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Encoding the Object Position for Assessment of Short Term Spatial Memory in Horses (*Equus caballus*)

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In this study, the detour problem was combined with the classic delayed-response task to investigate equine short-term spatial memory. Test subjects were eight female horses, divided into two groups (A and B) of four subjects each. The motivating object was made to move and disappear behind one of two identical obstacles in a two-point-choice apparatus. After a 10 s (Group A) or 30 s (Group B) delay the animal was released to seek the object. Both groups made more correct (14.8 ± 1.3 for Group A and 13.5 ± 3.1 for Group B, mean ± SD) than incorrect choices (5.3 ± 1.3 for Group A and 6.5 ± 3.1 for Group B, mean ± SD) and the performance of each group was significantly above chance level (z = 4.14, p = 0.000, for Group A and z = 3.02, p = 0.002, for Group B). Therefore, tested animals were able to recover the object by approaching the correct obstacle after 10 s or 30 s delays, showing that they had encoded and recovered from memory the existence of the target object and its location.

The ability to navigate around obstacles has been investigated extensively in the study of animal cognition by using an obstacle placed between the target object and the animal (Zucca, Antonelli, & Vallortigara, 2005), already described in horses (Baragli, Paoletti, Vitale, Sighieri, & Reddon, 2011). The "detour task" requires the animal to distance itself from the target object while detouring around the obstacle in order to reach it (Wynne & Laguet, 2004). The use of opaque obstacles (in which the object disappears from the animal's view) requires animals to maintain a sort of "memory" of the location of the disappeared object (Zucca, Antonelli, & Vallortigara, 2005). This is of particular interest for comparative cognitive research as a natural example of "delayed response" (Zucca, Antonelli, & Vallortigara, 2005).

Therefore, detour tests are often combined with the classic delayed-response task to compare the duration of short-term memory in different species (Hunter, 1913; cited in Vallortigara, Regolin, Rigoni, & Zanforlin, 1998). This paradigm has been used to assess short term memory in dogs (Fiset, Beaulieu, & Landry, 2003), cats (Fiset & Doré, 2006) and domestic chicks (Regolin, Rugani, Pagni, & Vallortigara, 2005; Vallortigara et al., 1998).

In recent years, several scientific papers have highlighted the cognitive abilities of horses. Horses can distinguish the social affiliation and social rank of their conspecifics (Krueger & Heinze, 2008) and they take into account the social rank of conspecifics to decide their feeding strategy (Krueger & Flauger, 2008). Moreover, horses appear to comprehend some human pointing gestures (Maros, Gácsi & Miklósi, 2008) and follow some human-given cues (Krueger, Flauger,

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Farmer, & Maros, 2010; Proops & McComb, 2010). Horses' ability to "learn to learn" was reported in several studies (Bear, Potter, & Friend, 1984; Fiske & Potter, 1979; McCall, Potter, & Friend, 1981) and discrimination and categorization learning has also been demonstrated in horses (Dixon, 1970; Flannery, 1997; Gardner, 1937; Hanggi, 1999, 2003; Mader & Price, 1980); one study reports that horses are able to remember categories and concepts for several years (Hanggi & Ingersoll, 2009).

Nicol (2002) states that horses are said to have excellent memories, as indicated by the fact that horses remember a maze which they have learnt to criterion after an interval of 1 week (Marinier & Alexander, 1994) and that horses that have acquired an operant response perform the same response after an interval of 1 month (Wolff & Hausberger, 1996).

However, specific studies on equine short-term memory are limited and contradictory. McLean (2004) was able to train twelve horses to approach food using a two-choice task with immediate release, but subjects could not perform above chance level when a 10 s delay was interposed between observation of food being dispensed and release of the subject. Therefore, the author suggested that short-term memory in the horse is severely limited in duration. These results also differ from those of Murphy (2009) in which eight horses performed above chance level with a 12 s delay. More recently Hanggi (2010) investigated whether four horses could recall the location of a food objective in a two-choice delayed response test finding that one horse was able to recover food after 20 s and another did so after 30 s. All these authors (Hanggi, 2010; McLean, 2004; Murphy, 2009) used the same animals to check different delays. Donkeys seem to be capable of finding the correct location of a hidden object after a 30 s delay, without having been tested with minor delays (Baragli, Paoletti, Vitale, & Sighieri, 2011).

The aim of this study was to investigate equine short-term spatial memory by the use of delayed responses when horses detour an opaque obstacle, avoiding testing the same subjects with different delays.

Method

Subjects

Test subjects comprised eight female horses of mixed breed (age 9.3 ± 3.5 years) that were randomly divided in two groups (Group A and Group B) of four subjects each. The horses were used as receivers in the embryo transfer program of the Department of Veterinary Clinical Sciences (University of Pisa, Italy) and were kept in a paddock (75 x 75 m), with *ad libitum* hay and water.

Materials and Procedure

The study was divided into two phases (Pre-Test and Spatial Memory test) and took place in a square enclosure. Initially, the horses' ability to resolve a detour problem (Pre-Test, PT) with an opaque obstacle was evaluated. The obstacle, made of wood shaving bales (consisting of a long side, with two oblique sides at 45° angles with respect to the long side), was placed in the middle of the testing area. Halfway along the long side of the obstacle, at ground level, an opening allowed the target object (food) to be pulled through and disappear toward the outer part of the obstacle. The food (300 g of cereal flakes) was put in a white bucket and placed on a square trolley that could be pulled by a brown rope fastened to the trolley. In the PT the animal was brought to stand in front of the obstacle, where the experimenter held it by the halter. The bucket of food on the trolley was directly in front of the animal. The horse was allowed to eat a small amount of food from the bucket, and as it was eating a second experimenter (located outside the enclosure behind the wooden panel) slowly pulled the trolley from the other side of the obstacle. Thus the food bucket "disappeared," after which the animal was freed. Every time the horse arrived at the food behind the obstacle, the test was considered to have been completed successfully. To reinforce this behavior, the animal was permitted to eat some food from the bucket on the other side of the obstacle. If the subject did not perform the detour within 5 min the test was considered unsuccessful. The PT ended when the animal regained the food bucket in three-out of five trials. This was the criterion for each animal to be admitted to the Spatial Memory test (SMt) in which the motivating object was made to move and disappear behind one of two identical obstacles while the animal watched. In the Pre-Test the trolley was pulled directly through the only obstacle, while in the Spatial Memory test it was necessary to use two pulleys since the angle of the two obstacles was not in line with the experimenter behind the wooden panel.

In the SMt, two obstacles identical to the previous ones in shape, size and functional characteristics were placed in the middle of the testing area (Fig. 1). Behind each obstacle, a wooden box was placed against the aperture through which the trolley passed; the part resting against the obstacle was open, so that the trolley with the food bucket disappeared inside the box. The animal was led to the starting area and held by the halter by two experimenters (one on the left and one on the right). A third person offered the subject the food bucket, then drew it away taking care that the bucket remained in sight of the animal; he then placed it on the trolley, 3 m in front of the starting area. From outside the enclosure, another experimenter pulled the trolley with the bucket behind one of the two obstacles. After the bucket disappeared, the horse remained in the starting area, held by the two experimenters for either 10 s (Group A) or 30 s (Group B), after which it was released. When the horse was released the experimenters immediately left the enclosure. The test ended when the animal went around one of the two obstacles in search of food.

The SMt comprised a total of 20 trials divided into five sessions of four trials each. Each day a single session of trials was administered to each horse. The obstacle behind which the food bucket disappeared varied from trial to trial following a semi-random sequence (Fellows, 1967). Each trial ended when the animal detoured either of the two obstacles (i.e. when the animal turned its head towards the back of the obstacle, once it had walked past it). Group A performed both PT and SMt (with a 10 s delay) first, to verify that the procedure was adequate for the study of spatial memory. After this Group B performed the same protocol with a 30 s delay in the SMt. The 30 s delay period was chosen arbitrarily since previous data (Murphy, 2009) had reported that horses are able to recover food after a 12 s delay. We used a different group of animals to check different delays following the criterion of Regolin et al. (2005).

Differently from the PT, this time the animal could not eat food directly from the bucket, neither behind the correct nor the wrong obstacle, since in this phase the bucket disappeared into the wooden box behind each obstacle. Variable reinforcement was used and one cereal biscuit was put on a plastic plate on each wooden box, so the biscuit could be found behind both the correct and the wrong obstacles. The presence of the biscuit on the wooden box was determined by a coin toss (yes or no) but not more than twice in succession. This method of reinforcement was chosen in order to maintain the horse's motivation for seeking the bucket, and to avoid conditioned responses (learning effect).

Whether the choice was correct or wrong, the test was considered valid if the subject went around the obstacle within 5 min. In the SMt inside the wooden box located behind the wrong obstacle there was an identical bucket containing the same amount of food present in the bucket on the trolley. Moreover, the experimenter closed the bucket with an airtight cover the moment it was placed on the trolley. These strategies ensured that the horse would not be guided by its sense of smell toward the correct choice. At the end of each trial, the animal was led out of the enclosure and kept for 5 min in an area where it could not see the apparatus being repositioned for the subsequent trial.

Altogether, eight people took part in the experiment; seven were trained to carry out the tasks necessary for executing both phases and they were rotated in the required positions so that no-one covered the same position for more than 1 day. The eighth person pulled the trolley behind the obstacle and was also trained in direct evaluation of the results (reported on a paper data sheet).

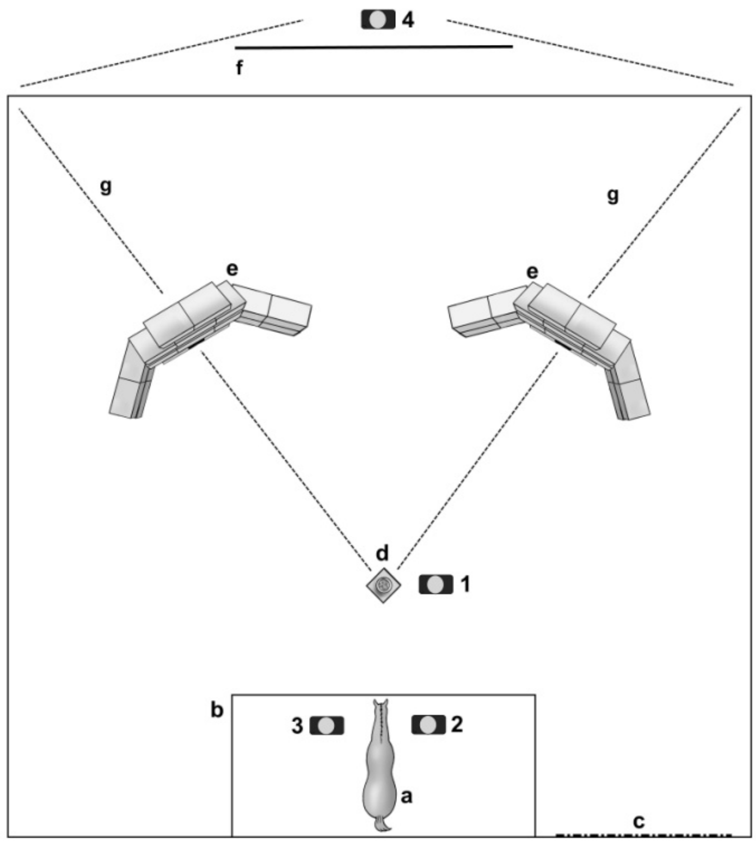

Figure 1. Apparatus in the Spatial Memory test: a) starting position of the horse, b) starting area, c) entrance gate, d) trolley with food bucket, e) obstacle, f) wooden panel, g) rope to pull trolley from opposite part of obstacle which was moved from one obstacle to the other during re-setting of the apparatus after each test. Distance d-b: at least 3 m and d-e: 5 m. In this phase there were four experimenters: 1) took bucket from trolley, approached horse, let it taste food, replaced bucket on trolley, 2) and 3) held horse by the halter, one on each side, 4) pulled trolley with food bucket.

Statistical Analysis

The number of correct vs incorrect choices was considered for each animal and for the two groups, and analyzed with a two-tailed Binomial test. The effects of groups, sessions and group/session interaction, and differences in performance over sessions were verified by the ANOVA tests. Data analysis was conducted using the SPSS® 17.0 statistical package (SPSS, Chicago, IL, USA).

Results

In the Pre-Test, horses of both groups met criteria by achieving three out of five of the detours; therefore all animals were admitted to the SMt. In the SMt both groups made more correct (14.8 ± 1.3 for Group A and 13.5 ± 3.1 for Group B, mean ± SD) than incorrect choices (5.3 ± 1.3 for Group A and 6.5 ± 3.1 for Group B, mean ± SD) and the performance of each group was significantly above chance level (see Table 1 for details). The two-way ANOVA reported no differences between groups (F(1, 39) = 0.7, p = 0.409), sessions (F(4, 39) = 1.4, p = 0.244) and the group/session interaction (F(4, 39) = 0.4, p = 0.842). The one-way ANOVA over the five sessions of trials also showed a non-significant effect of the trial sessions for both Group A (F(4, 15) = 0.87, p = .0503) and Group B (F(4, 15) = 0.92, p = 0.476).

Table 1
Number of correct and incorrect choices for both groups of horses.

Animal	Correct trials	Incorrect trials	Two-tailed Binomial Test
Group A (10-s delay)			
Horse 1	15	5	z = 2.01, p = 0.041
Horse 2	16	4	z = 2.46, p = 0.012
Horse 3	15	5	z = 2.01, p = 0.041
Horse 4	13	7	z = 1.12, p = 0.263
Total	**59**	**21**	**z = 4.14, *p* = 0.000**
Group B (30-s delay)			
Horse 1	15	5	z = 2.01, p = 0.041
Horse 2	14	6	z = 1.57, p = 0.115
Horse 3	16	4	z = 2.46, p = 0.012
Horse 4	9	11	z = - 0.22, p = 0.823
Total	**54**	**26**	**z = 3.02, *p* = 0.002**

Note: The number of correct and incorrect choices over total trials and the binomial test results are reported for Group A (10-s delay) and Group B (30-s delay).

Discussion

In this study we evaluated eight female horses for short-term spatial memory. In the test, the object disappeared behind one of two obstacles in front of the animal, and it had to remember behind which of the two obstacles the object had disappeared. Results during the Spatial Memory test suggested that horses had encoded and correctly recovered from their memory the spatial location of the hidden object.

If the horses had been able to understand the criterion of the task, follow the attractor’s smell or read the inadvertent cues of experimenters, their performance would have improved as the tasks progressed. However, no statistical difference was revealed over the progression of sessions, suggesting that no incremental or degradation learning effect was present. This may reflect the strategy of reinforcement used and also the strategy used to prevent the possible use of smell. Moreover, personnel were moved around frequently between trials, and it may have been difficult for horses to learn any inadvertent cues of experimenters. It appears that learning did not influence results, so we may hypothesize that our horses used their memory to recover the target object. Previous investigations on equine short-term memory (Hanggi, 2010; McLean, 2004; Murphy, 2009) did not check for a possible learning effect in the study groups. Therefore, authors cannot be sure that their horses solved the task solely by use of memory.

As indicated in the introduction section, several papers have reported that horses possess various cognitive skills (Baragli, Paoletti, Vitale, Sighieri, & Reddon, 2011; Hanggi & Ingersoll, 2009; Krueger et al., 2010; Krueger & Heinze, 2008; Mader & Price, 1980; Maros et al., 2008; McCall, Potter, & Friend, 1981; Proops & McComb, 2010; Proops, McComb, & Reby, 2009). These indications suggest that horses are indeed endowed with sophisticated and complex cognitive abilities. Therefore, it was surprising when recent research on short-term spatial memory suggested that horses may not have this memory perspective (McLean, 2004) or that they have a limited, but *trainable*, short-term memory capacity (Murphy, 2009). Negative or unexpected results, such as those reported by McLean (2004), could be due to the use of experimental methods that do not adequately take into consideration the horse's sensorial or neural capacities, or motivational factors (Hothersall & Nicol, 2007). This point is also underlined by the work of Martin, Zentall, & Lawrence (2006) in which the authors suggest that horses are capable of "learning to learn" but this ability depends on the type and method of stimulus administered. In another study it was observed that the horses performed better when a stimulus was presented at ground level and that performance decreased when the stimulus was at the height of 70 cm (Hall, Cassaday, & Derrington, 2003). Moreover, moving stimuli attract an animal's attention more easily than do motionless stimuli (see for review Washburn & Taglialtela, 2005).

Our data indicate that horses can retain a spatial memory for visual information for at least 30 s, like other animal species (Baragli, Paoletti, Vitale, & Sighieri, 2011; Fiset, Beaulieu, & Landry, 2003; Fiset & Doré, 2006; Regolin et al., 2005). This may help resolve the current controversy in the literature on equine short-term memory; even further studies are needed to define the retention limits of equine short-term memory.

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