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Effects of neonatal handling on subsequent manageability, reactivity and learning ability of foals

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Abstract

Behaviour is an important factor to be taken into account in the various uses of horses. Today horses are mainly used for sport and leisure activities. They should therefore be easy to manage, calm and not fearful. Early handling is known to improve manageability and learning ability and to reduce fearfulness in various species. It has become fashionable in the horse industry to use an early training procedure, referred to as ''imprint training'', which is said to produce durable if not permanent effects. However, no studies concerning the long-term effects of such neonatal handling have been carried out in horses.

The present study examines the short- and long-term effects of neonatal handling on manageability, general reactivity and learning ability of foals. Twenty-six Welsh foals were studied: 13 were handled daily for 14 days from birth and 13 were non-handled controls. The handling procedure consisted of fitting a halter, gently patting all parts of each foal's body, picking up feet and leading over 40 m. Two days, 3 months, 6 months and 1 year after the end of the handling period, foals underwent behavioural tests to measure their manageability and various aspects of their reactivity. The results showed that neonatal handling has only short-term effects on manageability: 2 days after the handling period, handled animals were significantly easier to handle than controls for the four parameters measured during this test (time to fit a halter, time to pick up feet, walk ratio that is time during which foal walks under constraint/total time measured during leading and number of defensive reactions). Two parameters (time to fit a halter and walk ratio) were still lower in handled foals than in non-handled foals 3 months later and only one 6 months later (walk ratio). One year later there was no difference between groups. In addition, there was no effect of handling on reactivity at any time of testing or in any of the tests (reaction to isolation from conspecifics, presence of a human,

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presence of a novel object and to a surprise effect). Finally, neonatal handling did not improve the spatial or discriminative learning abilities measured at 14 months of age. To conclude, the effects of neonatal handling are only temporary.

Keywords: Horse; Neonatal handling; Manageability; Emotional reactivity; Learning-ability; Human–animal relationship

1. Introduction

Behaviour is an important factor to be taken into account in the various uses of horses. Horses are nowadays used mainly for leisure and sport. They are often ridden by children and inexperienced riders and thus need to be calm, not fearful and easy to handle. A method which can provide such effects is therefore worthwhile. It has been proposed (Miller, 1991) that neonatal handling of horses could have permanent effects on reduction of fearfulness and improve ease of handling. However, the efficacy and durability of the effects remain unclear.

Numerous studies involving laboratory animals (for review: Denenberg, 1962), dogs (Fox and Stelzner, 1966) and more recently farm animals (for review: Rushen et al., 1999), including horses (Lansade et al., 2004) showed that handling can influence some aspects of animal behaviour such as fear of humans, manageability, general fearfulness and learning ability. Most authors found that early handling decreases fear of humans (poultry, Barnett et al., 1994; Jones and Waddington, 1993; Jones, 1994; pigs, Pearce et al., 1989; Tanida et al., 1995; rabbits, Hargreaves and Hutson, 1990; Podbersceck et al., 1991; foxes, Pedersen, 1993, 1994; dogs, Fox and Stelzner, 1966; cattle, Boissy and Bouissou, 1988; Boivin et al., 1992; sheep, Markowitz et al., 1998; goats, Boivin and Braastad, 1996; horses, McCann et al., 1988; Jezierski et al., 1999). Moreover, some studies showed that handling improves manageability, defined as the ease with which a person can impose a routine handling procedure such as the fitting a halter on horses (Heird et al., 1986; Mal and McCall, 1996; Jezierski et al., 1999). The effects of handling on general fearfulness have also been reported in various species (for review: Denenberg, 1962; Mason, 2000) although results are often contradictory (Lansade et al., 2004). Finally, it has been shown that early handling can improve later learning ability in several species such as rodents (Hebb, 1947; Bernstein, 1957) and rabbits (Anderson et al., 1972). Heird et al. (1981) and Heird et al. (1986) showed that horses handled for a few weeks after weaning performed better in a modified T-maze than non-handled horses.

The durability of the effects of handling varies between studies and according to species (foxes, Pedersen, 1994; cattle, Sato et al., 1981; Boissy and Bouissou, 1988; Boivin et al., 1992; goats, Boivin and Braastad, 1996; horses, Lansade et al., 2004; Simpson, 2002).

The diversity of results could be explained by the variety of protocols used. For example, duration of handling varied, with some authors handling animals from a few days (e.g. horses, Williams et al., 2002, 3 days; Simpson, 2002, 5 days; Mal et al., 1994, 7 days) to several months (e.g. horses, Heird et al., 1981, 4 days a week for 10 months, and Jezierski et al., 1999, 5 days a week for 14 months).

The period of life during which an animal is handled is also important. Some authors have suggested the possible existence of a ''sensitive'' or ''critical'' period in mammals during which the animal is more receptive to external stimuli and to handling. For instance, Scott (1992) reported that dogs must be handled from 3 to 12 weeks of life in order to be socialized. There are no effects of handling if contact is provided out of this period. The neonatal period is often considered to be a favourable period. Schaefaer (1963) found that handling rats during the first week of life is more effective than handling during any other period of life. Handling is more effective in pigs if it is provided from birth to 3 weeks of age rather than from 3 to 6 weeks or from 6 to 9 weeks (Hemsworth and Barnett, 1992).

Handling foals from birth to 42 days of age is more effective than handling them from 43 days of age to 84 days of age, suggesting the possible existence of a ''critical period'' within the first 42 days of life (Mal and McCall, 1996). A training method known as ''imprint training'' (Miller, 1991) has been widely promoted and used in the horse industry. Supporters of this method assert that neonatal handling has very long-term effects, lasting for several years, and suggest an imprinting-like process. Nevertheless, the efficacy of this method is debatable and the long-term durability of the possible effects has never been tested. The efficacy of the procedure was tested by Williams et al. (2002) and Simpson (2002), but the results were contradictory. Williams et al. (2002) handled 25 foals four times at 2, 12, 24 and 48 h after birth. The handling procedure consisted of patting all parts of the foal's body, fitting a halter, leading, rubbing the body with a plastic bag and spraying the foal. When tested at 1, 2 and 3 months of age, there was no difference between handled and non-handled foals, either in their reactions to stimuli used in the early training procedure or to a novel stimulus. Neonatal handling was therefore not effective in this study. Nevertheless, it is possible that 2 days of handling were not sufficient to produce any effects. Simpson (2002) handled seven foals from 2 to 8 h after birth and continued daily for 5 days. The handling procedure was similar to that used by Williams et al. (2002). When tested at 4 months of age, foals handled as neonates were more tractable and less reactive to stimuli with which they were familiar as neonates than non-handled foals. The efficacy of neonatal handling remains thus unclear. In addition, neither the long-term effects of neonatal handling on manageability nor the long-term effects on reactivity and learning ability have been tested in horses.

The initial aim of our study was therefore to examine the short-, middle- and long-term effects of limited neonatal handling on reactions to humans, manageability, general reactivity (reaction to novel objects, isolation, surprise effects) and learning ability. Moreover, if the effects were only temporary, it is possible that neonatal handling influence might lead animals handled as foals to respond more quickly to subsequent handling than animals not handled as foals. The second aim of this study was to test this hypothesis.

2. Animals, materials and methods

2.1. Animals and experimental groups

Twenty-six Welsh foals (6 males and 20 females) born in June 2000 were used. They were individually identified with ear tags. Foals were born outdoors without human

assistance. They were randomly allocated to one of two treatments: a ''handled group'' ($N = 13$; 4 males, 9 females) in which foals were handled daily (see below) from 6 ± 1 h after birth to 14 days of age and a ''control group'' ($N = 13$; 2 males, 11 females) in which foals were not handled, and received minimal human contact necessary for routine management.

Each dam-foal dyad from the handled group was individually penned every day from 5 to 7 h after birth and from 9:00 a.m. to 6:00 p.m. for the first 2 weeks of life, corresponding to the handling period. They were maintained outdoors during the night. Control foals and their dams remained outdoors during this period. From 2 weeks of age animals from both groups were maintained together outdoors until weaning. They were abruptly weaned at 5.5 months of age ($\pm$15 days). They were housed together indoors after weaning in an 8 m $\times$ 10 m pen for 3 months (winter period) and returned outdoors as one group from 9 to 14 months of age until the end of the experiment. When they were to be tested, foals were again placed in pens 24 h before the tests.

Apart from handling periods and tests, all foals received similar limited human contact necessary for routine husbandry: feeding when indoors, change of pasture and emergency veterinary care when necessary.

2.2. Handling procedure

The handling procedure followed as closely as possible the procedure used by Miller (1991) with minor modifications: probing the ears, inserting a finger up the nose, inserting a gloved, lubricated finger in the anus were omitted since Simpson (2002) showed they were not effective.

The first handling session was performed at 6 ± 1 h of age and lasted 45 min, divided into three periods of 15 min separated by two pauses of 10 min. On the following day foals were handled twice for 15 min (sessions 2 and 3, at 24 and 36 h of age, respectively). Foals were then handled 10 min twice a day until 4 days of age (sessions 4–7) and finally once a day until 14 days of age (sessions 8–18).

Handlers (4 women and 1 man) were all experienced and wore blue overalls. At the beginning of each handling session, the mare and her foal were placed in the same 4 m $\times$ 4 m pen and the mare was tethered in a corner of the box to avoid interference with her foal and the handler. The handler then quietly entered the pen, fitted the foal with a halter and gently patted all parts of its body: the head, shoulders, back, hindquarters and legs successively, for a total of 2 min. He picked up each of the forelegs and hindlegs. A white plastic bag was then shaken in front of the foal's head. Finally, each foal was taught to move forward and backwards with a pressure to the breast or the quarters and to walk 40 m from the pen to a specific point followed by its dam, both led by a helper. The four exercises: fitting with a halter, picking up feet, leading and shaking the plastic bag were each repeated within the same session until the foal no longer resisted or manifested avoidance or defensives reactions. Foals were then gently patted until the end of the session. The different exercises were introduced gradually: the handler placing a halter from the second session and the foal learning to walk from the seventh session onwards.

2.3. Behavioural tests

To assess the effects of handling (both short- and long-term) on reactions to humans, manageability and general reactivity or fearfulness, foals underwent the behavioural tests described below. Animals were subjected to this series of tests over a 2-day period at 16 days, 3, 6 and 12 months of age (±15 days). The tests at 6 months were conducted 3 weeks after weaning, since it has been shown that the stress induced by weaning is limited to 1 or 2 weeks (Houpt et al., 1984; McCall et al., 1985).

Between 14 and 15 months of age, animals underwent an experiment aimed at assessing the long-term effects of early handling on spatial and discriminative learning ability. This experiment required intensive daily contact between foals and handlers (including leading the foal without being haltered). We took this opportunity to see if extra-handling provided later in life had a differential effect depending on early experience of handling.

2.4. Tests performed at 16 days and 3, 6 and 12 months of age

Foals were exposed to three different sets of tests: open-field tests, handling tests and surprise tests. The open-field tests were performed on day 1 and handling tests and surprise tests were performed on day 2. The testing order was randomly assigned. The experimenter was an unfamiliar person for all tests. The mare was present and tethered in a corner of the test stall during the tests performed at 16 days and 3 months of age. A score was assigned based on agitation of the mare during the tests in order to compare behaviours of handled-foal mares and that of non-handled-foal mares that might influence the foal's behaviour (Table 1).

2.5. Handling tests

To assess manageability and the effects of the early handling regimen on later reactivity to humans, the above handling procedure was repeated.

As during the handling session, the foal was removed from its group and led (without being haltered) to a 4 m × 4 m pen, similar to those where handling was performed.

2.5.1. "Capture"

An unfamiliar handler tried to fit the foal with a halter. If he did not succeed within 3 min, a second person (helper) entered the pen. Both then had 5 min to catch the foal.

Table 1
Scores based on agitation of mares during tests

Score	Behaviour
0	No movement of feet
1	A few movements of feet (fewer than 15)
2	Continuous movement of feet
3	Continuous movement of feet and defensive reactions (kicking or rearing)

If the foal could not be caught in 5 min, the test was terminated. The time required to catch the foal was recorded. The foals that were not caught were not subjected to subsequent tests, they were given the maximum time allocated for the ''capture'' test (480 s) and for the ''feet picking up'' test (120 s) and we assigned them a ''walk ratio'' of 1 (see below).

2.5.2. "Feet picking up"

When haltered the foal was restrained by the helper. The handler attempted to pick up each foot beginning with the forefeet. The total time required to pick up the four feet was recorded. A maximum time of 120 s was allocated.

2.5.3. "Leading"

This test consisted of leading the foal along a 40 m corridor (maximum time allowed: 5 min). To assess ease of handling during leading in the corridor, we measured the time during which the foal walked under constraint (the rope was tight, foal had to be coaxed, i.e. it was gently slapped on the quarters when necessary, or it refused to walk despite coaxing) and the total time to cover the corridor. A ''walk ratio'', defined as time ''walking under constraint/total time'', was calculated. If the subject did not achieve the criterion (covering the corridor within 5 min), it was given a ratio of 1. Defensive reactions (rearing, kicking or attempting to bite) were recorded during each of the tests.

2.6. Surprise tests

Tests using different surprise effects were performed in the same pen with the same handler just after the handling test. The foal was restrained with a rope (5 m long) marked every 20 cm. The experimenter released the rope and let the foal move away when stimuli were presented. The flight distance after each stimulus was measured by the marks on the rope.

2.6.1. Stimulus known by handled foals

The experimenter shook the same white plastic bag used during the handling session in front of each foal's head for 3 s.

2.6.2. Stimulus unknown by handled foals

Three surprise effects were used. The experimenter suddenly placed a saddlecloth and a surcingle on the foal's back then drew them away. When the foal was still, powder was sprayed three times onto its shoulder (Orospray ND), and when the foal was still again a red and green umbrella was opened in front of the foal's head twice at a 2 s interval.

2.7. Open-field tests

These tests were only performed after weaning (at 6 and 12 months of age) since they required the foal to be alone in the pen. They were aimed at evaluating potential differences in general reactivity to novel situations.

We used situations classically reported to induce fear in ungulates, i.e. isolation from conspecifics, presence of an unfamiliar human and presence of a novel object. Similar tests have been designed and validated in sheep (Romeyer and Bouissou, 1992), cattle (Boissy and Bouissou, 1995) and adult horses (Viérin et al., 1998).

The open-field arena was a square pen (6 m × 6 m), divided into nine sectors of equal size by a grid painted on the floor. This pen had solid walls, and was separated from other pens containing animals by an empty pen on both sides, so that the foal being tested could not see other animals.

Each foal was individually and successively subjected to the following three fear-inducing situations, each lasting 2 min.

2.7.1. Isolation test

Behaviour was observed without any additional fear-inducing stimuli.

2.7.2. Human test

An unfamiliar experimenter quietly entered and stood stationary in sector 8, opposite the door.

2.7.3. Novel object test

An object, unknown to the foals, was introduced in sector 8 without human intervention (dropped from above one of the walls). A straw bale covered by an orange sheet (80 cm high) was used for the test performed 6 months after the end of the handling period and a blue plastic bag filled with straw (80 cm high) during the test performed 12 months following the handling period.

Observations were carried out from a hidden platform, 2 m high. Eleven behavioural parameters, previously interpreted as indicative of the presence or absence of fear (Viérin et al., 1998), were recorded using a tape recorder (Table 2). The data were subsequently transferred to a computer and analyzed using ''The Observer'' software (Noldus, 1991) which calculated frequencies, latencies and durations of the behavioural parameters.

Table 2
Parameters of behaviour measured during open-field tests

Parameters	Abbreviations
Time spent in squares 1–2–3 (s)	T123
Time spent in squares 7–8–9 (s)	T789
Squares entered (No)	SE
Immobilisation time (s)	IT
Neighing (No)	NN
Latency to first neighing (s)	LN
Defecation (No)	DF
Glance at the stimulus [a] (No)	GL
Latency of sniffing the stimulus [a] (s)	LS
Mean duration of sniffing [a] (s)	DS
Sniffing [a] (No)	SN

[a] Parameters of behaviour not recorded during isolation test.

2.8. Tests performed at 14 months of age

2.8.1. Learning ability

Fourteen months after the handling period, two different learning tasks were performed: a spatial learning task and a discriminative learning task. They lasted for 1 month, during which foals were in daily contact with humans: they were led (without being haltered) from one place to another eight times per day (10 ± 2 min per day).

The two learning tasks were performed in the 10 m $\times$ 8 m arena (Figs. 1 and 2) where foals had been living for 3 months after weaning. The food usually supplied to the horses (commercial food pellets) was used as the positive reinforcer for both learning tasks. In each of the two learning tasks, the foals were required to learn the location of the bucket containing food. The animals were underwent eight trials per day. The criterion for both tests was that the foal performed six correct responses per day on 3 consecutive days. Before each trial, the foal was led (without being haltered) to the starting box and then released in the arena. The maximum time allowed for each trial was 40 s.

On the first day of each learning test, all the buckets contained food. Each foal was placed in the arena three times for 3 min to become familiar with it. By the end of the familiarization period, all the horses had eaten from all the buckets.

2.8.2. Discriminative learning task

During the discriminative learning task, one end of the arena was divided in two compartments separated by a solid fence (1.5 m high $\times$ 2.5 m long, set perpendicular to the wall on the longitudinal centreline of the arena). Two buckets differing in colour and material (a grey metal bucket and a blue plastic bucket; 40 cm long $\times$ 20 cm wide $\times$ 20 cm deep) were placed in the centre of each compartment (Fig. 1). Only one bucket contained food. Discrimination was based on the presence of a relevant cue (bucket colour and material) and an ambiguous cue (spatial position). The spatial position of the bucket was

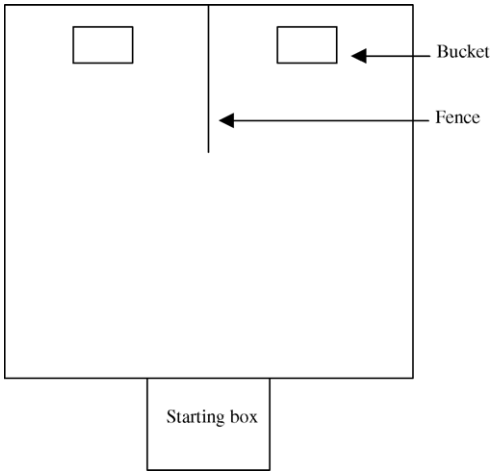

Fig. 1. Overhead view of the testing apparatus used for the discriminative learning test.

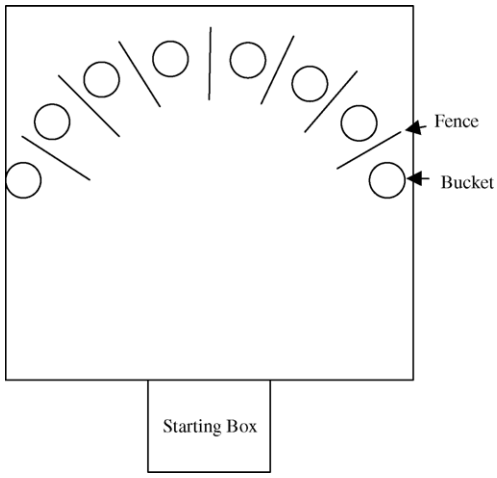

Fig. 2. Overhead view of the testing apparatus used for the spatial learning test.

randomly determined with the constraints that each stimulus had to appear in each position (left and right) an equal number of times per test session, and a maximum of three consecutive trials could occur without switching the stimulus position.

2.8.3. Spatial learning task

During the spatial learning task, one end of the arena was divided into eight compartments separated by seven fences (1.5 m high, 2.5 m long). A round white plastic bucket (diameter 30 cm, depth 30 cm) was placed in the centre of each compartment (Fig. 2). Only one compartment contained a bucket full of food, this compartment was always the same for a given horse.

2.9. "Capture test" performed before and after the learning period

The day before and the day after the learning task period, foals were subjected to a human test during which the handler tried to fit the foal with a halter. The procedure was exactly the same as described above (''capture test'').

2.10. Statistical analysis

Due to the small number of subjects and nature of measurements, non-parametric statistics were used. Data obtained for each group during the handling tests, surprise tests and learning tests were compared using the Mann–Whitney *U*-tests (Siegel, 1956).

Data from the ''Open-field tests'' were subjected to principal component analysis of variance (Frey and Pimentel, 1978) to determine the relationships between the various parameters measured. The Mann–Whitney *U*-test was used to compare the loadings from the two groups.

Values indicated in the text and the figures are medians, inter-quartile ranges, in the form of median [first quartile; third quartile], the *U* statistic and the critical value of *P*.

3. Results

3.1. Handling tests

When 16 days old (2 days after the end of the handling period), animals from the handled group were easier to handle than controls (Table 3). The time required to fit the foal with a halter and to pick up feet, the walk ratio and number of defensive reactions were significantly lower for the handled group than for the control group ($P < 0.001$, $P < 0.01$, $P < 0.05$, $P < 0.001$, respectively). At 3 months of age, the time required to fit a halter and the walk ratio were still significantly lower for the handled group than for the control group ($P < 0.05$; $P < 0.05$, respectively). The only significant difference at 6 months of age was a lower walk ratio for the handled group than for the control group ($P < 0.05$). At 12 months of age, there was no longer a significant difference between groups.

Table 3
Median ± inter-quartile of each group during handling tests and during known surprise tests

Parameters	Age	Treatment		*U*	*P*
		Handled	Controls		
Time required to fit halter (s)	16 days	19 [13; 24.5]	50 [40; 62]	1	***
	3 months	132 [35; 192.5]	261 [144; 430]	38	*
	6 months	170 [55; 242.5]	135 [105; 235]	75.5	NS
	1 year	52 [20; 57]	116 [25; 388]	39	NS
Time required to pick up feet (s)	16 days	30 [26; 42.5]	46 [40; 54.5]	35.5	**
	3 months	120 [48; 120]	120 [90; 120]	79	NS
	6 months	120 [120; 120]	120 [119; 120]	78	NS
	1 year	120 [49; 120]	120 [71; 120]	47	NS
Walk ratio[a]	16 days	0.24 [0.18; 0.29]	0.42 [0.3; 0.45]	45	*
	3 months	0.48 [0.12; 0.68]	0.9 [0.45; 0.97]	44.5	*
	6 months	0.29 [0.18; 0.41]	0.65 [0.39; 0.81]	37	*
	1 year	0.03 [0; 0.16]	0.13 [0; 0.96]	40	NS
Defensive reactions (No)	16 days	0 [0; 1.5]	5 [1; 6]	20.5	***
	3 months	1 [0; 3]	3 [0.5; 4]	53.5	NS
	6 months	1 [1; 2.5]	1 [0; 4]	64	NS
	1 year	1.5 [0; 4]	2 [0; 4]	34.5	NS
Flight distance measured during "plastic bag test" (m)	16 days	0.2 [0; 1]	1.5 [1; 2]	32.5	**
	3 months	0.7 [0.1; 1]	1 [0.2; 1]	79.5	NS
	6 months	0.5 [0; 1.25]	0.5 [0; 1.25]	81	NS
	1 year	0.5 [0; 1]	1 [0; 2.5]	38	NS

U statistics and *P* value are provided.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
[a] Walk ratio = "time during which foal walks under constraint"/total time.

3.2. Surprise tests

3.2.1. Stimulus known by handled foals

Two days after the handling period, the flight distance measured during the plastic bag shaking was significantly lower for animals in the handled group than for animals in the control group ($P < 0.01$). There was no difference during subsequent tests (Table 3).

3.2.2. Stimulus unknown by handled foals

The flight distances measured during the unknown surprise tests did not differ between groups for any test period or stimulus.

3.3. Mare behaviour

The scores for agitation in handled-foal mares and control-foal mares did not differ significantly at any point.

3.4. Open-field tests

Loadings of each variable on the factors of the principal component analyses are shown in Table 4.

3.4.1. Isolation test

During the ''isolation test'' performed at 6 and 12 months of age, the first factor of the principal component analysis explained 43% and 42% of the total variance, respectively. This factor could be interpreted as agitation induced by social isolation at 6 months of age or as an attempt to interact with conspecifics at 12 months of age (Table 4). The second factor explained 24% of total variance at 6 months of age and 27% at 12 months of age.

Table 4
PCA analysis, percent of total variability explained by component 1 and selection of behavioural parameters according to loading

Age	Test	Contribution of parameters on factor 1[a]	Contribution of parameters on factor 2[a]
6 months	Isolation	SE, 0.22; IT, 0.22; NN, 0.16; LN, 0.17; DF, 0.15	T123, 0.18; T789, 0.44
	Human	LS, 0.23; T789, 0.20; SN, 0.23; DS, 0.19	SE, 0.26; IT, 0.23; NN, 0.17; LN, 0.11
	Object	T123, 0.16; T789, 0.2; SN, 0.11; DS, 0.11	SE, 0.13; IT, 0.11; NN, 0.31; LN, 0.22
1 year	Isolation	T123, 0.21; T789, 0.2; NN, 0.24; LN, 0.17	SE, 0.42; IT, 0.32
	Human	LS, 0.16; T123, 0.17; T789, 0.21; SN, 0.16; DS, 0.11	SE, 0.12; IT, 0.15; NN, 0.17; LN, 0.27
	Object	LS, 0.11; T123, 0.1; T789, 0.17; NN, 0.1; GL, 0.13; SN, 0.17	SE, 0.34; IT, 0.29; LN, 0.15

[a] Contribution of parameters which are higher than 1/7 for isolation test and than 1/11 for others.

This could be interpreted as spatial position in the test arena at 6 months of age and agitation at 12 months of age (Table 4). Handled and control foals did not differ significantly on factor 1 or factor 2 at either 6 or 12 months of age.

3.4.2. Human test

During the ''human test'' performed at 6 and 12 months of age, the first factor explained 34% and 35% of total variance, respectively. This could be interpreted as motivation to interact with humans (Table 4). The second factor explained 23% and 26% of total variance, respectively, and could be interpreted as agitation (Table 4). The two groups did not differ significantly for either of these factors at any time of testing.

3.4.3. Novel object test

During the ''novel object test'' performed at 6 and 12 months of age, the first component explained 37% and 43% of total variance, respectively. This could be interpreted as motivation to interact with the object (Table 4). The second factor explained 22% and 19% of total variance, respectively, and could be interpreted as agitation (Table 4). The two groups did not differ significantly on these two factors for any period considered.

3.5. Learning task

Whatever the learning task (discriminative or spatial learning), the number of days required to reach the criteria did not differ between groups (*discriminative learning*: Handled 10[7;10]; Controls 11[8;12]/*spatial learning*: Handled 4[4;8]; Controls 4[4;9]).

3.6. ''Capture test'' before and after the learning tests

The time required to catch the foals was significantly lower after the learning test period than before (before 25.5[18;47.5]; after 14[10.5;26.5]; $Z = 2.09$; $P < 0.05$). Nevertheless, the two groups did not differ significantly, either before the learning period or after (*before*: Handled 24[22;43]; Controls 28[17;52]; *after*: Handled 15[11;30]; Controls 13[10;26]).

4. Discussion

The aims of this study were to examine the short- and long-term effects of neonatal handling (from 0 to 14 days of age) on manageability, general reactivity and learning ability, and to determine whether early handling can improve responses to subsequent handling.

Two days after the handling period, the four behavioural parameters measured during the handling test showed significant differences between animals of the handled group and controls. We can therefore conclude that the handling procedure used in this experiment was effective with respect to foal manageability, at least on a short-term basis. Three months after the handling period, handled foals were still somewhat easier to handle than controls since the time required to fit a halter and the walk ratio were significantly lower in

handled foals than in controls. Nevertheless, there was only one significant difference 6 months later (walk ratio) and there was no longer a difference between groups 12 months later. Our results thus indicate only temporary effects of neonatal handling on manageability. Hemsworth and Barnett (1992) in pigs, Boissy and Bouissou (1988) and Boivin et al. (1992) in cattle and Jones and Faure (1981) in hens also reported a decrease in the effects of handling with time.

Four studies have examined the effects of neonatal handling on manageability in horses. As early as 1 month after the end of the handling period, Williams et al. (2002) found no effects of neonatal handling on later manageability. Mal et al. (1994) tested their animals only 4 months later and found no effects either. In contrast, the results of the present study demonstrated clear differences soon after the handling period, which were still partially present 3 months later. These differences in results could be due to a difference in the amount of handling received by the foals. Williams et al. (2002) and Mal et al. (1994) handled foals for a shorter period than in our experiment (for only 2 and 7 days, respectively). On the other hand, our results are in accordance with those of Mal and McCall (1996) and Simpson (2002) who observed that foals handled during the neonatal period were more tractable during tests performed at 3 and 4 months of age, respectively. In addition, our study demonstrated that the effects of neonatal handling decreased rapidly between 3 months and 1 year of age.

Although the effects disappeared with time, we hypothesised that neonatal handling influence might lead animals handled as foals to respond more quickly to subsequent handling than animals that are not handled as foals. However, we found that human contact provided for 1 month during the learning task significantly improved foal manageability, but no more in handled foals than in controls.

We also studied the effects of neonatal handling on other behavioural aspects such as general reactivity. In the surprise test, in which the stimulus was known by handled foals (shaking the plastic bag), handled foals were less reactive than control foals just after the handling period, but not later. The ‘‘unknown surprise effects’’ and the three open-field test situations did not reveal any significant differences between groups. Therefore, early handling did not seem to affect general fearfulness: there was no generalisation from known stimuli to unknown stimuli. These results are consistent with those obtained in pigs by Hemsworth et al. (1986) during isolation tests, by Jones and Waddington (1993) in domestic hens during tonic immobility tests, by Heird et al. (1981) in horses using an emotionality score and by Mal et al. (1994) also in horses during a novel stimulus test. In contrast, other studies have found that handling reduces general emotionality and fearfulness, and not merely fear of humans. For instance, Fox and Stelzner (1966) reported that early handling reduces reactivity in isolation or with novel objects in dogs; Pedersen and Jeppesen (1990) and Pedersen (1994) observed that activity and exploration behaviour increased in red foxes handled from 2 to 8 weeks of age, as did Kersten et al. (1989) in rabbits handled from 10 to 20 days of age. Lansade et al. (2004) showed that handling horses during the 2 weeks just after weaning reduces reactivity in various situations such as isolation from conspecifics, presence of a human or a novel object.

Finally, we examined the effects of neonatal handling on later learning ability. Our results showed no long-term effects of neonatal handling on learning ability, either for the discrimination learning test or for the spatial learning test. In contrast, Heird et al. (1986)

found that foals handled for a few weeks (1–3 weeks) just after weaning displayed more correct responses in a simple place learning T-maze performed at 2 years of age than non-handled foals. These results suggest that handling just after weaning may more effectively improve learning ability than handling just after birth. However, in our experiment the learning tasks were performed only 14 months after the handling period, since they required daily human contact which could have significantly improved foal manageability and thus be a bias in the study of the long-term effects of neonatal handling. Therefore, we cannot exclude the possibility that neonatal handling has a short-term effect on learning ability.

To conclude, in contrast to Miller's hypothesis (1991), the results of the present study cannot be explained by an imprinting-like process which must be ''extremely stable and essentially irreversible'' (Hess, 1973; Creel and Albright, 1987). Nevertheless, the existence of a critical period in horse development (involved in the imprinting process) cannot be totally rejected. In the present study handling began between 5 and 7 h after birth, and may in fact have occurred after the end of the hypothetical critical period. It is accepted that in species with rapid development primary socialization of young (to the species) takes place through the imprinting phenomenon within a few hours following birth. Nevertheless, Williams et al. (2002) began to handle foals 2 h after birth and concluded that such early handling was ineffective. The hypothesis of the existence of an imprinting-like process in horses could thus probably be rejected.

From a practical point of view, it could be advantageous to begin handling at birth since it is easier, probably because very young foals are weaker, have fewer defensive reactions and may be less fearful of humans than older foals. However, since the effects of handling are only temporary, the handling procedure needs to be repeated regularly until the horse is broken in.

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