THE PUTATIVE WELFARE-REDUCING EFFECTS OF PREVENTING EQUINE STEREOTYPIC BEHAVIOUR

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Abstract

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The common practice of preventing equine stereotypic behaviour in the UK may be of concern, from a welfare perspective, if these behaviours constitute a coping response to a suboptimal environment. The aim of this study was to assess the putative function of these behaviours by measuring behavioural and physiological parameters i) before and after stereotypy prevention; ii) before and after stereotypy performance; and iii) in response to opiate antagonist (naloxone) administration.

The crib-strap significantly (P = 0.05) elevated mean plasma cortisol levels in crib-biting horses; a similar, although not significant trend (P = 0.07) was also observed for the weaving group during the anti-weave bar treatment. Both crib-strap and anti-weave bar significantly (P < 0.05) elevated plasma cortisol levels in the control horses. Although the latter result prevented a definite conclusion being drawn about the function of equine stereotypies, the results did indicate that the use of the crib-strap and anti-weave bar is stressful to the horse.

Plasma cortisol level was significantly (P = 0.04) higher immediately prior to the onset of stereotypy followed by a significant reduction post-stereotypy. This suggested that both cribbiting and weaving have a coping function to reduce stress levels in the animal.

Naloxone significantly reduced crib-biting by 84 per cent (P = 0.05) but it did not reduce weaving behaviour, indicating that crib-biting is a reward behaviour. However, resting behaviour was also significantly (P = 0.02) increased in crib-biting horses, suggesting that the stereotypy reduction was due to a sedative effect of the opiate antagonist. The latter was not measured, however, in control or weaving animals, and thus may be interpreted differently. The welfare implications of these results are discussed.

Keywords: animal welfare, equine, stereotypic behaviour

Introduction

In accordance with the current definition of stereotypic behaviour (a behaviour pattern that is repetitive, invariant and has no obvious goal or function; for review see Mason [1991]), equine stereotypic behaviours are considered here as crib-biting, weaving and box-walking. Based on archaeological evidence of excessive incisor wear associated with crib-biting, it can

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be concluded that these behaviours have been performed by the horse since the Middle Palaeolithic (Bahn 1980). The first written record of this behaviour was in 1696 in Sollysel's book *The Marechal Parfait*.

The clinical sequelae (incisor wear, strained ligaments) of these behaviours are relatively minor and there is little evidence to suggest that these behaviours are learned (Lindberg *et al* 1999). However, many horse owners regard stereotypies as unsightly 'contagious vices'. Horses that perform stereotypies are often ostracized within stables; they are banished from the premises altogether or attempts are made to physically prevent the performance of the behaviour (McBride & Long in press). For example, the crib-strap, (a leather strap with two pieces of metal hinged together at the mid-point) is often used to prevent crib-biting. Weaving may be prevented by the use of anti-weave bars on the door. This practice is often unsuccessful, since the animal steps back from the doorway and performs the behaviour inside the stable (McBride 2000). Box-walking is rarely prevented due to the impracticalities of immobilizing the horse within a stabled environment (McBride & Long in press).

Although the current definition of stereotypic behaviour states that it is without apparent function (for review see Mason [1991]), some evidence suggests that these behaviours may be acting as a coping response to a suboptimal environment (Cronin *et al* 1986; Kennes & De Rycke 1988; Cooper & Nicol 1991). This conclusion has been supported recently for equine stereotypy; significant increases in central nervous system (CNS) opioid activity (as indicated by significant decreases [P < 0.05] in pain threshold) after crib-biting, together with a significant decrease (P < 0.05) in heart rate have been reported (Lebelt 1998). The opioid activity was considered to facilitate a coping response by providing the animal with a reward experience, thereby reducing stress-levels and associated stress-related parameters (eg plasma cortisol level, heart rate). If this is correct, then preventing equine stereotypic behaviour, as is commonly attempted by people within the equine industry, may have serious welfare implications.

The first aim of this study was to investigate this putative function of equine stereotypic behaviour by i) measuring stress-related behavioural and physiological variables before and during the prevention of stereotypy using devices (crib-strap and anti-weave bar) that are currently in use in the UK; and by ii) measuring the same parameters before and after stereotypy performance.

A second approach was to determine the behavioural response to opiate antagonist administration. It has been stated (Dantzer 1991; Zanella et al 1996) that if CNS opioid activity is the mechanism by which stereotypy functions (ie it has reward characteristics), then blocking opioid receptors with opiate antagonists should cause increased stereotypic behaviour as the animal attempts to compensate for the lack of available endogenous opioid. The fact that opiate antagonists attenuate stereotypic behaviour in several species (Dodman et al 1987; Kennes et al 1988; Rushen et al 1990; Savory et al 1992; Schouten & Rushen 1992) is considered to disprove this hypothesis and to indicate instead, that it is endogenous opioids that are causing the behaviour. Thus, stereotypic behaviour may have no actual function and simply be the result of increased CNS opioid activity. However, this could be an oversimplification of the underlying neurochemistry involved. The motivation and the subsequent performance of reward behaviours, such as eating and sexual behaviour, can be increased in rodents by administering exogenous opioids (Majeed et al 1986; Mucha & Iversen 1986; Band & Hull 1990; Bakshi & Kelley 1994; Wise 1996) and attenuated by administering opiate antagonists (Giraudo et al 1993). Given that stereotypic behaviour can be induced (Mama et al 1992) and attenuated (Dodman et al 1987) by administering similar

agonists and antagonists, the foregoing suggests that stereotypy is also a reward behaviour and thus may have a putative coping function. Although an attenuating effect of administering opioid antagonist on crib-biting and weaving behaviour has already been reported (Dodman *et al* 1987; Nurnberg *et al* 1997), the method of behavioural assessment employed for the former study and the low number of experimental horses used for the latter study (eg n = 1 for weaving), requires that this work is repeated to ensure that any effect on stereotypy performance is either not due to chance, or is not due to an effect that is altering behavioural activity overall.

Methods

Animals

Crib-biting and weaving horses were identified within a population of 280 horses. Anecdotal reports from stable staff were used to identify potential crib-biting and weaving horses. Their performance of abnormal behaviours was then confirmed through direct observation. A total of four crib-biting and four weaving horses were identified; four control animals were recruited to match group sizes.

The general management and feed rations remained constant throughout the trial for all horses involved; 1.5kg Cavalry Mix (Dodson and Horrell Ltd, Kettering, UK), 125g chaff and 125g sugar beet per horse were given at 1200h and 1630h, and one hay net at 1800h. Horses were exercised for 1h day⁻¹ except on treatment days when no exercise was given.

Treatments and experimental design

All procedures were conducted under a UK Home Office Project Licence (PPL70/03175). Treatments are described in Table 1. Each treatment period lasted for 11h. This was based on similar studies using other species (Dantzer *et al* 1987; Terlouw *et al* 1991) and the availability of the horses. One weaving horse became unavailable during the course of the trial, thus n = 3 for the weaving group.

		Co	ntro	hor	ses		ib-bi rses	ting			eavin rses ^c	•
Treatment	Description	1	2	3	4	1	2	3	4	1	2	3
		Tr	eatm	ent s	eque	nce						
Saline	10ml saline ^a	1	2	3	4	1	3	2	3	1	3	2
Naloxone	Naloxone ^{ab} 0.02mgliveweightkg ⁻¹	2	3	4	1	2	1	3	2	2	1	3
Crib-strap	Crib-strap placed on the horse, 10ml saline ^a	3	4	1	2	3	2	1	1	-	-	-
Anti-weave bar	Anti-weave bar placed on the stable door, 10ml saline ^a	4	1	2	3	-	-	-	-	3	2	1

Table 1	Treatments and experimental design indicating treatment sequence
	for each horse.

^a administered by the right indwelling catheter, hourly.

^b ≈ 10 ml for a 500kg horse.

 $^{\circ}$ one weaving horse became unavailable during the course of the trial, therefore, n = 3 for the weaving group.

The sequence of treatments was randomized for each group of horses (Table 1) and all treatments were carried out in a loose box (4x4 m), to which the animals were familiar, at the same time each day (between 1000 and 2100h). Horses were brought to the loose box at 0700h for catheterization (Vygon, Ecouen, France) of the right and the left jugular veins. The time-interval between treatments for an individual horse was a minimum of 7 days. The

naloxone (Narcan®, Dupont Pharmaceuticals, Stevenage, UK) dose rate of $0.02 \text{mgkg}^{-1}\text{h}^{-1}$ was based on information gathered during a number of pilot studies (McBride 2000). Ten ml of 0.9% saline (Polyfusor®, Fresenius Kabi, Warrington, UK) was used as a control for the administration of the opiate antagonist; both were given intravenously via the right indwelling catheter. The experimental design was an incomplete Latin square; the control group received all 4 treatments, and the crib-biting and weaving group received 3 treatments each as shown in Table 1.

Measurements

Behavioural parameters

Behaviour was recorded using time-lapse video equipment (TVR-625 Ikegami, Cherstey, UK). Stereotypic behaviours were measured continuously as the total number of crib-bites or weaves during the 11h treatment period. The normal behaviours recorded are listed and defined in Table 2. These were measured using an instantaneous scan sampling technique (Bateson 1991), where samples were taken every 10min (66 per treatment); the tape was paused at 10min intervals (real time) and the behavioural state of the animal assessed and recorded subsequent to the experiment.

Behaviour	Description
Non-alert	Ear in relaxed state (not contracted to 90° or lying flat to the head); neck at angle $\leq 50^{\circ}$ to spine.
Alert	Ear contracted to 90° or lying flat to the head, neck at raised angle $\leq 50^{\circ}$ to spine
Resting	Eyelid and lower lip drooped, neck at lowered angle $\geq 30^{\circ}$ to spine
Sleeping	Eyelid closed, lower lip drooped, neck at lowered angle $\geq 30^{\circ}$ to spine
Standing	4 feet weight bearing
Leg-resting	3 feet weight bearing
Lying	Lying down
Walking	Walking
Eating	Eating
Drinking	Drinking
Crib-biting	Horse grips onto a fixed object using its incisor teeth, leans back onto hindquarters and contracts the strap muscle (contraction must be visible) of the neck to bring the head into an arched position. Air is sometimes taken into the oesophagus to produce a grunting sound.
Weaving	Lateral movement of the head and neck from side to side in a rhythmic repetitive manner with alternation of the weight onto the contralateral foreleg with respect to the position of the head.

Table 2Normal and stereotypic behaviours measured.

Physiological parameters

Plasma cortisol and beta-endorphin (hypothalamo-pituitary-adrenal [HPA] activity) levels and heart rate were recorded as indirect physiological measures of stress. Blood samples (7ml) were collected via the left, indwelling catheter into an EDTA vacutainer (NVS, Stokeon-Trent, UK) and centrifuged for 5min at 1600g. The plasma was then aliquoted into 1.5ml micro-centrifuge tubes (Elkay, Co Galway, Ireland) and stored at -20°C until assayed.

A frequent blood sampling protocol was considered necessary to give an accurate representation of mean hormonal levels. The primary constraint to this was the time required to take blood samples and to prepare and freeze the plasma aliquots; therefore, blood samples were taken every 20min.

Plasma cortisol level was measured using a previously described ELISA technique (Cooper *et al* 1989). Plasma beta-endorphin level was measured using an RIA technique previously described (Ralston *et al* 1988); this procedure was performed by Dr R Rodway, University of Leeds, UK. Heart rate (min⁻¹) was measured as beats per minute (bpm) using a Polar Sports TesterTM (Polar®, Kempele, Finland). Samples, rather than continuous measurements, were taken to give an overall mean heart rate measure during each treatment period: morning (1000-1200h), afternoon (1400-1600h) and evening (1900-2100h). Within these periods, the equipment was set to measure heart rate every 15sec.

Statistical Analyses

Repeated measurements of physiological parameters (heart rate and plasma cortisol and plasma beta-endorphin levels) were taken for each individual horse during the course of the treatment periods. The means \pm SD of these data were calculated to give a measure of central tendency and variability of the measured parameter for each animal. These mean values were then used to produce a measure of central tendency (mean) for each group of horses (control, weaving and crib-biting). The measure of variability in this instance to describe the variation within a sub-population of animals was mean \pm SEM. Similar summary statistics were applied to the stereotypic behaviour data.

Paired statistical analysis (Genstat® for Windows Release 4.1, Lawes Agricultural Trust, Rothamsted, UK) was used to compare treatment with saline values for each of the different parameters measured for each group of horses (control, crib-biting and weaving). Where the null hypothesis stated that there would be no change in the measured parameter (aspects of normal behaviour), a two-tailed analysis was employed. However, when the null-hypothesis stated that there would be no increase (eg for plasma cortisol levels, plasma beta-endorphin levels and heart rate) or decrease (for stereotypic behaviour) in the measured parameter, a one-tailed analysis was used. To facilitate the use of parametric statistics, percentage data (normal behaviour) were arcsine transformed; heteroscedastic (stereotypic behaviour) and skewed data (plasma cortisol levels) were log transformed to normalize the data distribution. Where data could not be normalized, equivalent non-parametric tests (Wilcoxon pairedsample test; Minitab® for Windows Release 12.22, Minitab LtdTM, Coventry, UK) were employed.

The effect of stereotypy performance on HPA activity was statistically analysed using physiological data pre- and post-stereotypy where the point of blood sampling had occurred at the start of a 15-20 min stereotypy bout. One-way repeated measures (ANOVA; Genstat® for Windows Release 4.1, Lawes Agricultural Trust, Rothamsted, UK) compared values of plasma cortisol levels and beta-endorphin levels at the point of stereotypy onset (pre-value 0-5 min) with other pre- (40min and 20min) and post- (40min and 20min) stereotypy values. Only five out of the seven stereotyping horses had performed stereotypy at the point of blood sampling during the course of the treatment period, and thus, only data from these animals were used in the analysis.

Results

Behavioural parameters

Normal behaviour

The mean $(\pm SEM)$ proportion of time spent performing aspects of normal behaviour during each treatment period, for each group of horses, are presented in Table 3.

The mean (\pm SEM) time spent performing non-alert behaviour by control horses significantly (P = 0.04) increased from 61.4 ± 6.0 per cent with the saline treatment to 75.0 ± 6.6 per cent with the crib-strap. The anti-weave bar also affected the behaviour of the control horses, significantly (P = 0.01) decreasing standing (from 66.1 ± 5.0 to $50.4 \pm 7.8\%$) and significantly (P = 0.02) increasing leg resting (from 32.6 ± 4.9 to $48.4 \pm 8.7\%$) relative to the saline treatment. The mean time spent resting by crib-biting horses significantly (P = 0.02) increased from $5.1 \pm 1.8\%$ with the saline-treatment to $20.4 \pm 3.0\%$ following naloxone administration. No other aspects of normal behaviour of the crib-biting or weaving horses were affected by the application of the different treatments.

Stereotypic behaviour

The frequency distribution of crib-bites and weaves (recorded continuously during the treatment periods) are presented as the mean number of crib-bites and weaves per 5min (Figures 1 and 2). To indicate the mean crib-biting and weaving rate during the treatment periods, the mean $(\pm SD)$ instances of each per hour are presented in Table 4.

One crib-biting horse (Horse 3) did not crib-bite during the saline or crib-biting treatment periods. However, overall mean crib-biting frequency was significantly reduced (P = 0.04) by 95.6 per cent (from 79.7 ± 64.8 during saline treatment to 3.3 ± 1.8) during the crib-strap treatment. In contrast, the mean frequency of weaving in the weaving group was not significantly affected by the anti-weave bar treatment.

The mean crib-biting frequency was significantly reduced (P = 0.05) by 84 per cent during the naloxone treatment (from 79.7 ± 64.8 to 13.1 ± 7.5 bites per hour) compared to the saline treatment. Mean weaving frequency was not significantly affected by the naloxone treatment (9.4 ± 3.3 weaves per hour during saline treatment compared to 12.8 ± 12.4 during naloxone treatment).

Physiological parameters

Mean values during the treatment periods

Heart rate

The mean \pm SD heart rate values for each individual horse and the mean \pm SEM value for each group of horses, during each treatment period, are presented in Table 5. No significant differences in mean heart rate were recorded between saline and other treatments for each group of horses.

Plasma cortisol

The mean \pm SD plasma cortisol levels for each individual horse and the mean \pm SEM value for each group of horses, during each treatment period are presented in Table 5.

Plasma cortisol levels of control horses were significantly elevated in response to both the anti-weave bar (mean \pm SEM; from 18.5 \pm 9.2 to 37.4 \pm 8.1 ngml⁻¹; P = 0.03) and crib-strap treatment (mean \pm SEM; from 18.5 \pm 9.2 to 34.0 \pm 10.0 ngml⁻¹; P = 0.05) as compared to the saline treatment. Similarly, plasma cortisol levels of crib-biting horses were significantly (P = 0.05) elevated during the crib-strap compared to saline treatment (mean \pm SEM; 33.8 \pm 6.7 to 48.7 \pm 8.2 ngml⁻¹). There was also a trend, although it was not significant, for higher plasma cortisol levels (P = 0.07) during the anti-weave bar treatment for the weaving horses (mean \pm SEM; 30.8 \pm 3.0 to 48.7 \pm 4.8 ngml⁻¹) compared to the saline treatment.

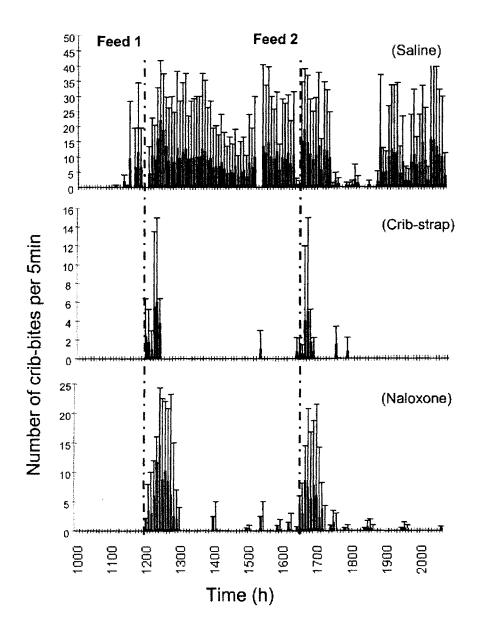


Figure 1Mean (±SD) crib-biting frequency (number per 5min) during the saline,
crib-biting and naloxone treatment

Plasma cortisol levels were not significantly affected by the administration of naloxone to any of the horse groups.

Plasma beta-endorphin

The mean \pm SD plasma beta-endorphin values for each individual horse and the mean \pm SEM value for each group of horses, during each treatment, are presented in Table 5.

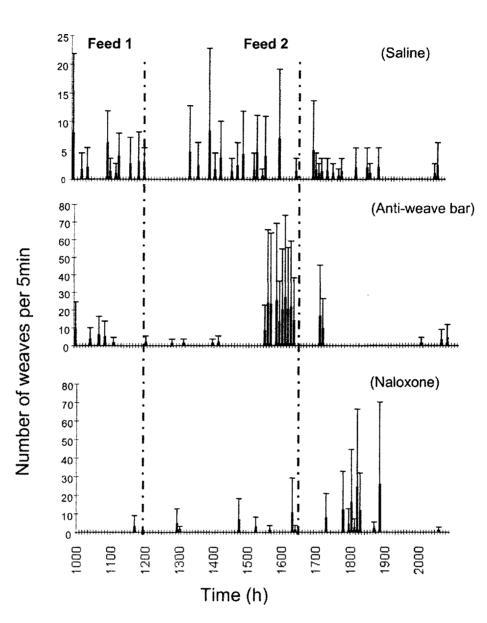


Figure 2 Mean (±SD) weaving frequency (number per 5min) during the saline, crib-biting and naloxone treatment

In control horses, wearing a crib-strap caused a significant elevation in plasma betaendorphin levels (P = 0.02); from 108.5 ± 53.6 during saline treatment to 135.3 ± 60.5 pgml⁻¹. The naloxone treatment significantly (P = 0.05) reduced plasma beta-endorphin levels in control horses when compared to the saline treatment (from 108.5 ± 53.6 to 70.3 ± 39.2 pgml⁻¹). No other treatment effect was measured for the other groups of horses when compared to the saline treatment.

		Conti	Control (n=4)			Crib-biting (n=4)	1=4)		Weaving ¹ (n=3)	= 3)
Treatment	Saline (%)	Naloxone (%)	Crib- strap (%)	Anti- weave bar (%)	Saline (%)	Naloxone (%)	Crib- strap(%)	Saline (%)	Naloxone (%)	Anti-weave bar (%)
Behaviour										
Alert	34.1 ± 5.6	26.8 ± 2.8	22.0 ± 7.3	28.5 ± 4.1	17.2 ±6.7	14.2 ± 7.8	14.6 ± 4.2	25.8 ± 8.7	16.7 ± 6.2	40.9 ± 5.3
Non-alert	61.4 ± 6.0	68.5 ± 3.5	75.0 ± 6.6*	65.9 ± 6.2	77.3 ±8.8	65.4 ± 9.7	79.0 ± 5.5	71.7 ± 9.7	77.3 ± 4.2	56.1 ± 4.7
Resting		4.7 ± 1.9	2.7 ± 1.6	5.6 ± 2.5	5.1 ±1.8	$20.4 \pm 3.0^{*}$	6.0 ± 1.1	2.5 ± 1.2	6.1 ± 5.2	3.0 ± 0.8
Sleeping		0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eating		44.4 ± 6.2	52.3 ± 11.0	34.0 ± 9.2	50.5 ±8.0	46.9 ± 2.8	62.0 ± 6.3	56.6 ± 11.0	42.4 ± 5.4	47.5 ± 2.2
Drinking	1.9 ± 1.4	0.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	1.9 ± 1.1	0.4 ± 0.4	1.6 ± 0.6	4.0 ± 1.6	3.0 ± 0.8	1.0 ± 0.4
Standing		61.6 ± 10.8	61.7 ± 10.7	$50.4 \pm 7.8^{*}$	49.5 ± 7.0	38.2 ± 6.0	47.8 ± 1.9	70.2 ± 8.8	49.5 ± 5.0	74.2 ± 8.4
Leg-resting		36.6±11.2	37.5 ± 10.5	$48.4 \pm 8.7*$	50.5 ± 7.0	60.6 ± 6.2	51.1 ± 2.3	29.8 ± 8.8	50.0 ± 4.7	23.2 ± 8.0
Lying		1.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.4	0.0 ± 0.0
Walking	1.3 ± 0.4	0.8 ± 0.8	0.8 ± 0.4	1.2 ± 1.0	0.0 ± 0.0	0.8 ± 0.4	1.1 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 1.6

Mean (±SD) individual and mean (±SEM) group number of crib-bites and weaves per hour during each	treatment (* $P \le 0.05$ significant difference from the saline treatment).
Table 4	ļ

Crib-bitin	g horses (mean ₁	orses (mean ±SD crib-bites h ⁻¹)	h ⁻¹)	Weav	ing horses (mea	Weaving horses (mean ±SD weaves h-1)	h-1)
Treatment	Saline	Naloxone	Naloxone Crib-strap	Treatment	Saline	Naloxone	Anti-weave bar
Horse 1	24.5 ± 49.3	$11.4 \pm 31.9 4.6 \pm 9.5$	4.6 ± 9.5	Horse 1	16.1 ± 15.6	37.6 ± 76.6	67.1 ± 117.4
Horse 2	273.5± 154.3	31.2 ± 53.8	7.7 ± 16.4	Horse 2	5.5 ± 8.8	0.2 ± 0.6	5.5 ± 12.5
Horse 3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	Horse 3	6.6 ± 9.6	0.5 ± 1.2	0.0 ± 0.0
Horse 4	20.7 ± 21.6	9.6 ± 9.8	0.9 ± 1.6	ł		·	·
Group mean ±SEM	79.7 ± 64.8	13.1 ± 7.5	$3.3 \pm 1.8^{*}$	Group mean ±SEM	9.4 ± 3.3	12.8 + 12.4	24.2 ± 21.5

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		פ	Control			Crio-Diting			weaving	
	Saline	Naloxone	Crib-strap	Anti-weave bar	Saline	Naloxone	Crib-strap	Saline	Naloxone	Anti-weave bar
Heart rate							i i k			
Horse 1	31.0 ± 4.4	30.9 ± 5.5	32.6 ± 4.3	29.8 ± 3.3	37.8 ± 2.0	34.0 ± 3.7	32.5 ± 4.7	42.2 ± 5.3	42.7 ± 5.7	35.9 ± 6.6
Horse 2	36.0 ± 6.4	37.4 ± 4.6	41.6 ± 5.6	35.5 ± 3.6	34.0 ± 8.0	32.7 ± 4.0	34.1 ± 3.4	36.4 ± 4.5	34.9 ± 4.3	38.8 ± 6.3
Horse 3	36.3 ± 4.9	34.3 ± 2.8	37.7 ± 4.9	36.8 ± 3.6	34.5 ± 4.6	31.4 ± 4.9	34.6 ± 5.0	32.2 ± 3.6	38.2 ± 5.2	31.8 ± 4.5
Horse 4	41.6 ± 5.0	37.5 ± 5.2	36.7 ± 5.1	40.1 ± 4.4	35.1 ± 4.2	37.2 ± 3.0	32.8 ± 4.2	·	•	ı
Gp mean ±SEM	36.2 ± 2.2	35.0 ± 1.6	37.1 ± 1.9	35.5 ± 2.1	35.4 ± 0.8	33.8 <u>±</u> 1.2	33.5 ± 0.5	36.9 ± 2.9	38.6 ± 2.3	35.5 ± 2.0
Plasma cortisol	tisol									
Horse I	21.5 ± 10.6	21.5 ± 5.5	23.4 ± 8.9	25.2 ± 6.3	21.4 ± 7.3	43.9 ± 14.9	24.7 ± 10.1	33.4 ± 10.2	17.0 ± 13.6	42.0 ±12.0
Horse 2	14.5 ± 5.6	18.3 ± 5.9	26.2 ± 10.9	22.1 ± 6.5	41.7 ± 10.9	27.6 ± 9.0	51.7 ± 25.2	23.9 ± 10.1	26.2 ± 8.9	59.8 ± 31.2
Horse 3	20.4 ± 10.4	26.4 ± 10.7	22.6 ± 8.3	54.4 ± 16.2	23.5 ± 7.4	22.3 ± 8.3	60.3 ± 19.3	35.2 ± 22.0	27.8 ± 10.8	44.4 ± 11.1
Horse 4	15.5 ± 7.7	88.1 ± 27.9	63.8 ± 37.3	47.7 ± 37.1	48.7 ± 34.7	27.6 ± 8.6	58.1 ± 28.4	•		•
Gp mean ±SEM	18.0 ± 1.74	38.6 ± 16.6	34.0 ± 10.0*	37.4 ± 8.1*	33.8 ± 6.7	30.4 ± 15.2	48.7 <u>±</u> 8.2*	30.8 ± 3.0	23.7 ± 2.9	48.7 ± 4.8
Plasma Bet:	Plasma Beta-endorphin									
Horse I	35.7 ±26.2	1.2 ± 6.5	51.3 ± 24.8	41.6 <u>±</u> 22.1	49.7 ± 17.0	<u>226.2447.8</u>	145.2 ± 43.6	349.0 ± 53.5	255.4 <u>+</u> 44.7	315.1±27.6
Horse 2	231.8 ± 1.4	151.6 ± 29.0	275.0 ± 39.2	342.6±60.3	200.6±27.3	275.9±53.4	204.0 ± 40.6	229.2 ± 53.0	232.2±52.2	237.0±30.0
Horse 3	3.4 ± 20.1	5.3 ± 30.7	18.7 ± 62.0	0.0 ± 0.0	260.5±39.1	246.9±33.0	184.8 ± 33.9	217.0 ± 31.1	182.9±62.2	111.8±18.3
Horse 4	163.0 ± 29.5	123.2±106.4	195.4 ± 30.2	140.9 ± 43.3	200.8 ± 66.9	138.7 ± 25.8	240.3 ± 77.8	•	•	•
Gp mean ±SFM	108.5 ± 53.6	70.3 ± 39.2*	135.3±60.5*	131.3±76.4	177.9±45.0	221.9±29.6	193.6 ± 19.8	265.0 ± 42.1	223.5±21.4	221.6±59.2

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Mean values before and after a bout of stereotypy

It was considered that the arrival and/or the ingestion of food could affect the physiological responses of the animals and, therefore, the data. Hence, pre- and post-stereotypy values were taken outwith the periods of meal delivery (excluding 1130-1230 and 1600-1700).

Heart rate

The sampling protocol provided insufficient data points to allow statistical comparison of pre- and post-stereotypy values.

Plasma cortisol

Plasma cortisol samples taken up to 40min before and 40min after a 15min bout of stereotypy are presented in Figure 3.

Plasma cortisol levels were significantly lower 40min after the start of the stereotypy bout compared to the pre-stereotypy values (for 0-5 min, falling from 45.3 \pm 19.2 to 28.7 \pm 11.8 ngml⁻¹; P = 0.006). Using parametric and non-parametric analysis, plasma cortisol levels also tended to be significantly (P = 0.05) lower 20min post-onset (45.3 \pm 19.2 ngml⁻¹) compared to pre-stereotypy values (from 0-5 min, 37.2 \pm 16.2 ngml⁻¹). Similarly, 40min pre- (from 34.9 \pm 12.9 to 45.3 \pm 19.2; P = 0.04) but not 20min pre-stereotypy (from 34.1 \pm 17.8 to 45.3 \pm 19.2; P = 0.14) values tended to be lower than 0-5 min pre-stereotypy values.

Plasma beta-endorphin

Plasma beta-endorphin samples up to 40min before and 40min after a 15min bout of stereotypy are presented graphically in Figure 3.

Pre-stereotypy (0-5 min) plasma beta-endorphin levels were not significantly different to any other pre- or post-stereotypy values.

Discussion

Compared to McGreevy *et al*'s (1995) survey of UK eventing and dressage horses, the prevalence of stereotypic animals amongst the 280 horses surveyed (1.4% in present study vs 7.5% eventing and 8.3% dressage crib-biters in McGreevy *et al* [1995]; 1.4% in present study vs 9.4% eventing and 9.5% dressage weavers in McGreevy *et al* [1995]) was relatively low. Redbo (1998) reported that more staff and, in turn, more human contact with horses within the stable environment reduced the prevalence of stereotypic behaviour. The nature of the stable environment in this present study (extensive uniform and tack cleaning, mucking out, feeding, inspections and tours) was such that long periods of time were spent by staff with the horses. Thus, this human-animal interaction may explain the low prevalence of stereotypies within this environment. Alternatively, the breed of horse used within this present study (Irish draught x Thoroughbred) may not be predisposed to performing stereotypies compared to the eventing and dressage horses sampled within the McGreevy *et al* (1995) study.

One horse identified as a crib-biter in the current study (Horse 3) did not perform cribbiting behaviour during any of the treatment periods. This horse was brought from another stable, and thus the novel environment may have attenuated the performance of the behaviour. The effect of novel environments in reducing the performance of stereotypic behaviour has been previously reported in other species (for review see Robins *et al* [1990]).

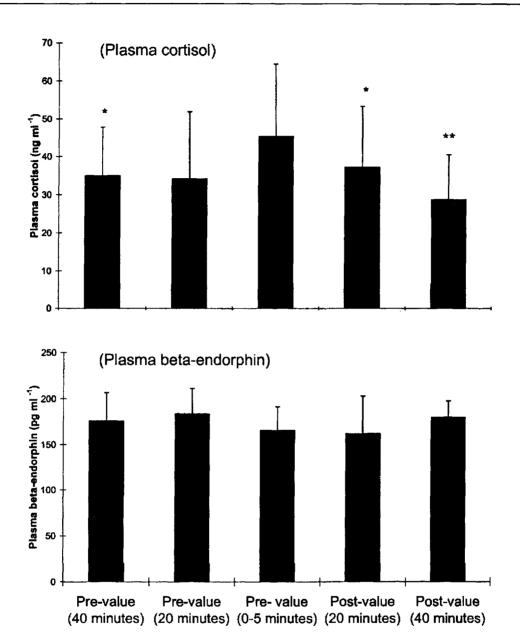


Figure 3 Mean (\pm SEM) pre- and post-stereotypy values for plasma cortisol levels and beta-endorphin levels for crib-biting and weaving horses combined (n=5) (*significantly [$P \le 0.05$] and **highly significantly [$P \le 0.001$] different from pre- stereotypy [0-5 min] values).

Physiological and behavioural consequences of stereotypy prevention

Our results showed that crib-biting was significantly (P = 0.04) decreased by 96 per cent during the crib-strap treatment compared to the saline treatment. This contrasted to weaving,

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which increased, although not significantly, compared to the saline treatment after the antiweave bar was installed. Thus, the crib-strap was more effective than the anti-weave bar in preventing stereotypic behaviour. This reflects the ability of weaving horses to stand back from the stable door and weave inside the stable.

Crib-biting and weaving horses had elevated plasma cortisol levels (P = 0.05 and P = 0.07 respectively) regardless of whether or not stereotypy was prevented during the crib-strap and anti-weave bar treatment. This suggests that either the prevention of stereotypy was not the cause of the stress-response (elevated plasma cortisol) or, performing stereotypy with the crib-strap and anti-weave bar present was stressful to the animal. However, plasma cortisol levels were also significantly elevated in the control horses during both the crib-strap (P = 0.05) and the anti-weave bar (P = 0.03) treatments, coupled with a significant rise (P = 0.02) in plasma beta-endorphin levels during the former treatment. This suggests that prevention of stereotypic behaviour did not elicit the stress-response but rather that it was caused by another factor common to all three groups of horses. A previous study (McGreevy & Nicol 1998) that prevented the performance of equine stereotypic behaviour, not by physically preventing the animal, but rather by removing surfaces for crib-biting, reported no effect on plasma cortisol levels. Together, these results suggest that the specific prevention of equine stereotypies is not stressful to the horse, which indicates that the behaviours do not help the animal to cope with its environment.

The use of the crib-strap or the anti-weave bar also had significant effects on the normal behaviour of the control horses but had no effects on the crib-biting or weaving animals. Stressors (eg intermittent electric shock) are known to reduce active behaviour (see Weiss et al [1981] for a review). Thus, the significant increase in time spent in a non-alert state by the control horses may reflect a stress-response to wearing the crib-strap. The significant increase in time spent leg-resting may indicate a similar reduction in behavioural activity caused by the stressful effects of having an anti-weave bar in the stable environment. The fact that these behavioural responses were not mirrored by the crib-biting and weaving horses may suggest that the crib-strap and the anti-weave bar were more stressful to the control animals. This is supported by the significant elevation in plasma beta-endorphin levels that was particular to that group of horses and is probably explained by the fact that crib-biting and weaving horses had already experienced, and thus had been habituated to, these devices. Overall, however, the physiological and behavioural data indicated that while the specific prevention of stereotypic behaviour may not induce a stress-response in the animal, the use of the crib-strap and the anti-weave bar per se are stressful to the horse. The use of these devices in terms of their effect on the animal's welfare is questionable.

It is also interesting to note that during the saline treatment, crib-biting and weaving were performed consistently throughout the treatment periods (Figures 1 and 2). However, during the crib-strap and anti-weave bar treatments, horses that continued to perform stereotypic behaviour performed 54.8 per cent of the total amount of daily crib-biting in the first 30min after meal 1 at 1200h and 34.4 per cent was performed within the first 30min after meal 2 at 1630h (Figure 1). For weaving horses, 71.4 per cent of the total amount of weaving was performed in the 60min prior to meal 2 (Figure 2). Thus, stereotypies, during a period of prevention, were performed only in response to specific causal stimuli; these stimuli were the anticipation of meal delivery for weaving and the consumption of food for crib-biting. These periods may indicate when the animal is most motivated to perform these behaviours. Horses trained to perform an operant task to receive a food reward will perform a grasping-type behaviour when the food is not presented (S D McBride personal observation). The morphology of this behaviour is similar to crib-biting and suggests that crib-biting may be

the manifestation of a continued eating motivation. The pre-feeding, locomotory nature of weaving may indicate that this behaviour originates from a continued motivation to acquire food.

Physiological consequences of stereotypy performance

Measuring HPA activity before and after stereotypy (15min bouts not performed in the context of food delivery or food consumption) showed that plasma cortisol levels became elevated prior to stereotypy performance and were lower 20 (P = 0.05) and 40 (P = 0.006) minutes afterwards. It was hypothesized at the outset that if equine stereotypy has a coping function then, during periods outside of meal delivery/food consumption, stress-related parameters should increase prior to the onset of stereotypy, followed by a reduction after the behaviour's performance. Our results support the view that both stereotypies (crib-biting and weaving) reduced HPA activity and thus they have a coping function. In contrast, no differences were measured between pre-stereotypy values (0-5 min) and other pre- and post-stereotypy values for plasma beta-endorphin levels. If the coping function of equine stereotypy is mediated via increased opioid activity, then the results show that this activity is not measurable within the peripheral blood system.

The behavioural and physiological consequences of opiate antagonist administration

The administration of naloxone caused a significant reduction (mean decrease of 84% between horses) in crib-biting behaviour compared to the saline treatment. This was less than the 100 per cent reduction reported by Dodman *et al* (1987), the difference may be explained by the fact that Dodman *et al* (1987) recorded stereotypic behaviour during 2 x 60min observations day⁻¹. The large variation in stereotypy frequency during the course of the day (Figures 1 and 2) indicates that sample measurements of stereotypic behaviour could generate misleading information.

The large reduction in crib-biting behaviour following administration of an opiate antagonist suggests that the underlying neurochemical mechanism of crib-biting behaviour is opioid-mediated. However, naloxone administration also significantly increased (P = 0.02) the amount of time spent resting (from 5.1 ± 1.8 to $20.4 \pm 3.0\%$) in crib-biting horses. This confirms the casual observation by Dodman *et al* (1987) of a sedative effect which could have contributed to or been wholly responsible for the reduction in crib-biting behaviour. However, this sedative effect was not apparent for control or weaving horses and this difference between groups may in fact indicate differences in opioid physiology (ie number of receptor sites and/or binding affinity). Animals continuously exposed to exogenous opioids can develop a state of physiological tolerance but, paradoxically, a state of behavioural sensitization (eg experience an increased sedative effect) to opiate antagonists (Crain & Shen 1992). Thus, the pronounced, sedative effect in crib-biting horses may in fact suggest that these animals have had a greater exposure to endogenous opioids and could support the view that this stereotypic behaviour is associated with endogenous opioid release.

Dodman *et al* (1987) reported no attenuation of weaving behaviour after naltrexone (0.04-0.05 mgkg⁻¹; intravenous) or nalmefene (0.08-2.0 mgkg⁻¹; subcutaneous) administration in one horse. In contrast, Nurnberg *et al* (1997) demonstrated a 30 per cent significant reduction in this behaviour after administering naltrexone orally (0.7mgkg⁻¹day⁻¹). The effect of opiate antagonist administration on weaving horses in the present study resulted in an overall increase in the mean weaving frequency by 36 per cent. However, the results were highly influenced by the low number of subjects involved (n = 3) and the values for Horse 1 (Table 4) which had a relatively high basal weaving frequency which more than doubled during the

naloxone treatment (from 16.1 ± 15.6 to 37.6 ± 76.6 weaves h⁻¹) when compared to the saline treatment. For weaving horses 2 and 3, weaving frequency was reduced by more than 90 per cent after antagonist administration. Thus, although these results must be treated with caution due to the low number of animals involved, one could infer that the underlying neurochemical mechanism of weaving is also opioid mediated but that there is a greater individual variation in the behavioural response to the antagonist. This variation may be explained by other neurotransmitters being involved in the underlying neurochemical mechanism of this stereotypic behaviour, as proposed by Nurnberg *et al* (1997), who attenuated weaving by administering serotonin (95% of baseline values) and dopamine (57% of baseline values) antagonists.

The initial hypothesis for this aspect of the study stated that, if equine stereotypies function as a reward behaviour, then the performance of these behaviours will be attenuated by the administration of an opiate antagonist. The results support this hypothesis for cribbiting behaviour but are inconclusive for weaving. Although the significant increase in resting behaviour by crib-biting horses following naloxone administration suggests that the change in behaviour was due to a sedative effect, the fact that this was particular only to cribbiting horses can in fact be argued to support the opposite view that crib-biting is opioid mediated. Overall, therefore, the results of the current study can be interpreted as supporting the view that crib-biting is a reward behaviour and, therefore, that this behaviour may have a coping function.

Animal welfare implications

The results of mean HPA activity measured during the crib-strap and anti-weave bar treatments, suggested that equine stereotypies do not have a coping function. This conclusion, however, was based primarily on the fact that a stress response was elicited from control as well as stereotypic animals. This should be treated cautiously, since the use of the crib-strap and anti-weave bar may be stressful to the horse for several concurrent reasons, one of which may be stereotypy prevention. The significant increase in HPA activity that was measured prior to 15min of stereotypy performance followed by a significant decrease post-stereotypy supports the opposite view; that both crib-biting and weaving horses have the ability to reduce HPA activity and thus function, to allow the animal to cope with a sub-optimal environment. In support of this conclusion, endogenous opioids appear to be involved in the underlying neurochemical mechanism for both crib-biting and weaving behaviour, although results from this study suggest that the link is greatest with the former of these equine stereotypies. This suggests that crib-biting is a reward behaviour and thus could have a coping function. The sedative effect measured in this study after opiate antagonist administration, for crib-biting horses only, may also support this conclusion.

The stress-response elicited by the use of the crib-strap and the anti-weave bar, in conjunction with data that indicate a coping function for equine stereotypies, suggest that serious questions need to be asked about the welfare implications of using such devices in the stable environment. In particular, whether the benefits of preventing clinical sequelae associated with equine stereotypies outweigh the potential welfare reducing effects of using the crib-strap and anti-weave bar.

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