



Is the left forelimb preference indicative of a stressful situation in horses?



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ABSTRACT

Evidence for behavioural and brain lateralisation is now widespread among the animal kingdom; lateralisation of limb use (pawedness) occurs in several mammals including both feral and domestic horses.

We investigated limb preferences in 14 Quarter Horse during different motor tasks (walking, stepping on and off a step, truck loading and unloading). Population lateralisation was observed in two tasks: horses preferentially used their left forelimb during truck loading and stepping off a step. The results also revealed that horses showed higher scores for anxious behaviours during truck loading suggesting that the use of the left forelimb in this task may reflect the main role of the right hemisphere in control of behaviour during stressful situation.

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1. Introduction

Limb preference has been reported in vertebrates (Vallortigara et al., 1999; Bisazza et al., 1998) including several mammals during different tasks (Rogers et al., 2013; Rogers and Andrew, 2002). Since each limb is controlled by the opposite brain hemisphere, limb preferences bias in vertebrates may indicate preferences to use the hemisphere opposite the preferred limb (Rogers, 2009; Vallortigara et al., 2011; Macneilage et al., 2009). In primates, the selective activation of one hemisphere is required to perform particular tasks, as for example the use of the right hemisphere/left-hand during difficult spatial tasks (Rogers et al., 2013). On the other side, limb preferences shown during simple tasks (i.e. tasks which can be performed easily and readily by either the right or the left hemisphere/limb) may reflect an individual's preference to activate a particular hemisphere, and hence to express a particular set of behaviour (Rogers et al., 2013). In non-human primates, for example, marmosets (*Callithrix jacchus*), which use preferentially their left hand to pickup pieces of food from the floor, are more fearful of novel environments (Cameron and Rogers, 1999) and fear-inducing stimuli (Gordon and Rogers, 2010).

In mammals, weaker paw preferences in tasks that required dogs to stabilise a container while licking food from it was associated to a strong stress response to certain sounds like thunderstorms (Branson and Rogers, 2006); dogs with no significant paw preferences to wipe a piece of tape from the nose also displayed higher levels of one of the stress hormones, adrenaline, some days after immunostimulation (Siniscalchi et al., 2010a).

Hemispheric specialisations in horses have been shown at both motor and sensory levels (McGreevy and Rogers, 2005; Austin and Rogers, 2012). Limb preferences have been reported in different breeds: in particular, a right bias for the leading limb used in initiation of galloping was observed in a large number of racehorses of various breeds at the population level (90%) (Williams and Norris, 2007). In addition, thoroughbred-racing horses showed a population bias to place the left forelimb in front of the other forelimb during grazing (McGreevy and Rogers, 2005). These two measures of limb preference appear to be independent of each other since Wells and Blache (2008) found no association between forelimb preference while grazing and limb preference while cantering. On the other hand in feral horses, Austin and Rogers (2012) showed no population preferences to place one forelimb in front of the other during grazing, stressing the hypothesis that limb preference in domestic horses may be entrained by human handling. Also similar to feral horses, the Przewalski horses (the closest undomesticated relative of *Equus caballus*) showed no significant forelimb preference (Austin and Rogers, 2014). In line with this work, McGreevy and Thomson (2005) found that standard bred trotting horses, which are handled on both sides, show a weaker leftwards

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population bias of forelimb preference while grazing than thoroughbreds, and Quarter Horses trained for cutting, which requires equal agility on the left and right sides, show no bias for the same measure. In addition, Wells and Blache (2008) reported a right population bias for forelimb preference while grazing in older horses (that had previously been ridden), whereas that was not the case for young horses (that had never been ridden).

Since motor laterality can be task-dependent and limb preference is associated with reactivity in different species (summarised by Rogers, 2009), here we investigated possible lateralisation in the first limb use in 14 Quarter Horse during different motor tasks (walking, stepping-off, stepping-on, truck loading and unloading) and its association with behavioural response (i.e. reactivity).

2. Materials and methods

2.1. Animals, management and experimental design

Fourteen Quarter Horse, homogeneous for live weight (500 ± 25 kg), body condition score (3 ± 0.25 arbitrary units; Martin-Rosset, 1990) and age (6 ± 1 years), were recruited in this study. All horses were in good health status and each had been handled and trained for reining competitions. The research was carried out in a horses-stall in Corato (Italy), horses were housed in a “open air” stable which contained 20 single stalls (10.5 m^2) in two rows, with a central aisle (3 m wide). All horses were kept in these individual stalls with constant access to water and were fed three times a day with commercial feed and locally grown oat hay. The amount of feed, feeding quality, and type remained constant over the experiment. Animals had been tamed under Italian tradition way at 2 years old and handled by their left side.

All horses were trained by the same person, and performed regular and light physical exercises during the whole study. They were accustomed to loading into a truck, travelling and unloading and they usually travelled at least once monthly to reach a competition or a trekking during spring and summer, but during the experiment they did not travel anywhere.

For the first part of the research, to study the Laterality Index of the horses, we filmed them during walking, stepping on and off a step. For walking, when the horse's forelimbs were level, the trainer (who was on the left side of the horse) would gently lead the horse with a load rope to move forward; the horse started walking 10 times on the central stall aisle in the straight on direction and the first forelimb lifted by the horse to move forward from a standing position was recorded. For the stepping-on and -off tasks, the same operator led subjects to step-on and off a step of 20 cm height. Horses were stopped at approximately 20 cm of distance of the step in order to achieve the starting position (i.e. the horse's forelimbs were level) and then invited to move on. The first forelimb lifted by the horse to step-on and -off from the starting position was recorded. All these events took part on the same day.

For the second part of the research, each horse, after been placed in the starting position (about 20 cm from the increasing truck ramp) was loaded into the truck by the trainer who was on the left side of the horse. Then the horse was positioned in the corner of the truck, with his head and tail towards the truck walls, his left side towards the inside of the truck and his right side towards the ramp. The horse was secured by the “log and rope” method and left one minute inside alone; see Mansmann and Woodie (1995). After 1 min, the trainer came into the truck, tied off and conducted the horse from the left side in the starting position of the climbing off task (20 cm before the decreasing ramp start). After the unloading, the horse was conducted into its own box.

Horses were tested once a week until a set of 10 trials \times each horse was collected.

Since horses had been tamed and handled during training and their daily life activities by their left side, the position of the trainer respect to the horse was not alternate during the whole experiment in order not to produce an alteration of the arousal state of the animals due to the fact of being worked on the side they have not been accustomed (i.e. their right side).

2.2. Heart rate

Heart rate (HR) was measured when horses were at rest in their stalls (Time I), after the loading into the truck (Time II) and after the unloading (Time III). Heart rate was measured by the trainer using a heart-rate monitor (Polar® T31) placing the Polar transmitter on the horse's coat at the level of the heart area.

2.3. Behavioural parameters

Videos of truck loading, standing into the truck and truck unloading (T0, T1, T2, respectively) were examined through a behavioural sampling ethogram and the frequency of typical stressor behaviours (Harewood and McGowan, 2005) were noted by eight trained observers who were blind to the testing paradigm. The adding of the single occurrences was calculating to obtain a final behavioural score (BS) (see Table 1).

2.4. Forelimb preference

First, a % response index (%Res) for the forelimb usage of each horse to get on the truck was calculated for all of the 10 trials, using the formula $\%Res = (L + R/L + R + N) \times 100$, where L and R signify respectively the number of left and right forelimb uses, and N signifies “no response” (i.e. if the horse did not get on the truck within 5 min after it was positioned in the starting position).

Lateral asymmetries in the use of the first forelimb during different tasks were than computed using two Indices:

$LI = (L - R/L + R)$, where L and R indicate, respectively, the number of times in which each horse used the left or the right forelimb as the first during different tasks

$LI_{(trials)} = (L - R/L + R)$, where L and R indicate respectively the number of left and right forelimb used as first of the total sample (14 horses) during every single trial.

For both indexes a score of 1.0 indicated exclusive use of the left forelimb as first and a score of -1.0 indicated exclusive use of the right forelimb as first. An LI score of 0 indicated equal numbers of

Table 1

Behavioural sampling ethogram using for noting down the occurrence of choose behavioural patterns.

Behaviour
Defecation
Urination
Head tossing
Sound emission
Licking/chewing
Explorative behaviour
Freezing
Stopping
Pawing
Jumping
Tail movement
Biting
Flight response
Shivering
Backward walking
Sliding
Back limb beating
Kicking

Modified from Harewood and McGowan (2005).

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