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Physiological correlates associated with cribbing behaviour in horses: changes in thermal threshold, heart rate, plasma β-endorphin and serotonin

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Summary

The aim of the present study was to obtain information on the possible mechanisms underlying cribbing behaviour in horses. To investigate the horse's responsiveness to an external stimulus, a device for telemetric measurement of thermal threshold, using the forelimb withdrawal reflex, was developed and validated. Measurements of thermal threshold took place in cribbing horses (n = 11) before and during cribbing periods. Heart rate was monitored continuously in the same horses. Blood samples were collected before and during cribbing periods as well and in age- and sex-matched control horses (n = 11). β-endorphin and cortisol were determined in plasma using radioimmunoassay techniques, serotonin was analysed by high performance liquid chromatography.

Compared with basal values, thermal threshold was significantly (P = 0.003) lower during cribbing periods. The mean difference was 4.9°C. Heart rate decreased significantly (P = 0.026) and showed a mean reduction of 2.4 beats/min during cribbing. Given the fact that arousal usually is associated with an increase in nociceptive threshold and in heart rate, the decrease in both during cribbing provide evidence that cribbing may reduce stress.

Cribbers showed 3 times higher basal β-endorphin levels than controls (mean 49.5 vs. 16.2 pmol/l, P = 0.006) and there was a trend for lower basal serotonin levels (mean 201.5 vs. 414.3 nmol/l, P = 0.07). These data indicate differences in cribber's endogenous opioid and serotonergic systems.

Introduction

Cribbing is a well recognised stereotypic behaviour pattern in the horse. It was mentioned in the literature as early as 1578 (Engelhardt 1990) and, since this time, there have been a large number of publications dealing with this behavioural problem usually referred to as a 'vice'.

Cribbing or crib-biting is the term for an oral based behaviour involving the horse grasping a fixed object with its incisor teeth and contracting the lower neck muscles to retract the larynx caudally. This movement is coincided with an in-rush of air through the crico-pharynx into the oesophagus producing the characteristic cribbing sound. Usually air is not swallowed but returns to the pharynx (McGreevy *et al.* 1995b). Since this particular behaviour pattern is repetitive, relatively invariant and has no obvious goal or function, it fulfils the criteria to be called a stereotypy (Mason 1991). The prevalence of cribbing in horses is 2.4–8.3% depending on breed, usage and management factors (Borroni and Canali 1993; McGreevy *et al.* 1995a,c; Luescher *et al.* 1998). There is no evidence that cribbing generally impairs the health of affected horses. Only 7% of all cribbers that underwent a surgical intervention were reported by their owners to have suffered from health problems such as recurrent colic or weight loss (Ritzberger-Matter and Kaegi 1998).

Although cribbing is a common behaviour abnormality with some economic significance, there is surprisingly little information concerning its underlying mechanisms. Therefore, the objective of the present study was to answer the following questions: 1) Are there differences between cribbers and noncribbers that could provide information about the causes of this stereotypy? 2) Are there measurable effects of cribbing sequences on horses that could provide information about the function of this behaviour pattern?

Materials and methods

Animals

Eleven cribbing horses (8 mares, 1 stallion, 2 geldings) of different breeds, age 8–24 years were used. The animals were single housed in conventional horse boxes in different riding stables in the surrounding of Munich and were exercised between one and 2 h/day. All horses were long term cribbers performing this behaviour pattern for at least 2 years. Eleven age- and sex-matched horses with no history of stereotypic behaviour, kept under similar housing conditions, were used as controls.

Study protocol

To minimise influences of circadian changes, all studies were carried out between 0830 and 1130 h. The cribbing horses were

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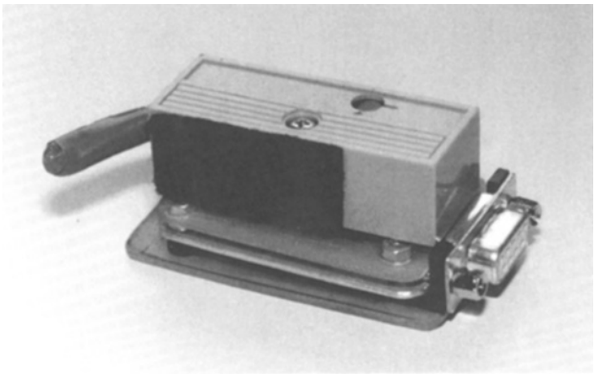

Fig 1: Sensor unit of the device for thermal threshold measurement consisting of a heating element, a thermocouple and a shake switch.

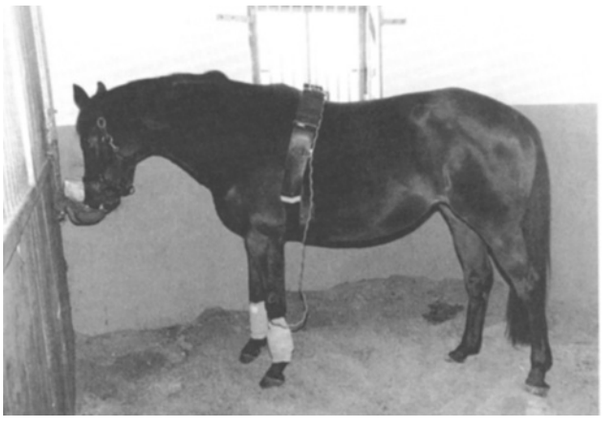

Fig 2: Equipment for remote controlled measurement of nociceptive threshold mounted on a cribbing horse.

videotaped in their home boxes and measurements of nociception and blood sampling took place under basal conditions (basal = no stereotypic behaviour for at least 30 min) and during cribbing periods (cribbing = cribbing for at least 15 min with no interruption longer than 2 min). Heart rate was recorded continuously in the cribbing horses during the complete observation period (0830–1130 h).

Blood samples of cribbers were collected by jugular puncture under conditions described above and, additionally, in the control horses under basal conditions (control). Blood withdrawal took place without restraining the horses and no aversive reaction to the procedure was observed. All basal samples of cribbers and control horses were collected between 0845 and 0930 h, the cribbing samples between 0945 and 1100 h.

Nociception

To study the horse's response to an external stimulus a device for remote controlled measurement of nociceptive threshold was developed and validated. The equipment consisted of a microprocessor based control unit, a sensor unit including a heating element, a thermocouple and a shake switch (Fig 1), an infrared receiver and an infrared transmitter. The control unit mounted on the horse's back using a common leather girth was connected with the infrared receiver and with the sensor unit placed under a bandage in the fetlock region of the left forelimb (Fig 2). After a measurement was released by the infrared transmitter, a steady increase in temperature was delivered to the fetlock region. As soon as the individual thermal threshold was reached the animal reacted by withdrawing its limb, a movement that activates the shake-switch integrated in the sensor unit and interrupts the heating process. The cut-off temperatures of several measurements recorded by the thermocouple were stored in the control unit. A hand-held computer with a commercial terminal software (Windows 3.1)[1] was used to export stored data from the control unit for further analysis and to define the maximum cut-off temperature and the temperature profile. During the present study, temperature increase was 1°C/s, the maximum cut-off temperature was set 5°C above the first measured cut-off temperature. To validate the procedure repeated measurements (3 times, 10 min intervals) were done in 5 mares on 2 consecutive days.

Depending on spontaneous behaviour of the individual horses, between 1 and 3 measurements were done under basal conditions and during cribbing periods respectively. Results of these measurements were grouped to a mean basal and mean cribbing value for each horse. Additionally, a statistical comparison between the first and the second individual basal measurement was performed to identify potential changes of thermal threshold caused by repeated measurements.

TABLE 1: Repeated measurements of thermal threshold (°C) in 5 mares on 2 consecutive days

Horse No	Day No	1	2	3
I	1	52.2	54.4	54.2
	2	59.7	58.7	58.8
II	1	54.1	53.8	56.5
	2	57.1	51.5	54.5
III	1	55.1	56.7	55.6
	2	61.6	63.9	63.5
IV	1	62.3	62.6	62.0
	2	58.0	59.0	59.6
V	1	63.9	65.2	62.2
	2	59.0	60.0	58.6

Heart rate

Heart rate was recorded continuously using the Polar Sport Tester[2], a device developed for use in human athletes. The electrodes were fixed at the left side of the thorax by means of the leather girth described above. The receiver was placed in a small pocket close to the transmitting electrode unit. Mean heart rate in beats/min (bpm) was stored in 5 s intervals. After transferring data to a PC by help of an interface it was analysed using the software package Polar heart rate analysing for Windows Ver. 5.0[2].

Basal heart rate was compared with data obtained during cribbing periods (mean 30 min before vs. mean of first 30 min during, a cribbing period). Additionally, a comparison between heart rate variability calculated as coefficient of variation (V%) during the periods described before was performed.

β-endorphin

Blood was collected in ice-cold polypropylene tubes containing EDTA (1 mg/ml). Samples were kept in crushed ice until they were centrifuged at 3,000 ***g*** for 10 min at 4°C. Aliquots of plasma were acidified by addition of acetic acid to a final concentration of 0.1 mol/l, placed on dry ice immediately and stored at -80°C until further processing.

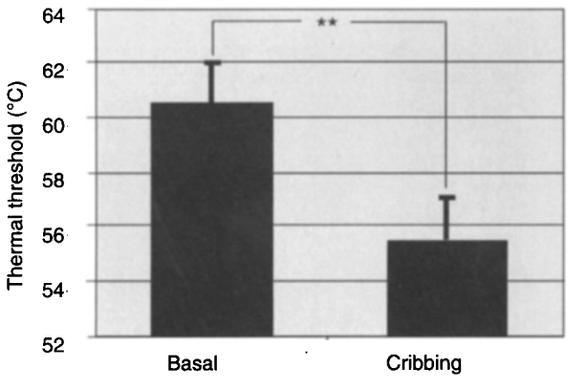

*Fig 3: Thermal threshold [°C] in dependence on behaviour of cribbing horses (n = 11). Results are presented as mean ± s.e. **Indicates a highly significant difference (P≤0.01).*

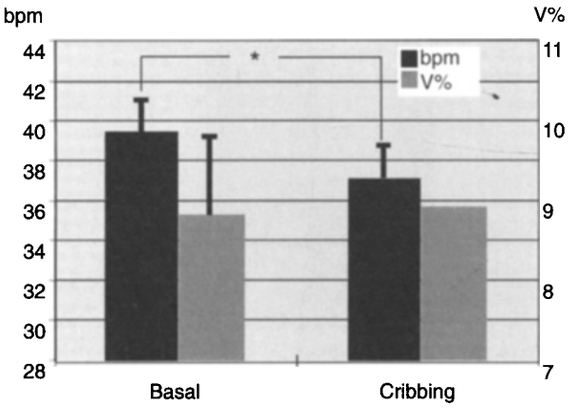

*Fig 4: Heart-rate frequency [bpm] and variability [V%] in dependence on behaviour of cribbing horses (n = 11). Results are presented as mean ± s.e. *Indicates a significant difference (P≤0.05).*

Peptides were extracted from plasma samples by reverse phase chromatography using 200 mg C_{18}- Sep-Columns[3] at 4°C using ice cold reagents. A vacuum manifold (FA-2100)[3], was used to control flow rate. After defrosting plasma samples were mixed with an equal amount of buffer A (0.1% trifluor acetic acid) before they were centrifuged at 24,000 ***g*** for 20 min at 4°C. After activation of the columns by passing 1 ml of 60% acetonitrile in buffer A (= buffer B) followed by 15 ml of buffer A, the samples were placed in the columns (flow rate = 1 ml/min). The columns were washed by 15 ml of buffer A, peptides were extracted by 3 ml of buffer B (flow rate = 1 ml/min) and dried using a vacuum concentrator (SpeedVac)[4]. Recovery was tested using radiolabelled β-endorphin[3] and showed to be 79.8%.

β-endorphin was measured using a commercial radioimmunoassay kit (RIK 8616)[3]. According to the supplier's information, the cross-reactivity with equine β-endorphin was 100%. β-endorphin concentrations were calculated using the software package MultiCalc 2.0[5]. Recovery of equine β-endorphin[3] added to horse double striped plasma was 68.3%. Intra-assay coefficient of variation was 4.6%. To avoid influence of inter-assay variation all samples were analysed in one assay.

Cortisol

Plasma cortisol concentrations were measured after ethanol extraction by a radioimmunoassay technique as previously described (Lebelt *et al.* 1996). Recovery of hydrocortisone[6] added to horse double stripped plasma was 81.6%. Intra-assay coefficient of variation was 3.2%. To avoid influence of inter-assay variation all samples were processed in one assay.

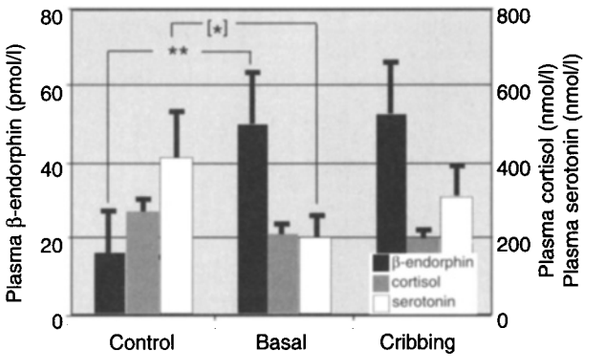

*Fig 5: Plasma β-endorphin, cortisol and serotonin levels in control horses (n = 11) and in cribbing horses (n = 11) in dependence on their behaviour ('basal' and 'cribbing'). Results are presented as mean ± s.e. **Indicates a highly significant difference (P≤0.01), [*] indicates a nonsignificant trend (P≤0.1).*

Serotonin

Plasma serotonin concentrations were determined by High Performance Liquid Chromatography (HPLC) with electrochemical detection (ECD). Measurements were performed in duplicates using a commercial HPLC kit[7] for plasma serotonin measurement consisting of a C18 reverse phase HPLC column and all reagents needed. N-methylserotonin is used as internal standard. Serotonin concentrations are calculated by help of the software package System Gold Ver. 7.11[8]. Recovery of serotonin[6] added to horse double stripped plasma was 93% (corrected for recovery of internal standard). Intra-assay coefficient of variation was 5.3%.

Statistical analysis

All statistical analysis was performed by two-tailed nonparametric test procedures using the software package WinStat Ver 3.1[9].

Comparison between cribbers and controls was done by Mann-Whitney U-test, comparison between the cribber's basal and cribbing values by Wilcoxon matched pair *t* test. The relationship between β-endorphin basal levels and age of the horses was investigated by Kendall's rank correlation coefficient. For all tests P≤0.05 was accepted as significant (*), P≤0.01 as highly significant (**) and P≤0.1 considered to indicate a trend ([*]).

All results are presented as mean ± s.e. The range is given in parenthesis.

Results

Nociception

The validation study showed marked inter-individual variation in thermal threshold and also intra-individual variation comparing the different experimental days (Table 1). But within one experimental day, no significant increase or decrease of

thermal threshold could by identified with repeated measurements (Friedman 2 way analysis of variance, 3 groups, 10 cases, $\chi r^2 = 0.667$, P = 0.717).

As shown in Figure 3, there was a highly significant difference in thermal threshold between basal and cribbing values with lower levels during cribbing periods (Wilcoxon matched pair *t* test, n = 11, z = -2.936, P = 0.003). The mean basal value was 60.5 ± 1.6°C (51.5–68.4°C) vs. 55.6 ± -1.6°C (49.1–66.7°C) during cribbing periods. The mean difference was -4.9 ± 0.9°C (-1.6 to -11.3°C).

No significant difference was found comparing the first with the second basal value (Wilcoxon matched pair *t* test, n = 9, z = -1.192, P = 0.233).

Heart rate

Mean heart frequency and heart-rate variation are shown in Figure 4. Heart rate recorded during cribbing periods was significantly lower when compared with basal values (Wilcoxon matched pair *t* test, n = 11, z = -2.223, P = 0.026). The mean basal heart rate was 39.4 ± 1.6 bpm (32.3–50.2 bpm) and 37.0 ± 1.1 bpm (30.5–51.4 bpm) during cribbing periods. The mean difference was -2.4 ± 0.8 bpm (-5.7 to +1.2 bpm).

There was no significant difference between heart rate variability comparing basal with cribbing periods (Wilcoxon matched pair *t* test, n = 11, z = -0.089, P = 0.929). The mean coefficient of variance during basal periods was 8.8 ± 1.0% (3.6–15.2%) and during cribbing periods 8.9 ± 0.9% (5.9–14.5%).

β-endorphin

Mean plasma β-endorphin levels are shown in Figure 5. There was a highly significant difference between basal β-endorphin levels in cribbers and controls with 3 times higher concentrations in the cribbers (Mann-Whitney U-test, n = 22, z = -2.725, P = 0.006). Mean basal plasma β-endorphin level in the cribbers was 49.5 ± 14.8 pmol/l (7.3–146.0 pmol/l) and in the controls 16.2 ± 10.3 pmol/l (0.3–124.6 pmol/l). No significant difference was found comparing the cribber's basal and cribbing β-endorphin values (Wilcoxon matched pair *t* test, n = 11, z = -0.357, P = 0.721). The mean β-endorphin concentration during cribbing periods was 52.7 ± 14.8 pmol/l (8.3–47.9 pmol/l).

There was a significant positive correlation between age and basal plasma β-endorphin concentrations as well in the cribbers (Kendall correlation coef., n = 11, (τ = 0.6, z = 2.569, P = 0.009) as in the controls (n = 11, (τ = 0.496, z = 2.123, P = 0.034).

Cortisol

Mean plasma cortisol levels are shown in Figure 5. There was no significant difference between basal values of cribbers and controls (Mann-Whitney U-test, n = 21, z = -1.197, P = 0.231) or between the cribbers basal and cribbing values (Wilcoxon matched pair *t* test, n = 10, z = -0.255, P = 0.799). Mean basal plasma cortisol level in the cribbers was 215.7 ± 21.3 nmol/l (130.9–350.2 nmol/l) and in the controls 267.6 ± 31.8 nmol/l (132.4–504.1 nmol/l). The mean cribbing value was 195.4 ± 13.5 nmol/l (138.6–260.1 nmol/l).

Serotonin

Mean plasma serotonin levels are shown in Figure 5. There was a trend indicating lower basal serotonin levels in cribbers compared with controls (Mann-Whitney U-test, n = 22, z = -1.806, P = 0.071). Mean basal plasma serotonin level in the cribbers was 201.5 ± 60.2 nmol/l (5.7–522.7 nmol/l) and in the controls 414.3 ± 109.5 nmol/l (70.4–1,249.6 nmol/l). No difference was found comparing the cribbers basal and cribbing values (Wilcoxon matched pair *t* test, n = 11, z = -1.245, P = 0.213). The mean serotonin concentration during cribbing periods was 307.6 ± 70.4 nmol/l (30.1–818.3 nmol/l).

Discussion

The aim of this study was to monitor different physiological parameters in cribbing horses. Measures were taken before and during cribbing periods and a comparison using age-matched control animals was carried out. By keeping the horses in a familiar environment and using video recording and a remote controlled device to access thermal threshold, the present work offered new approaches to study physiological responses associated with cribbing in horses. Perhaps, better information would have been obtained if more frequent blood sampling had been carried out, but it could have caused disturbance of spontaneous behaviour and have influenced the physiological parameters which were monitored.

Nociception

Changes in responsiveness in association with stereotypies have been proposed by Cronin (1985), Broom (1988) and Sambraus and Radke (1989). In the present study, measurement of thermal threshold was used as an example for the horse's reaction to an external stimulus. Unlike the data reported previously for other species (Rushen *et al.* 1990; Zanella 1992), measurements immediately during the abnormal behaviour performance were taken using a remote controlled device. It is probable that the appearance of an investigator using a hand-held equipment may have interrupted the cribbing periods or interfered with the animal's reaction to the stimulus.

Different methods to measure nociceptive reaction to determine the effectiveness of analgesic substances are described in the literature. Usually these procedures are based on a measurement of the latency to a defined thermal or mechanical stimulus (Pippi and Lumb 1979; Kammerling *et al.* 1985). As there is evidence that recording threshold instead of latency may be the more exact method (Nolan *et al.* 1987) devices measuring the reaction to a continuously increasing temperature or pressure have been developed (Nolan *et al.* 1987; Chambers *et al.* 1990). Based on a procedure described for the use in sheep (Nolan *et al.* 1987), the remote-controlled device used in this study has been constructed using the natural withdrawal reflex and a shake switch to interrupt the heating procedure. The equipment described here fulfils all ethical requirements for the measurement of nociceptive threshold formulated by Nolan *et al.* (1987): 1) the stimulus should be the minimum necessary to produce a measurable response, 2) the response should be a natural one to the animal, 3) the stimulus should terminate rapidly once the response has occurred and 4) there should be an upper cut-off of the stimulus to prevent tissue damage. Given the fact that, although marked differences in the individual threshold temperatures have been observed, there was no evidence indicating sensitisation or adaptation to repeated measurements, the technique described offers a reliable method for remote controlled measurement of thermal threshold in free moving horses.

Compared with basal values thermal threshold was significantly lower during cribbing periods. This result is in contrast to the previously postulated ideas of a reduced perception of aversive environmental stimuli during stereotypic behaviour patterns (Cronin 1985; Broom 1988; Sambraus and Radke 1989). However, the present data support findings in sows, showing a negative correlation between time spent performing stereotypic behaviour and tail flick latency (Rushen *et al.* 1990; Zanella 1992). Given the fact that stress or arousal is usually associated with an increase in nociceptive threshold (Amit and Galina 1986; Rodgers *et al.* 1988), the decrease during cribbing periods described here may indicate a state of reduced arousal.

Heart rate

During cribbing periods, heart rate was significantly lower compared with basal values. These results are in agreement with findings of Soussignan and Koch (1985) demonstrating a reduction of heart frequency during leg swinging, a stereotypic movement in children. Also sows were found to have lower heart rate immediately during bouts of stereotypic behaviour (Schouten and Rushen 1993). In contrast to the higher heart rate variability during stereotypic behaviour patterns of autistic humans reported by Hutt and Hutt (1978), no such change in association with cribbing could be identified in the present study.

Changes in heart rate reported here provide evidence for a lower level of arousal during cribbing periods in horses. This finding is supported by recently published preliminary results (Minero *et al.* 1996). The authors were able to demonstrate that heart frequency was lower when horses were cribbing compared to measures taken during other behavioural patterns. Furthermore, their observation that heart rate often rose before cribbing bouts and decreases after cribbing had started agrees with heart rate profiles in some of the cribbers included in the present study.

β-endorphin

Cribbing horses were found to have 3 times higher basal plasma β-endorphin levels when compared to an age- and sex-matched control group showing no stereotypic behaviour. The findings that opioid antagonists such as naloxone or naltrexone, are able to reduce cribbing behaviour (Dodman *et al.* 1987; McBride 1996) gave evidence that β-endorphin or other endogenous opioid peptides may be involved in the regulation of cribbing behaviour. In contrast to the results reported here a previously published work showed β-endorphin levels to be lower in cribbers compared to control horses (Gilham *et al.* 1994) whereas other studies found no differences (McGreevy and Nicol 1995; McBride 1996). These contradictory results may be due to the confounding effect of age on plasma β-endorphin levels in horses (Canali *et al.* 1996) that is confirmed by the present work. Comparisons in plasma β-endorphin levels are of significance only when age-matched control horses are used, a factor which was not taken into consideration in some former studies. An unpublished preliminary study on the relation between stereotypic behaviour in horses and β-endorphin levels using age matched controls supports the findings reported here (S. McDonnell, personal communication).

Although there are data indicating that endogenous opioids can cross the blood brain barrier (Banks and Kastin 1987), peripheral levels of β-endorphin may not reflect concentrations in the central nervous system (CNS) that should be associated with behavioural changes. Smith *et al.* (1986) were able to demonstrate that acute stress in sheep increases β-endorphin concentrations in plasma but not in cerebrospinal fluid (CSF). The lack of changes in plasma β–endorphin in association with the actual performance of cribbing behaviour does not necessarily exclude such changes in the CNS.

Given the fact that, also in horses plasma β-endorphin is a well established indicator of stress (McCarthy *et al.* 1993; Canali *et al.* 1996) the sustained high levels in cribbing horses may be interpreted as a result of chronic stress. There is some evidence for plasma β-endorphin to be an appropriate parameter to monitor states of chronic stress not detected by glucocorticoids (Janssen *et al.* 1995). Hence the elevated β-endorphin levels in cribbers might be interpreted as an indicator of poor welfare.

Cortisol

Plasma cortisol levels differed neither between cribbers and control horses nor between the cribber's basal and cribbing samples. Due to the general design of the present study it was not possible to collect a sufficient number of blood samples to obtain cortisol profiles. Although the influence of diurnal changes was minimised during the present study, basal cortisol levels in the horse are dependant on ultradian fluctuations (Irvine and Alexander 1994). As point samples may be able to reflect marked changes in situations of acute stress, but not slight changes in daytime profile, the present results are no proof that there is no change in cortisol in dependence on cribbing behaviour. There is some evidence from previous work for such changes in plasma cortisol but the results are contradictory. McGreevy and Nicol (1995) reported significantly higher basal cortisol levels in cribbing horses when compared with controls whereas McBride (1996) could not find a difference in basal concentrations.

Assuming that stereotypic behaviour may reduce stress, cortisol levels should increase when animals are prevented from performing this behaviour. McGreevy and Nicol (1995) were able to show that cribbing horses have significantly higher cortisol levels when prevented from cribbing and not offered an alternative occupation such as providing hay. Besides the methodological problems pointed out before, an explanation why there was no change in cortisol during the present study may be that the horses were not disturbed.

Given the fact that cribbers have marked elevated basal β-endorphin levels one should also consider that endogenous opioids could inhibit the hypothalamus-pituitary-adrenocortical (HPA) axis in horses (Alexander and Irvine 1995). Long term increase of β-endorphin as a result of chronic stress is thought to have an inhibitory effect on the adrenocortical reactivity (Janssen *et al.* 1995) which is why β-endorphin and not cortisol may be the more useful parameter to monitor situations of chronic stress.

Serotonin

Although this study failed to identify significant changes in serotonin levels, there was a trend for lower basal concentrations in cribbing horses compared to controls. There is some evidence from the literature that a deficiency of the neurotransmitter serotonin or 5-hydroxytryptamine (5-HT) may play an important role in the control of stereotypic behaviour. Most of these data is based on the use of serotonin agonists, inhibiting the transmitter re-uptake at central 5-HT receptors, in the treatment of obsessive-compulsive disorders in man (Rapoport 1988) and in certain kinds

of stereotypic behaviour in animals such as acral lick dermatitis in dogs (Goldberger and Rapoport 1991). In horses, administration of serotonin re-uptake inhibitors such as clomipramine as well as of l-tryptophan which is the natural precursor molecule from which serotonin is synthesised are reported to reduce stereotypic behaviour (McDonnell 1998). However, it remains unclear whether these are selective effects on stereotypic behaviour or just a result of a general sedative effect of these drugs.

Conclusions

Stereotypies in general may be an indicator of poor welfare in horses reflecting problems to cope with an inadequate environment (Broom and Kennedy 1993). Nevertheless, there is evidence from data presented here that the actual performance of an once established stereotypie may help horses to reduce arousal. Cribbing behaviour is associated with an decrease in both nociceptive threshold and heart rate. Assuming that this indicates a state of lower arousal, cribbing seems to have a positive effect on affected horses. As there is no evidence for a general reduction in the state of health caused by this stereotypic behaviour pattern symptomatic 'treatments' such as mechanical or surgical methods to prevent cribbing movements are at least doubtful from a welfare point of view.

The marked elevation of basal β-endorphin levels and the trend for lower basal serotonin concentrations in cribbing horses could provide evidence for an imbalance between their endogenous opioid and serotonergic systems as postulated by Dodman *et al.* (1997). As both can have modulating influence on dopaminergic neurons, an opioid-mediated activation and a lack of serotonin-mediated inhibition of central nervous dopaminergic pathways may be involved in the performance of stereotypic behaviour patterns. This hypothesis would explain the inhibitory effects of opioid antagonists (Dodman *et al.* 1987; McBride *et al.* 1996) and serotonin agonists (McDonnell 1998) on cribbing in horses. The question whether the high resistance of once established cribbing behaviour against environmental improvements may be related to such changes in neuroendocrinological systems needs further investigation.

Furthermore, the role of β-endorphin as a potential indicator of chronic stress should be taken into consideration for future work focusing on the causes for development and manifestation of stereotypic behaviour in the horse.

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Manufacturers' addresses

[1]Microsoft, Redmont, Washington, USA.
[2]Polar-Electro, Turku, Finland.
[3]Peninsula Laboratories, St. Helens, UK.
[4]Savant Instruments, Formingdale, USA.
[5]Wallach, Turku, Finland.
[6]Sigma Chemicals, Deisenhofen, Germany.
[7]Chromsystems, Munich, Germany.
[8]Beckman, Munich, Germany.
[9]Scientific Software Service, Berlin, Germany.

References

Alexander, S.L. and Irvine, C.H.G. (1995) The effect of naloxone administration on the secretion of corticotropin-releasing hormone, arginine vasopressin, and adrenocorticotropin in unperturbed horses. *Endocrin.* **136**, 5139-5147.

Amit, Z. and Galina, H.G. (1986) Stress-induced analgesia: adaptive pain suppression. *Physiol. Rev.* **66**, 1091-1120

Banks, W.A. and Kastin, A.J. (1987) Saturable transport of peptides across the blood-brain barrier. *Life Science* **41**, 1319-1338.

Borroni, A. and Canali, E. (1993) Behavioural problems in thoroughbred horses reared in Italy. In: *Proceedings of the International Congress on Applied Ethology*, Berlin. Eds: M. Nichelmann, H.K. Wierenga and S. Braun. KTBL, Darmstadt, Germany. pp 43-46.

Broom, D.M. (1988) The scientific assessment of farm animal welfare. *Appl. anim. Behav. Sci.* **20**, 5-19.

Broom, D.M. and Kennedy, M.J. (1993) Stereotypies in horses: their relevance to welfare and causation. *Equine vet. Educ.* **5**, 151-154

Canali, E., Ferrante, V., Matiello, S., Sacerdote, P., Lebelt, D. and Zanella, A.J. (1996) Plasma levels of β-endorphin and in vitro lymphocyte proliferation as indicators of welfare in horses in normal and restrained conditions. *Pferdeheilkunde* **12**, 415-418.

Chambers, J.P., Livingston, A. and Waterman, A.E. (1990) A device for thresholds in horses. *J. Ass. vet. Anaesth.* **17**, 42-44.

Cronin, G.M. (1985) *The Development and Significance of Abnormal Stereotyped Behaviour in Tethered Sows*. PhD Thesis, Agricultural University of Wageningen, The Netherlands.

Dodman, N.H., Shuster, L., Court, M.H. and Dixon, R. (1987) Investigation into the use of narcotic antagonists in the treatment of a stereotypic behaviour pattern (crib-biting) in the horse. *Am. J. vet. Res.* **48**, 311-319.

Dodman, N., Moon-Fanelli, A., Mertens, P., Fluger, S. and Stein, D. (1997) Animal models for obsessive compulsive disorders. In: *Obsessive Compulsive Disorders: Diagnosis, Etiology, Treatment.* Eds: D. Stein and E. Hollander. MDI, New York.

Engelhardt, B. (1990) *Zur Geschichte der Untugenden des Pferdes - Symptome, Ursachen, Behandlung and Forensik*. Diss. med. vet., University of Giessen, Germany.

Gilham, S.B., Dodman, N.H., Shuster, L., Kream, R. and Rand, W. (1994) The effect of diet on cribbing behavior and plasma β-endorphin in horses. *Appl. anim. Behav. Sci.* **41**, 147-153.

Goldberger, E. and Rapoport, J.L. (1991) Canine acral lick dermatitis. Response to the antiobsessional drug clomipramine. *J. Am. anim. Hosp. Ass.* **22**, 179-182.

Hutt, C. and Hutt, S.J. (1978) Heart rate variability - the adaptive consequences of individual differences and state changes. In: *Human Behaviour and Adaptation.* Eds: N. Blurton-Jones and V. Reynolds. Taylor and Francis Ltd., London. pp 171-189.

Irvine, C.H.G. and Alexander, S.L. (1994) Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domest. anim. Endocrinol.* **11**, 227-238.

Janssen, C., Helmond, F., Loyens, I., Schouten, W. and Wiegant, V. (1995) Chronic stress increases the opioid-mediated inhibition of the pituitary-adrenocortical response to acute social stress in pigs. *Endocrinol.* **136**, 1468-1473.

Kammerling, S.G., Weckman, T.J., DeQuick, D.J. and Tobin, T. (1985) A method for studying cutaneous pain perception and analgesia in horses. *J. Pharmacol. Meth.* **13**, 267-274.

Lebelt, D., Schönreiter, S. and Zanella, A.J. (1996) Salivary cortisol in stallions: the relationship with plasma levels, daytime profile and changes in response to semen collection. *Pferdeheilkunde* **12**, 411-414.

Luescher, A., Mckeown, D.B. and Dean, H. (1998) A cross-sectional study on compulsive behaviour (stable vices) in horses. *Equine vet. J., Suppl.* **27**, 14-18.

Mason, G. (1991) Stereotypie: a critical review. *Anim. Behav.* **41**, 1015-1037.

McBride, S. (1996) A comparison of physical and pharmacological treatments for stereotyped behaviour in the horse. In: *Proceedings of the 30th International Congress of the International Society for Applied Ethology*. Eds: I. Duncan, K. Widowski and D. Haley. University of Guelph, Canada. p 26.

McCarthy, R.N., Jeffcott, L.B., Clarke, I.J. (1993) Preliminary studies on the use of plasma β-endorphin in horses as an indicator of stress and pain. *J. equine vet. Sci.* **13**, 216-219.

McDonnell, S. (1998) Pharmacological aids to behavior modification in horses. *Equine vet. J., Suppl.* **27**, 50 (Abstr.).

McGreevy, P.D. and Nicol, C.J. (1995) Behavioural and physiological consequences associated with prevention of crib-biting. In: *Proceedings of the 29th International Congress of the International Society for Applied Ethology*. UFAW, London, UK. pp 135-136.

McGreevy, P.D., Cripps, P.J., French, N. P., Green, L.E. and Nicol, J. (1995a) Management factors associated with stereotypic and redirected behaviour in the Thoroughbred horse. *Equine vet. J.* **27**, 86-91.

McGreevy, P.D., Richardson, J.D., Nicol, C.J. and Lane, J.G. (1995b) Radiographic and endoscopic study of horses performing an oral based stereotypy. *Equine vet. J.* **27**, 92-95.

McGreevy, P.D., French, N.P. and Nicol, C.J. (1995c) The prevalence of abnormal behaviour in dressage, eventing and endurance horses in relation to stabling. *Vet. Rec.* **137**, 36-37.

Minero, M., Ödberg, F.O., Ferrante, V. and Canali, E. (1996) Preliminary results on the relationship between heart rate and cribbing in horses. In: *Measuring Behaviour '96*. Utrecht, The Netherlands.

Nolan, A., Livingston, A., Morris, R. and Waterman, A. (1987) Techniques for comparison of thermal and mechanical nociceptive stimuli in the sheep. *J. Pharmacol. Meth.* **17**, 39-49.

Pippi, N.L. and Lumb, W.V. (1979) Objective tests of analgesic drugs in ponies. *Am. J. vet. Res.* **40**, 1082-1086.

Rapoport, J.L. (1988) The neurobiology of obsessive-compulsive disorder. *J. Am. vet. med. Ass.* **260**, 2888-2890.

Ritzberger-Matter, G. and Kaegi, B. (1998) Retrospective analysis of the success rate of surgical treatment of aerophagia in horses at the Veterinary Surgical Clinic, University of Zurich. *Equine vet. J., Suppl.* **27**, 62 (Abstr.).

Rodgers, R.J. and Rendall, J.I. (1988) Environmentally induced analgesia: Situational factors, mechanisms and significance. In: *Endorphins, Opiates and Behavioural Processes*. Eds: R. Rodgers and S.J. Cooper. John Wiley and Sons Ltd, New York. pp 107-142.

Rushen, J., de Passillé, A.M. and Schouten, W. (1990) Stereotyped behaviour, endogenous opioids and post-feeding hypoalgesia in pigs. *Physiol. Behav.* **48**, 91-96.

Sambraus, H. and Radke, K. (1989) Zum "Weben" des Pferdes. *Dtsch. tieraerzth. Wschar.* **96**, 241-284.

Schouten, W. and Rushen, L. (1993) The role of endogenous opioids in stereotyped behaviour and in heart rate response in pigs. In: *Opioids in Farm Animals*. Ed: N. Parvizi. Landwirtschaftsverlag, Münster, Germany. pp 207-219.

Smith, R., Owens, P.C., Lovelock, M., Chan, E.-C. and Falconer, J. (1986) Acute haemorrhagic stress in conscious sheep elevates immunoreactive β-endorphin in plasma but not in cerebrospinal fluid. *Endocrin.* **118**, 2572-2576.

Soussignan, R. and Koch, P. (1985) Rhythmical stereotypies (leg-swinging) associated with reductions in heart-rate in normal school children. *Biol. Psychol.* **21**, 161-167.

Zanella, A.J. (1992) *Sow Welfare Indicators and Their Inter-Relationships*. PhD Thesis, University of Cambridge, UK.