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A few days of social separation affects yearling horses' response to emotional reactivity tests and enhances learning performance

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ABSTRACT

Learning performance is influenced by emotional reactivity, low reactivity being generally beneficial. Previous experiments show that emotional reactivity can be modified after a period of social isolation. We hypothesized that eleven days of isolation would affect yearlings' emotional reactivity and improve their learning abilities. Twenty-five yearlings were divided into two groups: 12 were continuously isolated for 11 days (isolated) and 13 stayed together (control). During the period of isolation, all yearlings underwent two learning tasks: a habituation procedure in which a novel object was presented for 120 s every day, either when the horse was alone (isolated) or with conspecifics (control); an instrumental learning task in which the yearling had to walk forwards or backwards to obtain a food reward. At the end of the isolation period, animals performed tests to assess aspects of emotional reactivity: reactivity to novelty, to humans, to social separation, to suddenness and to sensory stimuli. Results showed that isolated yearlings habituated more to the novel object than controls and performed better in the instrumental task. Moreover, they were less reactive to novelty, to social separation and to suddenness than controls. Overall, these data suggest that the better performance of isolated yearlings could be explained by a decrease in their emotional reactivity.

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1. Introduction

Emotional reactivity can vary according to fluctuations in the animal's biotic or abiotic environment. In view of the relationship between emotional reactivity and learning abilities (for a review: Mendl, 1999; Sandi and Pinelo-Nava, 2007), these changes could also induce modifications in learning abilities.

Some rearing conditions can involve a period of social separation, but how this influences emotional reactivity remains unclear. In rats, rearing young in social isolation enhances response to environmental novelty (e.g. Hall et al., 1997) and produces an anxiogenic profile (Weiss et al., 2004). Likewise, calves reared alone from 5 days to 3 months of age were more fearful when introduced to a novel social situation and when isolated in a novel arena (Jensen et al., 1997). By contrast, in a study with rodents, Gentsch et al. (1982) reported that several weeks of isolation, beginning after weaning, reduced locomotor activity when the animals were exposed to a new environment alone. Similarly, calves reared single from birth to 2 months of age were less stressed (bawled, tried to escape or pranced nervously less frequently) when isolated in a novel environment than those reared in groups (Purcell and Arave, 1991). Finally, horses housed alone after weaning exhibited less restless behaviour, fewer vocalisations and more exploratory behaviour in an Arena and Human Encounter test involving social separation, a novel environment and presence of a human (Søndergaard and Halekoh, 2003). However, as the tests used in these three experiments all involved a social component, we do not know whether isolation leads to a general reduction in emotional reactivity or only to a reduced response to social separation. Moreover, all these studies examined the impact of long periods of isolation, with no data about the influence of short periods of isolation.

Rearing animals in isolation can also affect their cognitive abilities. Some studies have found a negative influence of isolation on a variety of learning tasks in rodents. Rats isolated for 8 weeks after weaning acquired an active avoidance response less rapidly than socially housed rats (Labadze et al., 2006). Likewise, horses kept in individual stalls for several weeks acclimatized less readily to initial training for riding (Rivera et al., 2002), and in another study, horses housed individually for 3–8 months were more difficult to handle

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and train than horses housed in groups (Søndergaard and Ladewig, 2004). By contrast, other studies have reported that rearing animals for a long period in isolation facilitates learning, for example, a discriminative approach task (rodents: Harmer and Phillips, 1998b), an appetitive Pavlovian conditioning task (rodents: Harmer and Phillips, 1998a), and a maze learning task (rodents: Wongwitdecha and Marsden, 1996; calves: Purcell and Arave, 1991). The learning context may partly explain the different results. For instance, in the study of Purcell and Arave (1991), the maze learning task was performed in isolation. The authors suggested that the better performance of the isolated calves may have been due to lower separation anxiety rather than an improvement of their learning abilities. The kind of task, and particularly whether or not a human trainer is involved, could also affect the result. In horses, Rivera et al. (2002) explained that the unwanted behaviours observed in stalled horses during training could be due to a change in their relationship with humans (positive at first and more negative once training began). Finally, the length of the isolation period may also have an effect, but all the studies cited above examined the impact of long periods of isolation, and little is known about the impact of short periods of isolation.

The influence of emotional reactivity on learning performance has been well documented (for a review: Mendl, 1999; Sandi and Pinelo-Nava, 2007). Generally, a high level of fearfulness or anxiety impairs learning performance. In rats, the anxiety trait affects the ability to learn spatial orientation tasks: the most anxious rats showed slower acquisition and poorer memory in the water maze than less anxious animals (e.g. Herrero et al., 2006). In horses, the most fearful or reactive individuals take longer to learn various tasks, including instrumental conditioning tasks (Lindberg et al., 1999; Visser et al., 2003; Lansade and Simon, 2010), spatial learning tasks (Heird et al., 1986) and discriminative tasks (Fiske and Potter, 1979). This suggests that a period of isolation leading to a reduction in separation anxiety could improve learning performance.

Surprisingly, although some studies on horses have assessed the effect of long periods of social isolation on both emotional reactivity and learning abilities, the influence of short periods of social isolation on both processes is not known, even though horses are frequently exposed to this situation. In the present study, we hypothesized that a few days of isolation would change various aspects of emotional reactivity, such as reactivity to novelty, to suddenness, to humans and to isolation. We also hypothesized that it would modify the ability to learn certain tasks, often used in horse training, such as habituation to a novel object and instrumental conditioning (a forwards–backwards task). We conducted this study with yearlings, because horses are generally housed alone in individual boxes for the first time around this age.

2. Animals, materials and methods

2.1. Animals

The study involved 25 Welsh ponies (average height: 1.08 m), aged 10 months ± 1 month, reared under identical conditions at INRA Nouzilly (France). From birth to weaning, they lived in the same group with their mare in grass paddocks. They were weaned at 6 months ± 1 month (complete and definitive separation from the mother). The weanlings were kept together in a large stall and were turned out together in a paddock for 6 h per day. Animals received similar human contact, as required for routine husbandry, for example, feeding when indoors, change of pasture and veterinary care (deworming and vaccinations). During the change of pasture and the veterinary care, all the ponies were accustomed to being haltered.

The animals were randomly allocated to one of two groups matched for sex, age and sire (two stallions): isolated and control. One day before the start of the experiment, the 12 individuals (5 females and 7 males) in the isolated group were housed alone in a familiar 4 m × 5 m loose box (in which the horse is free) without any visual or tactile contact with each other. The 13 individuals (5 females and 8 males) in the control group were kept in a familiar 10 m × 7 m stall for the duration of the study. The stall size was in line with recommendations for the housing of horses [(2 × height at withers)2]. The size of the box and the stall differed but allowed the horses in both groups to perform trotting steps, roll and lie on their side. A smaller loose box, corresponding to the area per animal in the stall, would not have allowed these behaviours to occur, which could have introduced a bias in the study. All animals were housed on straw bedding, received concentrated feed (pellets) twice a day and hay once a day. Water was available ad libitum. During the experiment, each horse was turned out for half an hour a day, in its group or individually, in the same 50 m × 40 m dirt paddock.

The isolation period lasted 11 days, during which animals underwent the procedure of habituation to a novel object and to the forwards–backwards task. On day 11, emotional reactivity tests were performed. The learning tasks and the tests were carried out in different locations at each stage of the experiment. The procedures are described in the following section, and their timelines and locations are shown in Table 1. Two observers (always the same persons) simultaneously recorded the behaviours. All the tests were videotaped. The handlers and the observers were unfamiliar to the animals at the beginning of the experiment. Horses were weighed two days before isolation and at the end of the experiment (day 11) to evaluate a possible influence of treatment.

2.2. Habituation to a novel object

The aim of this procedure was to evaluate whether animals habituate to a novel object more efficiently when they are confronted by it alone (isolated group) or when they are with conspecifics (control group). The same object (a 90 cm × 40 cm green plastic bag filled with straw) was used for all phases of the procedure (evaluation before isolation, habituation, and evaluation after habituation).

2.2.1. Evaluation of reaction to a novel object before isolation (day −2)

To check that responses to the novel object used for the habituation procedure did not differ between groups prior to the experiment, animals were subjected to the following protocol two days before isolation. They were all tested alone in the corridor of the barn where they lived, which was converted into a 14 m × 3 m test arena (Fig. 1A).

In the first phase, horses underwent a habituation period during which they were trained to go from a starting line to a zone containing a familiar food bucket, placed 8 m away (Fig. 1A). The subject was led to this bucket to eat. Once it had eaten some pellets, it was taken back behind the starting line. After that, it was free to return to the bucket on its own. This was repeated four times. In the second phase of evaluation, the novel object was placed 50 cm from the bucket so that the horse had to approach the object in order to eat from the bucket. As in the first phase, the horse was free to return or not to the food bucket. The time the horse spent inside the area with the novel object (3 m × 3 m) was recorded, up to 120 s.

2.2.2. Habituation procedure (day 1 to day 9)

The habituation procedure started the day after the ponies were isolated. Seven learning sessions were conducted with a 2-day break (Table 1). They were performed in the environment where the horses lived during the experiment. Horses in the isolated group

Table 1
Experimental schedule.

Horses housed together		Isolated lived alone in individual loose boxes / Controls lived together in a large stall												
D-2	D-1	**D0**	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
Evaluation F/B			F/B S1	F/B S2	F/B S3	F/B S4	F/B S5			F/B S6	F/B S7	F/B S8	
Evaluation No			HNo S1	HNo S2	HNo S3	HNo S4	HNo S5			HNo S6	HNo S7	Evaluation No	
													Reactivity test

S = learning session; D = day; F/B: forwards–backwards task; HNo: habituation to a novel object.
On day 0, the animals were allocated to one of two treatments: isolated and control. All the tests were performed by all animals, but sometimes in different locations, depending on the group:
(dark grey) Performed in a familiar test arena.
(black) Performed in a novel test arena.
(light grey) Performed in their own individual loose box (isolated group) or in their own stall with the group (control group).

were tested alone in their individual loose boxes. Those in the control group were tested together and simultaneously in their large stall, so each one was observed in a group. The loose boxes and stall were permanently equipped with eating troughs, with one trough per horse. Animals ate their food in these troughs every morning and evening from the start of the experiment.

From day 1, the objects used during the evaluation phase (the green plastic bags filled with straw) were placed 50 cm from each animal's trough for 120 s at feeding time every evening. For the control group, the objects (one per horse) were placed simultaneously in the stall. The animals had to approach the object in order to reach their food. In each session, the time taken to eat from the feed bucket for the first time was recorded.

During the third session, an isolated horse caught its hoof in the object and panicked. Thereafter this horse refused to approach the object and was therefore excluded from analyses of sessions 3–7.

2.2.3. Evaluation of reaction to the object after the habituation procedure (day 10)

To evaluate the efficacy of these seven habituation sessions, all the horses were tested individually on day 10 under the same conditions, with the same object, in the same arena (Fig. 1A), and following exactly the same procedure as the one used for the pre-isolation evaluation. The time spent in the area close to the object was recorded.

2.3. Forwards–backwards task

2.3.1. Evaluation of response to the commands used during the task, before isolation (day −2)

Two days before isolation, the animals' response to the commands used during the learning procedure was evaluated to check whether there was any difference between the two groups (Table 1). Horses were tested in the corridor that was used to evaluate the reaction to the novel object (Fig. 1B). The experimenter stood still for 2 s on the left of the horse, holding it with a leading rein while an observer stood motionless in front of it at one end of the corridor. Horses were asked to walk forwards or backwards in response to visual, vocal and tactile commands. There were two trials (one forward and one backward walking). Seven levels of command were defined, from a visual signal (level 0) to a helper pushing the pony as hard as possible (level 6; see Table 2). The trial began with a command that was a visual sign made by the observer with his arm (level 0; Table 2). If the horse responded successfully, i.e. took two steps forwards or backwards, the trial ended. If not, the

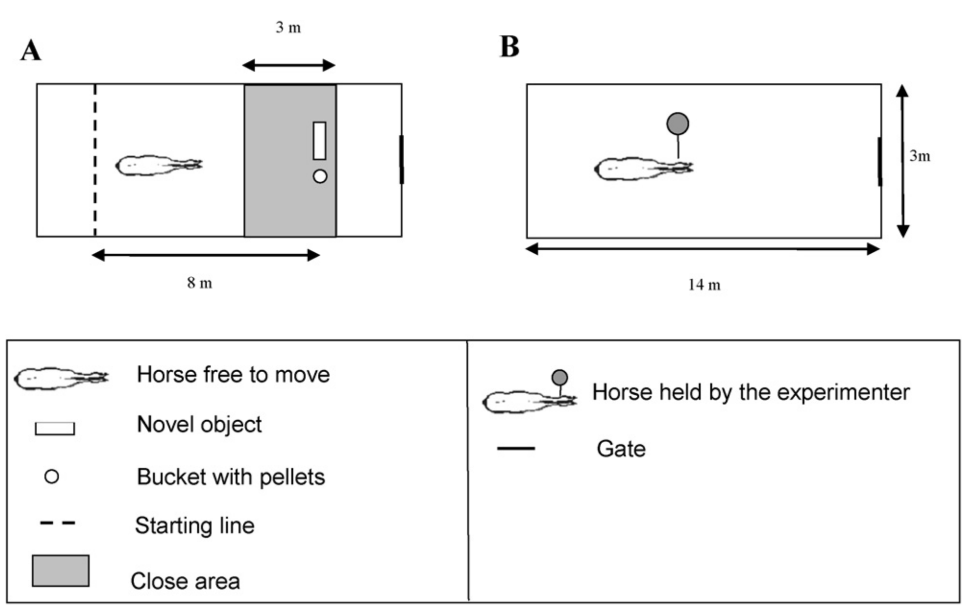

Fig. 1. Familiar test arena used for the novel object test (A) and for the forwards–backwards test (B).

Table 2
Levels of command used for the forwards–backwards task.

	Level of command	Walk forward	Walk backward
Excellent levels	Level 0	The observer moved his arm up from a horizontal to a vertical position	The observer moved his arm down from a horizontal to a downward position along his side
	Level 1	The handler said “walk”	The handler said “back”
	Level 2	The handler said “walk” and walked one step forward without increasing the tension on the leading rein	The handler said “back” and pivoted his body in order to face the pony
	Level 3	The handler said “walk” and exerted a slight pressure on the leading rein	The handler said “back” and placed his hand on the pony’s chest
	Level 4	The handler said “walk”, stretched his arm and pull on the leading rein	The handler said “back” and pushed the pony’s chest gently with his arm
	Level 5	The handler said “walk” and pull strongly on the leading rein using his body weight	The handler said “back” and pushed on the pony’s chest with his body weight
	Level 6	The handler said “walk” and pull the leading rein as hard as possible while one of the observers push the hindquarters	The handler said “back” and pushed the chest as hard as possible, helped by one of the observers

level of command was increased every 2 s until the pony responded correctly. We recorded the level of command at which each horse responded, as shown in Table 2.

2.3.2. Learning procedure (day 1 to day 9)

The learning procedure started the day after isolation and consisted of seven learning sessions (Table 1). The procedure was similar to the one described above, but a handful of pellets was given to the pony when it responded correctly (walking at least two steps forwards or backwards). Each session consisted of 16 trials comprised randomly of eight forwards walking and eight backwards walking. The intensity level (0–6) at which the horse responded was recorded for the 16 trials. Levels 0–2 were defined as excellent. We recorded the number of excellent levels performed in each session. The number of horses in each group that responded at least twice at level 0 during any session was also recorded. We chose this criterion because more than 95% of horses responded at least once at level 0 during a session, and thus the variability between individuals for this parameter was insufficient for statistical analysis. The inter-trial interval was 20 s, and behaviours were recorded during this time (number of neighs, defecations, looking at the observer, and walking backwards spontaneously).

2.3.3. Session performed in a novel arena (day 10)

Session 8 was performed in a novel arena (8.10 m × 2.70 m) to determine how the horses responded to the learning procedures under the stressful condition induced by novelty.

2.4. Emotional reactivity tests

Eleven days after isolation, reactivity tests developed by Lansade et al. (2003, 2008a,b,c) and Lansade and Bouissou (2008) were used to assess aspects of emotional reactivity: reactivity to novelty, to humans, to isolation, to suddenness, and to sensory stimuli.

All the tests were carried out in an 8.10 m × 2.70 m arena (Fig. 2). Two observers were hidden behind a dark window. An audience horse was tied up outside the arena, visible to the tested individual, to avoid social isolation interfering with the other characteristics under study. This audience horse was chosen for its calmness. Behavioural tests were carried out in the same order for a total period of approximately 30 min per horse. The behaviours observed during these tests were those that have been found to be reliable indicators of horses’ reactivity based on their stability over time and across situations (Lansade et al., 2003, 2008a,b,c; Lansade and Bouissou, 2008). Details of the relevant behaviours are given for each test procedure. In addition, we recorded the frequency of neighing throughout the tests.

2.4.1. Novel environment test

To determine reactivity to a novel environment, the horse was led to a test arena in an unfamiliar building. The time taken to cover the distance from the entrance of the building to the arena gate (14 m) was recorded. The horse was then left in the arena for 300 s and the frequency of defecations was recorded.

2.4.2. Sound test

This test involved the emission of a brief sound (fundamental frequency: 1000 Hz, maximal frequency: 1000 Hz, intensity: 70 db, duration: 1.5 s) when the horse was totally motionless, with its head lowered towards the floor. The response was coded in binary form: no reaction (absolutely no movement) or reaction (ears, head or body turned towards the sound transmitter).

2.4.3. Passive human test

To characterize reactivity to humans, an unfamiliar experimenter (always the same person) entered the arena and stayed motionless beside a wall for 180 s (Fig. 2). The frequency of sniffing or nibbling the person was recorded.

2.4.4. Tactile sensitivity test

To measure reactivity to tactile stimuli, von Frey filaments of various strengths were applied to the base of the horses’ withers (von Frey filaments, Stoelting, IL, USA). These filaments consist of a hard plastic body connected to a nylon thread and are calibrated to exert a specific force on the skin, ranging from 0.008 g to 300 g. They were applied perpendicular to the animal’s skin for 1 s, until the nylon filament started to bend. Trembling of the platisma muscle was recorded as a measure of tactile sensitivity. In the first phase of the test, which was carried out after the passive human test, a 0.008 g filament was applied to the right side of the horse, followed 60 s later by a 300 g filament to the left. In the second phase, after the novel area test, a 0.02 g filament was applied to the horse’s right side and a 1 g filament to its left.

2.4.5. Social isolation test

To characterize reactivity to isolation, the audience horse was removed from sight and sound of the tested horse for 90 s. The frequency of neighing was recorded. In order to measure locomotor activity, we divided the test pen into six sectors of equal size (Fig. 2) and recorded the number of sectors crossed and the frequency of trotting steps.

2.4.6. Novel area test

For this test, which characterizes reactivity to novelty, the floor of the novel arena was divided into three 2.7 m × 2.7 m zones: a departure zone, an intermediate zone and an arrival zone (Fig. 2). The arrival zone contained a familiar bucket containing pellets.

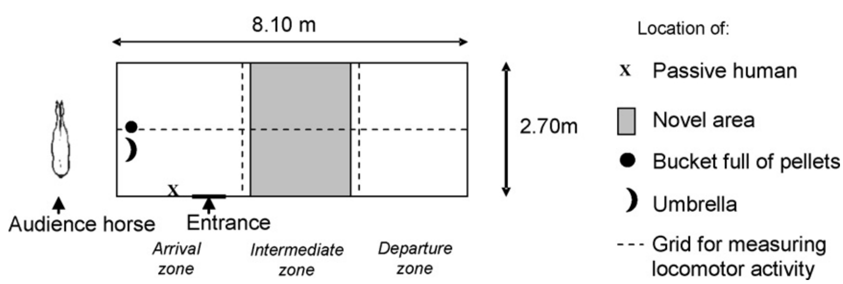

Fig. 2. Layout for the emotional reactivity tests in the novel arena.

Immediately prior to the test, each horse underwent a habituation phase during which an experimenter led it by the halter to the departure zone and released it so that it was free to go to the arrival zone to eat. This was repeated three times. For the test itself, a pink carpet (2 m × 2.7 m) was placed in the intermediate zone. As during the habituation phase, the experimenter released the horse in the departure zone and recorded the time it took to put one hoof on the carpet. If the horse did not cross the area within 180 s, the test was terminated and a time of 181 s was assigned.

2.4.7. Suddenness test

In this test, which characterizes reactivity to suddenness, the experimenter shook a black umbrella in front of the animal while it was eating. A bucket of pellets was placed near the entrance (Fig. 2). After the animal had had its head in the bucket for 3 s, the experimenter opened the umbrella. The time taken to return to the bucket was recorded, up to 180 s. However, because many horses did not return within that time, we analysed the proportion of horses in each group that went back within the 180 s.

2.4.8. Locomotor activity

The test pen was divided into six sectors of equal size (Fig. 2). An observer recorded in real time the number of sectors crossed by the horse during the novel environment test, the passive human test and the social isolation test.

From day 1 to day 11, when the experimenter took a control horse out of the stable for testing, he had contact with all the control horses. To balance the time spent with a human between groups, the experimenter walked around each loose box of the isolated group before taking out a control horse.

2.5. Statistical analyses

The data were analysed with XLStats software (Addinsoft Software, Paris, France). The parameters are presented at the end of each section describing the learning and testing procedures. We checked the distribution of the data using the Lilliefors test. As almost none of the parameters were normally distributed, we decided to use non-parametric tests. Wilcoxon tests were used for within-group comparisons, and a Mann–Whitney test and a *Z*-test for between-group comparisons. To avoid analysing too many parameters, the behaviours measured during the inter-trial interval of the forwards–backwards task were pooled across all the sessions (number of neighs, defecations, looking at the observer, and walking backwards spontaneously). Two-tailed tests were used. *P*-values smaller than or equal to 0.05 were considered significant, and values between 0.05 and 0.10 as a trend.

2.6. Ethical note

Experiments were conducted under licence from the French Ministry of Agriculture (no. 37-125). All the tests in this study were non-invasive. Box and stall sizes complied with recommendations for the housing of horses. After the experiments, the female ponies were kept at INRA for breeding and the males were sold for riding activities.

3. Results

3.1. Horse weight

The horses' weight did not differ between groups, either before (mean ± standard deviation: controls: 176 ± 39; isolated: 201 ± 33) or after isolation (controls: 178 ± 41; isolated: 204 ± 32).

3.2. Learning tasks

3.2.1. Habituation to a novel object

Before the experimental phase (day −2), the time spent in the area close to the novel object did not differ significantly between groups.

During the habituation procedure, horses in both groups went to the feed bucket close to the novel object significantly faster during session 7 than session 1 (Wilcoxon test: controls: $Z = -3.059$, $P < 0.001$; isolated: $Z = -3.059$, $P < 0.001$). During sessions 1, 2 and 7, the control horses took significantly longer to access food close to the novel object than the isolated animals, and during sessions 4 and 5 this difference showed a trend (Mann–Whitney test: S1: $U = 39.5$, $P = 0.02$; S2: $U = 43$, $P = 0.05$; S3: $U = 68$, *NS*; S4: $U = 41$, $P = 0.07$; S5: $U = 41.5$, $P = 0.08$; S6: $U = 48.5$, *NS*; S7: $U = 37.5$, $P = 0.04$; Fig. 3).

When the reaction to the novel object was evaluated individually in the same test arena (on day −2 and day 10), horses in both groups spent significantly longer in the area close to the object after than before the learning procedure (Wilcoxon test: controls: $Z = -2.93$, $P = 0.01$; isolated: $Z = -3.06$, $P < 0.001$). On day 10, post-habituation evaluation revealed that the control horses spent significantly less time than the isolated animals in the area close to the object (Mann–Whitney test: $U = 30.5$, $P = 0.008$; Fig. 4).

3.2.2. Forwards–backwards learning task

Before the experimental phase (day −2), there was no difference between groups in the level of the command at which the horses responded.

Horses in both groups achieved significantly more excellent levels during session 7 than session 1 (Wilcoxon test: controls: $Z = -3.18$, $P = 0.002$; isolated: $Z = -3.06$, $P = 0.002$).

From sessions 1 to 5, the sum of excellent levels for the forwards/backwards learning task did not significantly differ between groups (Fig. 5). However, differences appeared from session 6, with the control horses achieving significantly fewer excellent levels than the isolated animals (Mann–Whitney test: S6: $U = 36$, $P = 0.004$; S7: $U = 36$, $P = 0.004$). Moreover, the proportion of horses that responded at least twice at level 0 during a session tended to be

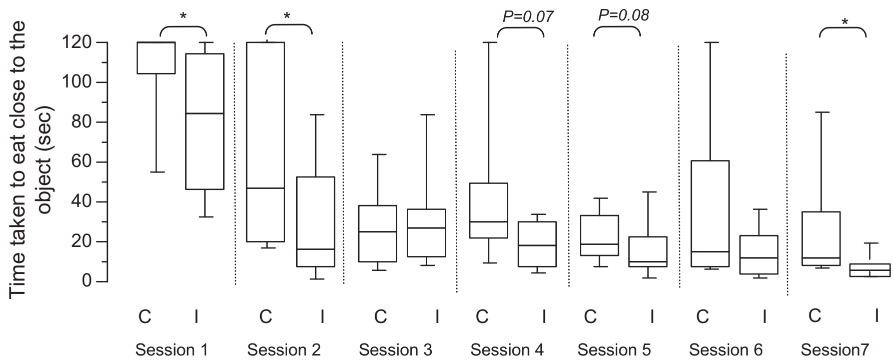

Fig. 3. Habituation to a novel object: median time taken to eat for the first time close to the object by horses with conspecifics (C group) and by isolated horses (I group) during seven habituation sessions. Sessions 1 and 2: controls, $N = 13$; isolated, $N = 12$; Sessions 3–7: controls, $N = 13$, isolated: $N = 11$. $^*P \leq 0.05$; Mann–Whitney test.

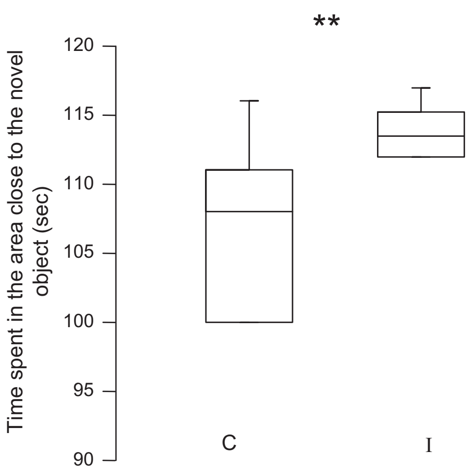

Fig. 4. Reaction to a novel object assessed on day 10 after seven habituation sessions: median time spent in the area close to the novel object by horses with conspecifics (C group) and by isolated horses (I group). $^{**}P \leq 0.01$; Mann–Whitney test.

lower in the control group than in the isolated group (controls: 2 out of 13; isolated: 6 out of 12; Z test: $Z = -1.85$, $P = 0.06$). In addition, the control horses tended to neigh and defecate more frequently than the isolated animals during all the sessions (Mann–Whitney test: $U = 108.5$, $P = 0.09$ and $U = 110.5$, $P = 0.07$ respectively).

When horses were put in a novel arena on day 10, control horses tended to perform with significantly fewer excellent levels than isolated animals (Mann–Whitney test: S8: $U = 46$, $P = 0.08$; Fig. 5).

3.3. Emotional reactivity tests

3.3.1. Novel environment test

The controls took significantly longer than the isolated horses to enter the novel arena and they defecated significantly more in this arena (Mann–Whitney test: $U = 139$, $P < 0.001$, Fig. 6A; $U = 36.5$, $P = 0.01$, Fig. 6B respectively). The frequency of neighs did not significantly differ between groups.

3.3.2. Sound test

Significantly more horses reacted to sound in the control group than in the isolated group (controls: 11 out of 13; isolated: 5 out of 12; proportion Z test: $Z = 2.23$, $P = 0.02$). No horses neighed during this short test.

3.3.3. Passive human test

The frequency of sniffing and nibbling the person and the frequency of neighs did not significantly differ between groups.

3.3.4. Tactile sensitivity test

The number of times the horses responded to the von Frey filaments did not significantly differ between groups. No horses neighed during this short test.

3.3.5. Social isolation test

The control horses neighed and trotted significantly more frequently than isolated animals when they were separated from the audience horse (Mann–Whitney test: $U = 35$; $P = 0.01$, Fig. 6C; $U = 36$, $P = 0.01$, Fig. 6D, respectively). They also crossed significantly more sectors than the isolated animals ($U = 36$, $P = 0.02$, Fig. 6E).

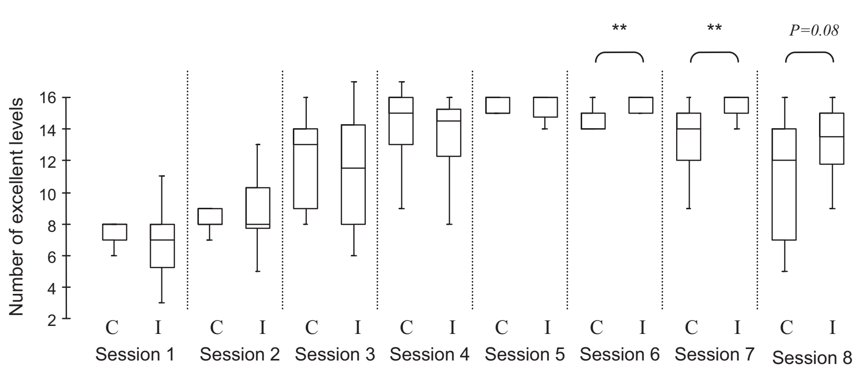

Fig. 5. Forwards–backwards task: median number of excellent levels (levels 0 + 1 + 2) performed during eight sessions by horses with conspecifics (C group) and by isolated horses (I group). $^{**}P \leq 0.01$; Mann–Whitney test.

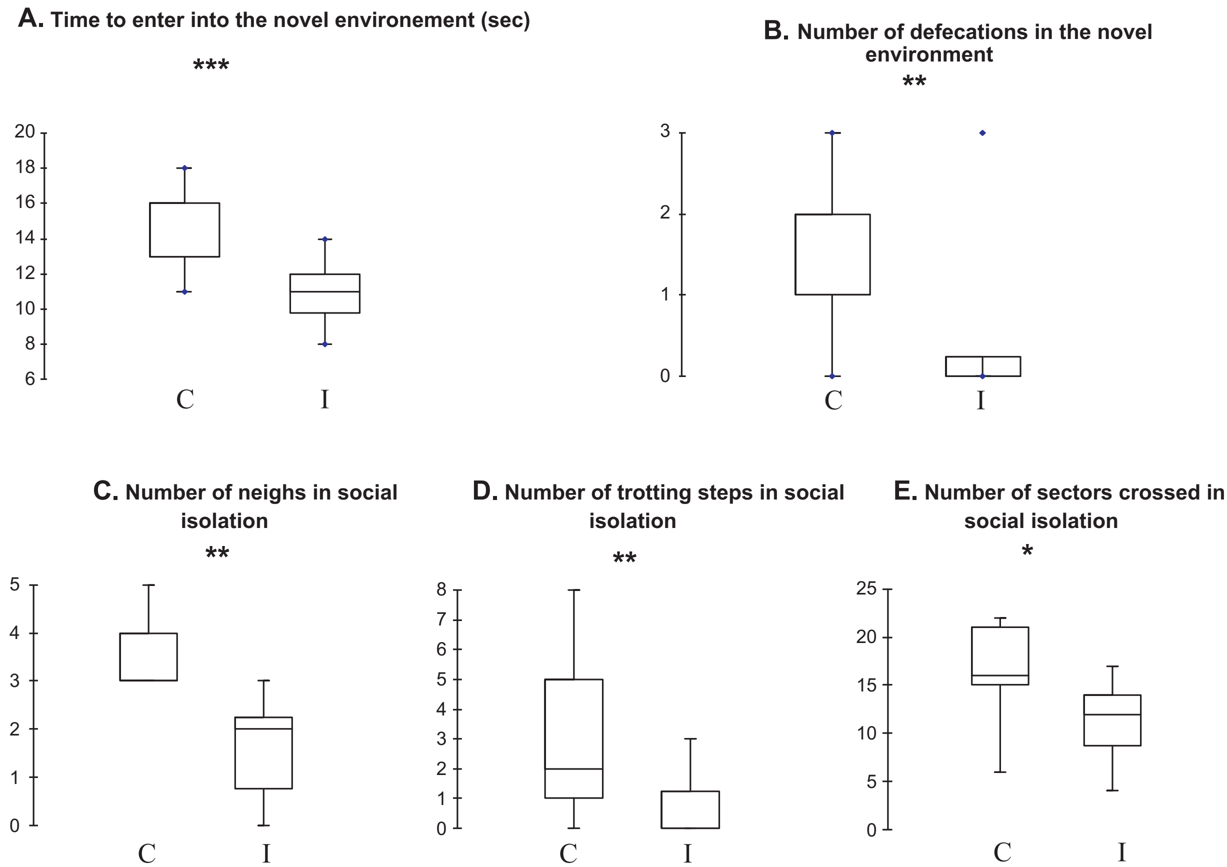

Fig. 6. Emotional reactivity of horses with conspecifics (C group) and of isolated horses (I group). Median (A) time to enter the novel environment; (B) number of defecations in the novel environment; (C) number of neighs in social isolation; (D) number of trotting steps in social isolation; (E) number of sectors crossed in social isolation. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; Mann–Whitney test.

3.3.6. Novel area test

The time taken by the horse to put one hoof on the carpet did not significantly differ between groups. There was no significant difference in the frequency of neighs.

3.3.7. Suddenness test

The proportion of horses that went back to the feed bucket after the surprise effect tended to be lower in the control group than in the isolated group (controls: 8 out of 13; isolated: 11 out of 12; proportion Z test: $Z = -1.76$, $P = 0.07$). No horses neighed during this test.

3.3.8. Locomotor activity

The number of sectors crossed by the horses during the reactivity tests did not significantly differ between groups.

4. Discussion

This is the first study to characterize the effects of a short isolation period on emotional reactivity and learning performance in yearlings, and two main results were obtained. We found that 11 days of isolation decreased emotional reactivity, as assessed by reactivity to separation, sound, suddenness and novelty, while learning performance improved, as evaluated by habituation to a novel object and by a forwards–backwards task.

4.1. A short period of isolation reduces emotional reactivity

Isolated horses reacted less than control horses in four out of seven tests measuring emotional reactivity: the novel environment test, the sound test, the social isolation test and the suddenness test. During the novel environment test, isolated horses entered the arena more quickly and defecated less frequently than controls. During the sound test, fewer isolated horses reacted than controls. During the isolation test, they neighed and trotted less frequently and crossed fewer sectors than the control horses. Finally, they tended to be less disturbed by the suddenness effect, more horses in the isolated group returning to the feed bucket after the umbrella had been shaken than in the control group.

Our results are in line with previous experiments describing a decrease in emotional reactivity after a period of isolation. For instance, in rodents, Gentsch et al. (1982) reported that isolation reduced emotional response to a novel situation. In ungulates, Purcell and Arave (1991) also found that calves reared alone were less stressed in a novel environment than those reared in a group. In horses, it has already been reported that single-housed horses display less restless behaviour, fewer vocalisations and more explorative behaviours in the Arena and Human Encounter test (Søndergaard and Halekoh, 2003; Søndergaard and Ladewig, 2004). Nevertheless, whether the effects obtained in the present study are as strong and long-lasting as those obtained after long-term social deprivation remains to be tested.

The lower reactivity of isolated horses in the social isolation test could first be related to the fact that they were used to being separated from their conspecifics. This lower reactivity to social separation could also explain the reduced reaction in the novel environment, sound and surprise tests. However, these tests were carried out in the presence of an audience animal, which would have limited the separation effect, even if we cannot be sure that individuals in the two groups perceived it in the same way.

Moreover, the controls did not neigh significantly more frequently than isolated horses during these tests, indicating that the differences were not only due to a reaction to separation. We therefore believe that differences between isolated and control horses are not just the consequence of lower reactivity to isolation, but also of lower reactivity to non-social stimulation, such as novelty and suddenness.

Secondly, the lower reactivity of isolated horses could also be due to apathy or learned helplessness, induced by the stress of isolation, as reported in several species reared alone (e.g. prairie voles: Grippo et al., 2007). However, the duration of the isolation period in the present experiment was shorter than those used in the studies reporting this type of phenomenon. Moreover, other behavioural parameters such as locomotor activity, sensory sensitivity and body weight were not affected by these 11 days of isolation, suggesting that isolated horses were not in a depressive-like state. However, further physiological measures, together with hedonism measures and cognitive bias tests (Mendl et al., 2009), are required to examine this issue further.

The most likely explanation for the reduced emotional reactivity of isolated horses is that isolation renders the individual less fearful. This decrease in fearfulness may be due to the fact that isolated animals are no longer under the influence of the group and habituate more efficiently to the stimuli of their environment. This hypothesis is supported by the results obtained during habituation to a novel object. In this test, horses from the isolated group were alone when exposed to the novel object, whereas horses from the control group were with their conspecifics. During five out of seven habituation sessions, horses in the isolated group took less time getting to their food which was close to the novel object than control horses did, and they spent more time near this object after the habituation procedure (on day 10). It is possible that horses living in a group perceive the stress of their conspecifics, which increases their own level of stress. A contagion of fear within groups has been described in ravens who approached a novel object faster when tested alone than when they were with a conspecific (Stöewe et al., 2006). In cattle, the state of stress can be perceived through olfactory cues contained in the urine of conspecifics, and can consequently become contagious (Boissy et al., 1998).

On the other hand, contradictory studies have reported that the presence of conspecifics could also encourage exploration of the environment through a social facilitation process (for reviews: Nicol, 1995, 2006) and inhibit stress responses through social buffering (for a review: Boissy, 1998). In fact, both processes (contagion of fear or facilitation process) are probably dependent on the social status and gender of individuals in the group and particularly of the demonstrators (for a review of the gender effect: Choleris and Kavaliers, 1999). In our experiment, the animals were yearlings, and the group effect may have been different in a group comprising animals of different ages.

4.2. *A short period of isolation improves learning*

The second aim of this study was to determine the effect of isolation on learning performance. In addition to greater habituation to a novel stimulus, isolation improved learning in the forwards–backwards task. In sessions 6 and 7, isolated horses achieved more excellent levels of performance than control horses, and this difference tended to be maintained during session 8, carried out under stressful conditions in a novel arena. They also tended to respond more frequently to a visual cue (level 0) than controls.

The better performance of isolated horses could be due to the effect of isolation on reactivity. As explained above, isolated horses had become less fearful and gregarious than the controls by the end of the experiment. They also neighed and defecated significantly less frequently during learning sessions, suggesting that they were less disturbed. According to Lansade and Simon (2010), less fearful horses perform this learning task better than more fearful animals. Thus, the better performance of isolated horses may be a consequence of the effect of isolation on reactivity. The positive influence of a low level of emotional reactivity or fearfulness on learning performance has been established in various species (e.g. passive avoidance in Japanese quail chicks: Richard et al., 2000; active avoidance and spatial learning in rats: Brush et al., 1985; Herrero et al., 2006; and in working dogs: Svartberg, 2002), including horses (instrumental conditioning tasks: Lindberg et al., 1999; Visser et al., 2003; spatial learning tasks: Heird et al., 1986; discriminative tasks: Fiske and Potter, 1979). However, Lansade and Simon (2010) found that less fearful horses performed better in the backwards–forwards task, but less well in an active avoidance task involving negative reinforcement. Thus, it is possible that isolation has a different influence on other types of learning.

Moreover, the first time horses are socially isolated could be considered as a period of reorganization associated with stress. According to Bateson (1979), this could result in animals being particularly sensitive to their environment. This specific sensitivity could make isolated animals more attentive to certain cues, and in particular to the commands given by the observer or handler. It is also consistent with findings that animals are susceptible to handling during the days following weaning (cattle: Boivin et al., 1992; goats: Boivin and Braastad, 1996; horses: Lansade et al., 2004), which is also a period of behavioural reorganization associated with stress and particular sensitivity to external stimuli.

For practical purposes, the period when yearlings are housed in individual loose boxes (e.g. after a sale, deworming, or when pasture is unavailable) can be an appropriate time to handle them, to teach them instrumental learning tasks and habituate them to new stimuli. This short period of isolation can reduce the anxiety associated with subsequent training, by habituating the horse to being away from conspecifics, and may improve welfare in the long term, but also the safety of handlers or riders. This could be particularly relevant for animals that are highly reactive and thus more likely to be slaughtered if no solution is found. However, maintaining horses in individual boxes for a long time is not recommended, because it can lead to restriction of the natural behavioural repertoire, to aggressive or stereotypic behaviour, and to impaired welfare (e.g. Christensen et al., 2002; Heleski et al., 2002; Kiley-Worthington, 1990). Thus, it is very important to establish the optimum duration of isolation needed to enhance subsequent training.

5. Conclusions

Our study shows for the first time that a short isolation period has a beneficial effect on emotional reactivity and learning abilities. In particular, isolation appears to make horses less fearful, thereby improving their ability to learn, which has relevance for horse training. However, further experiments are required to understand how isolation influences horses' responses and learning abilities and to determine whether these effects are long-term. It would also be interesting to extend these results to groups of horses of various ages, and to determine whether isolation is beneficial for other types of learning.

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