Hucul horses’ learning abilities in different learning tests and the association with behaviour, food motivation and fearfulness

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ABSTRACT

Research on learning in domestic animals is often based on food-rewarded tasks. It is still unclear however, how much intrinsic factors such as food motivation and fearfulness influence performance in food-rewarded operant tasks. This study aimed to investigate the association between learning ability in different tests, food motivation and fearfulness. Twenty-three young, naive Hucal horses were tested. The learning tests included a negative reinforcement test (NR), where the horses were trained to yield to rope and hand pressure; a clicker test (CT), where the horses were taught to follow a target for a food reward; and a visual discrimination test (VD), where the horses had to learn to recognise a correct bucket (containing food). Food motivation was estimated through a free feeding test, where the latency to finish eating a standard meal was measured. In the fear test, a plastic bag was waved in front of the horse in a standardised manner to induce a startle response. The escape distance and latency to resume eating was measured. Further, heart rate (bmp) and salivary cortisol (ng/ml) were measured before and after each learning test and the fear test. All horses reached the learning criterion in the learning tests (number of sessions to reach criterion (mean ± SD): NR 1.87 ± 0.63; CT 1.74 ± 0.96; VD 5.00 ± 1.71). No correlations were found in learning performance between the different learning tests, and food motivation did not appear to influence the speed of learning. Behavioural reactions in the fear test correlated moderately to performance in the CT test (e.g. sessions to reach criterion and escape distance, r = 0.43, P = 0.04), suggesting that fearfulness may affect performance in some types of learning tests. In line with previous studies, we conclude that learning performance differed between the various types of tests. We further conclude that food motivation – measured as latency to consume a meal – did not influence performance in the food-rewarded tests. This may suggest either that the small food rewards delivered in the tests triggered food motivation in all horses regardless of general food motivation; that our test was inappropriate for assessment of food motivation; or that other intrinsic and extrinsic factors govern performance in learning tests.

1. Introduction

A horse’s learning ability is one of the most important characteristics that define its usefulness to humans (Heird et al., 1981; Olczak et al., 2016). However, both intrinsic and extrinsic factors may influence learning performance. This is important for horse professionals as well as scientists to acknowledge to optimise performance and produce reliable research results. For example, learning can be influenced by life experiences (Heird et al., 1981; Lansade et al., 2012) and temperamental traits, e.g. more fearful individuals were found to perform at a lower level (Christensen et al., 2012; Valenchon et al., 2013b). Furthermore, it has been suggested that learning is task dependent as no correlations were found in the previous literature between tests that use disparate types of reinforcement: positive reinforcement (PR) vs negative reinforcement (NR) (e.g., Christensen et al., 2021; Lansade and Simon, 2010; Visser et al., 2003), and evaluations of learning performance should preferably be based on more than one test. In addition, some studies found that PR may enhance learning. For example, Hendriksen et al. (2011) horses were trained for trailer loading and it was shown that horses trained with PR and a target learned faster and exhibited less stress responses than horses trained with NR. Further, learning a new, potentially frightening task with combined reinforcement (NR + PR)
may result in calmer animals (Heleski et al., 2008) or a higher level of exploratory behaviour (Innes and McBride, 2008) when compared with NR. However, no differences were found in the speed of learning between groups (Heleski et al., 2008; Innes and McBride, 2008).

It has also been observed that motivation may be disrupted if horses face a too long delay in the access to the reward (Ahrendt et al., 2012; Valenchon et al., 2013a). In this study, we exposed horses to three different types of tests; one based on PR where the horses were to learn to follow a target; one based on NR where the horses were to learn to yield to rope and hand pressure (both tests have previously been used for horses, e.g. Christensen et al., 2012); and a PR-based visual discrimination test, which has previously been used to measure learning in a range of species (e.g. goats: Langbein et al., 2006; horses: Christensen et al., 2021).

As motivation is a key intrinsic factor during learning, it is important to understand how horses’ motivation affects their performance in various learning tests. Food is the most commonly used reinforcer in experimental studies on horses and during training based on positive reinforcement (e.g. clicker training), and food motivation was suggested as one of the factors that might have influenced learning in previous studies (Valenchon et al., 2013a; Lindberg et al., 1999). To the authors’ knowledge, no studies have investigated the association between food motivation and learning in horses. Food motivation can be assessed in various ways and was found to correlate across different methods by Olczak et al. (2018). Accordingly, a free feeding test, where horses’ latency to consume a meal is recorded, was suggested as the simplest method to estimate food motivation in horses (Olczak et al., 2018).

Attractiveness of a reward also influences an individual’s motivation to work for it (Lansade et al., 2013). For example, in Ninomiya et al. (2007), horses were trained to push a button for a food reward. It was reported that a change in the type of reward from hay to pelleted feed increased operant responses, and a reversed change from pelleted feed to hay significantly decreased the number of rewards obtained by the horses. Due to this variety of factors influencing horses’ performance in learning tests, it is important to investigate the various intrinsic and extrinsic factors that act on learning performance in interplay with each other.

In this study, we aimed to measure different types of learning in horses and the association to food motivation, fearfulness as well as behavioural and physiological reactions during testing. We had the following specific aims and hypotheses:

1. To investigate associations in performance variables between three different learning tests. The hypothesis was that there is no association between tests because they measure different types of learning.
2. To assess food motivation and investigate its association to performance in food-rewarded tests. We hypothesised that highly food-motivated horses would perform better in learning tests with food as a reward.
3. To measure fear responses towards a sudden stimulus and investigate the association to learning performance. We hypothesised that more fearful horses would have reduced learning performance and show more nervous behaviour and higher physiological reactions indicating stress during the learning tests.

2. Methodology

The experiment was carried out in The National Research Institute of Animal Production with approval of local Ethics Committee on Animal Experimentation KRA1_188_2015.

2.1. Horses, environment, handling

Twenty-five naive Hucul horses (13 fillies and 12 colts, aged 15–26 months) that were familiar with people only from basic management were initially included in the experiment. Two fillies were later excluded due to strong separation anxiety, i.e., n = 23. All horses belonged to The National Research Institute of Animal Production and were born and kept in Institute’s Odrzechowa Experimental Station. Fillies and colts were kept separately.

Water and hay were available ad libitum both in the stable and in the paddock, and oats were fed individually once a day after testing, according to the routine in the stud. Colts were always tested between 8:00 am and 2:00 pm and fillies between 2:30 pm and 6:30 pm. Colts were kept in group boxes, and were released on the paddock after testing, for minimum 8 h. Fillies were on the paddocks before testing from 7:30 am to 2:00 pm. They were kept in a group tie stall (i.e. 2–4 horses were tied up next to each other).

Basic management involved feeding, tying up, and moving of groups between paddocks and stables. The horses received regular veterinary checks, hoof care and were regularly checked for body condition by a zoo-technician. The horses were not trained to respond to light human-given lead-rope signals.

2.2. Experimental setup

The horses were exposed to three learning tests and one fear test. In addition, three food motivation tests were conducted, and the results published in Olczak et al. (2018). As we were interested in correlations, the test order was initially drawn at random and then carried out in the same order for each horse to minimise the influence of carry-over effects (e.g. if one test affects responses in the next). The tests lasted 1–5 days each, and each horse was tested once per day. The testing period took 3 months in total, with 3–4 days break between tests. The order of habituation and testing was: (1) habituation to the trainer and equipment, (2) negative reinforcement learning test, (3) habituation to outdoor test arena, (4) food motivation lever test (Olczak et al., 2018), (5) clicker training test, (6) food motivation jumping test (Olczak et al., 2018), (7) visual discrimination test, (8) free feeding test, (9) fear test (to avoid escalation of fear this test was planned as the final one).

Each horse was tested at the same time every day (± 20 min) to account for the daily rhythm in e.g. cortisol secretion and potential fluctuations in food motivation.

2.2.1. Food motivation

Three different attempts to measure food motivation in the horses were conducted (two operant tests – lever pressing and jumping – and a free feeding test). The tests and the results are described in detail in

K. Olczak et al.
Olczak et al. (2018), where it is also suggested to use the simple free feeding test (FF) as a measure of food motivation. Thus, only this test is included in the analysis here. In this test, the horses were given 1.5 l of oats and the time (min) was recorded for the horses to finish the meal. If a horse did not finish the meal, a result of 30 min was assigned.

2.2.2. Learning tests

2.2.2.1. Negative reinforcement test [NR]. Using negative reinforcement (pressure and release), the horses were taught to perform three steps forwards and three steps backwards in response to light lead rope and hand pressure. The trainer used different levels of signalling as described in Table 1. In each trial, the signal given to the horse started at level 1, and if the horse did not respond, the next level was applied. Special attention was made to ensure that the pressure was released immediately after the correct response. When more steps were required, pressure was released instantly after the first step was made and if required, pressure was re-applied to motivate the horse to take a second step.

As the horses were prone to follow the trainer, going backwards was evaluated as the learning criterion. The test was considered as completed when a horse performed three steps backwards in response to level 1 or 2. The behaviour had to be repeated five times in a row. Horses were tested once per day (one session lasting 10 min.). The horses were tested until they met the learning criterion or up to six training sessions. The number of required sessions and the number of signals (i.e. how many times the trainer gave signals to the horse until it passed the learning criterion) were measured.

2.2.2.2. Clicker test [CT]. The horses were taught to take three steps forwards and three steps backwards as a response to following a target. Initially, using classical conditioning, the horses were introduced to a clicker sound associated with a reward (a small handful of oats). In an initial conditioning phase, the trainer clicked and immediately gave the animal the reward in ten repetitions. The test started immediately after the conditioning phase. A telescopic target (Terry Ryan Click Stick, 6 – 58 cm long often used in dog training) was introduced and the horse was trained to follow the target, using 11 stages as described in Table 2. Initially, if the horse touched the target by himself, he received a click and reinforcement. If the horse did not show interest in the target, the experimenter brought it gently in contact with the muzzle (Table 2).

Each time a horse touched the target with his muzzle, he was immediately rewarded with a click and treat. Each of the stages was performed in ten successful (i.e., the horse received a treat) repetitions and then the next level started. If the horse lost interest and did not touch the target in 15 s, the target was moved around to get the horse’s attention. If the horse still showed no interest, it received a reminder (identical to step 1). The horses were trained in 15 min daily training sessions. The learning criterion was that the horse took three steps forwards and three steps backwards following the target. The number of reminders needed and the number of training sessions per individual were recorded.

2.2.2.3. Visual discrimination test [VD]. Two buckets that differed in shape, size and colour were used: one bucket was round and white (25 cm diameter, 15 cm height), the second bucket was rectangular, orange and larger (50 cm × 35 cm). At first, the horse was led to the two buckets placed next to each other, both filled with oats. To avoid neophobia, the horse was allowed to eat from both buckets. One of the two buckets was then randomly assigned for each horse as “correct bucket” and this one contained food during the test, while the second was emptied. The arena was divided with a transparent barrier and the buckets were placed on each side of the barrier. The horse was led to the empty bucket, then to the correct bucket and allowed to eat a mouthful of oats. The horse was then released at the release point to freely choose a bucket (Fig. 1). Depending on the horse’s reaction, one of the following actions was taken:

a. Correct bucket: the horse was allowed to eat a mouthful and was led back to the starting point. Buckets were changed according to Table 3 while the horse was turned around.

b. Incorrect bucket: the horse was led back to the starting point and the buckets remained in the same position. After four wrong choices the horse was led to both buckets again and allowed to eat from the correct bucket (after three reminders the test was terminated).

c. No response: the horse had 1 min to make a choice. If no choice was made, i.e. the horse did not approach any of the buckets, the procedure described for point “b” was applied.

There were 10 changes of the bucket position (Table 3).

The test was not time-limited and one session lasted until the horse had completed all 10 bucket position changes, or until it failed the test (no response three times in a row) as described before. The learning criterion was that the horse reached 80% correct choices during one testing session, which is considered a result above chance (Steffens et al., 2020). The horses received one test session with 10 bucket changes per day. The only exceptions, due to time constraints, were horses that required the highest number of sessions (five horses). For those horses, the last two sessions were done on the same day with minimum one hour break between sessions. The number of training sessions required, the

<table>
<thead>
<tr>
<th>Level</th>
<th>Backwards</th>
<th>Forwards</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The handler stood facing the horse and said “back” and put a hand up while taking a small step towards the horse</td>
<td>The handler stood facing the horse with hands at his chest level, said “walk” and gave a hand signal: lowering a straight arm next to the body with slight move toward the move direction (without any pressure on the lead rope)</td>
</tr>
<tr>
<td>2</td>
<td>The handler said “back” and placed his hand on the horse’s chest</td>
<td>The handler said “walk” with a hand signal and slightly pulled the lead rope.</td>
</tr>
<tr>
<td>3</td>
<td>The handler said “back” and pushed the horse’s chest gently</td>
<td>The handler said “walk” with a hand signal and pulled the lead rope</td>
</tr>
<tr>
<td>4</td>
<td>The handler said “back” and pushed harder on the horse’s chest</td>
<td>The handler said “walk” with a hand signal and pulled harder on the lead rope</td>
</tr>
<tr>
<td>5</td>
<td>The handler said “back” and pushed the chest harder, using his bodyweight, possibly helped by one of the observers until the horse showed a correct response.</td>
<td>The handler said “walk” with a hand signal and pulled the lead rope as much as possible while one of the observers pushed the hindquarter, until the horse showed a correct response.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The target was slowly brought to the muzzle of the horse</td>
</tr>
<tr>
<td>2</td>
<td>The target was placed approx. 10 cm from the muzzle in front of the horse</td>
</tr>
<tr>
<td>3</td>
<td>The target was placed approx. 50 cm from the muzzle in front of the horse</td>
</tr>
<tr>
<td>4</td>
<td>The target was moved around the experimenter in a fixed pattern (right, left, down and up), approx. 50 cm from the horse’s muzzle</td>
</tr>
<tr>
<td>5</td>
<td>The experimenter moved with the target approx. 1 m to encourage the horse to take one step forward</td>
</tr>
<tr>
<td>6</td>
<td>The target was placed low to encourage the horse to lower its head</td>
</tr>
<tr>
<td>7</td>
<td>The target was placed between the horse’s front legs so that one step backwards was required</td>
</tr>
<tr>
<td>8</td>
<td>The experimenter moved with the target approx. 2 m to encourage the horse to take two steps forward</td>
</tr>
<tr>
<td>9</td>
<td>The target was placed between the horse’s front legs and moved backwards with the horse so that two steps backward were required</td>
</tr>
<tr>
<td>10</td>
<td>The experimenter moved with the target approx. 3 m to encourage the horse to take three steps forward</td>
</tr>
<tr>
<td>11</td>
<td>The target was placed between horse’s front legs and moved further backwards as the horse moved, so that three steps backward were required</td>
</tr>
</tbody>
</table>

Table 1
Levels of signalling used in the negative reinforcement test.

Table 2
Stages of training in the clicker test.
total number of errors, and number of ‘no response’ were recorded.

2.2.3. Fear test [FT]

A bucket of oats was placed in the corner of the arena close to the entrance. To enable escape distance measurements, the arena was divided into seven zones (50 cm each; i.e. zone 0 = 0–0.5 m, zone 1 = 0.5–1 m, zone 2 = 1–1.5 m [...]). To avoid disturbance in horses’ behaviour, stones were used as neutral marks of zones. Each horse was led to the bucket and the rope was detached so it could move freely. To induce a fear reaction, a combination of novelty, suddenness, movement and sound was used (a white, 30 × 40 cm, plastic bag attached to a whip, held by an experimenter). Special care was taken to ensure that the horses did not see the plastic bag before. While the horse was eating calmly, the plastic bag was suddenly waved once, close to (0.5 m) the side of the tested animal, after which the bag was again hidden behind the experimenter. The test lasted until the horse returned to the food and

Table 3
Pattern of bucket changes in the visual discrimination test.

<table>
<thead>
<tr>
<th>Trial</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct bucket initially left</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>right</td>
<td>left</td>
</tr>
<tr>
<td>Correct bucket initially right</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
<td>right</td>
<td>left</td>
</tr>
</tbody>
</table>

Fig. 1. Visual discrimination test. Experimental setup with the location of the two different buckets. The horse was led straight towards the midline barrier and released while walking so it was free to choose a bucket.
resumed feeding. The escape distance (zones) and latency (seconds) to start eating again were measured.

2.3. Behavioural observations

All tests were recorded on HD videos (Cannon 500D) for a later recording of nervousness/stress-related behaviour. The following behaviours were observed for the total duration of all tests and recorded in seconds per minute of the test: stable orientation (i.e. fence pacing on the side closest to the stable and/or attempt to push the fence for NR test toward box/stall), alert (head up intently oriented and body tensed), running (trot and/or canter). Running was not measured in the visual discrimination test, as this behaviour could be natural for this test (i.e. when a horse runs toward a bucket with food).

2.4. Physiological recordings

Polar RS400 heart rate monitor with Polar equine electrodes was used to record heart rate (HR) as beats per minute (bpm). To ensure proper measurements, the winter coat was clipped and gel (ZelPol for ecg and USG) between skin and electrodes was applied. The HR was measured during the learning and fear tests, and for 10 min before each test (pre-test HR). The mean (HRavg) and maximum (HRmax) were later calculated from the data obtained. As the horses required different numbers of testing sessions to meet the learning criteria, only the HR from the first testing session was used in the analysis.

Saliva samples were collected on the first day of the learning and fear tests: 10 min before and 10 min after each test using Salivette cotton swaps. The samples were cooled down immediately at 4°C, and then frozen at −20°C for later analysis. Before analysis, the samples were thawed and centrifuged at 2500 rpm for 10 min. Salivary cortisol levels were determined employing a commercially available enzyme-linked immunosorbent assay - the DEMEDITEC Cortisol free in Saliva ELISA Kit. The kit is based on the principle of competitive binding. The intra-assay coefficient of variation was 5.8%, the inter-assay variation was 6.2% and the sensitivity was 24 pg/ml.

2.5. Data analysis

Statistical analysis was done with STATISTICA 13.1. The normality of data distribution was tested with the Shapiro – Wilk test.

To investigate the effects of testing on physiological parameters, HR values before and during the tests as well as cortisol levels before and after the tests were analysed. Normally distributed data were analysed with the t-test for dependent samples. Data that did not fulfill the criteria for the test (non-normal distribution) were analysed with the non-parametric Wilcoxon signed rank test. Heart rates were normally distributed. Cortisol responses were normally distributed for NR and FT, but for CT and VD the data did not follow a normal distribution.

Correlations between variables within and between tests were analysed with the Spearman rank correlation at significance level of P < 0.05.

Based on the free feeding test results (i.e. latency to finish the meal), the horses were divided into three groups: 25% with assumed lowest food motivation (i.e. a long latency to finish the meal), 50% middle and 25% with assumed highest food motivation (i.e. the shortest latency to finish the meal). Also, the free test was used to group horses (based on escape distance): 25% with lowest fearfulness, 50% middle and 25% with highest fearfulness (i.e. the longest escape distance). For both food motivation and fearfulness, the groups of horses with the 25% of lowest and highest results were compared with a U Mann-Whitney test in terms of their learning performance.

To enable an analysis of behaviour indicative of nervousness/stress during testing, the duration of the recorded behaviours (stable orientation, alert and running) were pooled.

3. Results

Habitation to the outdoor arena required 2.43 ± 0.90 training sessions.

3.1. Food motivation (FF)

All horses finished the meal (latency to finish eating, min), (median): 11.5 [9; 13]). Based on the latencies, the horses were grouped into a high food motivation group (latency < 9 min), a middle group (latency 9–13 min), and a low food motivation group (latency > 13 min).

3.2. Learning tests

Results of the learning tests together with physiological parameters (heart rate and cortisol) are presented in Table 4.

3.2.1. Negative reinforcement (NR)

The number of required training sessions to reach the criterion varied from one to three and the number of signals applied varied from 6 to 55, median 24 [18; 32]. As going backwards three steps in response to a light tactile or visual signal should be performed five times to fulfil the criterion for this test (i.e. in five subsequent repetitions) it was clear that two horses were very fast learners as they required only 6 and 7 signals respectively.

Table 4

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>cortisol (ng/ml)</th>
<th>Heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training sessions</td>
<td>1.87 ± 0.63</td>
<td>47.6 ± 6.18</td>
</tr>
<tr>
<td>CT</td>
<td>2 [1; 2]</td>
<td>49.8 [44.5; 53.5]</td>
</tr>
<tr>
<td>VD</td>
<td>1.74 ± 0.96</td>
<td>50.9 ± 10.1</td>
</tr>
<tr>
<td>2 [1; 2]</td>
<td>52.8 [48.8; 60.2]</td>
<td></td>
</tr>
<tr>
<td>N = 23</td>
<td>6 [5; 6]</td>
<td>60.5 [52.0; 68.0]</td>
</tr>
<tr>
<td>N = 23</td>
<td>1.92 ± 1.38</td>
<td>58.3 ± 8.19</td>
</tr>
<tr>
<td>N = 23</td>
<td>1.75 [0.53; 1.53]</td>
<td>60.8 ± 9.50</td>
</tr>
<tr>
<td>N = 23</td>
<td>3.15</td>
<td>61.5 [53.2; 61.5]</td>
</tr>
<tr>
<td>N = 23</td>
<td>66.0</td>
<td>63.0</td>
</tr>
</tbody>
</table>
3.2.2. Clicker test (CT)

In this test, the horses were trained to follow a target to perform three steps forwards and three steps backwards. The number of testing sessions (Table 4) correlated positively with the number of reminders (median 4 [2; 9], P = 0.01), while reminders correlated positively with duration of nervousness (r = 0.49, P = 0.02). There was a significant difference between HRavg before and during testing (t = 3.08, P = 0.01), but no significant difference in salivary cortisol before and after test was found (Z = 1.09, P = 0.27), and no correlations between HRavg, nor HRmax and salivary cortisol were found (r = -0.11, P = 0.62 and r = 0.23, P = 0.29).

3.2.3. Visual discrimination test (VD)

In the visual discrimination test, all horses passed the criterion of 80% correct responses (Table 4). Number of errors and number of ‘no response’ recorded in this test were (median [25%; 75%]): 13.0 [9; 23] and 0 [0; 2], respectively. No significant difference was found between HRavg before and during testing (t = 0.15, P < 0.88), nor between pre- and post-test salivary cortisol level (Z = 0.83, P = 0.41). Neither HRmax, nor HRavg correlated with salivary cortisol (r = 0.03, P = 0.92 and r = -0.42, P = 0.06). The number of testing sessions correlated positively with the number of times the horses did not respond (r = 0.86, P = 0.03). Also, nervousness correlated with number of errors (r = 0.49, P = 0.02) and HRavg (r = 0.45, P = 0.04).

3.3. Fear test (FT)

In the fear test, the heart rate (mean ± SD, bpm, HRavg: 60.1 ± 10.7), HRmax (120.3 ± 26.5), latency to resume eating (sec, 26.34 ± 26.08) and escape distance (zone, 1.95 ± 1.06) were recorded. HRmax and latency to resume eating correlated with escape distance (r = 0.55, P = 0.01; r = 0.58, P < 0.01, respectively), HRavg correlated with latency to resume eating (r = 0.44, P = 0.04), but not with escape distance (r = 0.41, P = 0.07). Further, the latency to resume eating correlated with duration of nervousness (1.85 [1.21; 3.13], r = 0.53, P = 0.01), and nervousness correlated with HRavg (r = 0.58, P = 0.01) and HRmax (r = 0.45, P = 0.03). The HRavg before the test (49.5 ± 6.11) differed significantly from HRavg during the test (t = -7.03, P < 0.001).

Salivary cortisol levels did not differ before and after the test (before: 1.54 ± 0.79, after: 1.73 ± 1.16; t = -0.78, P = 0.44).

Based on escape distance, the horses were divided into three groups: low fearfulness group (zone 0–1), a middle group (zone 2) and high fearfulness group (zone 3–4).

3.4. Food motivation, fearfulness and learning

No correlations between learning tests were found for any of the learning parameters: e.g. testing sessions NR and CT (r = -0.23, P = 0.38); NR and VD (r = -0.27, P = 0.30), CT and VD (r = 0.02, P = 0.94).

Both the number of testing sessions (CT) and number of reminders (CT) correlated with escape distance (FT), respectively: r = 0.43, P = 0.04 and r = 0.61, P < 0.001, but not with the latency to resume eating (r = 0.17, P = 0.43 and r = 0.41, P = 0.05). No other correlations were found between learning performance parameters and fear responses; neither between the latency to finish the meal in the FF test and learning performance parameters. Comparison of the groups with highest and lowest fearfulness (based on escape distance in FT) revealed differences between groups in term of reminders in CT: 25% most fearful: median 18 vs. 25% least fearful: median 18, U = 7, P = 0.01. No differences were found between the most and the least fearful horses for the other parameters in any of the learning tests: e.g. number of testing sessions: NR (U = 20, P = 0.24), CT (U = 24, P = 0.18), VD (U = 26, P = 0.59).

No statistically significant differences were found in any of the learning tests between horses with high and low food motivation (based on FT). For example, the number of testing sessions: NR (U = 15, P = 0.68), CT (U = 14.5, P = 0.63), VD (U = 15, P = 0.68). Finally, salivary cortisol levels did not correlate between the tests, whereas average heart rates did (Table 5).

4. Discussion

In this study, we found a lack of association in horses’ performance across different learning tests, which confirms our hypothesis that each test measured divergent types of learning. This finding is consistent with previous results (e.g. Christensen et al., 2012; Lansade and Simion, 2010). One reason for this lack of association in performance, even between learning tests within the same reinforcement regime, i.e. the clicker and the visual discrimination tests, could be related to differences in test design. For example, in the clicker test, the horses had to focus on the target in the presence of the trainer, who attempted to gain the horses’ attention, while in the visual discrimination test, the horses were let loose with no interaction with people except when led to the starting point. The possibility to move freely in the visual discrimination test possibly made some horses more prone to distraction by other stimuli in the environment.

Although we did not find significant differences in learning (in any of the learning tests) between horses with an apparently high vs. a lower food motivation, we cannot exclude the importance of food motivation. The Hucul horse is a primitive breed that evolved in mountain areas, where hard environments and limited food sources made them very keen on feed and they are generally considered to have a high food motivation (Kwiecińska-Olszewska, 2019). Lindberg et al. (1999) found that non-warble horses learned an operant task quicker than warmblood horses and suggested that this was caused by the former being more motivated to search for food. In this study, twenty out of twenty-three horses passed the learning criterion in the clicker test within just one or two testing sessions. This indicates that all horses were motivated to work for food and that positive reinforcement is an effective type of training for these horses. However, this low variation also makes associations between tests less likely and we recommend further studies on various types of horses and using more demanding learning tasks leading to a larger variation in performance. We also recommend that future studies include behavioural indicators of frustration and ‘over-arousal’ caused by the use of food rewards during learning tests, which may act to impair attention and learning in highly food-motivated horses. It would also be interesting to include manipulation of food motivation, i.e. by measuring learning performance in hungry vs. satiated horses as well as altering the value of the reward (e.g. hay vs. concentrates; Ninomiya et al., 2007). Furthermore, as both motivation and fearfulness can affect learning, it is likely that for some animals, disturbing external environmental stimuli can overshadow the presence of food, while for other animals, the opportunity to obtain food may overshadow environmental stimuli. It is known that more incentive stimuli overshadows weaker stimuli (McLean, 2008), but these mechanisms are not yet well-studied in horses. To really understand these processes, it would be interesting to compare four groups of horses: (1) low level of fearfulness and high food motivation, (2) highly fearful with high food motivation, (3) low level of fearfulness and low food motivation, (4) highly fearful with low food motivation.

It has previously been suggested that fearfulness may inhibit learning (Christensen et al., 2012), as horses’ attention will switch from the task to disturbing environmental stimuli (Mendl, 1999). Contrary to our expectations, there were no correlations between learning performance parameters in the negative reinforcement test and responses in the fear test. In the clicker test however, the number of sessions and the number of reminders correlated moderately with escape distance in the fear test. To investigate this relationship further, we compared the 25% most fearful horses with the 25% least fearful horses based on their escape distance, and found that the least fearful horses needed significantly
fewer reminders in the clicker test, whereas there was no difference in the other learning performance parameters. What is more, the number of reminders correlated positively with nervousness during the task, reflecting that reminders were applied when the horses were distracted and lost focus on the target. Also in the visual discrimination test, the number of errors correlated with nervousness during the test. These findings suggest that nervous behaviour within a learning test is more directly related to performance, rather than general fearfulness. It would be interesting to further investigate the effects of the horse being controlled by a human handler (i.e., under ‘stimulus-control’) vs. being loose during a learning test, as a loose horse has more freedom to express motivated behaviour and may be more distracted by environmental stimuli.

In the current study, the lack of difference between fearful and less fearful horses likely also reflect the low variation in the learning performance parameters as well as generally weak fear responses in Hucul horses. These horses originated in the Carpathian Mountains where flight responses are hampered, and their fear responses may be different to responses shown by horses originating from more open areas (Budyžynski et al., 1991, 1995). Budzyńska (2014) described different coping strategies of horses: active (flight-flight response) and passive (behavioural inhibition) in relation to adaptation to different environments, and it is likely that Hucul horses tend to show a more passive coping strategy. In addition, lower reactivity of non-warmblood horses has already been reported in several studies (e.g. Lindberg et al., 1999; Gorecka-Bruzda et al., 2011; Rørvang et al., 2015). Thus, the fear test used in this study could be too weak to induce strong reactions and high variability. Nevertheless, the HR increased significantly from the pre-test level in the fear test, whereas there was no significant difference in salivary cortisol before and after the fear test. These results could relate to the short test duration or a too low intensity of the test.

In Lansade and Simon (2010) it was reported that fearfulness may enhance performance in an avoidance test, but in a forward-backward test less fearful horses performed better. In their study, the horses were taught to respond to a tactile or vocal signal for a food reward, i.e. combined reinforcement was used. It is important to mention that their horses were adult and already handled which could significantly influence their results. In our study, we did two tests where the horses were taught to go backwards. In the negative reinforcement test, pressure was applied and released instantaneously following a correct response, while in the clicker test, only positive reinforcement was used. Signals to go backwards were different as in the first test the trainer’s hand was high, whereas in the clicker test the trainer’s hand and target were placed between the horses’ front legs. There was no correlation in performance in the two tests, which may indicate individual predispositions to type of learning.

The learning tests did not significantly increase salivary cortisol levels from pre-test levels, except in the negative reinforcement test, which was conducted as the first test and therefore could be experienced as more stressful (Gorecka-Bruzda et al., 2017). There was no corresponding increase in heart rate however, which likely reflects a low level of movement in this test. In contrast, significant increases in heart rate from pre-test values were found in the clicker training. In this test, the horses were able to move freely, thus the average heart rate response could reflect physiological responses to movement rather than stress. As evident from Table 4, the pre-test heart rate was higher for VD, which may relate to anticipatory behaviour as the VD test was conducted as one of the last tests, following a number of food-rewarded tasks (Section 2.2).

In line with previous studies, we conclude that learning performance differed between the various types of tests, and that food motivation – measured as latency to consume a meal – did not influence performance in food-rewarded tests. This may suggest either that the small food rewards triggered food motivation in all horses regardless of their general food motivation; that our test was inappropriate for assessment of food motivation; or that other intrinsic and extrinsic factors govern performance in learning tests. Further research is needed on the influence of food motivation on performance in food rewarded learning tasks.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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