Physiological and Behavioral Consequences Associated With Short-Term Prevention of Crib-Biting in Horses

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MCGREEVY, P. D. AND C. J. NICOL. *Physiological and behavioral consequences associated with short-term prevention of crib-biting in horses.* PHYSIOL BEHAV **65**(1) 15–23, 1998.—Crib-biting in the horse is frequently prevented in the short-term by horse-owners using physical means. Because it has been proposed that crib-biting may function to reduce stress, the effect of prevention of crib-biting and/or eating on the behaviour, heart rate, and plasma cortisol and β-endorphin concentrations was measured in six crib-biting and six normal horses. When crib-biters were unable to crib-bite, they showed an increase in ingestive behaviour. When crib-biters were prevented from crib-biting and eating, a relative stasis in the motility of the foregut occurred, suggesting that normal gut function in these animals depends on ad libitum access to food and to suitable crib-biting substrates. There was no significant difference in the mean baseline levels of normal and crib-biting horses but, contrary to expectations, β-endorphin levels were higher in crib-biting horses than in normal horses when crib-biting was prevented. Mean baseline levels of cortisol were higher, under a variety of test and control conditions, in crib-biting than in normal horses, but there was no significant rise in cortisol levels in crib-biters during periods when crib-biting was prevented, suggesting that the function of this oral stereotypy does not lie in stress-reduction.   © 1998 Elsevier Science Inc.

Stereotypy   Horse   Crib-biting β-endorphin   Cortisol

CRIB-BITING is an oral stereotypy reported in 5.5% of young racehorses (*Equus caballus*) (38). During crib-biting, the horse seizes a fixed object with its incisors and pulls back, drawing air into its cranial oesophagus while emitting a characteristic grunt (39). The behaviour is repetitive and invariant in form.

As with other abnormal behaviors in captive and domestic animals, the suggested proximate functions of stereotypies vary considerably. Suggestions which imply the absence of suffering, at least once the stereotypy has become established, have included "coping" with environmental stressors (3,4), "self-organization" of time-budgets (9), "adaptation" to aversive conditions (36) and "de-arousal" (54). Despite a persistent lack of semantic clarity, these possibilities have contributed to the general hypothesis that stereotypies may be "stress-coping" mechanisms (34).

Experimental studies have examined the effects of stereotypy prevention under controlled conditions to assess the validity of the stress-coping hypothesis. In response to cage modifications that prevented jumping, stereotypic bank voles, *Clethrionomys glareolus*, showed sustained elevations in corticosterone levels compared to nonstereotypic individuals (26). Because it is not clear to what extent these cage modifications may have prevented other behaviors, these results should be interpreted with care. In contrast, no change in plasma cortisol levels was detected in tethered sows, *Sus*

scrofa, after the prevention of stereotypic chain manipulations (51). Similarly, it has been found (56) that after 3 days of stereotypy prevention, the corticosterone levels of stereotypic wire-gnawing mice had returned to pretreatment levels after a transient rise observed on Day 1 of prevention. The transient rise may have reflected an initial response to changes in the cages, which subsequently disappeared as new behavioral patterns developed.

Caution is required in the interpretation of the results of prevention studies in relation to the stress-coping hypothesis. A rise in stress parameters during a period of behavioral prevention does not necessarily indicate that the function of that behaviour is stress reduction. For example, prevention of sexual behaviour could cause stress, even though its function is not stress reduction. However, it is likely that a stress response would accompany the prevention of a behaviour that was important in stress reduction. Thus, although it is important to measure the physiological consequences of preventing a stereotypic behaviour it is equally important to compare the results with those obtained by preventing nonstereotypic behaviors.

Stress-coping is not the sole function that has been ascribed to the crib-biting stereotypy in horses. Many stereotypies can be related to their motivational origin and morphology (39,43,28,42). Because crib-biting is an oral behaviour that involves activity of

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the teeth and lips as well as distension of the oesophagus, it may have an additional or alternative regulatory function in meeting unsatisfied foraging needs (52,39,31). It has also been suggested that the behaviour may result from gastrointestinal problems (19).

Despite the fact that the functional significance of crib-biting has yet to be established, prevention of the behaviour is regularly attempted by horse-owners. Short-term prevention of crib-biting can be achieved by the application of a cribbing-collar that restricts the horse's ability to arch its neck (17,13), housing animals in projectionless boxes, or electrifying any ledges in a loose-box (1). Longer-term prevention is not always successful but generally involves the excision of the nerves and/or muscles of the ventral neck (14) or the fistulation of the buccal cavity (24).

The overall aim of the current study was to determine whether the prevention of crib-biting resulted in physiological or behavioral changes that favoured either the stress-coping or digestive regulation hypothesis. To achieve this aim the study employed standard techniques to assess heart rate, behaviour, cortisol, and β-endorphin. A technique that has been established to assess gut transit time in humans (50), dogs, cats (K. Papassouliotis, Aspects of small intestinal function in cats and dogs, University of Bristol, 1994, Unpublished thesis). and calves (30) was adapted for equine use in this study.

Cortisol was measured as it is widely accepted as a stress parameter (P. M. Taylor, Some aspects of the stress response to anaesthesia and surgery in the horse, University of Cambridge, 1987, Unpublished thesis), and it rises reliably in horses in response to arousing and aversive stimuli (35,18).

The relevance of peripheral plasma levels of β-endorphin to central neural activity remains controversial (32). The possible involvement of β-endorphin in reinforcement (6,47) and the facilitation of equine stereotypy (7), the association of increased levels of β-endorphin with ingestion of palatable food (11), and the suggestion that palatable rations cause elevation of the pain threshold and increases in morphine potency (45,23) prompted its inclusion in our study. The suggestion that β-endorphin is a useful indicator of stress in the horse (15 and P. M. Taylor, Some aspects of the stress response to anaesthesia and surgery in the horse, University of Cambridge, 1987, Unpublished thesis) led us to hypothesise that levels of β-endorphin would rise when crib-biting is prevented.

The specific aims of the current study were to:

1) determine baseline plasma levels of cortisol and β-endorphin in normal and stereotypic horses. This was important because physiological parameters may vary in horses that behave differently; e.g., crib-biters have been reported to have lower than normal β-endorphin levels (11).

2) examine the physiological and behavioral responses that occur when horses are subjected to short-term prevention of crib-biting. We predicted that if crib-biting has a current stress-coping function, its prevention would result in an increase in cortisol levels, an increase in β-endorphin levels, and an increase in heart rate. If it has a digestive function, its prevention would accompany a reduction in gut motility. The experiment was also designed to investigate the possibility that eating hay and crib-biting are partial substitutes for one another. Therefore, it was important to determine the full effects of prevention by simultaneously removing the opportunity to perform both behaviors.

3) examine the physiological and behavioral responses that occur when horses are subjected to short-term prevention of a nonstereotypic behaviour (eating hay). This aimed to control for the general effects of removing an oral behaviour. However, it was accepted that with its nutritive function, eating hay could not be considered an absolute control for crib-biting, which is non-nutritive.

MATERIALS AND METHODS

Animals and Their Accommodation

Six stereotypic (crib-biting) and six normal Thoroughbred geldings were hired from members of the public (mean age of crib-biters: 8.4 years; mean age of normal horses: 6.8 years). No data were available on the exact time of onset of the crib-biting stereotypy in the thoroughbreds sampled, but all had had the stereotypy for a minimum of 12 months. They were all racehorses that were accustomed to frequent travelling and to housing in a variety of stables and loose-boxes. They were managed on-pasture and were fed hay for 5 days before the start of the experiment.

They were tested in three replicates of two stereotypic and two normal horses. The members of each replicate were mixed together in a paddock for 5 days before the start of the treatments. On treatment days, the four horses in each replicate were housed individually in adjacent loose-boxes (Tubby Boxes, Lillingstone Lovell, Buckingham) within a large naturally lit building. To prevent crib-biters from seizing projections with their teeth, all raised surfaces in the loose-boxes were covered with Perspex sheeting. The modifications allowed neighboring horses to see one another and the observer to monitor the horses' behaviour during the test. Each loose-box had a sliding door which was also covered with Perspex to eliminate any horizontal projections.

Two purpose-built removable "cribbing bars," made from planks of wood (65 × 15 × 4 cm) and housed in cast iron brackets, were bolted 1 m apart to the back wall in each box at a height of 1 m from the floor. The horses were assigned to one of four loose-boxes in a random order. Hay was fed ad libitum from a haynet suspended between the two cribbing bars. No concentrated food was given. Water was available at all times from an automatic waterer which was fixed close to the floor on the front wall to avoid its being used as a crib-biting substrate. The boxes were bedded with shavings and were cleaned every morning while the horses spent an hour (0800 to 0900 hours) in a paddock together. They returned to their stables at 0900 hours.

Experimental Procedure

The experiment was conducted over 10 consecutive days for each replicate. Days 1–2 were "acclimatisation days," whereas Days 3–10 were treatment days. Each treatment was imposed for 2 days, but physiological and behavioral recording was conducted only on Day 2 of each treatment. Each horse received all four treatments in an order determined by random selection.

On each treatment day, between 0900 hours and 1500 hours, horses were prevented from: 1) crib-biting (CBprev) by the removal of the cribbing bars with the haynet *in situ*; or 2) eating hay (Hprev) by the removal of the haynet with the cribbing bars *in situ*; or 3) crib-biting and eating hay (CB/Hprev) by the removal of the haynet and the cribbing bars.

Additionally, each horse acted as its own control by being deprived of none of these resources (N). The cribbing bar and haynet were both present during this treatment.

During the acclimatisation period horses were accustomed to the stable and to the wearing of heart rate monitors (Polar Sport Tester PE3000, Bodycare Products Ltd., Kenilworth, Warwickshire), which were attached to proprietary girth bands between 0800 and 1500 hours each day. On Days 1 and 5, 2.1 × 13.3 mm catheters (Angiocath, Becton Dickinson) were inserted into alternate jugular veins through a 1-mL subcutaneous (s.c.) volume of local anesthetic. Each catheter remained in place for 4 days allowing easy blood withdrawal during the experiment.

At 0900 hours on Day 2 of each treatment, the horses were fitted with heart-rate receivers which recorded the heart rate every 15 s. Blood samples for cortisol, β-endorphin and sulfapyridine assays were taken on Day 2 of each treatment, at 0900 hours (these were labeled "baseline responses") and every hour for the next 6 h ("treatment responses"). Behaviour was also recorded on Day 2 of each treatment for 50 min, between each blood-sampling session. At 1500 hours the girth bands were removed, and the resources were returned to the loose-boxes. Catheters were removed at this stage on Days 4 and 8.

β-Endorphin

Plasma β-endorphin assays were performed by a commercial laboratory using RIA kits (Sigma), (46). The reported range for β-endorphin in Thoroughbreds is 48.62 ± 5.02 pmol/liter (before exercise) to 98.0 ± 30.8 pmol/liter (after exercise) (S. Luna, Equine opioid, endocrine and metabolic responses to anaesthesia, exercise, transport and acupuncture, University of Cambridge, 1993, Unpublished Thesis). β-Endorphin levels in horses fluctuate with a diurnal rhythm that peaks at 0900 hours (15).

Cortisol

The cortisol assay was performed by a commercial laboratory using RIA kits (Sigma), (21,44). The reported range for cortisol in Thoroughbreds is 201 ± 73 nmol/liter (before exercise) to 301 ± 125 nmol/liter (after exercise) (2). Cortisol levels follow a circadian rhythm in the horse (8,31) and are generally at their peak during the sampling period (0900–1500 hours) used in this study.

Determination of Oro-Caecal Transit Times

The horses were given a paste of 26 g of sulfasalazine (Salazopyrin, Kabi Pharmacia Ltd, Milton Keynes, Bucks) milled with 30.5 g of mint sweet (Polo, Nestle Rowntree, York) by mouth. The first blood samples (7 mL into a vacutainer primed with oxalate fluoride) were immediately withdrawn via the i.v. catheters. Sequential samples were taken every hour for 6 h.

Sulfapyridine is produced by the action of large bowel flora on orally administered sulfasalazine. High-performance liquid chromatography (HPLC) was employed to detect sulfapyridine in the plasma samples, using the method described below.

Reagents. A solution containing 10 mg/mL of sulfamethazine (supplied by Sigma) as internal standard was prepared in citrate/phosphate buffer, pH 5.0. Buffer was prepared freshly each day from 24.25 mL of 0.2 M citric acid and 25.75 mL of 0.4 M disodium hydrogen phosphate.

Chromatographic conditions. The separation was performed on a C-18 Bondapak column (25 cm, 10-mm particle size, Waters Associates). Compounds were eluted with a solvent of 1% acetic acid and methanol (77:23) at a flow rate of 2.5 mL/min. A Waters pump model 6000 A and a fixed wavelength detector (Applied Chromatography Systems Model 750/10a) were used with a detection wavelength of 254 nm. The assay is linear up to 25 mg/mL with a limit of reliable detection and relative standard deviation of typically 0.2 mg/mL and 2–3%, respectively. The retention times of sulfapyridine and sulfamethazine were 3.1 and 4.8 min, respectively. Quantitation was effected by measuring the peak height ratio of sulfapyridine to internal standard and reference to a calibration curve.

Extraction procedures. The internal standard (1 mL) was added to 1 mL of plasma and 3 mL of diethyl ether/dichloromethane (6:4) in a 10-mL glass tube. The tubes were mechanically shaken for 15 min and then centrifuged for 10 min at 2,000 rpm (rotor radius 7.5 cm). The organic layer was separated and evaporated to dryness under nitrogen.

In all of the assays, the threshold of first appearance was set at the point where the ratio of sulfapyridine to the internal standard (sulfamethazine) reached 0.5.

Behaviour

Scan sampling of each horse's behaviour was performed every minute for the 50 min between each episode of blood-sampling. This gave a total of 300 samples for each horse undergoing each treatment. The following behaviors were recorded:

Crib-biting
Eating (chewing hay or pulling hay from the hay-net)
Drinking (head in watering device or standing beside device with water dribbling from mouth)
Rubbing (rhythmic scratching of body against structure of loose-box)
Resting (lying or relaxed standing posture with ears to the side)
Looking out (alert posture with ears forward facing in any direction other than toward the back wall).
Pacing (any locomotion)
Defaecating
Urinating

Analysis

Repeated measures ANOVA was used to assess the effects of repeated hourly sampling and repeated daily sampling and to determine whether there were differences in responses (heart rate, behaviour, cortisol, and β-endorphin) of normal and stereotypic horses.

Paired *t*-tests (two-tailed) were used to compare baseline responses to the means of each of the three separate treatment responses, separately for the stereotypic horses and for the normal horses. Paired *t*-tests (two-tailed) were also used to compare control treatment (N) responses with each of the three separate treatment responses (CBprev, Hprev, CB/Hprev) in the normal and stereotypic horses. Mann–Whitney tests were used to compare the latency of appearance of sulfapyridine in plasma between normal and stereotypic horses separately for each treatment, as these data did not meet the requirements for parametric analysis.

Using separate data from the two treatments that allowed crib-biting, Spearman Rank correlation coefficients were calculated for stereotypic horses to determine whether there was any temporal association between frequency of crib-biting and plasma levels of β-endorphin or between frequency of crib-biting and plasma levels of cortisol. Representative data for treatments N and Hprev are shown in Fig. 5.

RESULTS

β-Endorphin

A blocked catheter caused a loss of data for one stereotypic horse during treatment CBprev. Ten (2.9%) samples were contaminated during assaying and were discarded from the data-set.

For all horses, there was a marked fall in β-endorphin levels over the six samples taken hourly each day $[F(5,15) = 12.89; p < 0.0001]$, but there was no significant effect of daily test order, $F(3,37) = 1.45$; NS. Normal horses showed a diurnal peak in plasma β-endorphin at 0900 hours in line with previous findings (15). Predictably lower β-endorphin levels were then seen in all subsequent hourly means for these horses (Fig. 1a). β-Endorphin levels were also high at 0900 hours for crib-biters but the same pattern of decline was not observed, particularly in treatments CB/Hprev and Hprev, where mean response levels were very similar to baseline levels (Fig. 1b).
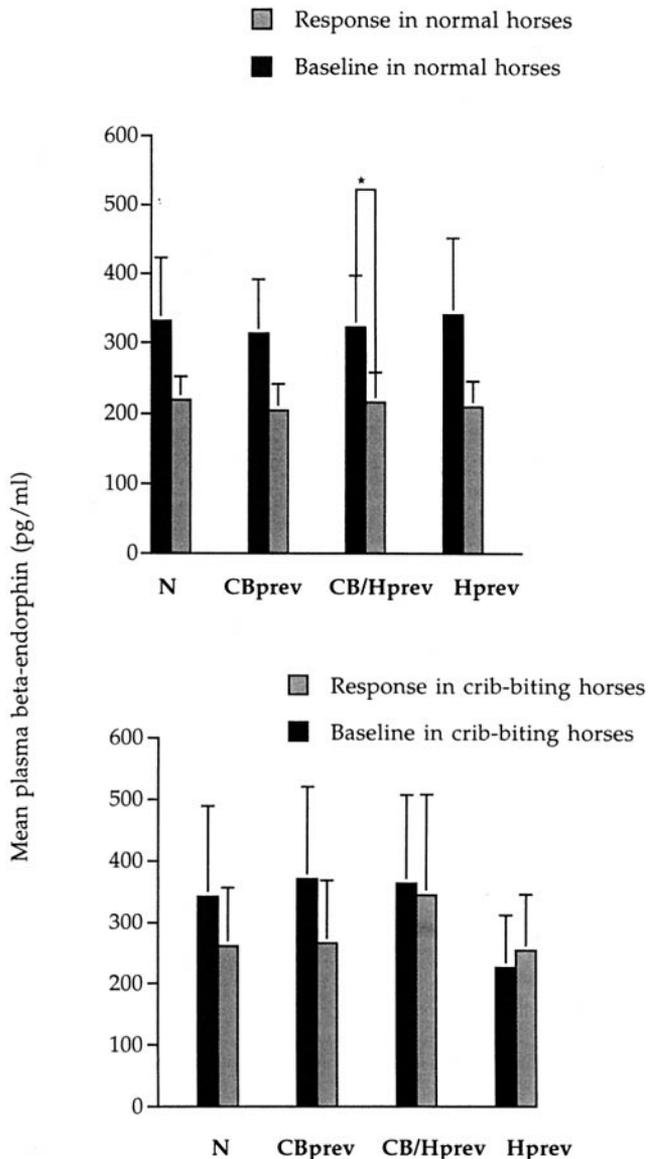
The mean baseline level of plasma β-endorphin was not sig-

FIG. 1 (a, b) Mean concentrations (SE) of plasma β-endorphin in normal ($n = 6$) and crib-biting horses ($n = 6$). Note that baseline is pretreatment reading (0900 hours) on day of that treatment.

nificantly higher in the stereotypic (325 ± 33.7 pg/mL) than in the normal horses (326 ± 5.86 pg/mL). However, the mean response level of plasma β-endorphin in all treatments was significantly higher in the stereotypic (281 ± 21.4 pg/mL) than in the normal horses (212 ± 3.29 pg/mL), $F(1,6) = 10.22$; $p = 0.02$ (Fig. 1, a and b).

There were no significant differences between the β-endorphin responses of stereotypic and normal horses during the control treatment and any of the three prevention treatments. However, during treatment CBprev there was a significant interaction between the two groups of subjects and the repeated measures of hourly β-endorphin sampling [$F(5) = 2.51$; $p = 0.048$]. β-Endorphin levels in stereotypic horses showed a gradual decline over the first 5 h of sampling followed by a rise at Hour 6, whereas normal horses showed a rapid decline from baseline to Hour 1 (Fig. 2).

The comparison of individual baseline responses with individual treatment responses showed a significant fall in plasma β-endorphin for treatment CB/Hprev in normal horses, $t(5) = 2.64$; $p < 0.05$ (Fig. 1a). There were no significant differences between baseline and treatment values for treatments N, CBprev and Hprev for normal horses or between baseline and treatment values for any treatment for stereotypic horses.

When the responses of individual horses during treatments CBprev, CB/Hprev and Hprev were separately compared with their responses during treatment N by paired t-tests, there were no significant differences.

Cortisol Levels

For all horses, there was no significant change in cortisol levels over the six samples taken hourly each day, but there was a marked fall with advancing daily test order [(3,43) = 1.45; $p < 0.05$].

The mean baseline level of plasma cortisol was significantly higher in the stereotypic animals (142.42 ± 13.78 nmol/liter) than in the normal horses (96.30 ± 15.58 nmol/liter) [$F(1,6) = 21.1$; $p < 0.005$]. Similarly, the mean treatment response level of plasma cortisol was significantly higher in the stereotypic animals (190.89 ± 22.78 nmol/liter) than in the normal horses (112.31 ± 6.56 nmol/liter) [$F(1,6) = 11$; $p < 0.05$] (Fig. 3, a and b).

The comparison of individual baseline responses with individual treatment responses showed a significant rise in plasma cortisol for treatment CB/Hprev in normal horses [$t(5) = -5.31$, $p < 0.01$] and for treatment CB/Hprev in stereotypic horses [$t(5) = -2.66$; $p < 0.05$]. There were no significant differences between baseline and treatment values for treatments N, CBprev and Hprev.

The cortisol concentrations of stereotypic horses were significantly higher ($p < 0.05$) than those of normal horses during CB/Hprev, N and Hprev [$F(1,9) = 6.49$, $p = 0.03$; $F(1,9) = 5.50$; $p = 0.04$; and $F(1,9) = 8.47$; $p = 0.02$, respectively] but not CBprev.

When the responses of horses during treatments CBprev, CB/Hprev and Hprev were compared separately with their responses during treatment N by paired t-tests, no significant differences were identified. However, in stereotypic horses, there was a trend toward higher levels of cortisol during CB/Hprev than during N [$t(5) = 2.34$, $p = 0.066$].

Heart Rate

Mean heart rates for all treatments for crib-biters and normal horses were 33.19 ± 0.90 and 35.31 ± 0.90, respectively, which did not differ significantly.

For all horses, two-factor repeated measure ANOVA showed significant falls in heart rates over the 6 h of each daily test [$F(1,5) = 4.43$; $p < 0.001$] but no significant change over repeated days. There were no significant effects of treatment on heart rate.

Oro-Caecal Transit Time

Times to the first appearance of salazopyrine ranged from 120 to 300 min. During treatments N, CBprev and Hprev, oro-caecal transit times for normal horses were 170 min $\pm$ 24.08, 150 min $\pm$ 20.49 and 190 min $\pm$ 28.64, respectively. These were not significantly different from those of stereotypic horses which were 190 min $\pm$ 36.06, 180 min $\pm$ 37.95, and 190 min $\pm$ 28.64, respectively.

During treatment CB/Hprev, significantly longer mean oro-caecal transit times were found in stereotypic horses (220 min $\pm$ 20, Mann–Whitney $p = 0.03$) than in normal horses (150 min $\pm$ 13.42).
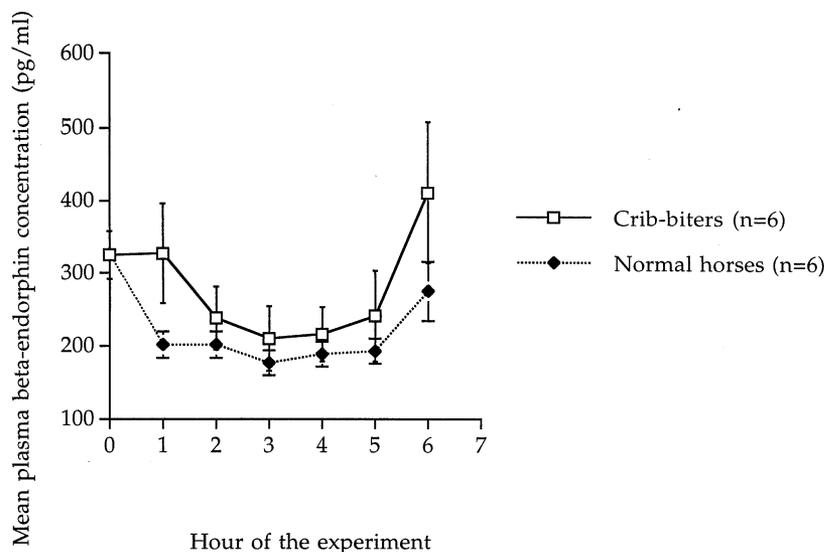
FIG. 2. Levels of β-endorphin during treatment CBprev.

Behaviour

During the control period N, crib-biters spent 20.2% ± 4.1% of their time crib-biting, 39.8% ± 1.2% eating and 29.8% ± 1.1% resting, whereas normal horses spent 42.3% ± 3.6% of their time eating and 41.5% ± 7.3% resting. Drinking, urinating, pacing, rubbing and looking out each took up less than 5% of the time budgets of both groups.

A three-factor repeated measures ANOVA showed a significant difference between the duration of eating behaviour of stereotypic and normal horses only in treatment CBprev during which crib-biters spent 42.08 ± 2.01% and normal horses 30.09 ± 8.38% of their time eating [$F(1,10) = 11$, $p < 0.01$ (Fig. 4)]. There were no significant effects of group or treatment on other behaviors.

No new stereotypies or conflict behaviors, such as displacement activities (pawing, head-tossing, kicking) were observed in the "prev" conditions.

Relationship between Stereotypy and Endocrinological Activity

There were no significant temporal correlations between crib-biting and plasma β-endorphin levels or between crib-biting and plasma cortisol levels during any treatment.

DISCUSSION

β-Endorphin

Baseline Differences

There were no significant differences between the baseline levels of β-endorphins in crib-biters and normal horses. In contrast, it was reported (11) that levels of β-endorphin in crib-biters were less than half of those of normal horses; it was suggested that either plasma levels are too peripheral to be a useful reflection of critical central levels of endorphin or that stereotyping is a response of individuals that are hypersensitive to the central release of β-endorphin. The same study (11) provided few details of the housing and management of their subjects although it seems that their horses were fed grain rations which were withheld on the morning of each study. The horses in our study were fed no concentrate ration. The difference in baseline values between our horses and those used in the previous study (11) may, therefore, reflect differences in the response of crib-biters to a concentrate feeding regime. The baseline levels in our study may also reflect a response to stimuli encountered in the paddock before catching or the effect of being caught. Social, agonistic, and kinetic behaviors are more easily expressed in the paddock than in the stable (28). Eating and crib-biting also occurred in the paddock where grass was available and consumed in preference to hay. Palatable foods are associated with increases in the rate of crib-biting in horses (29,11). In the current study, if the palatability of grass alone was sufficient to cause a bout of crib-biting, then one would expect to observe more stereotypy in crib-biters when they are at-pasture than when they are stabled and fed hay. This is not the case and, therefore, there must be other factors involved in the initiation of a bout of crib-biting.

β-Endorphin Responses to Treatment

The mean treatment response of crib-biting horses was significantly higher than that of normal horses, due partly to a significant fall in β-endorphin during one treatment. The greatest variations in β-endorphin levels were seen in the responses of crib-biters. However, there is negative correlation between the inhibiting effect of naloxone and the time since development of stereotypy in pigs and bank voles. Therefore, the role of endorphins in the stereotypies of these species is thought to decrease with the age of the stereotypy (5,25). This phenomenon may have increased variability in our data because we used horses of various ages. It may be that the stereotypic horses in this study had gone beyond the stage of stereotypy development that involves β-endorphin.

Interestingly, β-endorphin levels fell during most treatments relative to baseline levels, whereas cortisol levels rose. This finding lends weight to the suggestion that cortisol and endorphins are not released together (22). Furthermore, these findings support the suggestion (53) that elevated cortisol levels in humans with obsessive-compulsive disorders may exert a negative feedback effect on β-endorphin release. The fall in β-endorphin levels during most treatments relative to baseline levels taken at 0900 hours may be
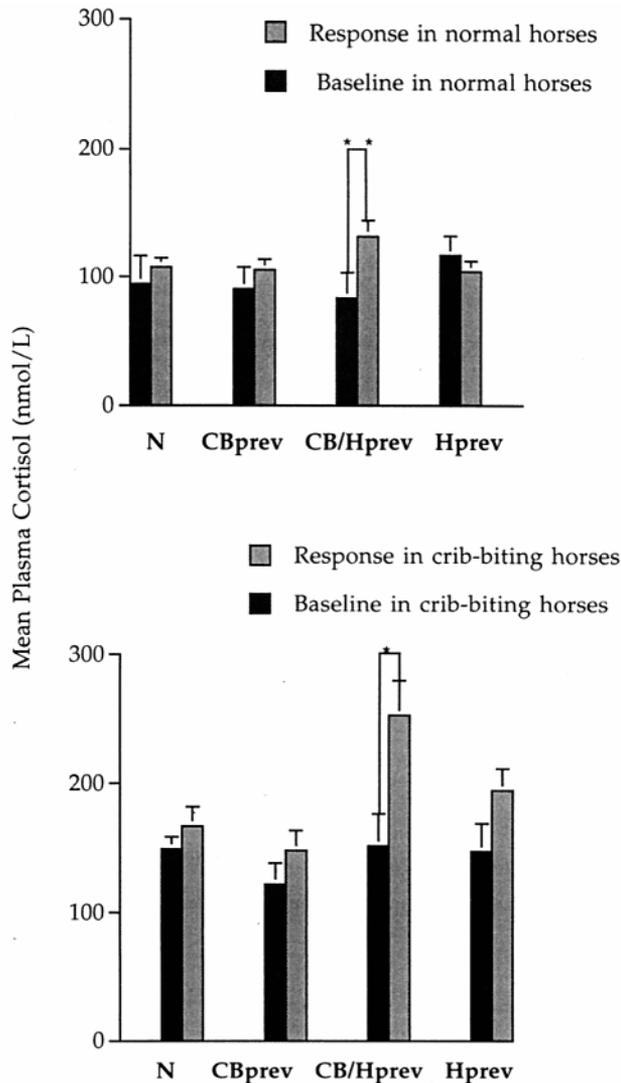
FIG. 3 (a, b) Mean concentrations ($\pm$ SE) of plasma cortisol in normal ($n = 6$) and crib-biting horses ($n = 6$). Note that baseline is pretreatment reading (0900) on day of that treatment.

FIG. 4. Percentage of time budget spent in each behaviour during treatment CBprev.

due to a diurnal effect because the daily peak is expected at this time (15). The absence of a predicted fall in the β-endorphin levels of crib-biters during treatments CB/Hprev and Hprev may indicate that these horses have difficulty in adapting to the removal of food.

Alternatively, the crib-biters may have found the 0900 hours move from the paddock to the stable more stressful than the normal horses, which exhibited a rapid decline in β-endorphin between baseline and Hour 1 (Fig. 2).

Because equine -endorphin levels have been shown to rise in response to a number of stressors (37), rises during all salient prevention treatments might be expected. Unexpectedly, however, normal horses showed no rise in β-endorphin in response to the removal of hay, and crib-biters showed no rise in response to any treatment. The stressors examined in previous equine studies where β-endorphin was measured (36 and P. M. Taylor, Some aspects of the stress response to anaesthesia and surgery in the horse, University of Cambridge, 1987, Unpublished thesis).) may
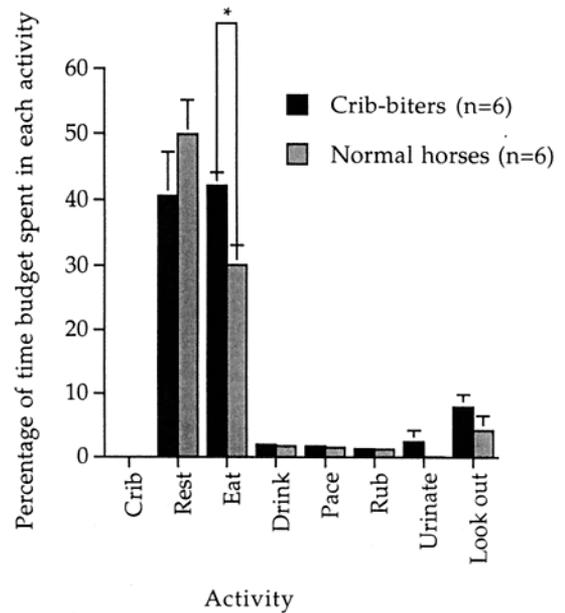
have been more severe and unfamiliar than those in the current study. Limited-forage feeding regimes and attempts to prevent crib-biting using cribbing collars are common. Our subjects may thus have had experience of restrictions related to those imposed in the experiment and may have adapted in some way. This would explain the lack of a significant rise in β-endorphin levels in animals exposed to an apparent stressor, much as chronically lame horses are reported to have β-endorphin levels within the normal range (37).

There was no significant difference in β-endorphin between treatments N and Hprev for either crib-biters or normal horses. Whereas a link has been identified between highly palatable food and endorphin release (7,44,47,23), our result supports the suggestion that the palatability of hay is not sufficient to stimulate endorphin release (7). The relationship between foods of varying palatability, circulating β-endorphin levels, and crib-biting seems to be less simple than has been suggested (11). The role of the novelty and physical nature of foodstuffs, precedent periods of starvation, and other peri-prandial stressors has to be addressed in future models of this behaviour.

It is suggested that the difference between the β-endorphin levels found in the crib-biters in previous studies (11, $n = 5$) and the current study ($n = 6$) are the product of biologic variability. Therefore, a further survey of the plasma levels of this opioid in larger cohorts of normal and stereotypic Thoroughbreds is required before its role in the facilitation of stereotypy can be more fully understood.

Cortisol

Baseline Differences

The mean baseline levels of plasma cortisol were significantly higher in crib-biters than in normal horses. These data do not at first suggest that crib-biting is functioning to reduce stress. However, longitudinal studies would be required to establish whether
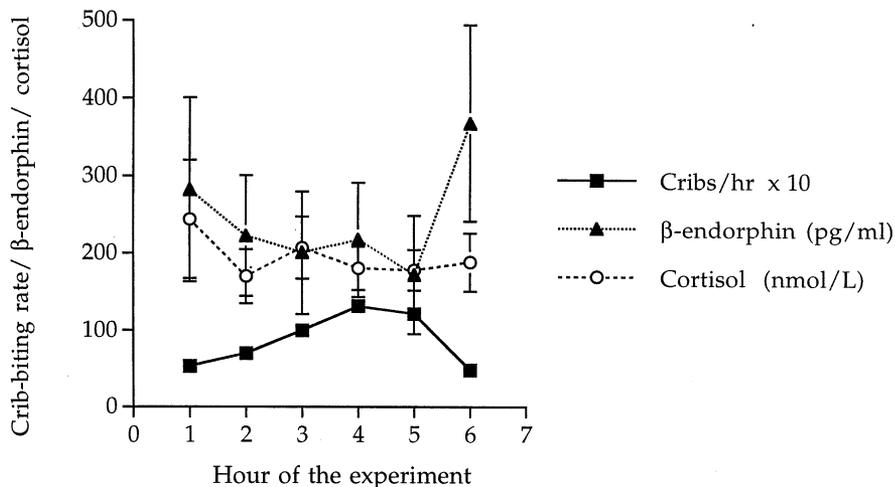
FIG. 5. Mean crib-biting rate and levels of plasma β-endorphin and cortisol crib-biters ($n = 6$) during treatment Hprev.

the development of crib-biting had reduced cortisol levels from even higher original levels in these horses.

It is interesting that this difference should cause no overt clinical signs. Exogenous corticosterone administered in the long-term is associated with immune-suppression, catabolism, and a predisposition to laminitis (55). Other causes of raised plasma cortisol, e.g., Cushing's Syndrome, are usually associated with obvious clinical changes in the long-term (12). The fact that both normal and stereotypic horses in the current study had cortisol levels within the normal range may account for the absence of any clinical signs associated with raised cortisol in our subjects. Crib-biters may be genetically more stress-susceptible animals that adapt to elevated cortisol levels at the time when the stereotypy is developing.

Cortisol Responses to Treatment

The marked fall in plasma cortisol that all horses showed with advancing daily test order is likely due to habituation to the experimental procedure.

Removal of the cribbing bar alone was not associated with a rise in plasma cortisol in normal or in stereotypic horses. However, the crib-biters ate significantly more than normal horses during treatment CBprev. Perhaps cortisol in stereotypic horses remained stable during this treatment because they were able to eat and, therefore, met their needs for oral stimulation.

The treatment which imposed the greatest restrictions on the stereotypic horses, i.e., CB/Hprev, was associated with the highest mean cortisol response. Although the stereotypic horses started from a significantly higher baseline value, this treatment elicited a significant increase that seems to be of biologic significance. Intriguingly, a similar peak response was noted for normal horses during this restriction. The removal of the cribbing bar would seem to be of no consequence to a horse that did not interact with it. However, it may be that removal of food and stable furniture may combine to cause stress in any horse.

In normal horses, hay removal was associated with a cortisol response that was lower than the baseline level for those days of treatment. It is interesting to compare these results with those from calves, having been deprived of food for 18 h, that showed a significant cortisol response only when food was re-presented (27).

Six hours without food may not cause stress in animals that are accustomed to intermittent feeding.

Heart Rate

A decreased heart rate during crib-biting bouts has been reported (33,41), but no similar relationship was detected over the 6 h of the current deprivation periods. The significant falls in heart rates over the 6 h of each daily test concur with previous studies in which heart rate was shown to peak at 0900 hours (15).

Oro-Caecal Transit Time

Oro-caecal transit time was remarkably consistent between most of the 12 subjects, except during CB/Hprev when it was associated with a later time of first appearance in the stereotypic horses. This implies that a relative stasis occurs in the motility of crib-biters' foreguts when they are unable to crib-bite and unable to eat.

Physical flushing by saliva and hyperactivity of the gastro-colic reflex (10) may be associated with crib-biting, and these factors may quicken the transit of ingesta in the foregut. As a result, gut motility may be restored to levels seen when crib-biting is allowed. This implies that crib-biting has a digestive function. Although it is interesting to consider the relationship between stressors and gut motility, there are insufficient data available to draw useful links between cortisol and gut motility in the horse.

Work in humans (16) indicates that examination stress, measured by elevations in serum cortisol, is associated with increased gastrointestinal symptoms, including diarrhea, without altering oro-caecal transit.

Behaviour

During treatment CBprev, there was significantly more eating in crib-biters than in normal horses. Although the time spent eating did not fully match the time that would have been spent crib-biting, it may be that eating has a compensatory effect for thwarted crib-biters. This may explain why significant changes in β-endorphin and cortisol were not detected during this treatment.

CONCLUSIONS

It has been suggested that, in the same way that increased sucking motivation (shown by non-nutritive sucking in calves) occurs after milk flow (49), positive feedback may underlie many feeding and drinking stereotypies (20,48). This may explain the effect on oro-caecal activity and trends toward an elevated stress response that food deprivation can have in combination with prevention of an oral stereotypy. Both crib-biting and feeding may function to supplement oral feedback and withdrawal of both is sufficient to cause a stress response in stereotypers which seem to be particularly sensitive individuals.

The current study showed that plasma cortisol levels in crib-biters were higher than normal horses under a variety of treatments. These data do not support the hypothesis that crib-biting has a coping function. However, studies of changes in cortisol level at the time that crib-biting was first developing would be required to exclude the possibility that crib-biting resulted in a lowering of plasma cortisol, albeit to a level still higher than that of normal horses. The significant rises in plasma cortisol that occurred, relative to baseline levels, in crib-biters prevented from eating and crib-biting, suggests that these two activities combine to facilitate homeostasis in stereotypic animals. The significant reduction in oro-caecal motility that occurred in crib-biters prevented from eating and crib-biting suggests that normal gut function in these animals is affected by oral activity, and that eating and crib-biting may be partial substitutes for each other. Crib-biting did not directly affect plasma levels of β-endorphins.

The absence of a significant rise in cortisol levels in crib-biters that were transiently prevented from performing this stereotypy suggests that the function of this oral stereotypy does not lie solely in stress-reduction. This result supports previous findings in sows that were prevented from stereotypic chain-manipulating (51) and in the conclusions of workers who examined the functional significance of stereotypic wire-gnawing in mice (56).

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