horses are more stress sensitive and less flexible when coping with stress than control horses.

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