



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

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ARTICLE INFO

Article history:

Received 24 October 2011

Received in revised form 23 April 2012

Accepted 5 June 2012

Keywords:

Fearfulness
Habituation
Instrumental
Isolation
Memory
Training

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.

This article is part of a Special Issue entitled: insert SI title.

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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 \pm 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 \pm 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 \times 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 \times 7 m stall for the duration of the study. The stall size was in line with recommendations for the housing of horses [(2 \times 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 \times 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 \times 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 \times 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 \times 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

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