Fearfulness in horses: A temperament trait stable across time and situations

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Lea Lansade
French Horse and Riding Institute

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Fearfulness in horses: A temperament trait stable across time and situations

Léa Lansade [a,*], Marie-France Bouissou [a], Hans W. Erhard [b]

[a] Laboratoire de Comportement, Neurobiologie et Adaptation, UMR 6175 Physiologie de la Reproduction et des Comportements, INRA-CNRS-Université de Tours-Haras Nationaux, F-37380 Nouzilly, France
[b] Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, Scotland, United Kingdom

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Abstract

The purpose of this study was to test the existence of a ‘‘fearfulness’’ trait in horses, by testing the stability across situations and over time of the responses to different fear-eliciting situations. It was also to identify which behavioural parameters are the best indicators of this trait. Sixty-six Welsh ponies and 44 Anglo-Arab horses were successively tested at 8 months and 1.5 years of age. Of these, 33 Welsh ponies and 21 Anglo-Arabs were also tested at 2.5 years of age. At each age, they were subjected to four test situations. The first test involved the introduction of a novel object in the test pen (novel object test). In the second test, a novel area was placed in the pen between the horse and a bucket of food, to determine the time the horse took to cross the area (novel area test). Finally, the third test consisted in suddenly opening an umbrella in front of the horse while it was eating (surprise test). During these tests, many behavioural parameters were recorded. A fourth test consisted of a surprise test during which the horse was held by a handler while its heart rate was measured. Spearman correlations were used to identify links between behavioural parameters measured during different tests and between different ages.

Reactions to the first three tests showed consistency between them and over time. Among all the behavioural parameters measured during these tests, some presented high stability over time and were well correlated with behaviours expressed during other tests, indicating they are the best indicators of a fearfulness trait: the frequency of licking/nibbling the novel object, the time to put one foot on the novel area and to eat from a bucket placed just behind it, and the flight distance and the time to eat under the opened umbrella. The stability across responses expressed in various fear-eliciting events and over time from 8 months to 2.5 years of age suggests the existence of a ‘fearfulness’ trait in horses.

The different indexes of heart rate measured or calculated during the surprise effect present limited stability over time and almost no correlation with the behavioural parameters measured during the other

* Corresponding author. Tel.: +33 2 4742 7279; fax: +33 2 4742 7743.
E-mail address: lansade@tours.inra.fr (L. Lansade).

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three tests. We conclude that, in contrast to the previously mentioned behaviours, these are not reliable measures of a temperament trait.

From a practical point of view, this study shows that it is possible to identify a horse's level of fearfulness as early as 8 months of age using the first three tests.

Keywords: Horse; Temperament; Fearfulness; Novelty; Suddenness; Behavioural tests

1. Introduction

The behavioural reactions expressed by individuals confronted with particular situations are strongly affected by their temperament. Temperament is generally considered as a set of behavioural tendencies, present early in life and relatively stable across various kinds of situations and over the course of time (Bates, 1987; Goldsmith et al., 1987). This stability means that an individual's behaviour in specific situations is predictable to a certain extent. Fearfulness is considered as one of the basic traits of an individual's temperament. While fear is an emotional state induced by the perception of actual danger (for a review, see Forkman et al., 2007), fearfulness can be defined as a predisposition to react in a similar manner to various fear-provoking events. Fearfulness can be inferred from the behavioural states expressed by the individual in potentially frightening situations. It has been frequently studied in farm animals as it influences many other behaviours, such as social interactions, maternal behaviour or learning ability, as well as productivity and welfare (for reviews, see Bolles and Fanselow, 1980; Gray, 1987; von Borell, 1995; Jones, 1996; Rosen and Schulkin, 1998; Boissy, 1998; McMillan, 1999; Maestripieri, 1999; Erhard and Schouten, 2001).

In horses, temperament can strongly determine the animal's usability. Buckley et al. (2004) conducted interviews with Pony Club members and concluded that a horse's temperament (quietness, reliability, safety) is the most important characteristic of a Pony Club horse, before its size, soundness, purchase price, etc. Excessive fear reactions limit the use of horses in many situations, and can even, in certain cases, render the horse dangerous. On the other hand, an excessively low level of fearfulness may be inconvenient in some equestrian disciplines, even if this aspect has so far attracted little attention. Consequently, the development of tests to assess a horse's temperament early in life, and particularly a possible fearfulness trait, would be particularly useful. For this purpose, it is necessary to explore the stability of the individual's reactions over time and in different frightening situations.

Stability of fear reactions over time has been reported in many species (for a review: Forkman et al., 2007). In post-weanling horses, Visser et al. (2001) reported a short-term consistency (1 month) of behaviour during a novel object test conducted at 9 and 20 months of age, but found that only a few behavioural parameters showed consistency between 9 and 20 months of age. Therefore, to our knowledge, the long-term consistency of fear reactions has never been convincingly demonstrated in the horse.

The search for stability of fear reactions across situations has also been reported (for a review: Forkman et al., 2007). This requires the observation of a number of individuals in various potentially frightening situations. In horses, Wolff et al. (1997) found correlations between two neophobia tests: an unknown object test and a bridge test, suggesting the existence of a trait of fearfulness. Using open-field tests, some authors have described correlations between reactions to novelty, to suddenness and to social isolation, and concluded the existence of a general trait of 'fearfulness' (e.g. sheep: Romeyer and Bouissou, 1992; Vandenheede et al., 1998; Viérin and Bouissou, 2003; horses: Viérin et al., 1998). However, in these cases a bias exists, since the correlations were established between situations involving different stimuli, but all involving some common fear-inducing element, such as social separation or novelty of the pen. The correlations may thus have been due to these common elements. In the present study, we chose to test the animals in an environment which does not provoke any particular fear reactions apart from those to the tested stimulus: horses were tested in a familiar environment, in the presence of conspecifics, and after a period of habituation to the test procedure. In this way, there were theoretically no common elements (such as social separation or novelty of the test pen) between the different tests.

Fear states can be expressed through behavioural responses, but also through physiological changes, and many studies have used heart rate measurements to characterise fearfulness (for a review: von Borell et al., 2007). In horses, Visser et al. (2002) showed that heart rate variables measured during a novel object test and a handling test may quantify certain aspects of a horse's temperament, such as fearfulness, since these measures were consistent between 9 months and 21 months of age. It would be interesting to examine whether there is a relationship between behavioural states and heart rate observed during fearful situations. Measuring the heart rate to predict a horse's level of fearfulness may be useful in practice because it is non-invasive and is easy to use by a person inexperienced in behavioural observations.

The purpose of this study was to determine whether it is possible to infer a trait of fearfulness from the measurement of behavioural and physiological responses (heart rate) expressed by individuals confronted with specific frightening situations. For this purpose, we tested the two characteristics of a trait: stability over time and across situations. We tested the animals in two situations involving novelty and in two situations involving suddenness, and we repeated these situations every year, from 8 months to 2.5 years of age. Many behavioural and physiological parameters were recorded during these situations. The first step was to test the stability over time of these parameters by calculating correlations between parameters measured from 8 months to 2.5 years of age. The second step was to determine the stability across situations of the parameters: for each age, we calculated the correlations between parameters measured in the four different fear-inducing tests. These two steps also allowed us to identify the most reliable indicators of this trait, i.e. the behavioural or physiological parameters which show the best stability over time and across situations.

This paper is part of a more general study which attempts to assess several possible temperament traits in horses such as gregariousness (Lansade et al., 2008), reactivity to humans (Lansade and Bouissou, in press) or activity level (Lansade, 2005; Lansade et al., 2006).

2. Animals, material and methods

2.1. Animals

One hundred and ten horses were used, divided into four groups. They originally comprised 22 Anglo-Arab horses (AA01) and 33 Welsh ponies (W01) born in 2001, and a further 22 Anglo-Arab horses (AA02) and 33 Welsh ponies (W02) born in 2002. Of those, five horses had to be excluded from the study due to illness ($N = 2$) or following a bone fracture ($N = 3$). The number of horses tested at each age is presented in Table 1. The animals of the two breeds were born and lived in two different places.

All animals were maintained on pasture with their dam until 6 ± 1 months of age when they were weaned. Males were castrated around 12 months of age. The animals were housed indoors from 6 to 11

Table 1
Age and number of horses in the two different breeds (AA: Anglo-Arab; W: Welsh Pony) and two different birth years (01: 2001; 02: 2002) at the three test periods

	2002	2003	2004
AA01	8 months 22 (22)	1.5 years 21 (20)	2.5 years 21 (20)
AA02		8 months 22 (21)	1.5 years 19 (19)
W01	8 months 33 (26)	1.5 years 33 (33)	2.5 years 33 (21)
W02		8 months 33 (32)	1.5 years 32 (22)

The numbers in brackets represent the number of horses that had their heart rate measured in the surprise test.

months, 20 to 23 months, and 32 to 35 months, corresponding both to the winter period and the test periods. Outside these periods, they were kept on pasture.

The Anglo-Arab horses were housed individually on sawdust bedding, in a 6 m × 3 m box adjacent to an individual 3 m × 3 m outside area (Fig. 1). Boxes were separated from each other by metal hurdles so that the horses could see all the other animals in the barn and interact with their neighbours (sniffing, licking, biting or grooming, etc.). The outside area was separated from the inside area of the box by a door. When the horses were in the outside area and the door was closed, they could not see inside.

The Welsh ponies were housed together in a straw-bedded, 15 m × 15 m group pen and were moved to an outdoor paddock every day for 4 h.

All the test animals were fed twice a day with commercial pellets and hay, except for the test periods involving feeding motivation during which they were fed three times a day, so that all the animals had a similar level of feeding motivation whenever they were tested. Water was available ad libitum.

Outside the test periods, the animals were maintained on pasture. They received similar, limited human contact necessary for routine husbandry: feeding when indoors, changes of pasture and any emergency veterinary care.

2.2. Experimental procedure

In order to examine only the reactions specific to the stimulus studied, we attempted to test animals in a context as neutral as possible: tests were performed in a familiar place and animals were habituated to the experimental procedure for a minimum of 3 days immediately before the tests.

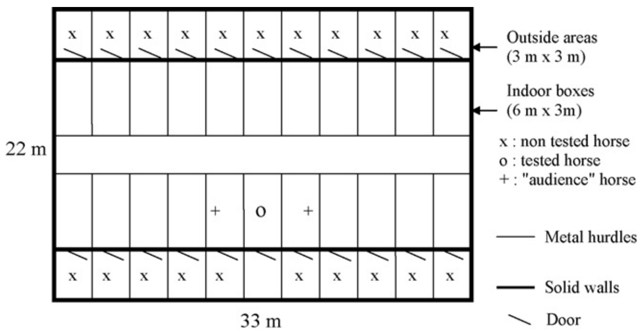

Fig. 1. Layout of the building where the Anglo-Arab horses were housed and tested.

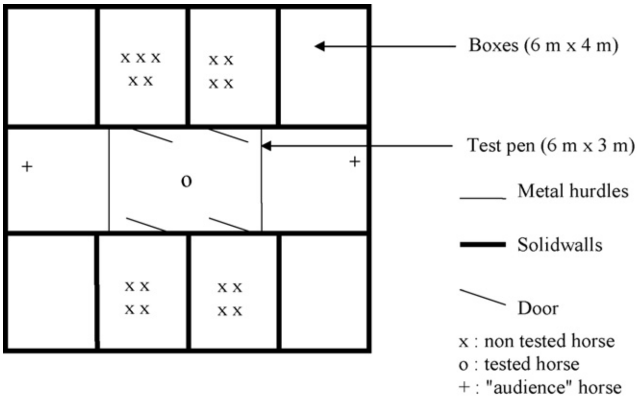

Fig. 2. Layout of the building where the Welsh ponies were housed and tested.

Anglo-Arab horses were tested in their own boxes while non-test horses were kept in their outside areas (Fig. 1). Welsh ponies were tested in a pen constructed in the corridor of a barn close to the pen where they lived (Fig. 2). In both cases, the test pens were 6 m long and 3 m wide. During the test period, the Welsh ponies were randomly moved, in groups of four or five, to four boxes (4 m $\times$ 6 m) adjacent to the test pen, for 4 h per day. In both breeds, when a horse was tested, it was moved by a handler from the outside part of its box (Anglo-Arab) or from its box (Welsh) and placed in the test pen, and the test began immediately. The other horses and the tested horse could not see each other.

Horses were randomly assigned to a testing order for each test. During the habituation phase and the different tests, the experimenters were hidden behind a one-way mirror. To avoid the test horse being alone in the pen during the habituation phase and the tests, two ‘‘audience’’ horses were attached at each side of the test pen, at a distance of about 3 m (Figs. 1 and 2). These were non-experimental animals and the same audience horses were always used for each group. They were unfamiliar to the test horses at the beginning of the study. They were chosen because they were known to be particularly calm and likely to stand still throughout the test period. They were habituated to the test situations before the experiment. During the tests, we also checked that they did not show any special reaction towards the fearful stimuli in the test pen.

2.2.1. Habituation to the test pen

This phase consisted of habituating the horse to being led into the test pen and to staying in it without any particular reaction. The test horses were placed in this situation daily for 5 min until they no longer manifested the following behaviours for three consecutives days: neighing, defecating, trotting or galloping. A single occurrence of one of these four behaviours during the three consecutives days was tolerated. For example, if a horse neighed only once during three consecutive days, but never defecated, trotted or galloped, we considered it habituated. The number of days required for habituation was between 3 and 6 days.

2.2.2. Test situations

Animals were subjected to four test situations. Animals born in 2001 (AA01 and W01) were tested at 8 months, 1.5 and 2.5 years of age, while those born in 2002 (AA02 and W02) were only tested at the ages of 8 months and 1.5 years (Table 1).

These tests consisted of potentially fear-eliciting situations, involving either a novel or a sudden stimulus. Suddenness and novelty are considered to be the most stressful stimuli (see Forkman et al., 2007). Moreover, horses are frequently confronted with them when used for riding. All the tests took place in the test pens described above. The tests will be presented in the same order they were carried out. Habituation to the test pen began on day 1, followed by the novel object test, the novel area test, the surprise test-horse free in the pen, and the surprise test-horse held, were carried out on days 12, 39, 40 and 41–45, respectively.

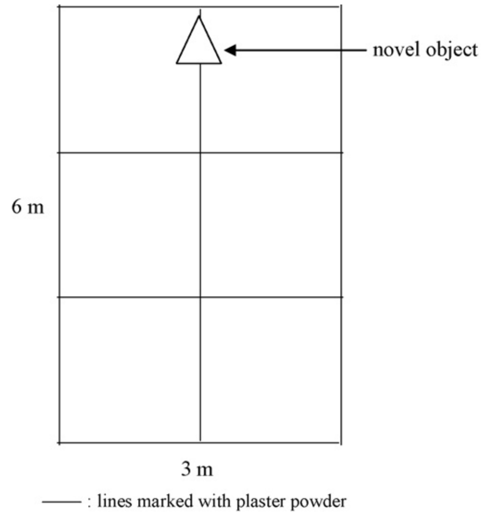

Fig. 3. Test-layout of the novel object test.

2.2.2.1. Novel stimuli

2.2.2.1.1. Novel object test. An object, unknown to the horses, was placed across the width of the pen before the animal entered it (Fig. 3). A different novel object was used for each step of the study. At 8 months, 1.5 years and 2.5 years of age, they were respectively: three green plastic bags (1 m tall) filled with straw, six white plastic bags (0.20 m long) suspended from a black plastic wire normally used for electric fences (0.60 m in diameter, 0.80 m above the ground), and 10 plastic folders of different colors (blue, green, red, purple) suspended by string from the same wire. To evaluate the area covered by the horse, the pen was divided in six sectors of equal size traced with plaster powder (Fig. 3). Horses were habituated to these lines during the habituation period, and they did not show any particular reaction to them during the test. The location of the horse was noted continuously enabling us to measure the ‘‘number of sectors entered’’ by the animal, which was an indicator of locomotion during the test. The test lasted 5 min, during which 17 other behavioural parameters were recorded (Table 2).

2.2.2.1.2. Novel area test. Three zones were marked on the floor of the test pen using plaster powder (Fig. 4). The first zone (1.50 m $\times$ 3 m) corresponded to a ‘‘starting zone’’, the second to a ‘‘central zone’’ (3 m $\times$ 3 m), while the third (1.50 m $\times$ 3 m) corresponded to an ‘‘arrival zone’’ and contained a bucket full of food (commercial pelleted food, part of the horses’ usual diet).

Habituation. This phase aimed at habituating the test animal to going to the arrival zone containing the food. An experimenter entered the pen with the test animal, led it (without being haltered) to the starting zone and then released it. The handler then immediately entered an adjacent box, out of the horse’s sight. The time recording started when the horse’s two forelegs crossed the line delimiting the starting zone. The animal had 40 s to cross the line into the arrival zone containing the food and to eat. If it did not succeed, the experimenter led it again to the starting zone (the horse was not fed its normal ration in this case). It was offered eight trials per day. The criterion for this habituation was that the horse performed seven correct trials per day on three consecutive days.

Test. The test was carried out on the day after the horse reached the criterion level. A novel area (3 m $\times$ 2 m) was placed in the middle of the central zone (Fig. 4), differing at each test stage. At 8 months, 1.5 and 2.5 years of age, it consisted respectively of: a white sheet of jute canvas, a plastic cover, and a wooden plank. The procedure was the same as the one used previously: the animal was led to the starting zone, released, and the time recording started when its two forelegs crossed the starting line. However, in this phase, the animal had 5 min to cross the arrival line and to eat, with only one trial. The test ended when the horse ate from the bucket, or after 5 min if it did not eat. Nineteen behavioural parameters were measured during this phase (Table 2).

Table 2
Parameters and definitions of behaviour recorded during the tests

Parameters and definitions when necessary	Novel object	Novel area	Surprise test, foal free
Glances at the stimulus (l, f) the horse stands still, with elevated neck, head and ears oriented in direction of the stimulus	X	X	X
Sniffing the stimulus (l, f)	X	X	X[a]
Licking/nibbling the stimulus (l, f)	X	X	X[a]
Contact with the stimulus (d)	X		
Time to put one foot on the novel area		X	
Time spent near the stimulus		X	
Time to eat		X	X
Flight distance			X
Startle responses (f)	X[a]	X[a]	
Neighing (l, f)	X[a]	X[a]	X[a]
Defecation (l, f)	X[a]	X[a]	X[a]
Trotting (f)	X[a]	X[a]	X[a]
Scrapping the floor with the foot (f)	X[a]	X[a]	X[a]
Vigilant position (f) the horse stands still, with elevated neck, head and ears oriented anywhere except in direction of the stimulus	X	X	X
Sniffing the floor (f)	X	X	X
Blowing (f) forceful expulsion of air through the nostrils preceded by a raspy inhalation sound	X	X	X
Sectors entered (n)	X		

l: latency, f: frequency, d: duration, n: number. 'X' indicates if the parameter was measured during the test or not.
[a] Parameters expressed by less than 15% of animals.

2.2.2.2. Sudden stimuli

The sudden stimuli consisted of the opening of a familiar black umbrella. Identical umbrellas were placed in front of each box for 1 month before the tests in order to familiarize the animals with them.

2.2.2.2.1. Surprise test, horse free in the pen. Before the animal entered the pen, the same bucket of food which was used in the ''novel area'' test was placed in the box, 1 m from the hurdles (Fig. 5). A closed umbrella was placed between two bars of the hurdle, 1 m above the bucket. The animal was released in the pen and when it had eaten for three consecutive seconds from the bucket, the umbrella was automatically opened and the time started. The test ended when the horse went back to the bucket and ate again for three consecutives seconds. The maximum time allowed was 5 min; after that time, the test ended. Seventeen

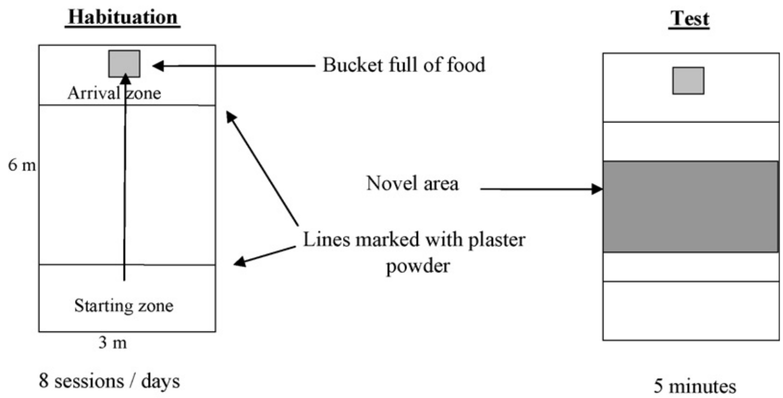

Fig. 4. Test-layout of the novel area test.

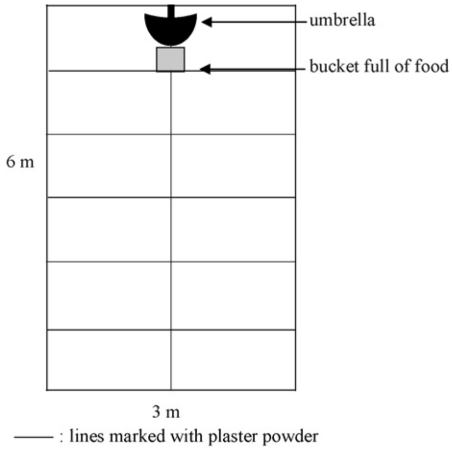

Fig. 5. Test-layout of the surprise test.

behavioural parameters were noted during the test (Table 2). The test was filmed, and the flight distance was measured on the video using lines marked every meter on the floor of the test pen (Fig. 5).

2.2.2.2.2. Surprise test, horse held, and heart rate measurement. During this test, the horse was held to limit the influence of locomotor activity on the heart rate.

When the animal was brought into the pen, the handler tried to fit it with a halter and fit it with a heart rate monitoring system maintained with a surcingle (Polar Accurex Plus) set to record heart rate every 5 s. The experimenter led the horse to the middle of the pen and tried to hold it still throughout the test. An assistant holding a closed umbrella behind his back stood 1.5 m from the horse. The recording began, and 5 min later, the assistant suddenly opened the umbrella twice, 1 m in front of the horse's head, then closed it and hid it again. The total duration of this test was 8 min. This test could not be performed on all the animals because of the difficulty of fitting certain animals with the equipment due to their violent defensive reactions. Therefore, the experimenter was given a maximum of 30 min to catch the horse and fit the equipment. If he did not succeed in this time, because the horse's reactions were too dangerous, the test was abandoned. The number of horses subjected to the entire test is presented in Table 1.

As animals presented various reactions when they were fitted with the equipment (some stayed relatively calm whereas others had strong defensive reactions), there was a wide variation in their heart rates measured before the surprise effect. To limit this effect, we analysed the change in heart rate before and after the opening of the umbrella. To do so, we measured the minimum and maximum heart rate and calculated the average during the 5 min before the opening of the umbrella. After that, several indexes were calculated: the difference between the maximum heart rate and the average, the difference between the maximum and the minimum, the percentage of the maximum as a function of the minimum or the average.

2.3. Statistical analyses

The statistical analyses used in this study are strictly identical to those used by Lansade et al. (2008) and Lansade and Bouissou (in press). The same protocol was repeated twice in two groups (AA02 and W02) and three times in the others (AA01 and W01). We did not combine the data from these different time points and groups (for justification, see Lansade et al., 2008; Lansade and Bouissou, in press).

Some behavioural parameters were expressed by less than 15% of animals and were not taken into account in the statistical analysis; they are presented in Table 2. Two different analyses were performed:

the first concerned the links between parameters measured at different ages (stability over time) and the second was aimed at quantifying the relationship between parameters of the different tests at the same point in time (stability across situations: inter-test correlations). To study the relationship between parameters, Spearman (rank) correlations were calculated, since they are more robust to data non-normality than the Pearson correlations. A correlation was considered to be statistically significant when its p-value was less than 0.05.

The relationship between variables (stability across situations) was calculated for 10 separate sets of data (Groups AA01 and W01 were tested three times, and groups AA02 and W02 were tested twice, resulting in 10 sets). The number of significant positive or negative correlations out of 10 is presented in matrix form showing only the variables which are correlated with one or more variable from the other tests. On the other hand, investigating stability over time involves fewer possible correlations, and the significant correlations ($p < 0.05$) are therefore presented in a table with the Spearman correlation coefficient (R-value). To investigate stability over time, eight correlation possibilities were calculated (8 months vs. 1.5 years for all four groups, 8 months vs. 2.5 years and 1.5 years vs. 2.5 years for groups W01 and AA01). No parameter was established to accept or reject the hypothesis of stability, on the assumption that stability can be measured on a gradient, with some variables never correlated over time or with each other, and some which are correlated in 100% of cases.

3. Results

3.1. Stability over time: search for correlations between parameters measured at different ages

The R-values of these correlations are presented in Table 3.

3.1.1. Novel object test

The parameters which were the most often correlated over time are the frequencies of licking/nibbling (in 8 units out of 8), the duration of contact and the frequency of glances (in 7 units out of 8), the latency of licking/nibbling and the frequency and latency of sniffing (in 6 units out of 8), and the frequency of blowing (in 4 units out of 8). Three other parameters were sometimes correlated between ages, but less frequently (Table 3).

3.1.2. Novel area test

The parameters which were the most often correlated over time were the time to put one foot on the novel area and the time to eat (in 8 units out of 8), followed by the frequency of glances (in 7 units), the frequency of sniffing the floor (in 6 units), the frequency of vigilant position (in 5 units). Five other parameters present few correlations over time and are presented in Table 3.

3.1.3. Surprise test, horse free

The parameters which were the most often correlated over time were the flight distance and the time to eat (in 7 units out of 8), followed by the frequency of glances (in 6 units). The other three parameters presented in the table were correlated less frequently.

3.1.4. Surprise test, horse held

The parameters which were the most often correlated over time were the ‘‘maximum heart rate’’, the ‘‘maximum–average heart rate’’ and the ‘‘maximum–minimum heart rate’’, but only in 4 units out of 8. The other two parameters were only correlated in 3 units.

Table 3
Stability over time, search for correlations between parameters measured at different ages

	Number of significant correlations out of 8	W01			W02 (8 months to 1.5 years)	AA01			AA02 (8 months to 1.5 years)
		8 months to 1.5 years	1.5 years to 2.5 years	8 months to 2.5 years		8 months to 1.5 years	1.5 years to 2.5 years	8 months to 2.5 years	
Novel object test									
Licking/nibbling (f)	8	0.76	0.59	0.60	0.51	0.47	0.60	0.58	0.41
Contact (d)	7	0.75	0.60	0.71	0.51	0.40	0.59	0.41	
Glances (f)	7	0.56	0.56	0.61	0.38	0.46	0.70	0.61	
Licking/nibbling (l)	6	0.61	0.67	0.48		0.43	0.45	0.41	
Sniffing (l)	6	0.65	0.58	0.61	0.49	0.51	0.67		
Sniffing (f)	6	0.68	0.61	0.66		0.53	0.61	0.44	
Blowing (f)	4	0.54	0.58	0.44	0.35				
Sectors entered (n)	2			0.37				0.44	
Glances (l)	1							0.46	
Vigilant position (f)	1				0.36				
Novel area test									
Time to put one foot	8	0.71	0.69	0.75	0.50	0.61	0.66	0.64	0.57
Time to eat	8	0.79	0.60	0.67	0.55	0.55	0.46	0.51	0.65
Glances (f)	7	0.54	0.66	0.69		0.53	0.65	0.59	0.52
Sniffing the floor (f)	6	0.69	0.80	0.67	0.58	0.55			0.60
Vigilant position (f)	5	0.70	0.66	0.55	0.54	0.53			
Licking/nibbling (f)	3		0.38			0.48			0.56
Sniffing (l)	3		0.68	0.66					0.42
Sniffing (f)	2		0.51		0.38				
Time near the area	2		0.71	0.35					
Licking/nibbling (l)	1								0.52
Surprise test, horse free									
Flight distance	7	0.35	0.61		0.56	0.62	0.79	0.58	0.37
Time to eat	7	0.36	0.69		0.48	0.74	0.65	0.62	0.52
Glances (f)	6		0.61		0.58	0.58	0.68	0.62	0.50
Sniffing the floor (f)	3		0.57		0.37		0.50		

Table 3 (*Continued*)

	Number of significant correlations out of 8	W01			W02 (8 months to 1.5 years)	AA01			AA02 (8 months to 1.5 years)
		8 months to 1.5 years	1.5 years to 2.5 years	8 months to 2.5 years		8 months to 1.5 years	1.5 years to 2.5 years	8 months to 2.5 years	
Vigilant position (f)	2			0.36	0.41				
Blowing (f)	2				0.67				0.49
Surprise test, horse tethered									
Maximum HR	4	0.41		0.45		0.53	0.80		
Maximum HR–average HR	4	0.40		0.60		0.44	0.62		
Maximum HR–minimum HR	4	0.40		0.49		0.44	0.70		
Maximum HR × 100/minimum HR	3	0.44		0.56			0.50		
Maximum HR × 100/average HR	3	0.47		0.49	0.46				

This table presents the parameters which are correlated at less one time between two different ages. For each test, they are classified from the more frequently correlated to the less frequently correlated. The numbers presented in the table correspond to the spearman correlation coefficient (*R*-value). This number is presented only when the correlation was significant ($p < 0.05$). l: latency, f: frequency, d: duration, n: number, HR = heart rate.

3.2. Stability across situations: search for correlations between parameters measured during different tests (inter-test correlations)

3.2.1. Novel object test and novel area test

The more rapidly and frequently the horses licked/nibbled and sniffed the object, the more rapidly they put one foot on the area and ate, and to a certain extent the less they glanced at the area. The number of units in which these correlations appear are presented in Table 4 (see also Fig. 6 for illustrations of some of these correlations). Other parameters were correlated between these two tests, but less frequently.

Table 4
Stability across situations, search for correlations between parameters measured during the novel object test and the novel area test

number of significant correlations between ages → ↓		**Novel object test**				
		8/8	*6/8*	*6/8*	*6/8*	*4/8*
Novel area test		Frequency of licking / nibbling the object	Latency of licking / nibbling the object	Latency of sniffing the object	Frequency of sniffing the object	Frequency of blowing
8/8	Time to put one foot on the area	**6 (-)***	**7 (+)**	**7 (+)**	**5 (-)**	
8/8	Time to eat	**5 (-)**	**5 (+)**	**5 (+)**	**3 (-)**	
7/8	Frequency of glances at the area	**5 (-)**	**5 (+)**	**5 (+)**	**2 (-)**	
6/8	Frequency of sniffing the floor	**2 (-)**				**6 (+)**
5/8	Frequency of vigilant positions	**4 (-)**	**5 (+)**	**6 (+)**		**3 (+)**
3/8	Latency of sniffing the area	**5 (-)**	**2 (+)**	**5 (+)**	**3 (-)**	
0/8	Frequency of blowing					**7 (+)**

The numbers indicated in the table correspond to the number of significant correlations out of the 10 possibilities of correlations calculated between two parameters. When this number is equal to 0, the box is empty. The direction of the relationship is also indicated (+: positive correlation; −: negative correlation). Only the parameters which are correlated with one or more parameter of the other test are presented. The parameters are presented from the more stable over time to the less stable. Note the shades of grey: the darker the box, the more stable the parameter over time and the more frequently it is correlated with parameters of the other test.
*As an example of the results, some graphs are presented in Fig. 6 to illustrate some of these correlations.

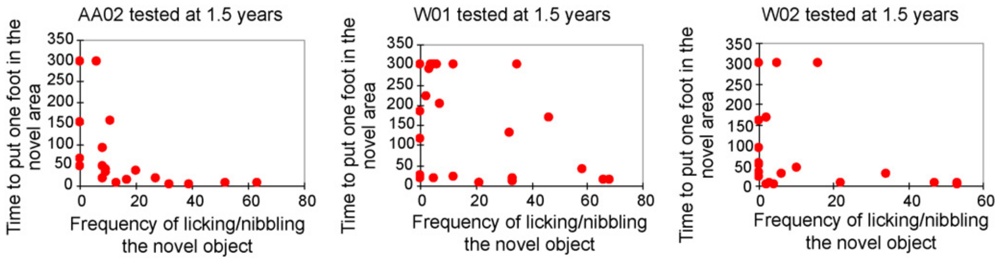

Fig. 6. Example of graphs presenting the relationships between parameters: relationships between the time to put one foot in the novel area and the frequency of licking/nibbling the novel object in group AA02, W01 and W02 tested at 1.5 years of age. The relationships were tested with Spearman's correlations with the following results: $R = -0.79$, $p < 0.0001$; $R = -0.51$, $p < 0.001$; $R = -0.58$, $p < 0.0001$, respectively. Of the four groups tested at 1.5 years of age, only group AA01 did not present significant correlation between these two parameters.

3.2.2. Novel object test and surprise test, horse free

The more rapidly and frequently the horses licked/nibbled and sniffed the object and the longer they spent in contact with it, the shorter the flight distance, and the faster they returned to eat after the surprise effect. Moreover, the more rapidly they licked/nibbled and sniffed the

Table 5
Stability across situations, search for correlations between parameters measured during the novel object test and the surprise test, horse free

			Novel object test					
	number of significant correlations between ages → ↓		8 / 8	7 / 8	6 / 8	6 / 8	6 / 8	4 / 8
			Frequency of licking/ nibbling the object	Duration of contact with the object	Latency of sniffing the object	Frequency of sniffing the object	Latency of licking/ nibbling the object	Frequency of blowing
Surprise test, horse free	7 / 8	Flight distance	7 (-)	5 (-)	6 (+)	5 (-)	7 (+)	2 (+)
	7 / 8	Time to eat	7 (-)	5 (-)	5 (+)	4 (-)	6 (+)	
	6 / 8	Frequency of glances at the umbrella	4 (-)	4 (-)	5 (+)	3 (-)	5 (+)	
	3 / 8	Frequency of sniffing the floor	1 (-)		5 (+)	3 (-)		4 (+)
	2 / 8	Frequency of blowing						6 (+)

The numbers indicated in the table correspond to the number of significant correlations out of the 10 possibilities of correlations calculated between two parameters. When this number is equal to 0, the box is empty. The direction of the relationship is also indicated (+: positive correlation; −: negative correlation). Only the parameters which are correlated with one or more parameter of the other test are presented. The parameters are presented from the more stable over time to the less stable. Note the shades of grey: the darker the box, the more stable the parameter over time and the more frequently it is correlated with parameters of the other test.

object, the less they glanced at the umbrella. These correlations appear generally in 5–7 units out of 10 (Table 5). Other parameters were correlated between these two tests, but less frequently.

3.2.3. Novel area and surprise test, horse free

The faster the horses put one foot on the area and ate in the novel area test, the shorter the flight distance and the faster they returned to eat during the surprise test. Moreover, the more they glanced during the novel area test, the shorter the flight distance, the faster they returned to eat and the more frequently they glanced at the umbrella and sniffed the floor during the surprise test. These correlations appear in 5–8 units out of 10 (Table 6).

It can be seen from the last three paragraphs that the parameters which are the most often correlated between the different tests are generally those which show the best stability over time (see Tables 4–6).

3.2.4. Surprise test, horse held and the other three tests

No parameters of the surprise test with the horse held were correlated with parameters of the novel area and the novel object test. There was only one correlation between the surprise test with the horse held and the surprise test with the horse free, namely, the ‘‘maximum–minimum heart rate’’ and flight distance. This correlation was significant in 5 units out of 10.

Table 6
Stability across situations, search for correlations between parameters measured during the novel area test and the surprise test, horse free

			Novel area test			
	number of significant correlations between ages	→	8/8	8/8	7/8	0/8
			Time to put one foot on the area	Time to eat	Frequency of glances at the area	Frequency of blowing
Surprise test, horse free	7/8	Flight distance	6 (+)	6 (+)	8 (+)	
	7/8	Time to eat	6 (+)	6 (+)	5 (+)	
	6/8	Frequency of glances at the umbrella	4 (+)	4(+)	5 (+)	
	3/8	Frequency of sniffing the floor			5 (+)	1 (+)
	2/8	Frequency of vigilant positions				5 (+)

The numbers indicated in the table correspond to the number of significant correlations out of the 10 possibilities of correlations calculated between two parameters. When this number is equal to 0, the box is empty. The direction of the relationship is also indicated (+: positive correlation; −: negative correlation). Only the parameters which are correlated with one or more parameter of the other test are presented. The parameters are presented from the more stable over time to the less stable. Note the shades of grey: the darker the box, the more stable the parameter over time and the more frequently it is correlated with parameters of the other test.

4. Discussion

The purpose of this paper was to examine whether a possible trait of fearfulness can be inferred from some of the behaviours expressed by horses during potentially fear-eliciting test situations.

Among the many behavioural parameters observed during the tests, we explored which ones presented stability over time and across situations. Sniffing or licking/nibbling the novel object, the times to put one foot on the novel area and to eat in the novel area test, the flight distance and the time to return to eat under the umbrella in the surprise test, as well as the glances at the stimulus in the novel area and the surprise tests presented such stability. The stability across situations of these parameters means that an animal which does not sniff, lick or nibble the novel object will look frequently at the novel area, will not put one foot on it or will not eat. It also has a long flight distance and eats after a long time during the surprise test. The stability over time means that an animal which shows these reactions at 8 months of age will also show them at 1.5 and 2.5 years of age.

These two aspects of stability mean that these behavioural parameters can be considered as the reflection of a trait of temperament. According to Gray (1987), Boissy (1998) and de Catanzaro (1999), the situations of novelty and suddenness used in this study are potentially fear inducing. Consequently, this trait can be called fearfulness. In addition, among the different parameters cited above, some of them showed better stability over time than others. These parameters were the frequency of licking/nibbling in the novel object test, the time to put one foot on the area and to eat in the novel area test, and the flight distance and the time to eat in the surprise test. The first three parameters were significantly correlated over time in all the units, and the last two parameters in 7 units out of 8. They can be considered as the best behavioural indicators of a trait of fearfulness in this study.

Concerning stability across situations, our results are in line with many studies which show links between reactions to different fear-inducing situations (rat: Broadhurst, 1957; Van der Staay et al., 1990; chicken: Jones, 1987, 1988; Jones and Mills, 1983; Japanese quail: Mills and Faure, 1986; dog: Goddard and Beilharz, 1984; cattle: Boissy and Bouissou, 1991; goat: Lyons et al., 1988; sheep: Romeyer and Bouissou, 1992; Vandenheede et al., 1998; Viérin and Bouissou, 2003 or horse: Viérin et al., 1998). However, in many of these studies, the correlations found between the different situations may be due to the existence of common elements in all of the tests situations, such as isolation or novel environment. This is theoretically not the case in the present study. However, while we checked that the audience animals did not express any particular behaviour related to the fearful stimuli, we cannot be sure that they did not influence the reactions of some test animals. Thus, we cannot exclude the possibility that this potential social effect may be considered as a ‘‘common element’’.

Concerning the stability over time, this is the first time that such long-term stability (from 8 months to 2.5 years of age) has been demonstrated in horses. Previously, Seaman et al. (2002) attempted to determine a consistency of responses to a novel object over a period of 18 days, but without success. Visser et al. (2001) also examined the stability of behaviours, but over a 1-year period. They found that only a few parameters were stable and they concluded that long-term stability could not be demonstrated convincingly. The low number of stable parameters over time found in the Visser et al. (2001) study may be explained by the fact that their tests involved several aspects of temperament, such as reaction to isolation, to humans or to restraint, in addition to the reactions to novel stimuli.

At this stage, we should point out that we deliberately only used experimental situations with a well-identified fear-eliciting event, and the responses were thus highly specific to the characteristics of the test conditions. Consequently, our results are likely to be different in conditions with less well-identified fear-eliciting events, for example, when novelty concerns the environment as a whole, or when suddenness involves a sound.

The other parameters measured during the tests, such as neighing, defecation, sniffing the floor, blowing or vigilant positions were either too rarely expressed to be analysed or were not sufficiently stable across time and situations. Thus, they cannot be considered as good indicators of a fearfulness trait in this study. Some authors, such as Viérin et al. (1998) in horses or Romeyer and Bouissou (1992) in sheep, interpreted these behaviours in terms of presence of fear. It is interesting to notice that unlike the above behaviours, these are less specific to the stimulus. Thus, it is possible that they could have been more involved as fear indicators in a novel arena design with no specifically located event than in our tests. Moreover, these behaviours can be expressed when the animal is frightened, but they are not sufficiently stable to be a specific reflection of a trait of temperament.

In addition to the study of behavioural parameters, we also investigated if heart rate can be used as an indicator of a trait of fearfulness. The different measures and indexes of the heart rate observed or calculated during the surprise test, when the horses were held, show poor stability over time and were never correlated with parameters of the novel object test or the novel area test. Only one of them was correlated with one parameter of the surprise test, when the horse was free (the ‘‘heart rate maximum–heart rate minimum’’ was correlated with the flight distance). Thus, due to the low stability of the different measures of heart rate, this test does not provide a valid measure of fearfulness under our conditions, particularly with young horses not used to being handled. An absence of links between behavioural and physiological responses such as defecation, corticoids and cardiac frequency has already been reported elsewhere (sheep: Torres-Hernandez and Hohenboken, 1979; cattle: de Passillé et al., 1995; goat: Lyons and Price, 1987; rat: Young and Leaton, 1994). Concerning stability over time, Visser et al. (2002) found that heart rate parameters measured during a fear-eliciting test (novel object test) was correlated between 9 and 21 months of age. In our study, these correlations over time were also found, but only in 4 units out of the 8 tested. This difference can probably be explained by the difference between our protocols: in Visser et al.’s study, horses were free during the recording whereas in our study they were held to avoid locomotor activity influencing the heart rate. Thus, the reaction to the presence of a human and to restraint may have strongly influenced their heart rate, particularly for the horses which were difficult to fit with the equipment due to their strong defensive reactions. Moreover, in contrast to the other three tests, the horses could not avoid the frightening stimuli. It is well known that the degree of control an animal can exert over its threatening environment by appropriate behaviour determines the perception the animal has of it and its reaction (Boissy, 1998). This was demonstrated by Weiss (1972) in rats which received an electric shock, with or without the possibility of stopping it. How well an individual can control the event, and possibly the extent to which it can act on the situation is moreover known to be one evaluation criteria involved in the development of emotional reactions (for a review: Désiré et al., 2002). Finally, the measure of heart rate we carried out may not be reliable: it is a very sensitive measure, which can be strongly influenced by many uncontrollable events such as the internal state (emotional or physiological) of the individual at the moment of the test, its locomotor activity or movements of its body or different external stimulations (e.g. sound in the stable) which can cause an increase in heart rate. In contrast to cardiac frequency, it is highly probable that other cardiac measures such as heart rate variability, used successfully by Visser et al. (2002), would be a better indicator of certain aspects of temperament if they are measured during appropriate conditions (for a review: von Borell et al., 2007).

Finally, this study also allowed us to examine if the stability of behaviour is a function of age. Many studies have found that the stability of behaviours increases with age. For example, in dogs, adult fearfulness could be predicted to some degree from fear reactions observed at 3 months of age, but the accuracy of the prediction improved with age (Goddard and Beilharz, 1984). In the present study, stability does not seem to change between 8 months and 2.5 years of age: there are not more significant correlations between 1.5 and 2.5 years than between 8 months and 1.5 years, and the values of R do not seem to increase with age. Lansade et al. (2007), studying the same horses as those used in the present experiment but from birth to 6 months of age (corresponding to the age of artificial weaning), found an age-dependent increase in stability during this period. Young foals expressed very few behaviours towards fear stimuli, neither approach reactions towards a novel object, nor avoidance reaction to a surprise effect. These reactions appeared progressively with age from birth to 6 months of age, but once these reactions appeared, they were stable over time. The present study complements this result by showing that behavioural stability may have reached a peak by about 6–8 months of age.

5. Conclusion

To conclude, this study shows that the level of fearfulness of an individual can be determined by observation of a few specific behaviours during short tests performed as early as 8 months of age. It is thus possible to select animals according to fearfulness from this age. The next goal will be to determine which level of fearfulness is appropriate for selection, depending on the specific use of the horse.

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