Reactivity to isolation and association with conspecifics: A temperament trait stable across time and situations

Article *in* Applied Animal Behaviour Science · February 2008

DOI: 10.1016/j.applanim.2007.03.003

3 authors, including:

Lea Lansade
French Horse and Riding Institute
36 PUBLICATIONS **402** CITATIONS

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Applied Animal Behaviour Science 109 (2008) 355–373
APPLIED ANIMAL BEHAVIOUR SCIENCE
www.elsevier.com/locate/applanim

Reactivity to isolation and association with conspecifics: 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, GB, United Kingdom*

Accepted 5 March 2007
Available online 10 April 2007

Abstract

A temperament trait is generally defined as individual differences in behaviour that are present early in life and relatively stable across situations and over time. The aim of this study was to test the existence of a trait «gregariousness» in horses, by testing the stability across situations and over time of the responses to different social events. Sixty-six Welsh ponies and 44 Anglo-Arab horses were successively tested at 8 months and 1.5 years of age. Among them, 33 ponies and 21 horses were also tested at 2.5 years of age. They were submitted to four test situations: isolation and separation from, attraction towards and passing conspecifics. We carried out the analysis using each of four test groups as a unit (e.g. 33 Welsh ponies born in 2001, tested in isolation).

Isolation and separation stood out as tests that showed a high consistency within test, across tests and across time. The most interesting behavioural parameter was the frequency of neighing, which was well correlated with other parameters measured in the same tests, such as defecation, locomotion and vigilance, as well as across the 3 years (e.g. for separation test: $0.41 < R < 0.61$). Therefore, the behaviour of neighing observed in separation or isolation tests as early as 8 months of age appears to be a good indicator of similar behaviour in similar situations later in life, but also of other behaviours which can render the horse difficult to use.

No parameter recorded during the attraction test presented stability across situations and time: the reactions to this test were not the expression of a stable characteristic of the individual and did not reflect the same characteristic as the three other tests.

Of the different parameters recorded during the passing conspecifics test, the time to cross the arrival line near conspecifics showed good stability across years ($0.35 < R < 0.68$). This parameter was also correlated

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

0168-1591/$ – see front matter
doi:10.1016/j.applanim.2007.03.003

with many others recorded during the same test, and also, to a certain extent, to the frequency of neighing in the isolation and separation tests.

This stability across responses expressed in various social contexts, and this stability over time, from 8 months to 2.5 years of age suggest the existence of a trait of gregariousness in the horse. From a practical point of view, that means it is possible to estimate the level of gregariousness of a horse as early as 8 months of age. Furthermore, additional analysis shows that gregariousness decreases with age.

Keywords: Horse; *Equus caballus*; Temperament; Trait; Isolation; Conspecific association; Stability

1. Introduction

Horses, like all domestic ungulates, are gregarious animals. Consequently, all events inducing separation from or confrontation with conspecifics can have a major impact on their behaviour. In many gregarious species, social isolation is known to induce severe psychological stress, which is expressed through behavioural and physiological changes: increase of vocalization, locomotion, heart rate or plasma cortisol concentration (cattle: Boissy and Le Neindre, 1997; sheep: Lyons et al., 1993; Cockram et al., 1994; Mears and Brown, 1997; Vandenheede et al., 1998; goats: Lyons et al., 1993; horses: Wolff et al., 1997; Mal et al., 1991).

Even though there is as yet no scientific study to confirm this, we propose that these situations can have a significant impact on the utilization of the horse. In equestrian activities, the horse is frequently led to be isolated from conspecifics or to pass beside other horses. Excessive reactions to these frequent kinds of situations can affect the concentration of the horse and render it difficult to use. The stress induced by the separation can also affect its welfare. Consequently, the possibility to select the horses as early as possible in function of their reactions to social separation and social attraction could be very useful.

The reactivity to social separation and/or to social attraction is often considered as a ''temperament trait'' (for a review, see Gosling and John, 1999). A temperament trait is generally defined as individual differences in behaviour that are present early in life and are relatively stable across various kinds of situations and over the course of time (Bates, 1987; Goldsmith et al., 1987). The reactivity to social separation and/or to social attraction is regrouped under various terms which are frequently used interchangeably, with little variation between their definitions. For example, the terms ''sociability'', ''social motivation'' or ''social attraction'' are used interchangeably to describe ''the tendency to graze close to their peers'' (Boissy and Dumont, 2002; Sibbald and Hooper, 2004 in sheep). The term ''social motivation'' is also defined as ''the tendency of a visually isolated animal to establish visual contact with, move towards and remain in proximity to a group of conspecifics'' (Launay et al., 1991 in chickens). This is a concept similar to the ''gregariousness'' defined by Wolff et al. (1997) in horses as the reactions to social separation. However, some of these terms are used to describe another type of tendency: the tendency to react to strangers (generally measured during social confrontation tests between familiar and unfamiliar partners). This is the case of the terms ''sociability'' (Svartberg and Forkman, 2002 in dogs), ''social behaviour'' (Tonissaar et al., 2004 in rats) or ''social reactivity'' (Perré et al., 2002 in domestic pullets). In this paper, we are interested only in the reactivity to social isolation, to social separation and to social attraction and we investigate whether these three correspond to a more general temperament trait in the horse, which we may call ''gregariousness''.

One approach to assess temperament traits which has become increasingly popular is to measure many behaviours in one or more situations or to carry out questionnaires regarding these behaviours and to use multivariate analyses, such as factor analysis or principal components analysis to extract two or three factors which are thought to represent important personality traits. This approach has been tested on several different taxonomic groups with very different ecological requirements including humans (Buss and Plomin, 1986; Goldsmith et al., 1987), rhesus monkeys (Stevenson-Hinde et al., 1980), octopuses (Mather and Anderson, 1993), cats (Lowe and Bradshaw, 2000), dogs (Svartberg and Forkman, 2002), sheep (Vandenheede et al., 1998; Viérin and Bouissou, 2001), goats (Lyons et al., 1988), pigs (Spoolder et al., 1996; Forkman et al., 1995), cattle (Grignard et al., 2001), donkeys (French, 1993) and horses (Viérin et al., 1998; Visser et al., 2001), and the two most commonly found axes, defined as traits, are activity/arousal and sociability. This is the quasi exclusive approach for the study of traits referring to social reactions. However, we do not think this approach is adequate to determine personality traits. Indeed, a trait is defined as a behavioural tendency, stable across situations and over time. Therefore this approach does not permit to determine if the dimensions found with the statistical analysis present stability. It is only a ''snapshot'' of the behaviour at a given time and in a given situation, which permits to extract some associated behaviours, but which does not demonstrate that they correspond to temperament traits. As pointed out by Tulloh (1961), Grandin (1993) or Seaman et al. (2002), it is possible that a large day-to-day variation in behaviour occurs. If behaviours used to describe temperament are not consistent over time, the animal may be assigned a different temperament score each time it is tested. It is the same problem if the behaviour is not consistent over situations in the same context. For this reason we used another approach, which consists of proposing the hypothesis of the existence of a trait and to test the stability across situations and over time of the different behaviours expressed by the animals in a specific context (in the present case, in social context). This approach has been commonly used for the assessment of a trait of fearfulness (for a review: Boissy, 1998), but very rarely for the assessment of a trait referring to social reactions. In horses, we can cite Seaman et al. (2002) who show that the responses of horses to an open-field arena (involving social isolation) is consistent over time during tests repeated three times with an average of 9 days between trials, suggesting the existence of a certain stability. However, in this study, stability between different situations referring to a social context (as open field test and behaviour during reintroduction in the social group) was not found and the stability over time was only demonstrated over a relatively short period.

Therefore, the aim of this study was to test the hypothesis of the possible existence of a general temperament trait, that we will call ''gregariousness'' by testing the two characteristics of a trait: stability across situations and stability over time. For this purpose, we tested the animals in two situations of social isolation (isolation and separation) and in two situations of social interactions (attraction to and passing between conspecifics), and we repeated these situations every year, from the age of 8 months to the age of 2.5 years. Among the many behavioural parameters we measured during these tests, we determined the more reliable behavioural indicators of the possible trait of gregariousness. These indicators are the behavioural parameters which show the best stability over time and across situations. From a practical point of view, these parameters could be used to assess the level of gregariousness of a horse. In the case of identification of a trait of gregariousness, we also tested effects of age and sex.

This paper is a part of a more general study which attempts to assess several possible temperament traits in horses such as fearfulness, reactivity to humans or activity level.

2. Animals, material and methods

2.1. Animals

One hundred and ten horses, divided in four groups were used. They originally comprised 22 Anglo-Arab horses (AA01) and 33 Welsh ponies (W01) born in 2001 and 22 different 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 after 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 at two different places. Anglo-Arab horses were individually identified with an electronic chip in the neck and Welsh ponies with an ear tag.

Animals of each group were maintained on pasture with their dam until 6 ± 1 months of age, when they were abruptly weaned (complete and definitive separation from the mother). Males were castrated around 12 months of age. From 6 to 11 months, 20 to 23 months and 32 to 35 months the animals were housed indoors corresponding both to the winter period and the period of tests.

Anglo-Arab horses were housed individually on sawdust bedding, 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 horses could see all the animals present 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 horses were in the outside area and when the door was closed, they could not see inside the barn.

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

They were fed twice a day with commercial pellets and hay, except for the period of tests involving a 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.

Table 1
Number of horses tested at the different ages

	Age		
	8 months	1.5 years	2.5 years
Welsh, born in 2001 (W01)	33	33	33
Welsh, born in 2002 (W02)	33	32	–
Anglo-Arab, born 2001 (AA01)	22	21	21
Anglo-Arab, born 2002 (AA02)	22	19	–

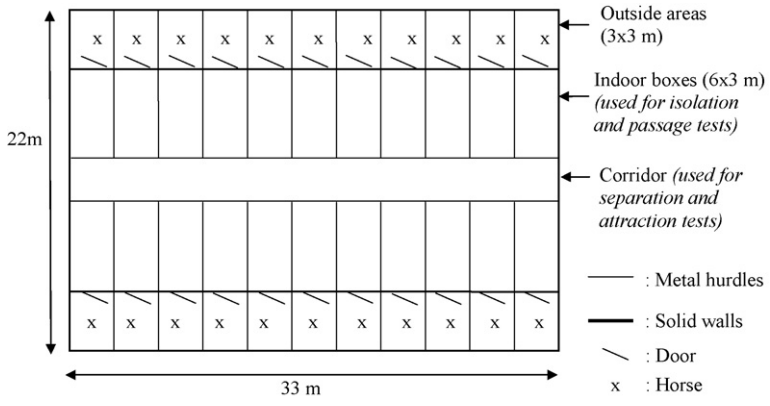

Fig. 1. Layout of the building where the Anglo-Arab horses were housed and tested.

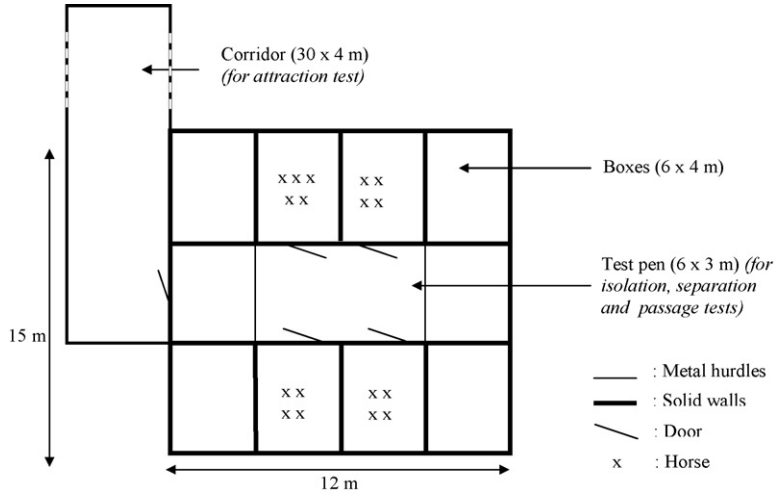

Fig. 2. Layout of the building where the Welsh ponies were housed and tested.

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

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 just before the tests.

Anglo-Arab horses were tested in the barn where they were living (Fig. 1). Welsh ponies were tested in a barn or a corridor adjacent to the pen where they were living. During the test period, the ponies were randomly placed into the four boxes (4 m × 6 m), in which the tests were later performed, for 4 h per day and in groups of 4 or 5 (Fig. 2). In both breeds, when a horse was tested, it was extracted from the outside part of its box (Anglo-Arab) or from its box (Welsh) by a handler and placed in the test pen and the test began immediately. The other horses could not see the test horse and vice versa.

Horses were randomly assigned to a testing order for each test. During the habituation and the different tests, the experimenters were hidden behind a one-way mirror. Two audience horses were used in the different habituations or tests. They were unfamiliar to the tested horses at the beginning of the study. They were chosen because they were known to be especially calm: they could stay motionless during the whole of the test periods.

2.2.1. Test situations

Animals were submitted to four test situations. Animals born in 2001 (AA01 and W01) were tested in these four situations at the age of 8 months, 1.5 and 2.5 years of age while the animals born in 2002 (AA02 and W02) were only tested at the age of 8 months and 1.5 years of age.

2.2.1.1. Isolation from conspecifics: "isolation test". This test consisted in isolating the horse from its conspecifics, without possibility to join them or to communicate with them, and to observe the reactions to isolation. Anglo-Arab horses were tested in their own boxes (whereas non test horses were kept in their outside areas, Fig. 1). Welsh ponies were tested in a pen constructed in the corridor of the barn (Fig. 2). In

both cases, the test pens were 6 m long and 3 m wide. To evaluate the area covered by the horse, they were divided in 6 sectors of equal size (1.5 m × 2 m) traced with plaster powder on the ground (on the sawdust in the case of Anglo-Arab horses, or on the concrete floor in the case of Welsh ponies).

Habituation. This phase consisted of habituating the horse to being led into the test pen and to staying in it without particular reactions. To avoid the tested horse being alone in the pen during habituation, two ''audience'' horses were attached on each side of the test pen, at about 4 m distance. The test horses were placed in this situation daily for 5 min, until they no longer manifested the following behaviours: neighing, defecations, trot or gallop, during three consecutives days. The presence of one occurrence of one of these four behaviours during the three consecutives days was tolerated. For example, if a horse neighed only once during three consecutives days, but never defecated, trotted or galloped, we considered it habituated.

Test. During the test, the two audience horses were absent. The test lasted 5 min during which 11 behavioural parameters were recorded (Table 2).

2.2.1.2. Separation from conspecifics: "separation test". Instead of moving the test horse away from conspecifics, in this test the conspecifics were moved away from the test horse (see below for more details).

Table 2
Behavioural parameters recorded during the tests

Parameters	Isolation	Separation	Attraction	Passage
Glance at the public horses (latency and frequency) the horse stands still, with elevated neck, head and ears oriented in direction of the stimulus			X[a]	X[b]
Sniffing the public horses (frequency) the horse stands still, with elevated neck, head and ears oriented anywhere except in the direction of the stimulus				X
Licking/nibbling the public horses (frequency)				X[a]
Neigh (latency and frequency)	X	X	X[c]	X[a,b]
Defecation (latency and frequency)	X	X	X[a]	X[a,b]
Trot (frequency)	X	X	X	X[a]
Scraping the floor with the foot (frequency)	X	X	X[a]	X[a]
Vigilant position (frequency)	X	X	X	X[a]
Sectors entered (number) test pens were divided into 6 (isolation and separation tests) or 5 (attraction test) sectors of equal size, marked on the floor with plaster powder. The number of sectors entered by the horse for the whole duration of the tests was quantified as an indicator of its locomotor activity	X	X	X	
Sniffing the floor/the apparatus (frequency)	X	X	X	X[a]
Snorting (frequency) forceful expulsion of air through the nostrils preceded by a raspy inhalation sound	X[a]	X[a]	X[a]	X[a]
Time spent near the stimulus (s) time spent in a zone of 2 m × 3 m, traced on the floor, just behind the audience horses. The horse is considered to be in the zone when both forelegs are in the zone		X		
Escape-attempt (frequency) the horse jumps on the hurdles delimiting the test pens	X[a]	X[a]	X[a]	X[a]
Time to cross the arrival line (s) time necessary for the two forelegs to cross the line delimiting the arrival zone			X	X
Stop in front of the horses (frequency)				X

[a] Behaviours expressed by less than 15% of the individuals in all 10 repetitions of the test.
[b] Only the frequency was measured.
[c] Behaviours expressed by less than 15% of the individuals in only 4 of the 10 repetitions of the test.

The test consisted of placing an audience horse near the test pen and then to remove it from the view of the test horse (without possibilities for the test horse to join it) in order to evaluate the response to social separation.

In both breeds, the animals were tested in a pen (6 m × 3 m) constructed in the corridor of the barn using metal hurdles.

Habituation. The habituation was similar to that described above for the isolation test, but the two audience horses were both present at only one end of the pen.

Test. The test lasted 3 min, consisting of two phases of 90 s each. During the first phase, the two audience horses were present at one end of the pen as during the habituation period: the animals did not manifest any particular behaviour (neighing, defecation, trot or gallop). During the second phase (separation), the audience horses were removed, and the test horse was alone. In the experiment conducted on Welsh ponies, during the phase 2, the audience horses were led away to a box adjacent to the corridor using a long lead rope attached to their halter, and were then out of sight of the test horse. This way, the test horse could not see the handler. In the experiment conducted on Anglo-Arabs, a sliding door separated the audience horses and the test horse. In the first phase, the door was open and the test horse could see the audience horses. In the second phase, the door was closed (with a system of ropes which also permit the experimenter to be hidden) and the test horse could not see the audience horse. Twelve behavioural parameters were recorded during phase 2 (Table 2).

2.2.1.3. Attraction towards conspecifics: "attraction test". This test consisted of isolating the test horse at one end of a corridor, with the opportunity to join the audience horses which were placed at the opposite end. The aim of this test was to assess the reaction to a social attraction. This test is similar to the "runway test" used in birds (Suarez and Gallup, 1983; Vallortigara, 1992; Mills et al., 1995; Jones et al., 2002).

The test took place in a long corridor (30 m × 4 m, Fig. 3) inside the barn (Anglo-Arab horses) or adjacent to the barn (Welsh ponies). Three zones were marked on the floor of the corridor using plaster powder. The first zone (4 m × 4 m) corresponded to a "starting zone" and the third one (4 m × 4 m) corresponded to an "arrival zone". In the second zone (22 m × 4 m), four wooden bars of 2 m long, resting on 80 cm high plastics cubes, were placed across the corridor, delimiting five sectors of equal size (Fig. 3). These bars prevented the test animals from walking in a straight line and hence increased the actual distance between the starting and the arrival zone.

Habituation. During the habituation period (5 min per day), the two audience horses were placed in two compartments constructed at the two ends of the corridor, and the test horse was allowed to move freely in the corridor for 2.5 min. After this time, the handler gently pushed the horse once (without the horse being

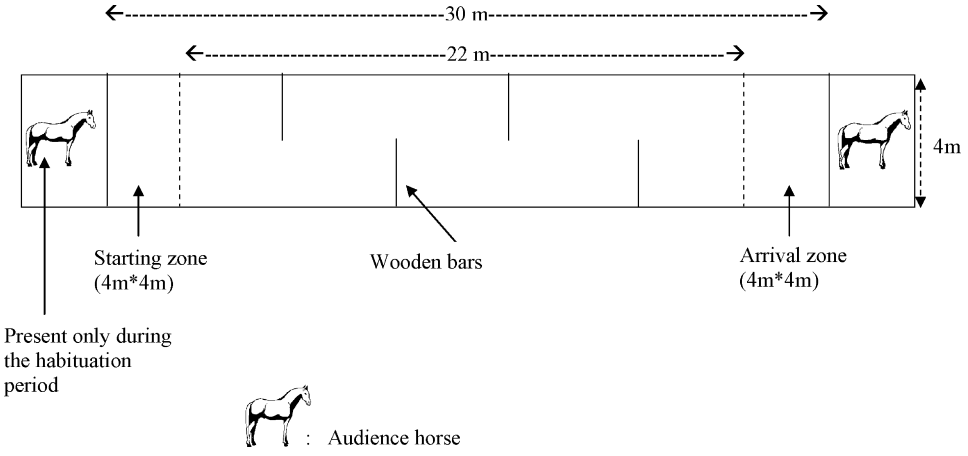

Fig. 3. Set-up for the attraction test.

haltered) from beginning to end of the corridor to familiarize it with every part of the apparatus, and then released it again for the rest of the habituation time. The criterion of habituation was identical to those described for the isolation test (neighing, defecate, trot or gallop no more than one time during three consecutives days). The number of days required for habituation was between 3 and 6 days.

Test. During the test, only one audience horse was present, placed in the compartment behind the arrival zone. The test animal was led into the starting zone by a handler (without being haltered) and was released in the corridor. Immediately, the handler entered an adjacent box to be hidden from the test horse. The time recording started when the horse's two forelegs crossed the line delimiting the starting zone. The animal had 90 s to cross the line into the arrival zone. If it did not cross the line in this time, the test was terminated and a ''time necessary to cross the arrival line'' of 91 s was assigned to the horse. Fourteen behavioural parameters were recorded during the test (Table 2).

2.2.1.4. Pass conspecifics: "passing test". This test consisted of making the horse go past the audience horses, with the possibility for the test horse to stop near them and to interact with them, in order to determine the reaction to a social attraction. This test involved a conflict of motivations between feeding on one hand and stopping to interact with conspecifics on the other hand. This kind of conflict has been used in sheep by Dumont and Boissy (2000), and Boissy and Dumont (2002).

The test took place in the corridor of the barn or in the box of the animal. Three zones were marked on the floor of the test pen (6 m × 3 m) using plaster powder (Fig. 4). The first zone (1.50 m × 3 m) corresponded to a ''starting zone''. The second zone corresponded to a ''central zone'' (3 m × 3 m). The third one (1.50 m × 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. During the habituation period, the two audience horses were tied 3 m away from the corridor (Fig. 4). An experimenter entered the pen with the test animal, led it (without being haltered) to the starting zone and then released it. Subsequently, the handler immediately entered an adjacent box to be hidden from the horse. 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. 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.

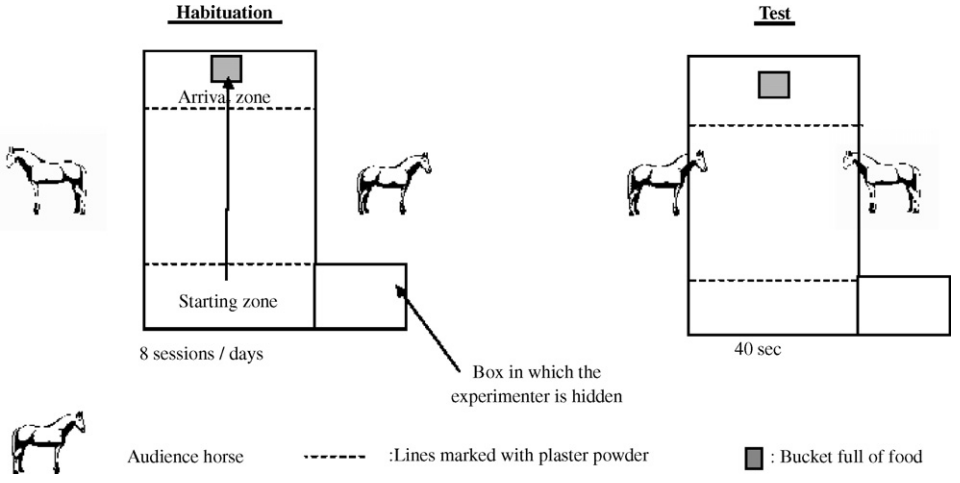

Fig. 4. Set-up for the passing test (starting and arrival zones: 1.5 m × 3 m; central zone: 3 m × 3 m).

Test. The test was carried out on the day after the horse reached the criterion. The two audience horses were tied alongside the corridor so that the test horse was able to touch them. The test animal had also 40 s to cross the arrival line, and 16 behavioural parameters were recorded (Table 2). Depending on group and age, up to 25% of the horses did not cross the arrival line in the allowed time. The others crossed the arrival line in a time ranging between 3 and 38 s.

2.2.2. Behavioural observations

The behavioural parameters recorded during the four test situations are presented and defined in Table 2.

2.3. Statistical analysis

In many studies which used the kind of tests used in the present experiment, the authors attempt to describe temperamental traits from numerous parameters using a PCA. The use of synthetic factors, obtained with a PCA, can be very useful to determine the links between different parameters. However, the use of the synthetic factors obtained with this kind of analysis does not allow us to answer our question: among the different behavioural parameters measured, which are the more stable across situations and over time, and thus are the best reflection of a temperamental trait? Therefore, we chose to analyse each of the parameters individually using correlation analyses.

The same protocol was repeated twice in two groups (AA02 and W02) and thrice in the others (AA01 and W01). We did not combine the data from these different time points and groups. The first reason is that the groups were not strictly tested in the same conditions since the two breeds were tested in different places and the two generations in different years. The second reason is that repeating several times the same statistical analysis (10 times for the search for links between parameters and 4 times for the search for consistency over time—see below) counterbalances the large number of correlations we performed into each analysis. The third reason is that obtaining the same result several times from analysis performed in several independent groups is more reliable than obtaining a conclusion from only one analysis performed in one group. A fourth reason is the possibility that links between behaviours might be specific to one group, but not another (Knapp and Moore, 1997).

Some behavioural parameters were expressed by less than 15% of animals, and, following Viérin and Bouissou (2003) were not taken into account in the statistical analysis; they are presented in the results. Then, three different analyses were performed: the first consisted of investigating the relationship between the behavioural parameters measured in the same test (intra-test correlations), 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) and the third concerned the links between parameters measured at different ages (stability over time). To study the relationship between parameters, Spearman (rank) correlations were calculated since they are more robust to non-normality of the data than the Pearson correlations. A correlation was considered to be statistically significant, when its p-value was smaller than 0.05.

Each test was repeated 10 times: the two breeds of horses born in 2001 were tested three times (at 8 months, 1.5 and 2.5 years) and those born in 2002, were tested two times (at 8 months and 1.5 years), resulting in 10 'units' of data. Therefore, the relationship between the parameters (stability across situations) was calculated in 10 separate units. Here we present the number of significant correlations out of 10 and the sign of the correlation. The results are presented in the form of a matrix. In the matrix, the number of correlations which were found significant in more than 5 units out of 10 is presented in bold. In this case, we also present the medians and inter-quartile ranges of the Spearman correlation coefficient (R-value) in the form of median [first quartile; third quartile], calculated for the 10 units. This way of presenting correlations has been proposed by Bayly et al. (2006). In the text, we present the number of significant correlations only when this number is equal or more than 5 (intra-test correlations) or 2 (inter-tests correlations).

In contrast, the search for stability over time involves fewer possible correlations, therefore the significant correlations ($p < 0.05$) are presented in table with the Spearman correlation coefficient (R-value).

2.4. Additional analyses: effect of age

We also tested the effect of age on social motivation. To avoid comparing too many variables, we performed the subsequent analyses only on the parameters which present the best stability over time and across situations of each test (except for the attraction test for which there is no stable parameter). These indicators are the frequency of neighing in the case of the isolation and separation tests, and the time to cross the arrival line in the case of the passing conspecifics test.

A Friedman test was used to compare these three parameters measured at different ages (8 months, 1.5 years and 2.5 years). Then, pair wise comparisons were done with a Wilcoxon test.

3. Results

3.1. Behaviours expressed by less than 15% of the individuals

3.1.1. Isolation and separation

In the isolation and separation tests, snorting and escape-attempts were expressed by less than 15% of the individuals, therefore they were not taken into account in the statistical analysis.

3.1.2. Attraction

In the attraction test, the behaviour of neighing was expressed by less than 15% of the individuals in 6 out of 10 units. All four units in which more than 15% of the individuals performed neighing behaviour were animals of 8 months of age. The correlation analyses for this behaviour were performed only in these four units. Glance at the audience horses, defecation, scraping the floor, snorting and escape-attempts were never sufficiently expressed to be analysed.

3.1.3. Passing conspecifics

In the passing conspecifics test, the only behaviours which were sufficiently expressed were the time to cross the arrival line, the frequencies of stops, glances at and sniffing the audience horses.

3.2. Correlations between parameters measured in the same test (intra-test correlations)

In this paragraph, we present the number of significant correlations only when this number is equal to or more than 5 units out of 10. For neighing and defecation, the latency and frequency were always negatively correlated. For the readability of the text and the tables, we only present the results for the frequency of these behaviours.

3.2.1. Isolation

In the isolation test, we found five relationships between behaviours that were significant in 5 or more of the 10 units. The more sectors an individual entered, the more often it defecated (number of units with significant correlations; median correlation coefficient and inter-quartile range: 6; R:0.43[0.36;0.50]), trotted (7; R:0.46[0.42;0.50]) and sniffed the floor (6; R:0.37[0.20;0.58]). The more often they neighed, the more often they defecated (6; R:0.41[0.15;0.49]) and the more often they performed vigilant behaviour (7; R:0.54[0.28;0.60]).

3.2.2. Separation

In the separation test, we found four relationships that were significantly correlated in 5 or more of the 10 units. The more sectors an individual entered, the more often it neighed

Table 3
Passage test, intra-test correlations

	Time to cross the arrival line	Frequency of glances at	Frequency of sniffing	Frequency of stops
Time to cross the arrival line	X	**6** (+) 0.61 *0.34*; *0.75*	**9** (+) 0.76 *0.46*; *0.83*	**9** (+) 0.59 *0.49*; *0.73*
Frequency of glances at		X	**7** (+) 0.54 *0.39*; *0.66*	**8** (+) 0.64 *0.52*; *0.75*
Frequency of sniffing			X	**10** (+) 0.85 *0.72*; *0.92*

The numbers indicated in the table correspond to the number of units out of 10 with significant correlations (bold), the direction of the relationship (+: positive correlation; −: negative correlation), the median correlation coefficient and inter-quartile range (italics).

(6; *R*:0.42[0.32;0.59]) and the more often it trotted (6; *R*:0.39[0.32;0.60]). Neighing and trotting were also positively correlated (6; *R*:0.40[0.36;0.53]). In 5 of the 10 units, horses that were more vigilant sniffed the floor less often (5; *R* −0.35[−0.26;−0.56]).

3.2.3. Attraction towards conspecifics

In the attraction test, only the time to cross the arrival line was correlated with other behaviour in five or more units. Individuals that were fast at crossing the arrival line trotted more often (5; *R*:−0.29[−0.10;−0.37]), sniffed the floor and the apparatus less often (6; *R*:0.53[0.34;0.66]) and were less vigilant (5; *R*:0.51[0.15;0.74]).

3.2.4. Passing conspecifics

All the parameters analysed for this test are correlated in at least six units (Table 3).

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

3.3.1. Isolation and separation

The frequency and latency of neighing are correlated between the isolation and the separation tests in the majority of units (in at least 5 units out of the 10 analyses performed; Table 4). In addition, in 4 units out of 10, the latency and frequency of defecation measured during the isolation test are correlated with the latency and frequency of neighing measured during the separation test, and the frequency of sniffing the floor is correlated between the two tests. The other parameters are correlated in less than 2 units out of 10.

3.3.2. Isolation and attraction towards conspecifics

No parameter of the isolation test is correlated with parameters of the attraction test in more than one unit out of 10.

3.3.3. Isolation and passing

No parameter of the isolation test is correlated with parameters of the passing conspecifics test in more than 4 units out of 10 (the majority of the units). However, in 4 units out of 10,

Table 4
Variables which are correlated between the isolation test and the separation test (inter-tests correlations)

	Isolation test	
	Latency of neighing	Frequency of neighing
Separation test		
Latency of neighing	**7** (+) 0.42 *0.33*; *0.49*	**5** (−) −0.42 *−0.50*; *−0.34*
Frequency of neighing	**8** (−) −0.46 *−0.51*; *−0.43*	**7** (+) 0.45 *0.38*; *0.52*

The numbers indicated in the table correspond to the number of units out of 10 with significant correlations (bold), the direction of the relationship (+: positive correlation; −: negative correlation), the median correlation coefficient and inter-quartile range (italics).

the longer the time required to cross the arrival line in the passing conspecifics test is, the higher the frequencies of neighing (R:0.13[0.017;0.45]) and vigilant positions (R:0.22[0.09;0.47]) in the isolation test are. The other parameters are correlated in fewer than 2 units out of 10.

3.3.4. Separation and attraction towards conspecifics

No parameter of the separation test is correlated with parameters of the attraction test in the majority of the units. However, in 3 units out of 10, the more the animals neigh in the separation test, the more rapidly they cross the arrival line of the attraction test. No other variables are correlated in more than 2 units out of 10.

3.3.5. Separation and passing

No parameters of these two tests are correlated in more than 2 units out 10.

3.3.6. Attraction and passing

No parameters of these two tests are correlated in more than 2 units out 10.

3.4. Stability over time

3.4.1. Isolation

Seven parameters are correlated over time at least in one case out of the 8 tested (Table 5), but it is the frequency of neighing which is the more often correlated between two different ages. This frequency is correlated in each group between 8 months of age and 1.5 years of age but never with the age of 2.5 years.

3.4.2. Separation

Nine parameters are correlated over time at least in one out of 8 units (Table 5). One of them is correlated in all of the units (neighing frequency) and two of them are correlated in at least four out of eight units (frequency of defecation and number of sectors entered).

Table 5
Stability over time

	Significant correlation (*n*)	W01			W02	AA01			AA02
		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	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
Isolation									
Neighing (f)	4	0.41			0.39	0.45			0.40
Neighing (l)	2	0.43				0.45			
Scraping floor (f)	2		0.51	0.42					
Sectors entered (n)	2					0.46			0.40
Defecation (f)	1		0.47						
Sniffing floor (f)	1		0.40						
Trot (f)	1					0.67			
Separation									
Neighing (f)	8	0.41	0.53	0.52	0.47	0.46	0.55	0.41	0.61
Sectors entered (n)	5	0.38	0.48			0.57		0.41	0.50
Defecation (f)	4		0.38	0.53	0.36	0.67			
Neighing (l)	3	0.48				0.45	0.48		
Defecation (l)	3		0.40	0.59		0.64			
Trot (f)	3			0.41	0.36		0.46		
Time near stimulus	2				0.46		0.55		
Scraping floor (f)	2	0.40	0.39						
Vigilant positions (f)	1						0.50		
Attraction									
Crossing arrival line (l)	1		0.39						
Trot (f)	1					0.64			
Neighing (f)	1/4		–	–		0.47	–	–	
Passage									
Crossing arrival line (l)	7	0.68	0.63	0.48	0.35	0.49	0.56	0.36	
Looking at audience (f)	3	0.37	0.47				0.46		
Sniffing audience (f)	2					0.63		0.50	
Stops (f)	2	0.40					0.43		

Table 6
Comparison of horses of different ages regarding frequency of neighing in the isolation and separation test, and the latency to cross the arrival line in the passing test (Wilcoxon tests)

	Significant changes	W01			W02	AA01			AA02
		8 months compared to 1.5 years	1.5 years compared to 2.5 years	8 months compared to 2.5 years	8 months compared to 1.5 years	8 months compared to 1.5 years	1.5 years compared to 2.5 years	8 months compared to 2.5 years	8 months compared to 1.5 years
Isolation, neighing (f)	Wilcoxon: 5 (decrease)	$>^{***}$	$>^{***}$	$>^{***}$	$>$, $P < 0.1$	$>^{**}$	$>$, $P < 0.1$	$>^{**}$	ns
Separation, neighing (f)	Wilcoxon: 5 (decrease)	$>^{***}$	ns	$>^{***}$	$>^{***}$	$>^{**}$	ns	$>^{***}$	ns
Passing, time to cross the line	Wilcoxon: 6 (decrease)	$<^{***}$	$<^{***}$	$<^{***}$	$<^{*}$	ns	$<^{**}$	$<^{**}$	ns

$>$: decrease; $<$: increase.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3.4.3. Attraction towards conspecifics

Few correlations between different ages were found. The time required for the horse to cross the arrival line and the frequencies of trot and neighing are correlated over time but only in one unit out of the 8 (time to cross the line and trot) or 4 (neighing) tested (Table 5).

3.4.4. Passing

The time to cross the arrival line is correlated over time in seven units out of the eight tested, the frequency of glances at the audience horses in three units and the frequencies of stop in front of the horses and sniffing the horses in two units (Table 5).

3.5. Additional analysis

3.5.1. Effect of age

The frequencies of neighing measured during the isolation and the separation tests decrease significantly between two successive ages in five units out of eight, whereas the time to cross the arrival line decreases in six units out of eight (p values are presented in Table 6).

4. Discussion

The purpose of this study was to test the hypothesis of the existence of a temperament trait, that we will call ''gregariousness''. To be considered a trait, individual differences in behaviour have to be consistent across time, and for the label 'gregariousness' to apply, these differences must also be consistent across several situations from within a social context.

For that purpose, we tested horses of two breeds in different situations, involving isolation and separation from, and attraction to or passing conspecifics. Some of these situations shared common characteristics. Isolation, for example, featured in the first three tests, while the possibility to join and eventually interact with other horses was only part of the last two tests, not the first two. These tests were carried out at three different ages, between 8 months and 2.5 years. We recorded many behavioural parameters currently described in these kinds of situations (Rushen, 1990; Lyons et al., 1993; Boissy and Le Neindre, 1997; Cockram et al., 1994; Vandenheede et al., 1998; Mal et al., 1991; Wolff et al., 1997; Viérin et al., 1998).

Some of these behaviours were not sufficiently expressed in the tests to be analysed and can, therefore, not be considered to be good indicators of the studied temperament trait. Examples are the frequencies of neighing and of defecating observed during the attraction and the passing conspecifics tests, as well as the frequencies of snorting and escape attempts carried out during all four tests. Neighing appears to be specific to a situation of isolation or separation from conspecifics, when the animal is unable to join them. It is almost not expressed in the two other tests. According to Waring (1983), neighing occurs usually when social partners are separated, and it is considered to be the contact call of horses (Klingel, 1972). Other studies, such as that of Wolff et al. (1997), show that it does not occur in frightening situations involving novelty or suddenness.

4.1. Isolation and separation tests

The two most correlated tests are the isolation test and the separation test. Of the different parameters recorded during these two tests, the neighing behaviour shows a relatively strong stability across situations and over time. Its frequency and latency are positively correlated

between these two tests in at least half of the tested units. Its frequency presents also stability over time: it is correlated between all of the ages and in all of the groups in the separation test. In the isolation test, it is correlated in all of the groups between 8 months and 1.5 years of age, but not later (at 2.5 years of age, few horses neigh in this test, resulting in a poor variability of responses at this age). This stability suggests that the neighing behaviour and particularly its frequency can be considered as an indicator of a temperament trait at 8 months and 1.5 years of age. In addition, the neighing behaviour is also correlated (intra-test correlations) with many other behaviours, such as defecation, locomotion or vigilant position expressed in situations of isolation or separation. This result is in accordance with Viérin et al. (1998), who also showed that the more a horse neighs, the more it defecates, moves in the pen and presents vigilant position. These behaviours can render the horse unsuitable for the practice of horse-riding unless managed and trained appropriately. Therefore, the behaviour of neighing observed in separation or isolation tests as early as 8 months of age appears to be a good indicator of similar behaviour in similar situations later in life, but also of other behaviours which may render the horse difficult to use.

Some others parameters measured during these two tests also present stability over time (such as locomotion and defecations measured during the separation test) and across situations (defecations in the isolation test and neighing in the separation test).

Stability over time and across these two tests of these different parameters (and particularly the behaviour of neighing), suggest the existence of an underlying temperament trait, which we will refer to as the "*reactivity to social isolation or separation from conspecifics*".

4.2. Passing conspecifics test

Of the different parameters recorded during the passing conspecifics test, the time to cross the arrival line shows a strong stability across years (in seven units out of the eight). This parameter is also correlated with many others recorded during the same test, such as the frequency of looking at and of sniffing the audience horses, or of stopping in front of them. Therefore, this time may reflect also a temperament trait, we may call: "*the propensity to walk past conspecifics*".

4.3. Attraction test

None of the responses observed in the attraction test showed stability across situations and over time. One possible explanation is, of course, that the attraction to other horses is not a temperament trait. A second potential explanation is that, while the underlying motivation may be stable, the set-up of the bars etc may have introduced an added factor, which was not stable across time. A highly motivated horse, for example, that does not see its way around the bars right away, may get very agitated (move a lot, neigh, eventually defecate), thus becoming less likely to find the way around the bars. The performance in the test may thus be related to how quickly a horse sees the solution to the problem. This means that a high level of attraction may lead to a short or a very long arrival time. If that is the problem with the test, then altering the task by replacing the bars placed across the corridor with some other means of slowing the horses down might be a solution.

4.4. Link between the different tests/traits

The time to cross the arrival line in the passing conspecifics test is positively correlated with the frequencies of neighing and of vigilant positions measured during the isolation test in 4 units

out of 10. It is not the majority of the units, therefore we cannot conclude these two tests measure strictly the same phenomenon, but they certainly measure some common characteristics. Therefore, we propose that a more general trait exists, which we call ''*gregariousness*'', and which may include ''*the reactivity to isolation or separation from conspecifics*'' and ''*the propensity to walk past conspecifics*''. The best indicators of this trait are the frequency of neighing during the isolation and separation tests and the time to cross the arrival line during the passing conspecifics test.

4.5. Effect of age

An effect of age was found on the three best indicators of social motivation. With increasing age, animals neigh less during the separation and isolation tests and cross less rapidly the arrival line during the passing conspecifics (differences between ages are statistically significant in 5 units out of 8 at least). This change could be explained by a process of habituation to the test, by a maturational process or by an experiential process independent of the tests. In the case of habituation, that would mean that a few minutes of tests every year would have been sufficient to habituate the animal to this situation. On the other hand, a similar apparent decrease of social motivation has been shown in other studies. Viérin and Bouissou (2003) show that 3–4 months old lambs react more in isolation than 5–6 months old. In horses, Wolff et al. (1997) observed that the oldest (3 years old) animals (especially the males) produced fewer vocalisations in the isolation test than the youngest. Their explanation for this result was that by this age, the horses (especially the males) have left the herd and may be less dependent on their strong social bonds. Moreover, we propose that the adult animals are less vulnerable to dangers (such as predators) than the young, therefore their survival are a little less dependent on their proximity to the group. This makes a maturational process more probably than one of mere habituation to the test.

5. Conclusion

To conclude, this study allowed us to identify a set of behaviours stable across situations and across years, and which we could thus consider as an indicator of a temperament trait of gregariousness. This trait includes ''the reactivity to social isolation or separation from conspecifics'' and a ''propensity to walk past conspecifics''. It can be inferred from the frequency of neighing measured during isolation or separation tests and the time to cross the arrival line in the passing conspecifics test, which seem to be the best indicators of this trait. We also showed that the trait ''gregariousness'' seems to decrease with age.

From a practical point of view, these results suggest that it is possible to assess a horse's gregariousness from as early as eight months of age. This assessment could allow the identification of horses who require careful management and appropriate training in order to become suitable riding horse.

Acknowledgments

The first author was supported by a PhD grant from the ''Region Centre'' and from the National French Stud: ''les Haras Nationaux''. This institute also funded this experiment. The authors are grateful to Guy Duchamp (INRA Nouzilly, France), Catherine Trillaud Geyl (Station expérimentale des Haras Nationaux, Chamberet, France) and their staff for allowing the use of

the animals and facilities. Thanks are due to the students Hélène Roche, Marjorie Coulon and Juliette Brisson for their participation in collecting the data.

References

Bates, J.E., 1987. Temperament in infancy. In: Osofsky, J.D. (Ed.), Handbook of Infant Development. 2nd ed. Wiley, New York, pp. 1101–1149.

Bayly, K.L., Evans, C.S., Taylor, A., 2006. Measuring social structure: a comparison of eight dominance indices. Behav. Process. 73, 1–12.

Boissy, A., 1998. Fear and fearfulness in determining behavior. In: Grandin, T. (Ed.), Genetics and the Behavior of Domestic Animals. Academic Press, San Diego, pp. 67–111.

Boissy, A., Dumont, B., 2002. Interactions between social and feeding motivations on the grazing behaviour of herbivores: sheep more easily split into subgroups with familiar peers. Appl. Anim. Behav. Sci. 79, 233–245.

Boissy, A., Le Neindre, P., 1997. Behavioral, cardiac and cortisol responses to brief peer separation and reunion in cattle. Phys. Behav. 61, 693–699.

Buss, A.H., Plomin, R., 1986. The EAS approach to temperament. In: Plomin, R., Dunn, J. (Eds.), The Study of Temperament: Changes, Continuities and Challenges. Lawrence Erlbaum, Hillsdale, NJ, pp. 67–77.

Cockram, M.S., Ranson, M., Imlah, P., Goddard, P.J., Burrells, C., Harkiss, G.D., 1994. The behavioural, endocrine and immune responses of sheep to isolation. Anim. Prod. 58, 389–399.

Dumont, B., Boissy, A., 2000. Grazing behaviour of sheep in a situation of conflict between feeding and social motivations. Behav. Process. 49, 131–138.

Forkman, B., Furuhaug, I.L., Jensen, P., 1995. Personality, coping patterns, and aggression in piglets. Appl. Anim. Behav. Sci. 45, 31–42.

French, J.M., 1993. Assessment of donkey temperament and the influence of home environment. Appl. Anim. Behav. Sci. 36, 249–257.

Goldsmith, H.H., Buss, H.H., Plomin, R., Rothbart, M.K., Thomas, A., Chess, S., Hinde, R.A., McCall, R.B., 1987. What is temperament? Four approaches. Child Dev. 58, 505–529.

Gosling, S.D., John, O.P., 1999. Personality dimension in non-human animals: a cross species review. Curr. Dir. Psychol. Sci. 8, 69–75.

Grandin, T., 1993. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36, 1–9.

Grignard, L., Boivin, X., Boissy, A., Le Neindre, P., 2001. Do beef cattle react consistently to different handling situations? Appl. Anim. Behav. Sci. 71, 263–276.

Jones, R.B., Marin, R.H., Satterlee, D.G., Cadd, G.G., 2002. Sociality in Japanese quail (*Coturnix japonica*) genetically selected for contrasting adrenocortical responsiveness. Appl. Anim. Behav. Sci. 75, 337–346.

Klingel, H., 1972. Das Verhalten der Pferde. In: Helmcke, H.G., Starck, D., Wermuth, H. (Eds.), Handbuch der Zoologie. Walter de Gruyter, Berlin, pp. 1–68.

Knapp, R., Moore, M.C., 1997. Male morphs in tree lizards have different testosterone responses to elevated levels of corticosterone. Gen. Comp. Endocrinol. 107, 273–279.

Launay, F., Mills, A.D., Faure, J.M., 1991. Social motivation in Japanese quail coturnix coturnix japonica chicks selected for high or low levels of treadmill behaviour. Behav. Process. 24, 95–110.

Lowe, S.E., Bradshaw, J.W.S., 2000. Ontogeny of individuality in the domestic cat in the home environment. Anim. Behav. 61, 231–237.

Lyons, D.M., Price, E.O., Moberg, G.P., 1988. Individual differences in temperament of domestic dairy goats: constancy and change. Anim. Behav. 36, 1323–1333.

Lyons, D.M., Price, E.O., Moberg, G.P., 1993. Social grouping tendencies and separation-induced distress in juvenile sheep and goats. Dev. Psychobiol. 26, 251–259.

Mal, M.E., Friend, T.H., Lay, D.C., Vogelsang, S.G., Jenkins, O.C., 1991. Behavioural responses of mares to short term confinement and social isolation. Appl. Anim. Behav. Sci. 31, 13–24.

Mather, J.A., Anderson, R.C., 1993. Personalities of octopus. J. Comp. Psychol. 107, 336–340.

Mears, C.J., Brown, F.A., 1997. Cortisol and ß-endorphin responses to physical and physiological stressors in lambs. Can. J. Anim. Sci. 77, 689–694.

Mills, A.D., Jones, R.B., Faure, J.M., 1995. Species specificity of social reinstatement in Japanese quail Coturnix japonica genetically selected for high or low levels of social reinstatement behaviour. Behav. Process. 34, 13–22.

Perré, Y., Wauters, A.M., Richard, M.-A., 2002. Influence of mothering on emotional and social reactivity of domestic pullets. Appl. Anim. Behav. Sci. 75, 133–146.

Rushen, J., 1990. Use of aversion-learning techniques to measure distress in sheep. Appl. Anim. Behav. Sci. 28, 3–14.
Seaman, S.C., Davidson, H.P.B., Waran, N.K., 2002. How reliable is temperament assessment in the domestic horse (*Equus caballus*)? Appl. Anim. Behav. Sci. 78, 175–191.
Sibbald, A.M., Hooper, R.J., 2004. Sociability and the willingness of individual sheep to move away from their companions in order to graze. Appl. Anim. Behav. Sci. 86, 51–62.
Spoolder, H.A.M., Burbidge, J.A., Lawrence, A.B., Simmins, P.H., Edwards, S.A., 1996. Individual behavioural differences in pigs: intra- and inter-test consistency. Appl. Anim. Behav. Sci. 49, 185–198.
Stevenson-Hinde, J., Stillwell-Barnes, R., Zunz, M., 1980. Individual differences in young rhesus monkeys: consistency and change. Primates 21, 498–509.
Suarez, S.D., Gallup, G.G., 1983. Social reinstatement and open-field testing in chickens. Anim. Learn. Behav. 11, 119–126.
Svartberg, K., Forkman, B., 2002. Personality traits in the domestic dog (*Canis familiaris*). Appl. Anim. Behav. Sci. 79, 133–155.
Tonissaar, M., Philips, M.A., Eller, M., Harro, J., 2004. Sociability trait and serotonin metabolism in the rat social interaction test. Neurosci. Lett. 367, 309–312.
Tulloh, N.M., 1961. Behaviour of cattle in yards. II. A study of temperament. Anim. Behav. 9, 25–30.
Vallortigara, G., 1992. Affiliation and aggression as related to gender in domestic chicks. J. Comp. Psychol. 106, 53–57.
Vandenheede, M., Bouissou, M.F., Picard, M., 1998. Interpretation of behavioural reactions of sheep towards fear-eliciting situations. Appl. Anim. Behav. Sci. 58, 293–310.
Viérin, M., Bouissou, M.F., 2001. Pregnancy is associated with low fear reactions in ewes. Phys. Behav. 72, 579–587.
Viérin, M., Bouissou, M.F., 2003. Responses of weaned lams to fear-eliciting situations: origin of individual differences. Dev. Psychobiol. 42, 131–147.
Viérin, M., Bouissou, M. F., Vandenheede, M., Trillaud-Geyl, C., Arnaud, G., 1998. Developpement d'une méthodologie destinée à mesurer les réactions de peur chez le cheval. In: 24ème journée d'étude de l'Institut du Cheval.
Visser, E.K., van Reenen, C.G., Hopster, H., Schilder, M.B.H., Knaap, J.H., Barneveld, A., Blokhuis, H.J., 2001. Quantifying aspects of young horses' temperament: consistency of behavioural variables. Appl. Anim. Behav. Sci. 74, 241–258.
Waring, G.H., 1983. Horse Behavior: The Behavioral Traits and Adaptations of Domestic and Wild Horses, Including Ponies. Noyes, Park Ridge, NJ.
Wolff, A., Hausberger, M., Le Scolan, N., 1997. Experimental tests to assess emotionality in horses. Behav. Process. 40, 209–221.