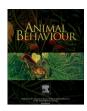
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Positive interactions lead to lasting positive memories in horses, *Equus caballus*

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Social relationships are important in social species. These relationships, based on repeated interactions, define each partner's expectations during the following encounters. The creation of a relationship implies high social cognitive abilities which require that each partner is able to associate the positive or negative content of an interaction with a specific partner and to recall this association. In this study, we tested the effects of repeated interactions on the memory kept by 23 young horses about humans, after 6 and 8 months of separation. The association of a reward with a learning task in an interactional context induced positive reactions towards humans during training. It also increased contact and interest, not only just after training, but also several months later, despite no further interaction with humans. In addition, this 'positive memory' of humans extended to novel persons. Overall, positive reinforcement enhanced learning and memorization of the task itself. These findings suggest remarkable social cognitive abilities that can be transposed from intraspecific to interspecific social contexts.

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The quality of social relationships within a group has an adaptive value (Silk 2007; Cameron et al. 2009). These relationships are built on repeated interactions, and are defined by each partner having expectations during subsequent encounters (Hinde 1979). Depending on the perceived valence (positive/negative) of the interactions, the relationship will range from confidence and reassurance to fear and stress, implying differential cerebral processing of the positive and negative emotions induced (Silberman & Weingartner 1986; Demaree et al. 2005). Therefore, the creation of a relationship implies high social cognitive abilities which require that each partner is able to associate the positive or negative emotional content of an interaction with a specific partner and to recall this association.

In the wild, horses generally live in family groups composed of an adult stallion, one to three unrelated mares and their young, although up to a third of groups may contain up to five stallions and up to 26 mares and their young (Feist & McCullough 1976; Linklater 2000; Boyd & Keiper 2005). The first social interactions occurring in a foal's life are with its dam, and often consist of feed-related interactions through the suckling behaviour. If some sort of interference occurs around that first suckling, the mother–young relationship may be impaired in the long term (Hausberger et al. 2007b). Thus, the first and strongest social bond in a horse's life, one uniting a foal with its dam, appears to be at least partly a feeding bond. The family group represents a rich social environment surrounding the young during its development (e.g. Boyd 1988; Feh 2005; Bourjade et al. 2009). Horses maintain long-term bonds with several members of their family group, but they also interact temporarily with members of other groups when forming herds (Waring 2003; Feh 2005). Equid social relationships are long lasting and, in some cases, lifelong (Linklater 2007).

In groups of horses, social preferences are expressed by a greater spatial proximity and frequent affiliative behaviours, such as mutual grooming (Clutton-Brock et al. 1976; Feh 1987; Sigurjónsdóttir et al. 2003; van Dierendonck et al. 2004). Opportunistic observations suggest that horses are able to recognize social partners (e.g. after a 1-year separation from their mares, stallions immediately distinguished and joined their former mates in a large herd with many other mares present; Feh 2005). Experimental studies have demonstrated that horses can distinguish the calls of nonkin group members from the calls of familiar individuals living

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in a neighbouring group and from the calls of strangers (Lemasson et al. 2009), and that they are even capable of cross-modal individual recognition (Proops et al. 2009). These recent findings suggest a process of social learning of vocal signatures and long-term memorization of the different degrees of social bonds maintained among horses (Lemasson et al. 2009; Proops et al. 2009).

It has been suggested that the social cognitive skills of horses to negotiate complex relationships may apply to human–animal interactions and relations (Kruger 2007; Linklater 2007; Murphy & Arkins 2007; Hausberger et al. 2008). However, while the memory of interactions is primarily associated with individual recognition, it has been shown in many species that in social contexts, the experience an animal has with a specific person may be generalized to other humans (poultry, *Gallus gallus*: Ghirlanda et al. 2002; pigs, *Sus scrofa*: Hemsworth et al. 1994, 1996; cattle, *Bos taurus*: Lensink et al. 2000; dogs, *Canis lupus familiaris*: Miklósi et al. 1998). In domestic horses, there is growing evidence that horses tend to show consistency in their reactions to different persons (Hausberger & Muller 2002; Henry et al. 2005; Lansade & Bouissou 2008; Fureix et al. 2009).

Positive human–animal relationships have often been used to investigate animal cognition (Davis 2002). Using a close relationship with their subjects, Boysen (1992) and Pepperberg (2000) have extended the boundaries of what is known about the mental abilities of chimpanzees, *Pan troglodytes*, and African grey parrots, *Psittacus erithacus*, respectively. However, once the relationship has become established, one may wonder what memory would be kept if the interactions were to cease and a prolonged separation were to occur.

Here, we used the human-horse relationship to investigate the ability of horses to build a relationship on the basis of short- and long-term memory of the interactions. Thus, we tested whether a series of positive interactions could create and influence the human-animal relationship in the long term, even after a prolonged separation. We used the horse training context which requires daily human-horse interactions, and provided food during the training sessions to make training a pleasurable experience for the animals. Horses' behaviour during training confirmed that the use of food rewards led them to perceive training sessions as positive interactions (see Results). We hypothesized that horses are able to build a bond and keep a long-term memory of the relationship, on the basis of repeated interactions. In addition, we tested whether horses, in this interspecific context, were also capable of building a general perception of humans on such a basis.

METHODS

Animals and Experimental Groups

We studied 20 Anglo-Arabian and three French Saddlebred horses (15 females and eight geldings). All horses were born and housed at the 'Station Expérimentale des Haras Nationaux' (SEHN), Chamberet, France. When 4-6 days old (in April-June 2006), foals were led with their dams to a large pasture where they stayed together until weaning at the age of 6 months. From weaning, horses were kept at pasture in randomly composed small groups (three groups of six and one group of five). Three weeks after weaning, they were pushed along fenced pathways into four large indoor stalls for winter; each weaning group was kept in an adjacent but separate stall. At the age of 7 ± 1 months, all males underwent a surgical castration procedure. Our training programme started in the spring when the horses were approximately 1-year old, and all yearlings were then released in a large pasture near the training arena. During the winter period following training, they were led back to their winter stables in randomly composed groups of five or six individuals. Before and during this experiment, additional human interventions were limited to hay distribution by caretakers twice a day during the winter periods.

Horses were randomly assigned to two treatment groups according to their sex and sire: horses trained with positive reinforcement (PR; N=11) and controls, trained with no reinforcement (C; N=12). The positive reinforcement was a food reward, which consisted of a few hand-given grain pellets. As the experiment was conducted in spring and summer, horses from both groups were kept together in a large pasture and were not given any supplementary feed.

Learning and Remembering

Training procedure

The aim of our training programme was to train yearlings to remain immobile without being held in response to a simple vocal command: 'reste!' (i.e. French for 'stay!'). Once this was achieved, the horses underwent various handling or veterinary procedures using the same vocal command. All horses were trained until completion of the training programme (Table 1). Before the start of training, all yearlings were habituated to being haltered and led in hand. Rewards were never used during this initial habituation.

Horses were trained 5 min/day, 5 days per week. During the two remaining days, they all stayed together in a pasture. On training days, the horses were pushed along fenced pathways from their pasture to a large indoor pen. They were then individually allowed to enter an adjacent training arena ($10.5 \times 15 \text{ m}$), separated from the pen by large doors, which prevented the horses from touching or seeing each other during training. During this experiment, horses would spontaneously approach the doors leading to the training arena and the experimenter only had to open the doors and let one horse in at a time. Training was performed by a single experimenter (woman, long dark hair). She dressed differently every day but always wore the same green coat. At the beginning of each training session, the experimenter opened the door of the training arena and waited for one horse to enter spontaneously. It was haltered and a lead rope was attached. Training then began for a 5 min session, after which the horse was released in an outdoor pen. At the end of the day, all the horses were released in their pasture.

During all training sessions, the horses' behaviour was continuously recorded using a digital voice recorder equipped with a microphone, to verify whether horses perceived positive reinforcement training as a positive interaction (e.g. positive/affiliative behaviours: sniffing, licking; versus negative/defence behaviours: biting, kicking or 'falling down' on the experimenter, which consists of the horse leaning heavily against, and letting itself fall down on, the experimenter while she was picking up a foot).

The experimental training programme involved learning to remain immobile in response to a vocal command and then accepting various handling or veterinary procedures. Training was divided into steps of increasing complexity as described in Table 1. The horse had to fulfil the performance criterion of each step to get to the next one, that is, it had to succeed three times consecutively in the given step. For example, a horse had to remain immobile on order while the experimenter brushed its whole body three times (trials) consecutively (step 12), before moving on to the next handling procedure (feet picking: steps 13-21). Horses were not limited in the number of steps they could complete successfully within a training session, which simply ended after 5 min. Each time they remained immobile for the required duration (steps 1–6) and accepted the handling procedure (steps 7–41), horses of the PR group received hand-given pellets as a reward and carried on with the training programme, whereas horses from the C group were

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