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A preliminary study of the effects of handling type on horses' emotional reactivity and the human–horse relationship

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ABSTRACT

Handling is a crucial component of the human–horse relationship. Here, we report data from an experiment conducted to assess and compare the effect of two training methods. Two groups of six Welsh mares were trained during four sessions of 50 min, one handled with traditional exercises (halter leading, grooming/brushing, lifting feet, lunging and pseudo-saddling (using only girth and saddle pad) and the second group with natural horsemanship exercises (desensitization, yielding to body pressure, lunging and free-lunging). Emotional reactivity (ER) and the human–horse relationship (HHR) were assessed both prior to and following handling. A social isolation test, a neophobia test and a bridge test were used to assess ER. HHR was assessed through test of spontaneous approach to, and forced approach by, an unknown human.

Horses' ER decreased after both types of handling as indicated by decreases in the occurrence of whinnying during stressful situations. Head movement (jerk/shake) was the most sensitive variable to handling type. In the spontaneous approach tests, horses in the traditional handling group showed higher latencies to approach a motionless person after handling than did the natural horsemanship group. Our study suggests that natural horsemanship exercises could be more efficient than traditional exercises for improving horses' HHR.

1. Introduction

A low-level of emotional reactivity among horses and positive human–horse relationships are crucial for improving safety, learning (Fiske and Potter, 1979; Hausberger et al., 2008; Le Scolan et al., 1997; Mader and Prince, 1980; Visser et al., 2003; Wolff and Hausberger, 1996), breeding performance, and resistance to disease (Bertrand, 2002). Emotional reactivity can be defined as a set of concomitant behaviour changes and physiological manifestations that occur in anxiety producing circumstances (Hall, 1934). The level of fear or adverse reaction (escape and aggression) shown may reflect the quality and characteristics of the human–horse relationship (Henry et al., 2005). Strong inter-individual differences regarding these two temperament traits have been demonstrated, and there is evidence that these traits are influenced by genetic (Hausberger and Muller, 2002; Le Scolan et al., 1997; Mader and Prince, 1980; Vierin et al., 1998; Wolff et al., 1997) and environmental factors (Hausberger et al., 1996, 1998, 2004; Søndergaard and Halekon, 2003; Søndergaard and Ladewig, 2004; Weeks and Beck, 1996).

Several studies suggest that handling exercises (touching and rubbing, halter-leading, lifting the horse's feet when the trainer takes hold of the fetlock) may both decrease a horse's emotional reactivity and improve its relationships with humans (Hada et al., 2001; Heird et al., 1986; McCann et al., 1988). Nevertheless handling has various consequences depending upon the age at which it is practised. Some authors have pointed out that handling early in a foal's life is likely to lead to an improvement in their subsequent manageability (Jeziershi et al., 1999; Lansade et al., 2004; Mal and McCall, 1996) and contribute to decreases in emotional reactivity (Visser et al., 2002). However, recent studies underline the weaknesses or short-term duration of effects when handling is practised before weaning (Lansade et al., 2005; Williams et al., 2002).

The way the animal perceives the environment (Maros et al., 2008) and human activities (i.e. handling) may also interact with

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the effects of handling. This could be linked with the effects of whether the patterns of handling used are consistent with the natural needs and repertoire (particularly of interactive behaviour) of horses. Therefore, it may be reasonable to predict that any different effects of handling styles may become evident when horses' behaviours in stressful situations are observed. On the other hand, the effects of some postures and attitudes currently used by horse handling experts, where individual experts may take opposite approaches (e.g. eye contact during catching), show no significant effects when tested in standardised conditions (Verrill and McDonnell, 2008). Such results challenge not only our knowledge of how animals perceive a stimulus (which may depend on the animal's temperament and the feelings of the human towards animals) but also the way the human–animal interaction develops (see Hausberger et al., 2008 for a review). Nevertheless, with age, horses' personality profiles increasingly appear to be a basic determinant of the behaviour observed (Hausberger and Richard-Yris, 2005; Henry et al., 2007).

Currently, there is a growing interest in natural horsemanship (term coined by McGreevy et al., 2005, hereafter referred as 'NH'). This handling style, said to be inspired by natural horses' behaviour, is based on an interpretation of the natural ethogram of the horse (McGreevy et al., 2005). Wild horses are known to deliver visual cues such as body language in order to communicate with congeners (Waring, 1983 in Goodwin, 1999). Accordingly NH techniques emphasise the importance of visual and gestural cues. This practise relies on instructions and interactions (i.e. motivation to move close to the trainer as in the round-pen technique) which are thought to be more suitable for horses than traditional procedures (traditional horsemanship, term used by McGreevy et al., 2005, hereafter referred as 'TH'). NH handling differs from TH firstly in that it uses visual signs and postures instead of vocal instructions, although 'silent' training is often required in TH riding. Secondly, when the horse gives the correct response, the NH handler ceases to apply an unpleasant stimulus (negative reinforcement). Note that negative reinforcement is a cornerstone of traditional handling although positive reinforcement (e.g. food reward) is increasingly used whether in the case of rescued equids (Innes and McBride, 2008) or to improve safety and welfare during equine practices (Waran et al., 2002). Another feature more typical of the NH approach consists in exposing the horse to a variety of visual, auditory, olfactory, and tactile stimuli in order that the horses undergo habituation and desensitization to these stimuli. The latter term is preferentially used by NH handlers although it would be more suited to the case of sensitized or hypersensitized animals (Hanggi, 2005). To our knowledge there are currently no experimental studies that have compared the efficiency of NH handling to that of TH handling. Improving emotional reactivity and the human–horse relationship is of importance for both the horses and their users, but before proposing a new method of management be widely used, we require clear tests of the approach's efficacy. Previous findings about handling effects in foals and young horses have demonstrated an improvement of both emotional reactivity and the human–horse relationship after handling (Heird et al., 1986; Lansade et al., 2004, 2005; Visser et al., 2002). Beyond this improvement, we expect a differential effect on emotional reactivity and the human–horse relationship between the two handling styles according the features that differentiate them. That is, because NH accounts for horses' natural behaviour to a greater degree, we expect it to lead to greater improvements in both behaviour types.

The emotional reactivity of two groups of horses was assessed with three tests, namely social isolation, neophobia and bridge-crossing, whereas the human–horse relationship was assessed with two additional tests: firstly, whether the horse spontaneously approached an unknown human entering the pen and staying motionless at its centre (voluntary approach, hereafter referred as 'VA'), and secondly, whether they approached and/or made contact with the same human entering the pen and trying to touch the horse (forced approach, hereafter referred as 'FA'). We discuss the results and the limitations of this preliminary study.

2. Materials and methods

The experiment was conducted at the stud farm Haras de Carmantran (Castelnau d'Estretefonds), in Southern France during autumn 2004. Animal care and experimental manipulations were in accordance with the rules of the French committee of animal experimentation ethics.

2.1. Subjects and experimental handling groups (Table 1)

The subject animals were 12 Welsh mares from A, B and C phenotypes referred as sections in the Welsh Stud Book (see Table 1), 2–10 years old, non-pregnant and without foals. They were all born and bred at the stud farm, where they lived all year round in a 2 ha pasture allowing natural social interactions, and for grouping behaviour to be expressed. They were fed grass *ad libitum*, hay daily, and apples three times a week from autumn to spring. Water was available *ad libitum*. Prior to the experiments, these horses were only used for reproduction, had little contact with humans (except for feeding and veterinary care) and were only trained to be haltered, led and trimmed. Accordingly, we considered the individuals included in this study to have had similar levels of experience with humans. It is also worth nothing that, as the mares were not used for riding, older mares were unlikely to be more familiar with humans than younger individuals. Horses were pseudo-randomly assigned to one of the two treatment groups of six animals, each group being trained using one of the two handling approaches. Two females that engaged regularly in aggressive interactions were assigned to different groups, while 2 individuals sharing an affiliative relationship were assigned to the same group. The age distribution of the two groups was similar (Mann Whitney test, $U = 13$, $P > 0.05$) although the age dispersion was higher in NH than in TH handling group (mean: 5, range: 3–5 and mean: 5, range:

Table 1
Composition of the experimental groups with age (years), Welsh type and name of sire.

Traditional horsemanship handling group				Natural horsemanship handling group			
N°	Age (years)	Section[a]	Sire	N°	Age (years)	Section[a]	Sire
1[b]	2	C	Orient la Bree	7	3	A	Padisha Carmantran
2[b]	2	C	Orient la Bree	8	3	B	Oxford Oliver
3	5	B	Oxford Oliver	9	4	A	Honeyman Cernin
4	5	B	Oxford Oliver	10[c]	4	C	Orient la Bree
5[c]	6	C	Orient la Bree	11	4	B	Nuage de la Brunie
6	10	A	Honeyman Cernin	12	5	B	Oxford Oliver

[a] A: Welsh Mountain pony, B: Welsh pony, C: Welsh pony of Cob type.
[b] Females that shared preferred associated relationships (grouped together).
[c] Females that interacted aggressively (kept apart).

2–10 respectively). During the experiment, the groups were maintained in two different 1.5 ha paddocks, apart from the stud farm's other horses.

2.2. Experimental procedure

The experiment was divided into four consecutive stages: (1) a familiarization period with the experimenters and experimental arenas, (2) emotional reactivity (hereafter called ER) and human–horse relationship (hereafter called HHR) tests prior to handling, (3) handling sessions and (4) ER and HHR tests following handling.

2.2.1. Familiarization

Although all horses had already been trained to be halter-led, they were first habituated to being caught and halter-led individually (one daily session of 10 min) during the first three days at the experimental site and in the pasture during the fourth day. All animals of each handling group were also given the opportunity to explore and forage together freely in the experimental area for 90 min. This experimental area consisted of 2 pens (diameters 18 and 14 m) in a rectangular and vegetated paddock (100 m × 40 m), familiar to the horses and visually isolated from adjacent pens by an opaque net.

2.2.2. ER and HHR tests

Horses were tested alone in all experimental tests. The same procedures were used to assess ER and HHR prior to and following handling. ER was assessed by three behavioural tests, adapted from Wolff et al. (1997). During the social isolation test, each horse was released into the pen for 5 min. During the neophobia test, each horse was introduced into the arena with an unknown object located on the ground at its centre (two blue pillows in a transparent plastic bag prior to handling and a white and green plastic dog crate following handling) for 5 min. During the bridge test, an unfamiliar person halter-led the horse (without body contact and in silence) from a starting line drawn in front of the pen's door, 9 m from a blue tarp (2 m × 3 m) which was to be crossed within a maximum time of five min, otherwise the test was stopped. During the ER tests, the horses' behaviour (see Table 2) was recorded with a digital video camera, and occurrences of specific behaviours were recorded using The Observer® software (Noldus).

Two behavioural tests adapted from Søndergaard and Halekon (2003) were used to assess HHR. During the first one, the voluntary approach (VA) test, soon after releasing a horse near the door of the pen, an unfamiliar person walked to the centre of the pen and remained motionless, with their arms by their sides, looking silently at the ground. The time the horse took to approach (horse's head within a distance of 1 m) and to touch the person were recorded. The test was stopped when the horse touched the person or after 3 min. During the human approach (forced approach: FA) test, the same person entered the pen silently and approached the horse at a rate of approximately one step per second, with their hands by their sides, looking at the horse's shoulder. If the horse stood/remained still, the person raised their right hand to allow the horse to sniff it and attempted to touch the horse's nose. The horse's behaviour was scored on the following scale: 1 = moved away from the person before he got within a 2-m range, 2 = stood still, 3 = sniffed the hand and 4 = the person could touch the horse's nose. The unfamiliar people were different for the HHR tests prior to and following handling.

Both prior to and following handling, social isolation, VA, neophobia and FA tests were conducted during the same session (day 1) in that order, while the bridge test was conducted during a second session (day 2). For practical reasons, the TH group was tested first each day and, for both groups, the order in which animals within a group were tested was randomly established for each handling session.

Table 2
Behaviours recorded during the social isolation, the neophobia and the bridge tests. For each test, behaviours were measured if current knowledge suggested that they were indications of anxiety. Galloping only occurred during the social isolation test. Levels of exploration walking near the object, object exploration and pawing the object were measured during the neophobia test. For the bridge test, the rate of occurrence (total number/duration of the test) was recorded.

Behavioural items	Description
Social isolation and neophobia tests	
Exploration standing	Standing still with the head low (nose bellow the withers—croup line)
Exploration walking	Quiet four-beat gait with the head low (sniffing the floor or not)
Exploration walking near the object	Bending the head in the direction of the object while walking near it
Pawing the floor	Striking the floor with a foreleg, with the head low
Pawing the object	Striking the object with a foreleg, with the head low
Object exploration	Standing still near the object with the head low (sniffing it or not)
Blow	Short non-vocal sound given during exhalation
Urination	Urination
Head	Jerking/shaking the head
Body shaking	Jerking the body
Attentive standing	Standing still with the head high (nose on or above the withers—croup line)
Attentive walking	Four-beat gait with the head high
Tail swishing	Jerking the tail quickly
Defecation	Defecation
Whinny	Vocalisation
Trot	Two-beat gait
Gallop	Three-beat gait
Trying to escape	Attempting to escape from the pen, putting the head between the fence bars and pushing
Bridge test	
Standing still	Stopping all movement and standing still during the bridge approach
Tail swishing	Jerking the tail quickly
Pushing around	Putting the head and the neck in front of the unfamiliar person during walking
Head low	Nose below the belly-line
Feet on the tarpaulin	Putting one or both foreleg on the tarpaulin, without actually crossing it
Startle	Startle with a hindleg flexion
Whinny	Vocalisation

2.2.3. Handling procedures

Two of us were involved in the handling (F.C.—TH group and P.M.—NH group). The aptitude level of the two trainers was more than that of a layperson as F.C. graduated in traditional horse riding and P.M. was teaching natural horsemanship as a professional. They had no previous contact with the trained horses, nor did they have contact with the horses included in the study outside the training sessions.

Handling involved four individual sessions of 50 min, and conversely to tests was conducted in parallel for both groups in their respective pens, separated visually by an opaque net. In order to avoid any dangerous responses from the horses induced by the stress of social isolation (Nicol et al., 2005), two old mares that were familiar to each tested horse, as well as hay and water, were present in the experimental paddock during handling.

The popularity of new methods of training and the fact that individual trainers interpret the precepts of different training methods idiosyncratically complicate the choice of exercises as characteristic of each type of handling. Some exercises are common to both types of training and thus we retained for each handling type only those exercises which seemed to us to best account for the philosophy they represented, that is human oriented for TH handling, and horse focused for NH handling (see Tables 3 and 4). For example,

Table 3
Training exercises and approximate timing used for the traditional horsemanship handling condition.

Traditional horsemanship handling	
Exercises[a]	Duration (min)
Halter lead from both left and right side of the horse, includes pauses where the horse is stroked.	6
Desensitization by rubbing the head, ears, neck then the hindquarters, first the left side then the right side	9
Brushing the forequarters then the hindquarters, first the left side than the right side	6
Lunging. The horse moves in circles around the trainer in both clockwise and anti-clockwise directions The trainer stands in the middle holding a rope (lunge line) that is attached to the horse's halter.	9
Training to pick up the forefeet when the trainer lifts the horse's foot by taking hold of their fetlock (picks up the feet)	2
Halter lead	1
Training to pick up the forefeet as previously described	1
Lunging as previously described	9
Placing the saddle pad on the horse back from each side	1
Training to walk with belly wrap (adjusted girth) held by the trainer in both clockwise and anti-clockwise directions.	2

[a] Stroking at the end of each exercise, two food rewards with one at the end of the session.

reward (i.e. positive reinforcement) does not exist in the communicative world of equids, while it is almost systematically used in experimental studies of learning in horses (Nicol, 2002). Positive reinforcement could also minimize the risk that the horses might learn undesirable behaviour performed to escape punishment perceived as unavoidable (Waran et al., 2002). On the other hand, negative reinforcement could be close to the learning process at work when horses learn how to avoid being chased (McGreevy and McLean, 2007) during aggressive bouts.

Table 4
Training exercises and approximate timing used for the natural horsemanship handling condition.

Natural horsemanship handling	
Exercises	Duration (min)
Desensitization	
- Forequarters then hindquarters by rubbing with hand, then with rope, then with stick	10
- Rubbing the feet first the forefeet then the hindfeet, from the two horse's sides.	3
- With a white plastic bag, rubbing the body on two sides then shaking the bag in front and behind the head, next the same with the plastic bag attached at the top of the stick	3
- With a survival blanket	3
- Body cue pressure at the head then at the hindquarter	2
Lunging with ending by returning to stand in front of the trainer after she bends over Circles at clockwise and anti-clockwise directions	3
Free lunging with asking the horse to change direction (from clockwise to anti-clockwise or vice versa) when the trainer moves backward	3
Learning to yield to the physical cue of pressure on the shoulder and hindquarter on both sides, next, learning to yield to the trainer pulling on their halter by moving their head across and down, and next to move their head sideways in response to pressure from the trainer's hands on the opposite side of their face.	6
Lunging as previously described	3
Free lunging as previously described	2
Desensitization of belly then the hindquarters by rubbing with a rope	2
Yielding to physical cue pressure on shoulder and hindquarter on both sides	2
Desensitization	
- On forequarter and hindquarter by rubbing with hand	1
- On tail hearth girth by rubbing with a rope	1
Lunging	1

TH group handling involved training exercises commonly cited (Ancelet, 2008) (Table 3): halter leading, body care (the horse was rubbed and brushed on both sides of the body), lifting of the feet, lunging and pseudo-saddling (only the girth and saddle pad placed on the horse). NH group exercises (Table 4) involved desensitization (the horse was rubbed on both sides of the body with the hand, a stick, and then a plastic bag until he no longer moved), yielding to physical pressure (the horse had to move its shoulders or croup when pushed with a finger on the shoulder or the belly respectively) and yielding to body cue pressure (i.e. without physical contact—it had to move its head when the handler shook his hands near the eyes and its croup when the handler bent over it), lunging and "free-lunging" (lunging without rope and only using postural instructions and vocal incentives. Note that some minor variations in the timing of these training exercises occurred according to an individual horse's response to the exercises. However, the total duration of a training session was maintained constant.

One major difference between the two types of training is that horses of the NH group were taught to stop their movement by returning to stand in front of the handler. Training this response by the horse is proposed by some to be the most significant difference between horses trained under NH and those trained under TH handling (Waran et al., 2002). NH also includes exercises intended to encourage a horse to remain close to the human (i.e. join-up in a round pen), these exercises are proposed to reinforce the focus on the handler. The two handling types differed also with respect to reward. The NH group was trained to perform a behavioural pattern which was rewarded by the cessation of an unpleasant stimulus (e.g. seeking for a quiet standing during an exposure to a frightening object—negative reinforcement). The probability of occurrence of the desired behaviour is therefore increased through operant (instrumental) conditioning (Hanggi, 2005). Conversely, the TH group was trained to exhibit the appropriate response by providing both pats and one or two food rewards per exercise (positive reinforcement). But note that although the reinforcers used in NH and TH training are dissimilar, both learning process come through operant conditioning. Horses are allowed to perform a "voluntary" behaviour which is maintained through its consequences. Although it was not planned prior to handling, the groups also differed with respect to vocal stimuli, i.e. the NH handler used vocal instructions less frequently. Two sessions per handler were video recorded in order to measure the frequency of vocal communication.

2.3. Data analyses

The behavioural responses recorded are given in Tables 5–8. For the ER and HHR tests, behavioural items not performed by ≥50% of the horses in each group both prior to and following handling were discarded as these behaviours were not typically sensitive to differences between handling.

In order to assess the effects of handling type, we calculated a performance index (Δ_i) for each behaviour measured. In order to achieve normal distribution of values, we based the index on the Log-transformed ratio rather on the difference between the level of that behaviour observed prior to handling and the level observed following handling:

$$\Delta_i = \mathrm{Log}\left(\frac{(N2_i + 1)}{(N1_i + 1)}\right) \quad (1)$$

where $N1_i$ and $N2_i$ are, respectively, the frequency (or duration, rate for the bridge test, see Table 7) of the behaviour i prior to and following handling. We added 1 to $N1$ and $N2$ in order to avoid zero values for the denominator. Δ_i varies from $-\infty$ to $+\infty$. Negative and positive values of Δ_i reflect a decrease, and an increase respec-

Table 5
Mean occurrences and durations (±SD) of behaviours recorded during social isolation test. (N): frequency; (D): duration (in seconds); *n*: number of performing horses.

	TH handling group						NH handling group					
	Before training			After training			Before training			After training		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
ISOLATION												
Exploration standing (N)	6.17	2.86	6	8.20	6.42	5	5	3.52	6	9.50	4.04	6
Exploration walking (N)	16	2.61	6	13.40	3.85	5	7.50	5.79	6	13.67	1.97	6
Attentive standing (N)	11.83	4.26	6	9	3.41	6	11.33	2.34	6	9.83	4.45	6
Urination (N)	6.33	5.77	3	1.50	0.71	2	5.67	5.68	6	3.33	4.04	3
Trot (N)	5.33	6.66	3	3	2.83	2	10	5.72	4	–	–	0
Gallop (N)	6	–	1	–	–	0	3	2.83	2	–	–	0
Whinny (N)	10.50	7.23	6	6	1.22	5	11.83	9.11	6	5	4.08	4
Blow (N)	2	1.55	6	3	1	3	1.25	0.50	4	2	1.41	5
Tail swishing (N)	*2*	*1.41*	*2*	*2*	*0*	*2*	*1.33*	*0.58*	*3*	*3*	–	*1*
Defecation (N)	1.25	0.50	4	1	0	3	1	0	2	1.50	0.71	2
Urination (N)	–	–	*0*	–	–	*0*	*1*	–	*1*	*1*	–	*1*
Pawing the floor (N)	*1*	–	*1*	–	–	*0*	*1*	–	*1*	*2*	*0*	*2*
Head movement (N)*	**1**	0	2	**2.25**	0.96	4	**3.75**	3.77	4	**1**	0	4
Body shaking (N)	–	–	*0*	*1*	–	*1*	*1*	–	*1*	*1*	–	*1*
Exploration standing (D)	28.20	15.09	6	57.8	46.08	5	20.37	16.33	6	56.07	31.27	6
Exploration walking (D)	127.93	48.81	6	92.27	38.39	5	90.57	48.57	6	123.11	50.99	6
Attentive standing (D)	102.22	42.62	6	169.21	78.96	6	127.61	62.75	6	112.81	61.02	6
Urination (D)	25.63	24.67	3	5.96	1.30	2	28.58	42.64	6	16.69	21.68	3
Trot (D)	54.55	88.29	3	12.06	16.04	2	48.31	33.23	4	–	–	0
Gallop (D)	*1.92*	–	*1*	–	–	*0*	*2.94*	*2.69*	*2*	–	–	0

Italics refer to behaviours that were displayed by fewer than half the horses before and after the handling.
* Handling group significant effect in the linear model ($P < 0.05$), values in bold.

tively in the frequency (or duration or rate as appropriate) of a given behaviour following handling, and $\Delta_i = 0$ a lack of change.

Data were analyzed using SPSS 1 ver. 12.0 (SPSS Inc., 2003) and R ver. 2.6.0 (R Development Core Team, 2007) software. Values of Δ_i (log transformed ratio) followed approximately a normal distribution. We used a linear model (R Development Core Team, 2007) to assess the relative efficiency of the two handling types (TH and NH) for each behavioural item. One sample *t*-tests (two-tailed) were used to detect whether Δ_i differed significantly from zero. Because multiple tests were performed on the same set of data, causing a risk of alpha inflation, a Bonferroni correction was applied to keep the type I error constant (Garcia, 2004; Nakagawa, 2004). Accordingly, the alpha probability of 5% was divided by the corresponding number of hypothesis tests (i.e. behavioural items analyzed, 8 for the isolation and neophobia tests, 4 for the bridge test and 2 for the VA test). So a result was considered statistically significant when *P* values were smaller than 0.0062, 0.0125 and 0.025 respectively. *P* values given in the text take in account the Bonferroni correction. Considering the risk of masking significant effects following the correction, we choose to report the cases of approximations to statistical significance (i.e. $0.05 < P \leq 0.1$ after Bonferroni correction).

Table 6
Mean number of occurrences and durations (±SD) of behaviours recorded during the neophobia test. (N): frequency; (D): duration (in seconds); *n*: number of performing horses.

	TH handling group						NH handling group					
	Before training			After training			Before training			After training		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
NEOPHOBIA												
Exploration standing (N)	6.20	1.64	5	6.17	4.96	6	4.60	1.52	5	4.33	1.97	6
Exploration walking (N)	14.33	2.88	6	9	9.87	6	13.17	2.48	6	8.33	3.20	6
Attentive standing (N)	11.5	4.85	6	8.17	1.94	6	11	4.20	6	9	3.79	6
Attentive walking (N)	2	1.41	2	–	–	0	5.75	6.95	4	2	1	3
Trot (N)	*8*	–	*1*	–	–	*0*	*4.50*	*2.12*	*2*	–	–	*0*
Object exploration (N)*	1.33	0.52	6	3.60	2.19	5	**1.20**	0.45	5	**3.50**	0.84	6
Whinny (N)*	5.83	3.19	6	3	2	5	**7.17**	6.11	6	**1.25**	0.50	4
Blow (N)	2.75	2.06	4	3	1.41	2	5.50	5.45	4	2	–	1
Tail swishing (N)	2.25	1.89	4	1	–	4	8	10.44	3	7	–	1
Defecation (N)	1.25	0.50	4	–	–	0	1	0	3	–	–	0
Urination (N)	*1*	*0*	*2*	*1*	–	*1*	*1*	*0*	*2*	–	–	*0*
Pawing the floor (N)	*1.50*	*0.71*	*2*	*1*	–	*1*	*2*	*0*	*2*	*2*	–	*1*
Head movement (N)	1.50	1	4	2.75	2.06	4	4.67	1.15	3	2	1.41	2
Body shaking (N)	–	–	*0*	–	–	*0*	*1.50*	*0.71*	*2*	–	–	*0*
Pawing the object (N)	–	–	*0*	*1.50*	*0.71*	*2*	*1*	–	*1*	–	–	*0*
Exploration standing (D)	33.33	4.99	5	46.21	52.16	6	28.86	20.95	5	63.35	76.27	6
Exploration walking (D)	139.95	51.15	6	64.89	55.56	6	125.57	45.31	6	61.93	27.88	6
Attentive standing (D)	115.28	60.09	6	137.72	88.37	6	122.31	55.02	6	139.32	72.18	6
Attentive walking (D)	3.22	1.67	2	–	–	0	27.27	40.29	4	9.16	7.29	3
Trot (D)	*65.24*	–	*1*	–	–	*0*	*20.96*	*15.16*	*2*	–	–	*0*
Object exploration (D)*	5.24	3.18	6	56.39	78.67	5	**3.91**	2.76	5	**31.07**	22.19	6

Italics refer to behaviours that were displayed by less than half the horses before and after the handling.
* One-sample *t*-test, $P < 0.05$, values in bold.

Table 7
Mean rates (±SD) of behaviours recorded during the bridge test. (R): rate; (D): duration; *n*: number of performing horses.

	TH handling group						NH handling group					
	Before training			After training			Before training			After training		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
BRIDGE TEST												
Standing still (R)	3.92	1.52	6	3.39	1.20	6	4.61	1.84	6	3.73	1.61	6
walking bouts (R)	3.89	1.56	6	3.39	1.20	6	4.61	1.84	6	3.69	1.64	6
Whinny (R)*	**1.88**	1.34	5	–	–	0	1.54	–	1	–	–	0
Startle (R)	*0.38*	–	*1*	–	–	*0*	*2.24*	*1.20*	*2*	–	–	*0*
Feet on the tarpaulin (R)	–	–	*0*	*0.40*	–	*1*	–	–	*0*	*0.20*	–	*1*
Starting line crossing[a]	2.02	1.21	6	2.73	1	6	2.21	1.09	6	2.41	1.25	6
Tail swishing (R)	*0.20*	–	*1*	*2.78*	–	*1*	–	–	*0*	*3.04*	–	*1*
Pushing around (R)	*0.20*	–	*1*	–	–	*0*	*1.54*	–	*1*	–	–	*0*
Head low (R)	2.48	1.27	2	2.26	1.26	4	3.93	–	1	5.43	2.84	2
Blow (R)	–	–	0	–	–	0	1.39	–	1	–	–	0
Standing still (D)	43.52	28.01	6	22.43	23.09	6	41.20	26.05	6	26.48	29.47	6
Time spent in the device (max.: 300 s)	92.67	115.46	6	66.17	114.58	6	39.33	25.81	6	71.17	112.76	6

[a] Number of starting line crossing (attempts) during the 5 min test duration; italics refer to behaviours that were displayed by fewer than half the horses before and after the handling.

* one-sample *t*-test, $P < 0.05$, values in bold.

Table 8
Mean durations and indices (±SD) recorded during HHR test. (T): time in seconds (max: 180 s); (I): index; *n*: number of performing horses.

	TH handling group						NH handling group					
	Before training			After training			Before training			After training		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
HHR												
VA 1 m (T)*	**38**	69.74	6	**68.17**	65.19	6	**32.83**	38.05	6	**16.33**	7.37	6
VA to contact (T)*	**49.33**	66.24	6	**111.33**	75.67	6	**82**	66.85	6	**28**	16.21	6
FA unknown human (I)	3.17	1.17	6	3.67	0.52	6	3	1.26	6	3.67	0.52	6

* Handling group significant effect in the linear model ($P < 0.05$), values in bold.

3. Results

Two sessions were video-taped for each handling condition, and the number of times the handler spoke to the horse was counted for each. The TH handler spoke 186 times in one session, and 208 times in the other, while the NH handler spoke less frequently: on 41 and 34 occasions in the two sessions analyzed. Whatever the training type, such speech was mostly used as a reward.

Mean (±SD) frequencies, duration and rate of behaviour performed during the ER and HHR tests are given in Tables 5–8. Prior to the horses' experience with training, we found no statistical differences between the groups in the level of occurrence of any behavioural item examined. As the horses had not had much previous experience being handled by humans, we expected an overall improvement of ER and HHR following handling. This was tested by comparing Δ_i to 0 (expected value if there was no change in behaviour from before to after handling). Then, we compared the specific effect of handling type for each test.

3.1. Emotional reactivity

3.1.1. Social isolation

Horses tended to vocalise less frequently after handling, although the difference was non significant for TH group (*t*-test: $t_5 = -1.22$, $P > 0.1$) and marginally significant for the NH group (*t*-test: $t_5 = -3.91$, $P = 0.088$) (Fig. 1). The effect of handling on head movement differed significantly between groups when horses were introduced alone into the test arena ($F_{1,10} = 7.26$, $P < 0.05$) (Fig. 1). The TH group performed more head movement after handling, while the opposite was true for the NH group (Table 5).

3.1.2. Neophobia

Horses in both groups showed similar trends (Fig. 2). After handling, they tended to be observed less frequently performing behaviours categorized as exploratory walking and attentively standing. They also spent more time investigating the object during the novel object test following training. There were significant changes in frequency of occurrence for behavioural items in the NH group. Horses in this group vocalised less frequently (NH: *t*-test:

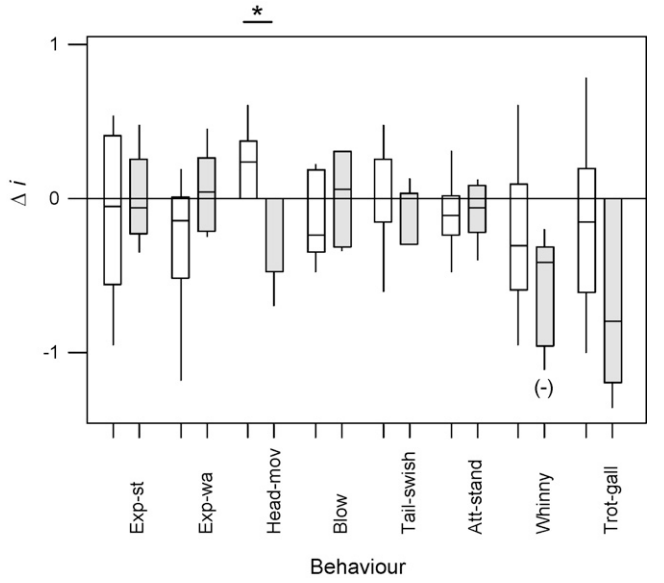

Fig. 1. Box-plot of the index (Δ_i) of change in behavioural occurrences between pre- and post-handling for the social isolation test. Exp-st: Exploration standing, Exp-wa: Exploration walking, Head-mov: Head movement, Tail-swish: Tail swishing, Att-stand: Attentive standing, Trot-gall: Trot and/or gallop. Open box: TH group, filled box: NH group. + and −: significant (*t*-test, $P \leq 0.05$) and (+) and (−): almost significant (*t*-test, $0.05 < P \leq 0.1$) departure from zero (alpha levels are corrected according inflation risk, see Section 2).

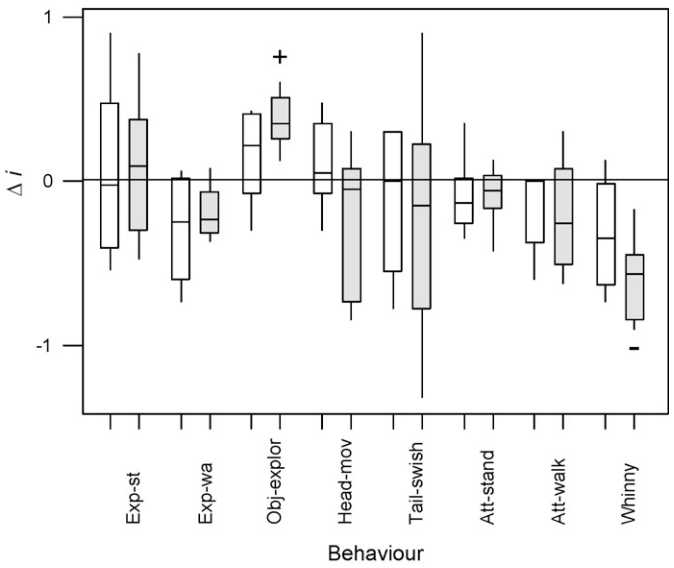

Fig. 2. Box-plot of the index (Δ_i) of change in behavioural occurrences and duration (D) between pre- and post-handling for the neophobia test. See Fig. 1 and Obj-explor: Object exploration, Head-mov: Head movement, Att-walk: Attentive walking.

$t_5 = -5.73$, $P < 0.05$; TH: *t*-test: $t_5 = -2.44$, $P = 0.48$) after handling. Additionally, they investigated the novel object more frequently during the neophobia test (NH: *t*-test: $t_5 = 5.46$, $P < 0.05$; TH: *t*-test: $t_5 = 1.39$, $P > 0.9$) (Fig. 2 and Table 6).

3.1.3. Bridge test

The horses of the TH group tended to vocalise less after being trained (Fig. 3 and Table 7), although it was only marginally significant (TH: *t*-test: $t_5 = -3.18$, $P = 0.096$). They also tended to cross the bridge more rapidly after being trained, although not significantly so (TH: *t*-test: $t_5 = -1.26$, $P > 0.9$). No change was found for the horses of the NH group (whinny: *t*-test: $t_5 = -1$, $P > 0.9$; time spent in the device: *t*-test: $t_5 = 0.27$, $P > 0.9$).

3.2. Human–horse relationship—VA and FA tests

When considering their reactions to the presence of a motionless and unfamiliar human in their pen, the groups differed substantially. Horses in the TH group increased their latencies to approach within 1 m and contact the unknown human conversely to horses

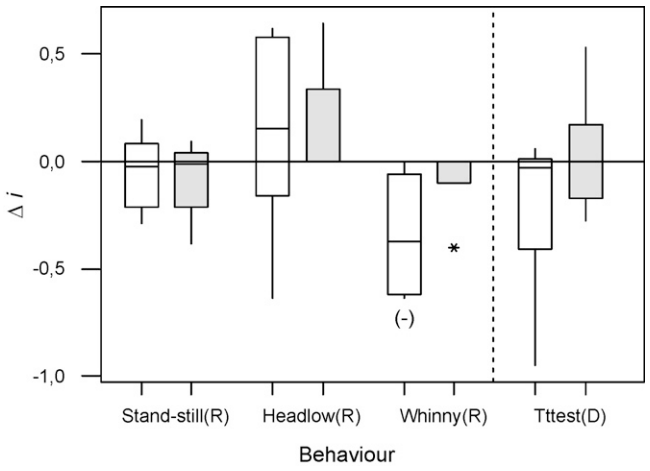

Fig. 3. Box-plot of the index (Δ_i) of change in behavioural occurrences and rate (R) between pre- and post-handling for the bridge test. See Fig. 1 and Tt test: time spent in the device (max: 5 mn).

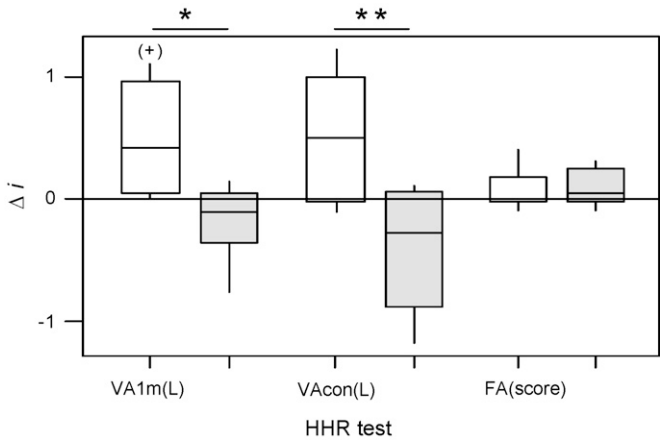

Fig. 4. Box-plot of the index (Δ_i) change in latencies (L) or scores for approach and contact with an unfamiliar human between pre- and post-handling for the HHR tests. VA 1m: Voluntary approach to 1 m, VA con: voluntary approach to contact, FA: forced approach.

in the NH group which approached and made contact with the human subject more rapidly after handling (approaching within 1 m: $F_{1,10} = 7.9$, $P < 0.05$; making contact with the human subject: $F_{1,10} = 8.7$, $P = 0.01$) (Fig. 4 and Table 8). The effect of the handling within each group remained, however, reduced as only the horses of the TH group tended to increase the time taken to approach within 1 m, although the difference is only marginally significant (TH: *t*-test: $t_5 = 2.5$, $P = 0.1$; NH: *t*-test: $t_5 = -1.34$, $P = 0.48$). There was no significant variation for the time spent to contact (TH: *t*-test: $t_5 = 2.26$, $P = 0.14$; NH: *t*-test: $t_5 = -1.90$, $P = 0.24$) (Fig. 4 and Table 8).

Regarding the FA test, the scores did not vary following handling for both groups (TH: *t*-test: $t_5 = 0.94$, $P = 0.39$; NH: *t*-test: $t_5 = 1.42$, $P = 0.21$) ((Fig. 4).

4. Discussion

The analyses revealed some differences between the effects of the two types of handling on the behaviour of the horses. These were mainly behaviours that can be seen as indicators of the quality of the human–horse relationship.

Our results must be interpreted cautiously because of the small sample sizes. Concerning general effects of handling, we observed that the ER of horses in both groups decreased after handling (i.e. horses were observed to gallop and whinny either less frequently, or not at all after handling). It has to be noted that defecation did not occur at all during the neophobia test after handling. Unexpectedly, in this latter test, horses of both groups tended to spend less time exploring the arena, and more time performing behaviours categorized as object exploration after handling. Our results are compatible with previous experimental studies showing that handling exercises reduce the stress experienced by horses (positive effect: Heird et al., 1986; Lansade et al., 2004, 2005; Visser et al., 2002) and so improve their emotional reaction when alone or confronted with novel situations. Indeed, the decrease in behaviours linked with stress (i.e. whinnying, defecation, trotting, galloping) may arise from habituation to both the training and testing environment and to manipulation by humans as has been seen in other species (for a review see Boivin et al., 2003).

Owing to the focus of the present study, the differences existing between the two handling groups were more enlightening. The main objective of the experiment was to detect any differences in the effects of the two types of handling on ER and HHR. Performance (i.e. the frequency, or duration of occurrence of a given behaviour) before handling was compared to that following handling When

measuring the change in behaviour in this way, whinnying and head movements were less frequent among horses in the NH group when isolated from their group, introduced in the arena and confronted with new objects, possibly indicating a reduction in anxiety. It is known that foals whinny to keep or get contact with their mother (Feh, 2005) and characterizes the period of weaning (Moons et al., 2005). When socially isolated, individuals often whinny to maintain or to regain contact with other horses (Cooper and Albentosa, 2005; Feh, 2005). Vocalisations are also associated with alertness (McDonnell, 2003) and performed in association with defecation, movement and the adoption of a vigilant posture in experimental situations of isolation and separation (Lansade et al., 2008a).

Head movements (i.e. head jerks) are described as a threat behaviour in social contexts (Goodwin, 2002) and associated with stress in horses used in equitherapy (Pyle, 2006). In our situation, as the horses were tested alone, we can consider head movement to be an indication of a conflict-related state. The reduction of these behaviours is consistent with the reduction in time spent trotting/galloping and the increase in time spent exploring the novel object.

The results concerning whinnying in the bridge test were inconclusive regarding the effect of handling type, because horses in the NH group did not neigh before handling. Accordingly, an observed improvement in ER was impossible, in contrast to what was observed for the TH group.

The results reveal that NH and TH training differ in their consequences for the human–horse relationship. Indeed, handling in the TH group lead to effects which were opposite to what was expected, as performance deteriorated. Horses took longer to approach the human following handling (five out of six horses). This trend could indicate a decrease in reactivity (Lansade et al., 2004) rather than an increase in fearfulness as all the horses eventually came close enough to touch the motionless human. In contrast, NH training seems to be beneficial as horses in this group approached the unknown human subject more rapidly following training. The influence of type of work on stereotypy is discussed by Hausberger et al. (2008) and the authors suggested that there may be a possible influence on subsequent relations between a given horse and humans. The beneficial effect of NH exercises was, however, restricted to the situation where horses were invited to approach the human subject spontaneously. When horses were subjected to an approach performed by an unknown human subject, no effect of handling or the type of training exercise was detected. This difference in the effect of handling types between the tests of voluntary and forced approach may be explained by the fact that the latter test is more intrusive than the former. Forced approaches by humans could be too stressful, inhibiting the positive effect mentioned above. From an ecological point of view, horses are prey animals so were dependent on adaptative behaviour patterns that enabled them to avoid predators. Thus, a moving subject heading towards an isolated horse can produce stress as it could mimic predator approach. Waring (2002) pointed out that when a stimulus object approaches a horse, avoidance behaviour soon becomes evident, while a motionless person is more readily approached and investigated than a walking one (Zeeb, 1963, cited in Waring, 2002). In the voluntary approach test, horses are allowed to express spontaneous responses, and this difference may allow the positive effect of NH handling to become evident. Moreover, the difference in methods employed to measure the horses' reactions in the voluntary approach compared to those employed to assess responses to the forced approach may account for the lack of significant differences between groups in the forced approach test. Alternatively, the results could also be explained by too little handling (four sessions) to detect differences between handling types.

As the HHR test involved unfamiliar people, this suggests that NH handling can be beneficial in the context of riding activities, which involve repeated exposures to different unfamiliar people. This suggests the following areas for future research (1) the way horses categorize humans according to previous experiences in terms of positive and negative stimuli (Hausberger et al., 2008) and (2) whether and when horses generalize across stimuli (Hanggi, 2005, Henry et al., 2005) particularly from a familiar trainer to unknown humans (Hausberger and Muller, 2002, Krueger, 2007); horses may not anticipate that an unfamiliar person will behave towards them in the same way as a known trainer. Generalization could be favoured through repeated exposure (habituation/desensitization) using a variety of unusual objects (Hanggi, 2005). To be complete, it must be noted that there is evidence that generalization across settings does not always occur, as studies have shown that horses that displayed following behaviour when trained in a round pen, did not show it when returned to a pasture (Krueger, 2007).

Some methodological points, however, may account for the differences in human-approach patterns in our experiment. Halter leading and lunging training was performed on the edge of the pen for both groups, but only horses in the NH group were taught to stop their movement by returning to stand in front of the handler. This could increase the likelihood that horses in the NH group would perform this type of movement subsequently, including during testing. Performing the VA test in a different place from the one where the horses were trained (e.g. a non-circular and non-familiar paddock, Søndergaard and Halekon, 2003) could allow us to confirm this possible handling effect. Further investigation of handling influence could seek to isolate the effects of the type of reward used, as well as whether and how frequently vocal communication was used by the handler.

Our study suggests that NH exercises could improve horses' HHR, whereas ER seems to be influenced similarly by the two types of handling tested. As Waran et al. (2002) underlined, although the welfare of horses trained through NH handling may not necessarily be better than that of traditionally trained horses, those receiving NH handling show a reduced tendency to make responses thought to indicate negative affective states, including panic. However, this preliminary study needs to be confirmed by further replications and longer periods of handling. Whether the effects detected in this experiment have long-term consequences also remains to be studied. In addition, we cannot exclude an effect of the handler's personality, given that the rider's personality has been suggested to have an effect on levels of cooperation between rider and horse (Visser et al., 2008). On the other hand, the horse's temperament (Lansade et al., 2007, 2008a) and sensoriality (i.e. sensory sensitivity—Lansade et al., 2008b) profile imply differential receptivity and sensitivity, respectively, of horses to handling instruction. As we have previously mentioned, horses have been demonstrated to be able to generalize from their daily contact with humans (i.e. caretaker) to an interaction with an unfamiliar person (Hausberger and Muller, 2002) and for some cognitive abilities (e.g. conceptualization of relative size—Hanggi, 2003) such generalization has also been shown to occur. Tests could be used to assess how horses categorize humans as positive, neutral or negative stimuli (Waiblinger et al., 2006).

In our experiment, the effects of variables specific to a given horse or handler (i.e. personality profile) could be a handicap in detecting the properties of handling *per se*. Possible ways to reduce this effect would be, for example, to train horses with only one handler, or alternatively to balance the handlers' training across groups. It would also be possible to homogenise experimental groups on the basis of the horses' behavioural profiles, since some temperament traits prove to be stable across time and situations (e.g. reactivity to humans: Lansade and Bouissou, 2008). Handling style primarily seems to affect the human–horse relationship rather than emotional reactivity. Hence, taking into account the temperament of

the horse and its relation with human personality appears to be a promising avenue of research in improving the operational value of human–horse relationships.

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