



# Negative versus positive reinforcement: An evaluation of training strategies for rehabilitated horses

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## Abstract

Rescued equids are often exposed to rehabilitation and training (or retraining) programmes to improve their physical and psychological well-being as well as to facilitate the re-homing process. Training uses either positive or negative reinforcement learning procedures and it is considered here that, there may be welfare implications associated with using the latter technique as it has the potential to overlay acute stress on animals with a chronic stress life history. The aim of this study, therefore, was to compare these training strategies (negative versus positive reinforcement) on equine behaviour and physiology as the first step in establishing an optimal rehabilitation approach (from a welfare perspective) for equids that have been subjected to chronic stress in the form of long-term neglect/cruelty. Over a 7-week period, 16 ponies (aged 6–18 months) were trained using either positive ('positive') ( $n = 8$ ) or negative reinforcement ('negative') ( $n = 8$ ) techniques to lead in hand, stand to be groomed, traverse an obstacle course and load into a trailer. Heart rate was measured (5 s intervals) on days 1 and 4 of each training week, 'Pre' (1 h), 'During' (0.5 h) and 'Post' (1 h) training session. Ethograms (10.00–20.00 h) outside of the training period were also compiled twice weekly. In addition, weekly arena tests (as a measure of reactivity) were also performed 1 week before and during the 7 weeks of training.

Results showed significant differences between the two training schedules for some measures during the latter stages of the trial and suggested that animals trained under a positive reinforcement schedule were more motivated to participate in the training sessions and exhibited more exploratory or 'trial and error' type behaviours in novel situations/environments. In this context, the incorporation of positive reinforcement schedules within a rehabilitation programme may be of benefit to the animal from a welfare perspective.

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## 1. Introduction

Within the UK, horses subjected to long-term neglect/cruelty (chronic stress) are often rescued by equine welfare charities. These animals normally proceed through a process of rehabilitation (to improve physical and psychological well-being) and, if possible, a regime of training (or retraining) to facilitate re-homing as a safe riding/companion animal and thereby improve the overall quality of life for the animal.

Within the various welfare organisations, different training strategies are often applied using either ‘conventional’ (e.g. British Horse Society) or ‘modern’ (e.g. Parelli, Monty Roberts, clicker training) techniques. These techniques can be categorised in terms of whether they apply negative or positive reinforcement schedules or a combination of both. Both forms of reinforcement involve associative learning (Gleitman, 2003), the linking of one stimulus to another and the association of actions with the attainment of something positive (e.g. food) or the avoidance of something the animal considers to be unpleasant (e.g. physical pressure or pain). In several species, it has been well documented that, for animals previously exposed to chronic stress, subsequent exposure to mild acute aversive stimuli will produce a heightened stress responses (e.g. O’Connor et al., 2004). The application of training techniques that employ negative reinforcement through the application of physical pressure may, therefore, be inappropriate for rescued horses that have a life history of chronic stress. Furthermore, horses exposed to sub-optimal husbandry conditions (Rivera et al., 2002) are also reported to be less receptive to negative reinforcement techniques. Negative reinforcement may thus also be an unproductive strategy of training (or retraining).

The aim of this study, therefore, was to compare the behavioural and physiological effects of negative versus positive reinforcement-based training techniques on horses that had been rescued from a situation of maltreatment. The first objective was to measure the physiological (heart rate) response of both groups in anticipation of, during and after the training sessions. The second objective was to examine both the behavioural and physiological (heart rate) responses of both groups to a series of validated temperament tests during the training period. The third objective was to obtain information about the general behavioural profile of horses in each training group during the training period.

## 2. Material and methods

### 2.1. *Animals and husbandry*

Sixteen Welsh sections A and B ponies (6–18 months), which had been rescued from three areas of common land in Wales (Gelligeir, Llangynidr and Glenmorang) were used in this experiment. The ponies were rescued on the grounds of abandonment and starvation due to overcrowding and, on arrival, were condition scored between one and three under the Equine Industry Welfare Guidelines (ADAS, 2002). Unwanted ponies are abandoned onto common land by previous owners and typically have a history of group (herding, loading) rather than individual (halter, bridle, saddle) handling. A pre-trial rehabilitation period between 2 and 5 months allowed the ponies to attain full physical health. This time period was spent grazing as a group in a 12-acre paddock with no human contact. One month prior to the experiment, ponies were herded into a barn and then individually stabled thereafter. Stables were in two blocks of six and one block of four and all ponies had visual contact with neighbouring conspecifics. All ponies were subject to the same husbandry procedures. Thirty days were spent desensitising the head and girth regions of all ponies so that head collar and surcingle could be applied. No other contact was given. Once the trial and training commenced all ponies were stabled individually from Monday morning to Friday afternoon and then returned to the paddock as a group during the week-end.

## 2.2. Training procedure

Animals were randomly assigned to either positive or negative training schedules, with groups balanced for both age (6–18 months) and sex (five females and three males/group). The training procedures commenced on week 2 of the trial. Positive reinforcement training (PR) was applied by employing the clicker method as previously described (Williams et al., 2004). In brief, the sound of the clicker was converted to a secondary conditioned reinforcer through pairing with a food reward (primary reinforcer). The click was then used to indicate the performance of a correct operant response where correct operant responses were predominantly in the context of ‘trial and error’ behaviour with minimal application of pressure to engage and direct the animal in this process.

The negative reinforcement training strategy (NR) involved the pony learning to perform tasks in order to avoid or alleviate an aversive pressure (application of a riding whip). This was achieved by first lightly touching the animal with the riding whip with increasing pressure until it responded with a near-correct response. This process was continued during the subsequent sessions until the correct response was attained.

Training started on week 2 of the trial with ponies trained for 30 min four times per week by equally experienced staff trainers. The time of training (morning or afternoon) and the trainer were randomly assigned throughout the trial. Training objectives included (a) leading in hand, (b) standing to be groomed, (c) picking up feet, (d) traversing an obstacle course and (e) loading into a trailer.

## 2.3. Measurements

### 2.3.1. Pre-, during and post-training heart rate measurements

Heart rate monitors (Polar, Finland) set to record at 5 s intervals, recorded measurements 1 h prior to (‘Pre-’), 30 min during (‘During’) and 1 h after (‘Post-’) the training session was completed.

### 2.3.2. Temperament test (arena test)

The temperament test was a variation of a previously validated arena test (Visser et al., 2001) and was carried out on all animals for the duration of the experiment (1 week before and 7 weeks during training) using an indoor arena (22.5 m × 5.5 m) with a concrete floor. A large open umbrella, used as the novel object, was suspended from the ceiling and held at 50 cm above the ground. During the test, each individual

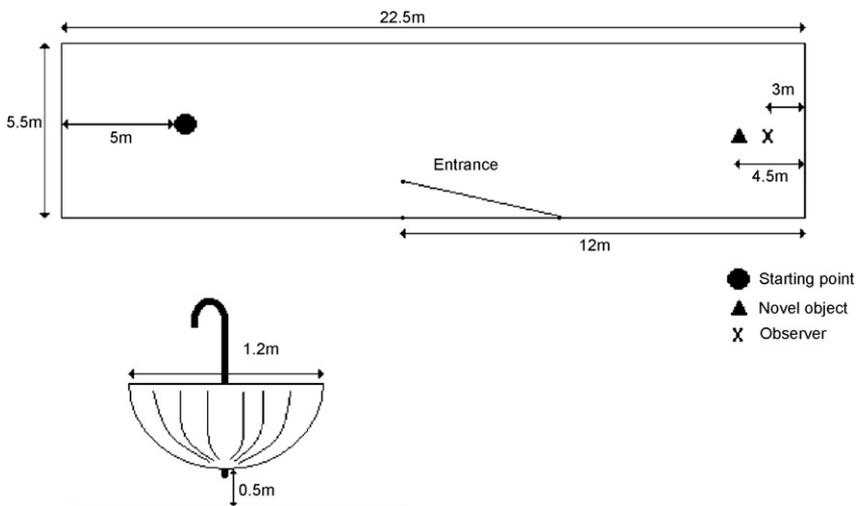


Fig. 1. Arena test area and novel object.

pony was led by the observer into the arena to a mark 13 m from the novel object. The pony was halted, the heart rate monitor (5 s intervals) was switched on and the pony was released for 6 min whilst the observer moved to a sitting position behind the novel object (Fig. 1). Heart rate was measured for the duration of the test. Behaviour was scored under four headings (general, tail position, vocalisation and novel object) in accordance with defined criteria (Table 1) with additional measurements of latency to touch observer/novel object and minimum distance from observer/novel object if not touched.

### 2.3.3. General behavioural profile (time budget)

From 1 week before and during the 7 weeks of training, behavioural observations (Table 2) were recorded twice weekly for 8 h (10.00 and 20.00 h), using instantaneous scan sampling at 5 min interval on those days. Observations were not taken during the training session.

### 2.3.4. Statistical analysis

The normal distribution of both heart rate and transformed (arcsin) behavioural data facilitated the use of parametric statistics. An ante-dependence test (Genstat; Release 4.2, 5th Edition; Lawes Agricultural Trust)

Table 1  
Arena test behavioural observation score sheet

Score	Description
<b>General score</b>	
1	Not nervous, stationary, resting hind leg or moving around oblivious to the novel object. Looking on ground for food with head at shoulder level.
2	Moving around at far end of arena, looking on ground for food. Stopping occasionally, raising head above shoulder level, looking at object.
3	Looking at object regularly, ears pricked. Walking/trotting adjacent to novel object, with sudden stops.
4	Looking at novel object directly with ears pricked. Vigorous walk/ trotting adjacent to object, near ear pointing towards object, looking at object with peripheral vision.
5	Vision directed towards object, ears pricked, abruptly raising and lowering head. Erratic movement. Muscles tensed, sweating, no movement towards object. Snorting through nostrils. Elevated head and tail, regular defecation.
<b>Tail position</b>	
1	Normal tail carrying position.
2	Tail position at horizontal back line.
3	Occasional elevation of tail.
4	Tail permanently elevated.
5	Tail elevated with flesh under-part visible.
<b>Vocalisation</b>	
1	No vocalisation.
2	Occasional.
3	Regular vocalisation.
4	Regular vocalisation with occasional snorting through nostrils.
5	Continual vocalisation and snorting through nostrils.
<b>Touching novel object</b>	
1	Nuzzling/ biting/ licking object/observer.
2	Object/observer touched, pony remains near object.
3	Object/observer touched with muzzle, pony moves calmly away.
4	Object/observer tentatively touched with muzzle with outstretched neck.
5	Object/observer tentatively touched with muzzle with outstretched neck, pony panics and quickly moves away.
6	Object/observer not touched.

Table 2  
Time budget; behavioural observation definitions

Behaviour	Definitions
Alert	Looking around with interest, ears pricked in direction of surrounding noise/activities.
Non-alert	Head shoulder level, ears not pricked, uninterested in surrounding noise/activities.
Pawing at hay	Using fore foot to paw at hay.
Locomotion	Not stationary, walking within stable, but not in direction of stable door.
Approach stable door	Movement directly towards stable door, with head at or over stable door.
Standing resting	Standing, resting hind leg, head shoulder level, eyelids drooping/eyes closed.
Recumbent sleeping	Not standing, eyes closed.
Feeding	Eating hay.

was subsequently used to assess statistical differences between treatments and to take into account the repeated measures nature of the data.

### 3. Results

#### 3.1. Pre-, during, and post-training heart rate values

Mean ( $\pm$ S.E.M.) 'Pre-', 'During' and 'Post-' training period heart rate values for each treatment ( $n = 8$ ) are presented in Fig. 2. There was a general trend of increased heart rate up to day 1 of week 3 for 'Pre-', 'During' and 'Post-' data with a subsequent decline by the end of the trial. Mean heart rate values were significantly higher ( $p < 0.05$ ) for the positive reinforcement treatment for the 'During' training measurement for week 7 (day 4) (d.f. = 1,  $F = 7.468$ ,  $p = 0.01$ , and for all three measurements in week 8 (day 1) (d.f. = 1,  $F = 4.662$ ,  $p = 0.03$ , d.f. = 1,  $F = 9.100$ ,  $p = 0.01$  and d.f. = 1,  $F = 6.740$ ,  $p = 0.01$ , respectively). No other significant differences between treatments for mean heart rate values were observed.

#### 3.2. Temperament test (arena test)

Arena test results showed similar trends of decreasing values in both mean weekly 'heart rate' and weekly 'behavioural score' for both training schedules (Table 3, Fig. 3). During week 8, the NR 'behavioural score' was significantly higher (d.f. = 1,  $F = 3.853$ ,  $p = 0.05$ ) compared to the PR treatment.

There was also a general trend for PR animals to approach faster (Table 3) and to come closer (Table 3, Fig. 4) to the novel object and observer compared to those within the NR treatment, reaching significance (d.f. = 1,  $F = 5.529$ ,  $p = 0.03$ ) and near significance (d.f. = 1,  $F = 3.487$ ,  $p = 0.08$ ) for distance from observer and novel object, respectively, in week 6.

No other significant differences for measured scores taken during the arena test between treatments were recorded.

#### 3.3. General behavioural profile (time budget)

Behavioural observations were calculated as a percentage of the total time spent performing each behaviour (Table 4). Overall, there was a decrease in percentage time ( $\pm$ S.E.M.) spent alert with a corresponding increase in time spent 'non-alert' during the course of the experiment (Table 4). Statistical analysis showed several significant differences between the treatments. At

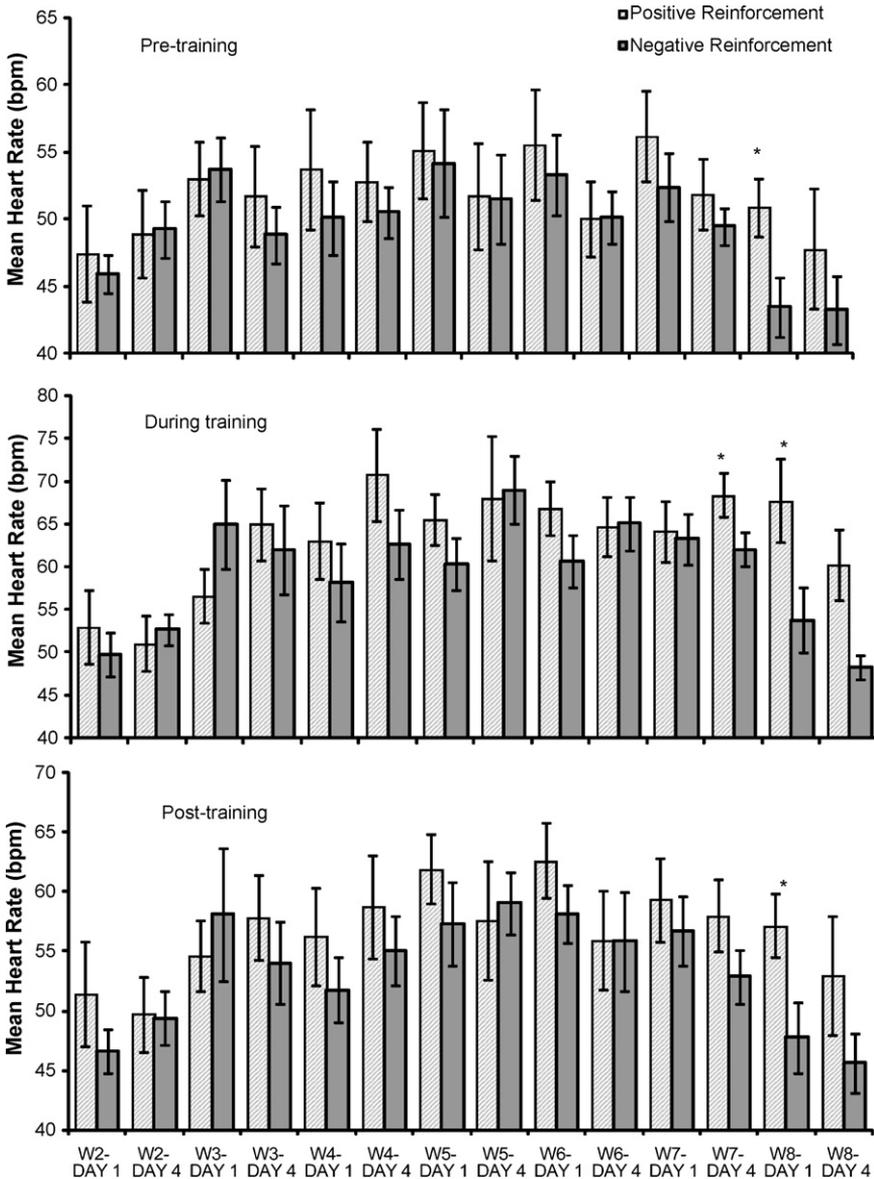


Fig. 2. Mean ( $\pm$ S.E.M.) heart rate (bpm) pre- during and post-training (weeks 2–8, days 1 and 4) for positive ( $n = 8$ ) and negative ( $n = 8$ ) reinforcement treatments (\* denotes  $p \leq 0.05$ ).

weeks 5 and 6 PR ponies approached the stable door (Table 4) significantly more than the NR ponies (d.f. = 1,  $F = 4.320$ ,  $p = 0.04$ , and d.f. = 1,  $F = 4.880$ ,  $p = 0.03$ , respectively) whereas NR ponies spent significantly more time standing resting in week 3 and recumbent sleeping in week 7 (Table 4) (d.f. = 1,  $F = 5.480$ ,  $p = 0.02$ , and d.f. = 1,  $F = 4.891$ ,  $p = 0.03$ , respectively). In week 6 the PR ponies spent significantly more time feeding than the NR ponies (Table 4) (d.f. = 1,  $F = 4.747$ ,  $p = 0.03$ ).

Table 3  
 Mean values ( $\pm$ S.E.M.) for measurements taken during the temperament (arena) test (weeks 1–8) for positive ( $n = 8$ ) and negative ( $n = 8$ ) reinforcement treatments

Measurement	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Weekly score	POS	7.7 $\pm$ 0.8	6.4 $\pm$ 0.7	6.5 $\pm$ 0.8	4.4 $\pm$ 0.8	3.9 $\pm$ 0.4	3.6 $\pm$ 0.4	3.6 $\pm$ 0.4	3.3 $\pm$ 0.2
	NEG	7.0 $\pm$ 0.7	6.8 $\pm$ 0.6	6.0 $\pm$ 0.6	4.6 $\pm$ 0.5	4.4 $\pm$ 0.4	4.1 $\pm$ 0.3	4.3 $\pm$ 0.2	4.4 $\pm$ 0.4*
Distance from observer (cm)	POS	275 $\pm$ 98.1	305.0 $\pm$ 115.8	265.0 $\pm$ 181.3	78.8 $\pm$ 69.9	28.1 $\pm$ 24.7	13.8 $\pm$ 12.4	51.9 $\pm$ 24.9	62.5 $\pm$ 32.4
	NEG	441.2 $\pm$ 120.4	220.0 $\pm$ 105.7	188.8 $\pm$ 87.8	197.5 $\pm$ 97.0	206.9 $\pm$ 124.6	137.5 $\pm$ 67.3	210.0 $\pm$ 101.3	217.5 $\pm$ 89.0
Distance from observer (cm)	POS	357.5 $\pm$ 117.6	425.0 $\pm$ 125.0	377.5 $\pm$ 192.2	168.8 $\pm$ 75.6	82.5 $\pm$ 44.9	40.0 $\pm$ 30.6	70.0 $\pm$ 37.2	87.5 $\pm$ 57.3
	NEG	617.5 $\pm$ 115.6	356.3 $\pm$ 115.0	326.3 $\pm$ 93.9	307.5 $\pm$ 110.5	287.5 $\pm$ 143.5	250.0 $\pm$ 81.8*	306.3 $\pm$ 119.6	298.8 $\pm$ 112.0
Time taken to touch object (s)	POS	319.0 $\pm$ 41.0	299.6 $\pm$ 41.5	246.8 $\pm$ 55.8	184.6 $\pm$ 55.0	201.3 $\pm$ 53.6	160.1 $\pm$ 52.3	265.5 $\pm$ 46.3	233.1 $\pm$ 48.4
	NEG	360.0 $\pm$ 0	298.1 $\pm$ 34.3	292.3 $\pm$ 44.4	292.1 $\pm$ 44.9	264.8 $\pm$ 47.6	291.9 $\pm$ 32.1	300.8 $\pm$ 22.9	337.4 $\pm$ 14.9
Time taken to touch observer (s)	POS	360.0 $\pm$ 0	360.0 $\pm$ 0	323.8 $\pm$ 33.5	297.3 $\pm$ 41.9	255.6 $\pm$ 50.5	213.5 $\pm$ 48.6	204.5 $\pm$ 53.0	188.4 $\pm$ 48.7
	NEG	360.0 $\pm$ 0	358.0 $\pm$ 2.0	323.1 $\pm$ 36.9	307.0 $\pm$ 37.2	269.6 $\pm$ 34.3	294.1 $\pm$ 43.1	324.4 $\pm$ 27.5	299.1 $\pm$ 42.1
Score if object touched	POS	4.9 $\pm$ 0.2	4.4 $\pm$ 0.6	4.0 $\pm$ 0.8	3.5 $\pm$ 0.7	3.6 $\pm$ 0.7	3.4 $\pm$ 0.6	3.8 $\pm$ 0.8	3.1 $\pm$ 0.8
	NEG	5.0 $\pm$ 0	4.6 $\pm$ 0.4	4.5 $\pm$ 0.5	4.3 $\pm$ 0.7	3.8 $\pm$ 0.7	4.3 $\pm$ 0.6	4.8 $\pm$ 0.3	4.4 $\pm$ 0.6
Score if observer touched	POS	5.0 $\pm$ 0	5.0 $\pm$ 0.5	4.5 $\pm$ 0.5	4.5 $\pm$ 0.5	4.1 $\pm$ 0.5	3.8 $\pm$ 0.6	3.4 $\pm$ 0.7	2.8 $\pm$ 0.7
	NEG	5.0 $\pm$ 0.6	4.5 $\pm$ 0.4	4.8 $\pm$ 0.4	4.6 $\pm$ 0.5	4.1 $\pm$ 0.5	4.4 $\pm$ 0.6	4.1 $\pm$ 0.6	3.9 $\pm$ 0.8
Mean heart rate (bpm)	POS	89.3 $\pm$ 9.5	85.2 $\pm$ 9.4	75.3 $\pm$ 6.3	68.8 $\pm$ 4.5	63.7 $\pm$ 4.6	64.8 $\pm$ 4.5	68.0 $\pm$ 4.1	51.2 $\pm$ 4.1
	NEG	96.1 $\pm$ 12.5	86.4 $\pm$ 9.6	78.8 $\pm$ 4.9	75.8 $\pm$ 6.1	65.3 $\pm$ 5.5	60.8 $\pm$ 3.6	61.1 $\pm$ 3.6	54.3 $\pm$ 5.0

\* Denotes  $p \leq 0.05$ .

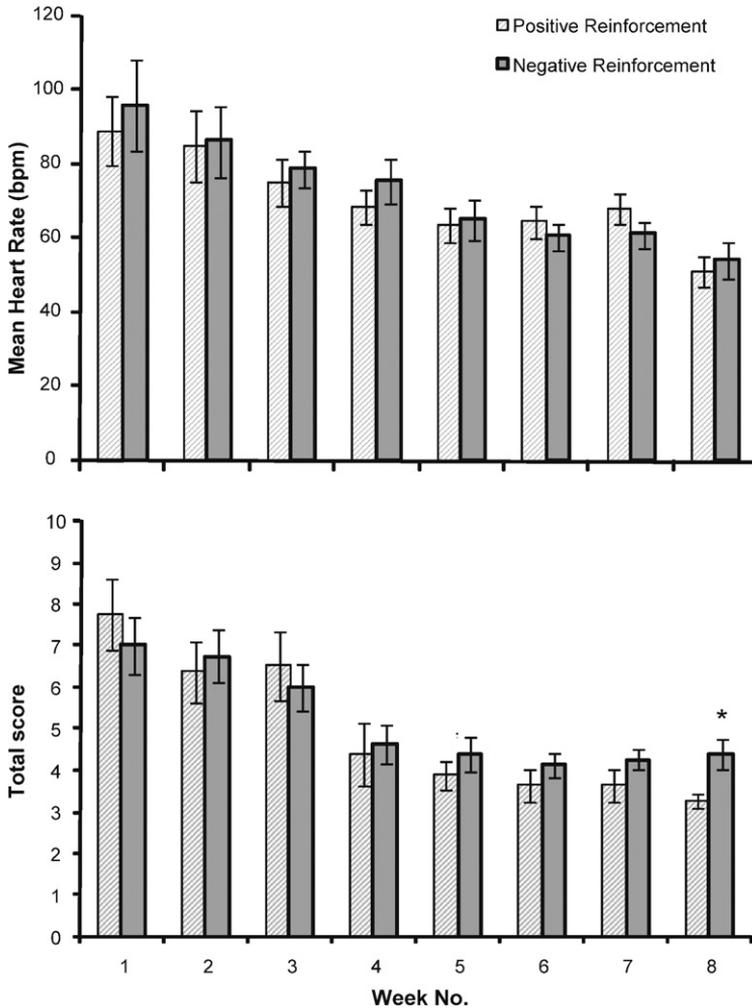


Fig. 3. Arena test: mean ( $\pm$ S.E.M.) heart rate and weekly score (weeks 1–8) for positive ( $n = 8$ ) and negative ( $n = 8$ ) reinforcement treatments (\* denotes  $p \leq 0.05$ ).

#### 4. Discussion

Prior to the study, the only human contact that the ponies had received was that associated with the rescue procedure, which, due to circumstances of the situation (the use of auditory and physical pressure and being loading into a confined and novel environment for the purposes of transportation) was most probably an aversive experience for the animals. In this context, it was anticipated that mean heart rate values for animals within both treatments would initially increase during the early stages of the trial due to human contact. This was indeed demonstrated by the results with a general trend of increased heart rate up to day 1 of week 3 of the trial before, during and after the training session. After week 3, there was reasonable individual variation in mean heart rate (until week 6, day 4) considered to reflect the individual emotionality in response to the learning tasks. The significantly greater mean heart rate of PR ponies in the latter stages of the

Table 4  
 Mean ( $\pm$ S.E.M.) percentage of time spent performing general behaviours (weeks 1–8) for positive ( $n = 8$ ) and negative ( $n = 8$ ) reinforcement treatments

Measurement	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Alert	POS	29.4 $\pm$ 2.8	31.9 $\pm$ 3.5	25.0 $\pm$ 4.7	19.8 $\pm$ 5.0	16.2 $\pm$ 2.9	10.6 $\pm$ 3.2	8.8 $\pm$ 3.4	10.4 $\pm$ 3.3
	NEG	35.6 $\pm$ 2.3	36.9 $\pm$ 3.3	22.3 $\pm$ 5.0	21.9 $\pm$ 3.7	8.0 $\pm$ 3.1	11.4 $\pm$ 3.4	13.2 $\pm$ 3.1	15.0 $\pm$ 3.8
Non-alert	POS	56.7 $\pm$ 3.4	51.1 $\pm$ 2.8	50.1 $\pm$ 2.6	58.7 $\pm$ 3.5	61.6 $\pm$ 3.7	65.9 $\pm$ 1.9	69.9 $\pm$ 3.7	72.3 $\pm$ 3.0
	NEG	52.6 $\pm$ 2.7	48.7 $\pm$ 3.0	55.0 $\pm$ 4.1	59.0 $\pm$ 3.2	65.5 $\pm$ 2.3	66.1 $\pm$ 2.2	67.9 $\pm$ 2.2	68.9 $\pm$ 4.0
Pawing at hay	POS	18.6 $\pm$ 2.9	1.8 $\pm$ 1.8	10.6 $\pm$ 3.2	1.8 $\pm$ 1.8	0 $\pm$ 0	5.4 $\pm$ 2.6	1.8 $\pm$ 1.8	8.6 $\pm$ 3.5
	NEG	19.4 $\pm$ 2.0	8.8 $\pm$ 3.4	13.0 $\pm$ 3.2	10.0 $\pm$ 4.1	12.3 $\pm$ 4.2	7.8 $\pm$ 3.8	13.0 $\pm$ 3.2	9.8 $\pm$ 3.0
Locomotion	POS	38.6 $\pm$ 2.1	17.6 $\pm$ 3.2	10.8 $\pm$ 4.3	20.5 $\pm$ 3.2	28.8 $\pm$ 2.9	25.7 $\pm$ 3.5	28.1 $\pm$ 3.4	33.0 $\pm$ 3.0
	NEG	38.9 $\pm$ 2.8	21.7 $\pm$ 3.4	20.5 $\pm$ 5.0	26.3 $\pm$ 3.2	37.1 $\pm$ 3.8	32.3 $\pm$ 2.1	35.7 $\pm$ 1.9	35.6 $\pm$ 2.8
Approach door	POS	10.2 $\pm$ 4.0	25.0 $\pm$ 2.5	29.6 $\pm$ 3.9	26.6 $\pm$ 5.3	20.4 $\pm$ 4.9	20.8 $\pm$ 4.4	24.7 $\pm$ 3.1	26.3 $\pm$ 5.5
	NEG	9.0 $\pm$ 4.4	24.8 $\pm$ 4.3	19.8 $\pm$ 3.9	23.1 $\pm$ 5.9	6.8 $\pm$ 3.5*	12.2 $\pm$ 4.6*	12.3 $\pm$ 5.2	11.6 $\pm$ 5.0
Standing resting	POS	5.8 $\pm$ 3.8	6.8 $\pm$ 3.5	4.4 $\pm$ 2.9	7.2 $\pm$ 2.7	10.8 $\pm$ 4.3	3.6 $\pm$ 2.4	9.4 $\pm$ 3.8	7.2 $\pm$ 2.7
	NEG	8.0 $\pm$ 3.1	6.2 $\pm$ 3.1	14.2 $\pm$ 2.3*	5.7 $\pm$ 3.8	13.2 $\pm$ 4.3	9.8 $\pm$ 3.0	7.2 $\pm$ 2.7	8.0 $\pm$ 3.1
Recumbent sleep	POS	3.6 $\pm$ 2.	9.4 $\pm$ 3.8	22.7 $\pm$ 2.3	16.8 $\pm$ 1.1	11.6 $\pm$ 4.4	17.8 $\pm$ 2.9	6.8 $\pm$ 3.5	1.80 $\pm$ 1.8
	NEG	1.8 $\pm$ 1.8	8.0 $\pm$ 3.1	14.6 $\pm$ 3.4	13.0 $\pm$ 3.2	11.2 $\pm$ 3.6	14.8 $\pm$ 2.6	10.4 $\pm$ 3.3*	4.4 $\pm$ 2.9
Feeding	POS	55.3 $\pm$ 2.5	57.9 $\pm$ 2.6	46.8 $\pm$ 1.8	56.0 $\pm$ 2.9	54.0 $\pm$ 3.4	57.0 $\pm$ 1.0*	56.3 $\pm$ 2.4	56.2 $\pm$ 2.2
	NEG	50.5 $\pm$ 1.5	57.3 $\pm$ 2.2	51.4 $\pm$ 1.6	54.3 $\pm$ 2.2	58.0 $\pm$ 2.7	52.3 $\pm$ 1.4	58.7 $\pm$ 1.6	51.5 $\pm$ 3.2

\* Denotes  $p \leq 0.05$ .

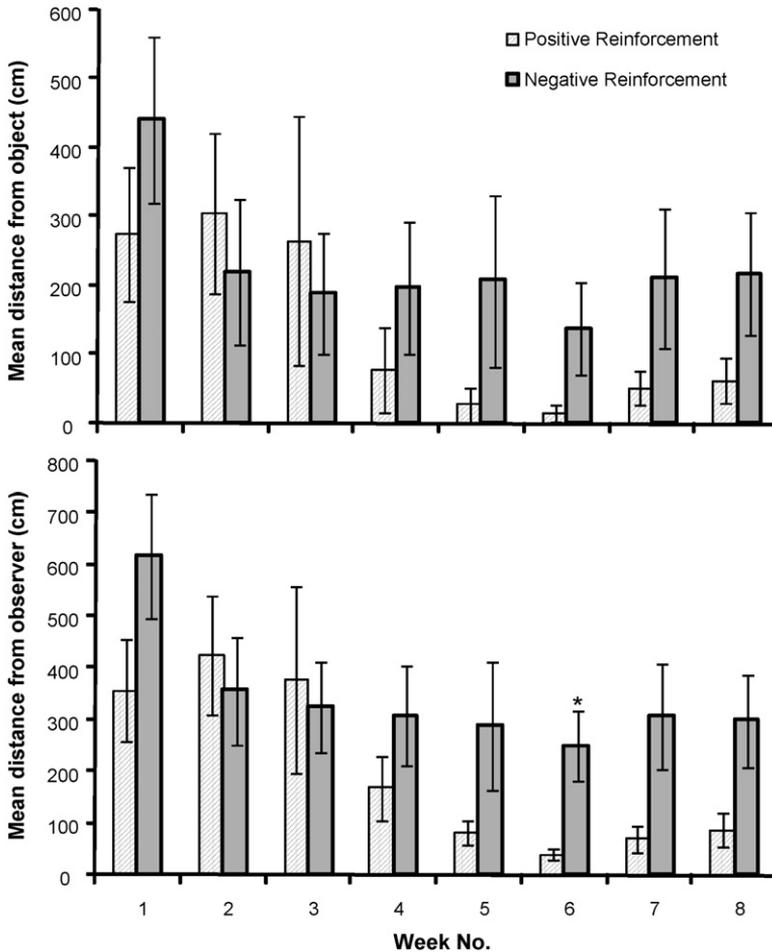


Fig. 4. Arena test: mean ( $\pm$ S.E.M.) distance from novel object and from observer (weeks 1–8) for positive ( $n = 8$ ) and negative ( $n = 8$ ) reinforcement treatments (\* denotes  $p \leq 0.05$ ).

trial (from week 7, day 1) could have suggested increased stress during the trial, however, it was also observed that these animals were extremely motivated to gain access to the training environment during these latter stages of the study. Thus, it was considered that the results indicated a positive rather than a negative emotional response during the latter part of the trial, i.e. increased adrenaline/noradrenaline release in anticipation of the food reward during the training, a response which is well documented (Wiepkema and Koolhaas, 1993).

During the course of the arena tests, both heart rate and behavioural observation scores showed a decrease in values for both PR and NR groups demonstrating progressive habituation to the arena environment for both groups of animals. Although there were very few significant differences between treatments for arena test measures, there was a very strong trend for PR ponies to come closer to the novel object/observer compared to NR animals from week 4 onwards with significance ( $p < 0.05$ ) between groups for distance from observer by week 6 (Table 3, Fig. 4). PR ponies, thus, may have habituated faster and were subsequently more inclined to

express explorative behaviour or, may have had altered perception of the human observer during the course of the trial. Conversely, NR ponies may have been generally less motivated to explore or, perhaps were perhaps more fearful of the situation and/or the human observer. The high behaviour score values obtained by the NR ponies (which were significantly different between groups by week 8) suggest that NR ponies did indeed remain more reactive during the 8-week period.

For the overall time budget of the animals, it is clear that the percentage of time spent alert for both treatments decreased after week 2 and with a simultaneous increase in percentage time spent non-alert for both treatments showing habituation to the stable environment for animals in both treatments. Although no significant results were evident, data in [Table 4](#) does also suggest that NR ponies spent a higher percentage of time than PR ponies pawing with the fore foot during the course of the trial. Performing this behaviour is often considered to be a sign of frustration and thus, the results could be interpreted as the negative reinforcement training affecting the animal's perception of the environment. It was also observed that PR ponies spent a higher percentage of time approaching the stable door, especially during the latter stages of the trial. This may again reflect anticipation for the training session or human contact linked to the attainment of food.

Overall, although there were relatively few significant differences between treatments, results did tend to suggest that, rather than negative reinforcement training being something that overtly compromises the animal's welfare, instead, the use of positive reinforcement training schedules may in fact confer active benefit to the animal. Animals under the latter regime tended to be more motivated to partake in rehabilitation activities and as a result of performing more explorative/'trial and error' type behaviours were more likely to attain the correct operant response. This motivation may possibly make the rehabilitation process a more positive experience for the animal. In addition, positive reinforcement resulted in the animal making more active contact with the human trainer. In the context of many of rescued animals coming from a background of human mistreatment, this again may be an additional advantage of positive reinforcement as a rehabilitation strategy. It may also explain the higher number of PR compared to NR ponies that met criteria for re-homing immediately after the completion of this study.

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