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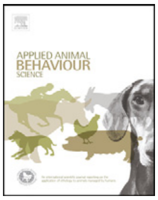

Does learning performance in horses relate to fearfulness, baseline stress hormone, and social rank?

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ABSTRACT

The ability of horses to learn and remember new tasks is fundamentally important for their use by humans. Fearfulness may, however, interfere with learning, because stimuli in the environment can overshadow signals from the rider or handler. In addition, prolonged high levels of stress hormones can affect neurons within the hippocampus; a brain region central to learning and memory. In a series of experiments, we aimed to investigate the link between performance in two learning tests, the baseline level of stress hormones, measured as faecal cortisol metabolites (FCM), fearfulness, and social rank. Twenty-five geldings (2 or 3 years old) pastured in one group were included in the study. The learning tests were performed by professional trainers and included a number of predefined stages during which the horses were gradually trained to perform exercises, using either negative (NR) or positive reinforcement (PR). Each of the learning tests lasted 3 days; 7 min/horse/day. The NR test was repeated in a novel environment. Performance, measured as final stage in the training programme, and heart rate (HR) were recorded. Faeces were collected on four separate days where the horses had been undisturbed at pasture for 48 h. Social rank was determined through observations of social interactions during feeding. The fear test was a novel object test during which behaviour and HR were recorded.

Performance in the NR and PR learning tests did not correlate. In the NR test, there was a significant, negative correlation between performance and HR in the novel environment ($r_S = -0.66$, $P < 0.001$, i.e. nervous horses had reduced performance), whereas there was no such correlation in the home environment (both NR and PR). Behavioural reactions in the fear test correlated significantly with performance in the NR test in the novel environment (e.g. object alertness and final stage: $r_S = -0.43$, $P = 0.04$), suggesting that performance under unfamiliar, stressful conditions may be predicted by behavioural responses in a fear test. There was a negative correlation between social rank and baseline stress hormones ($r_S = -0.43$, $P = 0.04$), i.e. high rank corresponded to low FCM concentrations, whereas neither rank nor FCM correlated with fearfulness or learning performance. We conclude that performance under stressful conditions is affected by activation of the sympathetic nervous system during training and related to behavioural responses in a standardised fear test. Learning performance in the home environment, however, appears unrelated to fearfulness, social rank and baseline FCM levels.

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1. Introduction

Learning has been defined as a process of adaptive changes in individual behaviour as a result of experience (Thorpe, 1963). One type of learning is instrumental or operant conditioning, where a response made by the animal is followed by a biologically relevant event (reinforcement or punishment) that changes either the probability of the behaviour recurring or some aspect of its form (Mills et al., 2010). An aversive stimulus (punishment) reduces the likelihood of the action being repeated, whilst a desired consequence (reinforcement) increases the likelihood. Positive reinforcement is the addition of a pleasant stimulus to reward a desired response, whereas negative reinforcement is characterised by subtraction of an aversive stimulus (e.g. pressure) to reward a desired response and thus make this response more likely in the future (Mills et al., 2010). Some reviews have highlighted the paradox that most studies of equine learning are based only on positive reinforcement, whilst much traditional equine training is based on negative reinforcement (Cooper, 2007; Murphy and Arkins, 2007). Similarly, the majority of learning tests have investigated e.g. maze or discrimination learning with visual or spatial cues, whereas typical training techniques for horses are based on tactile or auditory cues. If learning studies are to be more applicable, it appears relevant to use tests which to a higher degree reflect how horses are trained in practise.

Some previous studies have reported that horses' performance in different learning tests did not correlate (e.g. Lansade and Simon, 2010; Visser et al., 2003; Wolff and Hausberger, 1996). This lack of correlation between learning performance in different tests suggest that other characteristics, such as fearfulness, attention or motivation may govern success or failure in learning tests (Nicol, 2002). Increased learning by horses that are naturally calm may be due to reduced interference with the learning process, whereas fearful animals may shift attention away from the task, resulting in poor performance (Mendl, 1999; Nicol, 2002). Furthermore, fearfulness and stress may interfere with learning because prolonged high concentrations of glucocorticoids can impair both memory and learning skills as shown in several mammalian species; the link between stress hormones and these functional deficits is believed to relate to changes in the hippocampal formation (McEwen and Sapolsky, 1995; Morris, 2007). There is a large amount of scientific literature supporting the Yerkes-Dodson law, i.e. that cognitive function including learning has a biphasic relation (inverted U-shape) to stress levels; i.e. low or moderate concentrations of circulating glucocorticoids may enhance cognitive function, whilst high or prolonged elevations of these hormones can lead to cognitive disruption and performance below baseline levels (McEwen and Sapolsky, 1995; Mendl, 1999; Morris, 2007). Long-term stress of various housing or training conditions may therefore cause marked impairment of learning and memory abilities through the damaging actions of chronically elevated levels of glucocorticoids on brain structure.

In horses, no clear link between learning performance and fearfulness or stress has been demonstrated; Visser et al. (2003) indicated that emotionality, based on behavioural reactions during the learning test, may cause horses to be non-performers, whereas there was no simple relationship between heart rate, behaviour and learning performance in horses that did perform the task. Heird et al. (1986) found that emotionality, based on a subjective score of 1–4, did not correlate to maze test performance in 16 horses. They reported, however, that the least emotional horses (*n* not specified) ultimately achieved a higher level of performance. In contrast, Lansade and Simon (2010) found that fearfulness *enhanced* performance of ponies in an avoidance test based on positive punishment, but *decreased* performance in a negatively reinforced handling test. The handling test was, however, based on exercises (leading forward and backing) which the 36 participating 5–7 years old ponies that were accustomed to handling were likely to have experienced previously. The results may therefore reflect timing of reinforcement during previous handling rather than actual learning abilities.

There are to our knowledge no published studies of the effect of social status on fearfulness or baseline adrenocortical activity in horses, whereas some older studies failed to demonstrate effects of social dominance on learning in horses (reviewed by Nicol, 2002). We aimed to investigate learning performance in horses, evaluated through both positive and negative reinforcement in practical learning tests, and the link to fearfulness, baseline stress hormone levels as well as social status. The learning tests were developed in cooperation with professional trainers involving exercises that the young, participating horses had not previously experienced. We hypothesised that learning performance would be impaired by increased fearfulness and higher baseline stress hormone levels, which in turn would be influenced by social status.

2. Materials and methods

The study period was from July to October 2010. The study conformed to the 'Guidelines for ethical treatment of animals in applied animal behaviour and welfare research' by the ethics board of the International Society of Applied Ethology (www.applied-ethology.org).

2.1. Animals and management

Twenty-five Danish Warmblood geldings (18 3-year-olds and 7 2-year-olds) were used in the experiments. The horses arrived at the research institute approximately one month prior to the study and were pastured together day and night with free access to water, minerals and hay (available ad libitum in two feeding houses; 200 cm × 300 cm, 14 feeding places/house) as well as vegetation in an 8 ha pasture. The horses came from 8 private studs (2–8 horses/stud) and had been handled only for routine management at each stud. Upon arrival, the horses were habituated to halters and leading and to wearing an elastic girth with heart rate equipment as well as to being stroked on the body with a whip. Subsequently, the horses were habituated to social isolation in a closed, indoor test arena and to feeding from a feed container (with rolled oats and molasses), placed in the middle of the arena. A horse was considered as habituated to the test arena when

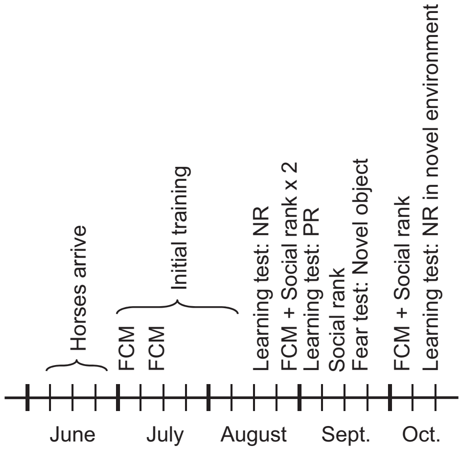

Fig. 1. Overview of data collection (FCM: collection of faeces for analysis of faecal cortisol metabolites; NR: negative reinforcement; PR: positive reinforcement; social rank: registrations of social interactions during a limited resource test).

it voluntarily entered the test arena, walked directly to the feed container and fed for at least 90 of 120 s. The initial handling and habituation lasted 1.5 months. An overview of the entire study, including the two learning tests, the fear test, collection of faeces and registrations of social behaviour is presented in Fig. 1.

2.2. Test environments

The fear test was carried out in the test arena (10 m × 10 m; Fig. 2) at one end of the stable. Next to the test arena was a corridor, separated from the arena by horizontal bars (Fig. 2; observer corridor). The same observer was present in this corridor during the initial habituation and during the fear test. Another part (18 m × 4 m) of the stable was used as a waiting area where hay was available ad libitum. At least two other horses were present in the waiting area during testing. The corridor between the test arena and the waiting area was used for the learning tests (Fig. 2). Furthermore, an outdoor area on the other side of the stable was used as a novel environment for the final learning test. Thus, the fear and learning tests were carried out in different areas to avoid frustration as the horses were trained to expect food in the test arena, which was used for the fear test.

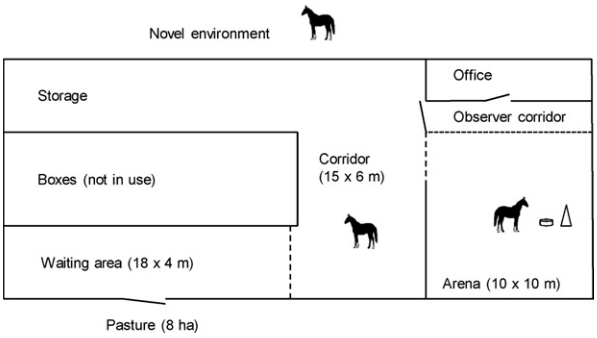

Fig. 2. Test environment. Horse figures represent test areas: arena: fear test (circle: feed container; triangle: novel object), corridor: learning tests (NR and PR), novel environment: learning test (NR).

The horses were exposed to the tests in the same order, because we aimed to investigate correlations in responses. Balancing the test order could cause disturbance due to carry over effects, i.e. a horse that is exposed to a clicker test before a negative reinforcement test is likely to anticipate food as a reward and may become frustrated due to the change in reinforcement regime.

2.3. Learning tests

The negative reinforcement (NR) test was performed by a professional trainer and consisted of five predefined stages (S1–S5; Table 1) during which the horses were trained to stand (S1) and move sideways by crossing their front (S2–S3) and hind legs (S4–S5) on signal from the trainer (lead pressure and gentle whip tapping). The stimulation was stopped immediately when the horses performed the requested response. Whenever a horse met the passing criterion for a given stage (Table 1) the trainer carried on with the next stage. Each horse was trained 7 min/day for 3 days with a one-day break between days 2 and 3. The horses always started on S1, irrespective of performance on previous days. The first 3-days test was performed in the home environment (Fig. 2; corridor) and the same training stages were later applied to the horses in a novel, outdoor environment containing several unknown objects (Fig. 2).

The positive reinforcement (PR) test was a clicker training test, performed in the home environment (Fig. 2; corridor) by an acknowledged clicker trainer. The test consisted of six predefined stages (S1–S6; Table 2) during which the horses were taught to touch a target (round plastic lid, Ø: 20 cm) with their muzzle for a click and a food reward (small pieces of carrot). The target was subsequently used to encourage the horses to perform different exercises such as stepping onto a wooden board (*h*: 2 cm, *l*: 200 cm, *w*: 120 cm) as well as searching the target on the ground, away from the trainer (S5; Table 2). Finally, at S6 the horses had to learn to touch a different target (traffic cone with white and orange stripes, 60 cm high) on the floor to gain a reward, and subsequently to seek this cone although it was placed away from the trainer. Similar to the NR, the test lasted 3 days (7 min/horse/day) with a one-day break between days 2 and 3, and horses always started on S1, irrespective of previous performance. The PR test was not repeated in a novel environment because the only accessible area could be used only once as a novel area (used for the NR test).

The trainers did not get any information about the horses prior to testing. Heart rate (HR) and performance, measured as the number of passed stages (and termed “final stage”) in the training programme, were recorded on each test day as well as the latency to complete each stage. Half numbers (.5) were used for horses that were halfway through a training stage when the test time ran out, and quarters (.25) for horses that just started, or nearly finished (.75) a stage. In this way we were able to distinguish between horses that e.g. just completed S3 (final

Table 1
Learning test: Negative reinforcement.

Stage	Description	Passing criterion
S1	*Parking (3 × 5 s).* The horse is trained to stand motionless whilst the trainer moves 2 m away and stay still for 5 s.	Three successive "parkings" of at least 5 s each.
S2	*Cross front legs (3 × 1 step to each side).* The horse is trained to take a step to the side on signal from the trainer (light whip tapping on the left/right shoulder).	Three correct responses to a light signal on each shoulder.
S3	*Cross front legs (3 × 3 steps to each side).* The horse is trained to take three steps to the side on signal from the trainer (light whip tapping on the left/right shoulder).	Three correct responses to a light signal on each shoulder.
S4	*Cross hind legs (3 × 3 steps to each side).* The horse is trained to take three steps to the side by crossing its hind legs on signal from the trainer (light whip tapping on the left/right hindquarters).	Three correct responses to a light signal on the left and right hindquarter.
S5	*Leg yield (3 × 5 steps to each side).* The horse is trained to walk sideways by crossing both front and hind legs in a smooth rhythm, at least five steps.	Three correct responses to a light signal on each side.

stage = 3.0) and horses which nearly completed S4 (final stage = 3.75) within the allocated test time.

2.4. Fear test

The day before the novel object test, all horses were exposed to the usual arena with a feed container for two 2-min sessions. This procedure enables us to measure baseline responses and thus ensure that all horses are sufficiently habituated to the test arena, i.e. walks directly to the feed container and stays there with a low HR (avg. 45–55 bpm). Furthermore, practising the entire test procedure (just without the test object) on the day before a test reduces the risk of reactions to the test procedure itself. On the test day, a pyramid-shaped object (tripod wrapped with a red and white plastic band, 120 cm high) was placed next to the feed container. This object was known to produce fear responses in young Danish Warmblood horses (Christensen et al., 2011). The test time started when the horse passed a line marking on the floor at a 90° angle to the entrance, i.e. just before the object became visible. In case a horse backed away from the entrance upon seeing the object, the handler gently led it forwards again. The handler did not enter the arena. Behavioural reactions (latency to eat, number of eating bouts, duration of alertness and sniffing the object; Christensen et al., 2011) and heart rate were registered during a 3-min exposure. One horse (H8) did not eat within the 3 min and in order to obtain a true latency rather than a censored data point, this horse was allowed to stay in the arena until feeding (at 270 s). After the test, the horses were habituated to the object to minimise carry-over effects.

2.5. Faecal cortisol metabolites

Faecal cortisol metabolites (FCM) were measured as a non-invasive parameter of adrenocortical activity (Möstl et al., 1999; Touma and Palme, 2005). Faecal samples were collected on four separate days (Fig. 1) between 8:00 and 10:00 a.m., directly after defecation on pasture by observers following the horses. Collection days were Mondays where the horses had been on pasture without disturbance, except for routine checking, for more than 48 h in order to measure baseline stress hormone

Table 2
Learning test: Positive reinforcement (clicker).

Stage	Description	Passing criterion
S1	*Seek target.* The horse is trained to touch the target with its muzzle for a click and a food reward. When the horse actively seeks the target in a middle position, the target is held in other positions, i.e. the horse has to lift or lower its head to touch the target.	10 rewards in a middle position as well as 3 rewards in a low, middle and high position.
S2	*Front legs on board (3 × 5 s).* The horse is trained to follow the target (i.e. rope pressure is not used) and to step onto a wooden board with its front legs and stay still for 5 s. The horse is subsequently backed down from the board before being encouraged to step up again.	3 completions.
S3	*Four legs on board (3 × 5 s).* The horse is trained to follow the target and stand still for 5 s with all four legs on the board. The horse is subsequently led forwards down from the board before being encouraged to step up again.	3 completions.
S4	*Backwards on board (3 × 5 s).* The horse is trained to walk backwards onto the board by placing the target between its front legs and rewarding it for stepping backwards. Direction is controlled by the use of the target only. The horse has to stand still for 5 s on the board and is subsequently led forwards down from the board before being encouraged to step up again.	3 completions.
S5	*Target on floor (×3).* The trainer throws the target on the floor and the horse has to approach and touch it for a reward. The target is thrown at an increasing distance from the trainer.	3 completions where the distance is at least 2 m from the trainer.
S6	*New target.* A cone is introduced on the floor and the horse is rewarded for touching the cone. When the horse has received 10 rewards for touching the cone, it is led to a starting line 3 m from the cone and released and has to return to the cone alone.	3 completions from the starting line.

levels. Samples were weighed (0.5 g) immediately after collection, frozen at −20 °C and sent on dry ice to the lab (Vienna). The samples were analysed as described in Merl et al. (2000) and Palme and Möstl (1997), using an 11-oxoaetiocholanolone enzyme immunoassay, validated for horses (Möstl et al., 1999). The inter-assay and intra-assay coefficients of variation were 9.5 and 11.3%, respectively. The sensitivity was 0.9 ng/g faeces.

2.6. Social rank

Social rank was determined through observations of social interactions on the pasture during four limited resource tests (4 × 1 h; Fig. 1). Three feed containers with attractive roughage (grass silage) were placed in the pasture, approximately 20 m apart. Three observers (standing 10 m from each container) registered all successful displacements (i.e. a receiver horse moved at least 3 m away from the feed container in response to a threat or an aggressive interaction from another horse) as well as the ID of the horses (initiator and receiver). Based on these observations, we were able to calculate the ratio of the number of horses each individual had displaced and the number of horses each individual had been displaced by; the higher the ratio, the higher the rank of the horse.

2.7. Data recording and analysis

HR was recorded with Polar Equine RS800 (Polar Electro OY, Kempele, Finland), which consisted of an Equine Wearlink and a W.I.N.D. transmitter and a wristwatch receiver. Water and gel were used to optimise the contact between electrode and skin. The HR monitoring equipment was fitted on the horse in the waiting area prior to testing, and the receiver stored data from the transmitter (R-R recordings). Subsequently, data were downloaded via a Polar Interface to a PC, using the software Polar ProTrainer, Equine edition, 5^{TM}. The average HR (HR avg) and the maximum HR (HR max) during the test sessions were determined for each horse.

In the novel object test, behaviour (Section 2.4) was recorded through direct observation using a hand-held computer (Workabout, PSION PLC, UK). Frequencies, durations and latencies were calculated using SAS 9.1 (www.sas.com). In the learning tests, the number of passed stages and the latency to pass each step were recorded manually by an observer.

Correlations in behaviour, performance and HR within and between tests were analysed using the Spearman Rank Order correlation (SigmaPlot11, www.systat.com). Furthermore, correlations between the test variables and FCM levels and social rank were tested. For analysis of differences in performance between days in the learning tests and between FCM collections (i.e. whether FCM concentrations differed significantly between collections) a one-way repeated measures ANOVA was used. Post hoc analysis was performed via the Holm–Sidak method (SigmaPlot11, www.systat.com).

Two of the 25 horses did not participate in all tests; H13 was euthanised on August 24th due to intestinal volvulus, i.e. he participated only in the first learning test. H19 was sold and picked up by his owner on September 15th, i.e. he did not participate in the last learning test (NR training in novel environment).

Table 3
HR and performance results (mean ± SE) from learning and fear tests.

Test	HR average (bpm)	HR max (bpm)	Performance (final stage)
Learning tests			
NR	52.8 ± 1.1	77.7 ± 2.5	3.1 ± 0.2
PR	52.6 ± 0.8	73.7 ± 1.6	4.4 ± 0.2
NR in novel environment	64.5 ± 2.9	94.3 ± 6.1	2.2 ± 0.2
Fear test			
Novel object	72.4 ± 2.6	106 ± 3.6	–

NR: negative reinforcement, PR: positive reinforcement.

3. Results

3.1. Learning tests

An overview of results (HR and performance) from the three learning tests is presented in Table 3. In the NR test, the range in final training stage was 1.5–5, i.e. the horse that performed at the lowest level (H15) completed S1 and half of S2, whereas the horse with the highest performance (H17) completed S5. In the PR test, the range was 3 (H5)–5.75 (H6). The horses generally completed fewer stages in the NR test in the novel environment and the range was 0 (H8, H9) to 4 (H22). In both learning tests, the horses' performance increased gradually across the three test days in the home environment (Fig. 3; PR: $F_{2,46} = 15.2$, $P < 0.001$ and NR: $F_{3,68} = 20.6$, $P < 0.001$), i.e. the horses appeared to remember the exercises from the previous test days and performed at a higher level on subsequent days. Performance on the first day of each learning test did not predict final performance on day 3 (NR: $r_S = 0.31$, $P = 0.125$ and PR: $r_S = 0.24$, $P = 0.248$), whereas there was a significant, positive correlation between performance on days 2 and 3 (NR: $r_S = 0.63$, $P < 0.001$ and PR: $r_S = 0.49$, $P = 0.015$). As expected, the latency to pass the first stage

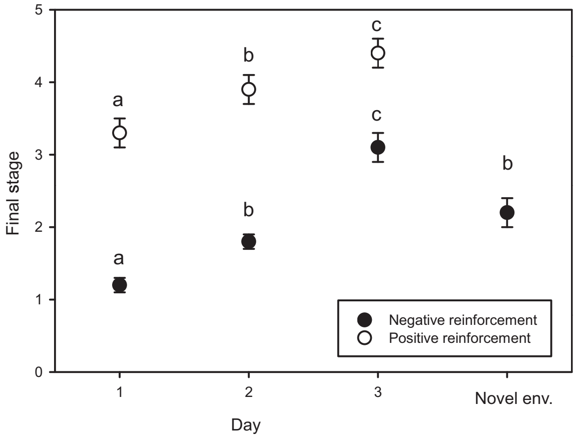

Fig. 3. Performance (final stage; mean ± SE) on the 3 days of each learning test and performance in the NR test in the novel environment. Different letters indicate significant differences within NR and PR. Comparisons between NR and PR are not relevant as the stages differ between the tests.

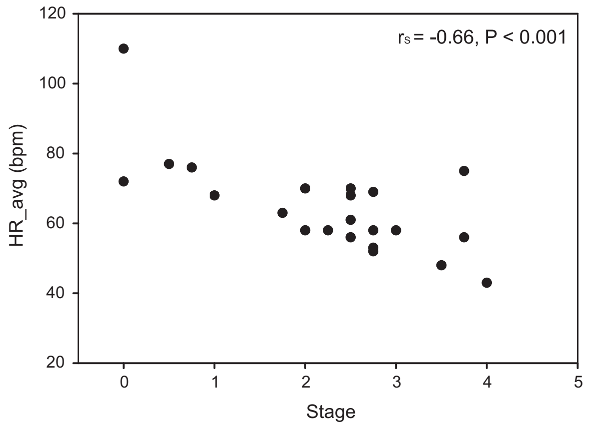

Fig. 4. Performance (final stage) and average heart rate during training of each horse in the NR test in the novel environment.

(S1) correlated negatively and significantly to final stage on day 1 (NR: $r_S = -0.85$, $P < 0.001$ and PR: $r_S = -0.87$, $P < 0.001$) and there was a weak tendency to a negative correlation with final stage on day 3 in the NR test ($r_S = -0.34$, $P = 0.098$), but no correlation to final stage on day 3 in the PR test.

There were significant negative correlations between performance and HR in the NR test in the novel environment (HR avg: Fig. 4; HR max: $r_S = -0.59$, $P = 0.004$), whereas there were no such correlations in the home environment (both NR and PR). There were also no correlations in performance, nor HR, between the NR and the PR test. Surprisingly, there was also no correlation in performance between the NR test in the home vs. in the novel environment ($r_S = 0.10$, $P = 0.653$).

3.2. Fear test

Behavioural reactions (duration of alertness and sniffing the object, latency to eat, number of eating bouts) and HR correlated significantly within the novel object test, except average HR and number of eating bouts, where there was a tendency to a negative correlation (Table 4). Interestingly, the behavioural variables in the novel object test correlated significantly to performance in the NR test in the novel environment (Table 5). The maximum HR in the novel object test correlated significantly to both performance (Table 5) and average HR ($r_S = 0.42$, $P = 0.049$) in the novel environment, whereas the average HR in the novel object test did not correlate to performance, nor HR, in the novel environment. There were no correlations between behavioural reactions in the novel object test and performance in the NR and PR tests in the home environment.

3.3. Faecal cortisol metabolites (FCM)

FCM levels are shown in Fig. 5. The two collections in July had higher cortisol metabolite concentrations, compared to the two later collections (one way RM ANOVA: $F_{3,68} = 7.85$, $P < 0.001$). There were significant correlations between almost all collection days, i.e. horses that had high FCM levels in one collection also tended to have high FCM levels on the other collection days. Due to the higher FCM levels in July, the average of the first two FCM collections (initial period) as well as the average of the last two collections (test period) were correlated separately to performance in the learning and fear tests; the only significant correlation was between FCM in the initial period and performance in the NR test in the novel environment ($r_S = -0.56$, $P = 0.006$).

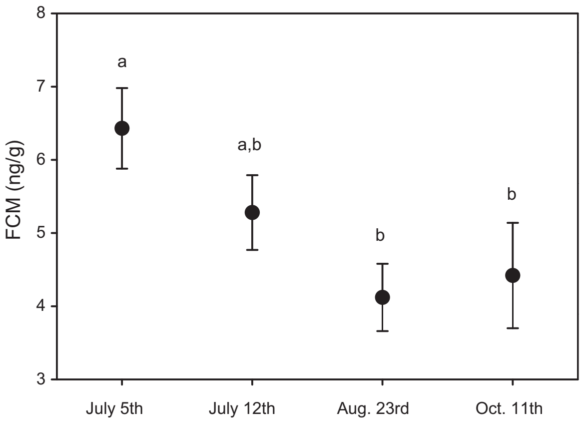

Fig. 5. Concentrations of faecal cortisol metabolites (FCM; mean ± SE) on the four collection days. Different letters indicate significant differences between days.

3.4. Social status

No opposing displacements were observed, i.e. if Horse A was observed to displace Horse B then Horse B never displaced Horse A, indicating that the dominance hierarchy was stable two months after establishment of the group. The most dominant horse in the group was observed to displace 21 other horses in the group and was not displaced by any. The second highest ranking horse was observed to displace 17 other horses and was displaced by one (the top ranking), whereas the two horses with the lowest displacement ratio were not observed to displace any other horses in the group and were observed to be displaced by eight other horses each, i.e. these horse were both assigned a ratio of 0. The lowest ranking horses were frequently observed to stay at the periphery of the area with feed containers once they had been displaced, whereas middle ranking horses typically approached another container if they had been displaced from one container. A few high ranking horses were observed to monopolise containers, although the containers were large enough for several horses to feed simultaneously. The observations in relation to social rank were carried out in late August to October and rank ratio was correlated to the average of the two FCM collections in the same period (August 23rd and October 11th). Interestingly, there was a significant, negative correlation between FCM level and rank ratio ($r_S = -0.43$, $P = 0.035$), indicating that higher ranking horses had lower FCM values. There were no correlation between rank and performance in the learning and fear tests.

Table 4
Spearman correlations (r_S) between variables in the novel object test.

	HR max (bpm)	Latency to eat (s)	Eating bouts (freq)	Alertness (s)	Sniffing (s)
HR avg	0.73 $P < 0.001$	0.66 $P < 0.001$	−0.35 $P = 0.094$	0.62 $P = 0.001$	−0.44 $P = 0.033$
HR max		0.52 $P = 0.010$	−0.45 $P = 0.027$	0.56 $P = 0.005$	−0.63 $P = 0.001$
Latency to eat			−0.76 $P < 0.001$	0.93 $P < 0.001$	−0.54 $P = 0.006$
Eating bouts				−0.75 $P < 0.001$	0.53 $P = 0.008$
Alertness					−0.61 $P = 0.002$

4. Discussion

Our results demonstrate that performance in a practical learning test under unfamiliar and stressful conditions can be predicted by behavioural reactions in a standardised fear test, performed in the home environment. The reported reactions in the novel object test were strongly correlated to reactions in three other types of fear tests, including exposure to sudden and tactile stimuli (Christensen, under review), suggesting that the responses reflected an underlying trait. Thus the predisposition to react to frightening stimuli appears to influence horse performance in a novel environment. Additionally, our results suggest that performance is influenced by immediate activation of the sympathetic nervous system because an increased heart rate corresponded to a poor performance in the novel environment. It is likely that fearfulness interferes with performance because stimuli in a novel environment can overshadow signals from the handler, i.e. the motivation of the horse to respond to environmental stimuli is greater than its motivation to respond to human signals. Indeed, one obvious response to a threatening or challenging situation or stimulus is to shift attention away from the task at hand to focus more on the threat (Mendl, 1999).

This study applied relatively short training sessions (7 min/horse/day) and yet we found a significant increase in day-to-day performance within the two learning tests. There was no correlation between performance in the two learning tests, indicating that learning ability is task-dependent or that horses are motivated by different reinforcement regimes. Similar results were obtained by Visser et al. (2003) and Lansade and Simon (2010); both studies compared performance in an active avoidance test to performance in either (1) a positively reinforced test (feeding based; Visser et al., 2003) or (2) a negatively reinforced handling test (Lansade and Simon, 2010). Active avoidance tests may, however, be less suitable for testing practical learning abilities in horses for the following reasons; (i) Horses that tend to flee from the aversive stimulation, i.e. possibly the most fearful or most active horses, are more likely to accidentally enter the requested compartment and thus learn by trial-and-error; (ii) If a delay is included between brief periods of the aversive stimulation (as in Lansade and Simon (2010) where a 3 s break was included between the 1 s air puffs), the stimulation is likely to act as positive punishment of any coincidental behaviour that was shown just prior to the stimulation, and the horse will be less likely to perform this particular behaviour again. If the horse still does not accidentally perform the requested response (bar crossing) in the next trial another random behaviour will be punished. This random application of punishment is likely to cause fear and frustration which in turn may lead fearful animals to try to escape the punishment. Thus we suggest that the positive correlation between fearfulness and performance reported by Lansade and Simon (2010) may relate to fear responses during the task, rather than reflecting a beneficial effect of fearfulness on learning.

Visser et al. (2003) found that emotionality might have caused horses to be non-performers in their study, but the reported higher mean HR in non-performers could also be due to frustration in horses that were unable to complete the task. For horses that performed the learning tasks, no correlation was found between HR, HRV and performance and as the horses had been habituated to the test environment prior to testing (K. Visser, personal communication), this finding corresponds to the results of the present study where HR and performance was unrelated in the home environment. No horses were classified as non-performers in the present study; probably because the horses were cued towards the requested response through adjustment of stimulation intensity, i.e. starting with a mild stimulation, which was removed when the horse performed parts of a correct response. Although learning tests with a human trainer may better reflect the way horses are trained in

Table 5
Spearman correlations (r_S) between performance (final stage) in the negative reinforcement (NR) test in the novel environment and behavioural and HR reactions in the novel object test.

	Latency to eat (s)	Eating bouts (freq)	Alertness (s)	Sniffing (s)	HR avg(bpm)	HR max (bpm)
Final stage (NR, novel)	−0.36 $P = 0.088$	0.45 $P = 0.031$	−0.43 $P = 0.040$	0.62 $P = 0.002$	−0.21 $P = 0.324$	−0.50 $P = 0.015$

practise, inclusion of a human can also be problematic as the test results will depend on the handler, e.g. timing of pressure and release, and the handler may be biased towards certain types of horses.

Our study failed to demonstrate significant effects of baseline stress hormones (FCM) on learning in horses. Rather, our results indicated that stress sensitivity (i.e. the increase in FCM sampled after transition to a new environment and mixing with new horses) predicted impaired learning performance under stressful conditions (novel environment). It should be noted that the horses' baseline concentrations of glucocorticoids during the actual testing period were low, reflecting that the horses were kept under low stress conditions (24 h on pasture in a stable social group). Two studies on horses in training stables used the same FCM analysis method as in the present study, and the first reported a median baseline value of 6.7 ng/g (before onset of training; 40 Quarter horses, age 2 years, all sexes; Gorgasser et al., 2007). The second study reported mean levels of 5.7 and 5.3 ng/g (60 Danish Warmblood, age 3–19 years; all sexes; Poulsen et al., 2011). These levels are similar to the two first collections in our study, where the horses might have been affected by the recent transfer to the research institute and group formation. It would be highly interesting to further investigate the effects of long-term stressors, such as repeated mixing, suboptimal housing or training, on horse cognition, including learning and memory.

Our results support a relation between social rank and FCM values in horses. Social rank has previously been shown to be a predictor of glucocorticoid levels in both animals and humans (Creel, 2001; Hellhammer et al., 1997; Sapolsky et al., 1997), and the results have led to the formulation of two main hypotheses; the "stress of domination" hypothesis which predicts higher glucocorticoid levels in dominant individuals, because dominants fight more than subordinates to maintain their position (Creel et al., 1996; Mooring et al., 2006). On the other hand, the "subordination stress" hypothesis is explained by the fact that subordinates may experience greater harassment and less control than dominants, which can lead to elevated glucocorticoid secretion (Blanchard et al., 1993; Shively, 1998). These two hypotheses suggest that social stress may be experienced by both high- and low-ranking individuals and varies as a function of social organisation and behavioural traits associated with high and low rank as well as with the stability of the social hierarchy (Creel, 2001; Sapolsky, 2005). In stable hierarchies, subordinates are more physically and psychologically harassed, they lack social control and predictability, and need to work harder to obtain resources than dominants. In unstable hierarchies, however, dominant individuals are in the centre of the social tensions, and thus experience more stress than subordinates. In our study, the determination of social status was based upon registrations of successful displacements during test periods with limited resources. This method was chosen because we believed it to better reflect the social status of a horse rather than basing rank determination on the mere frequency of aggressive interactions. Some high ranking horses frequently used only mild threats (or none at all) to displace all other horses from a feed container, whereas other horses were sometimes observed to show fierce aggression towards horses that were lower (or close to their own position) in the hierarchy. We found a strong correlation between the results from the limited resource test and those obtained through field observations of social interactions (Ahrendt and Christensen, 2012), and the lack of opposing displacements suggests that the hierarchy was relatively stable. There was a significant negative correlation between rank and FCM levels indicating that a higher social status was associated with a decreased level of cortisol metabolites. The horses had access to two feeding houses with hay ad libitum but there was insufficient space for all 25 horses to eat at the same time because high ranking horses typically monopolised a full side of a feeding house and lower ranking horses were often waiting for an opportunity to eat. Thus, our results support the "subordination stress" hypothesis, typical for a stable hierarchy. Furthermore, we found that social rank was unrelated to performance in the fear and learning tests. Similar results were obtained for horses in visual discrimination (Mader and Price, 1980), simple maze (Haag et al., 1980; Houpt et al., 1982), and avoidance learning tests (Haag et al., 1980).

In conclusion, we found that learning performance in a novel, but not in a known, environment was related to fear reactions in a standardised fear test. We further found that baseline stress hormone levels were related to rank but not to learning performance. The horses' performance in different learning tests did not correlate, indicating that learning is task dependent and/or that horses are motivated differently by negative and positive reinforcement. Further studies on equine learning could with advantage apply standardised tests based on practical training and focus on whether stress reactivity (as indicated in our study) or stress due to management or training may interfere with learning and memory.

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References

Ahrendt, L.P., Christensen, J.W. Limited-resource test as an alternative to field observations in determination of hierarchy. Proc. 8th Int. Equitation Science Conference, 18–20 July 2012, Edinburgh, www.equitationscience.com.

Blanchard, D.C., Sakai, R.R., McEwen, B., Weiss, S.M., Blanchard, R.J., 1993. Subordination stress – behavioral, brain and neuroendocrine correlates. Behav. Brain Res. 58, 113–121.

Christensen, J.W. Object habituation in horses: the effect of voluntary vs. negatively reinforced approach to frightening stimuli, under review.

Christensen, J.W., Zharkikh, T., Chovaux, E., 2011. Object recognition and generalisation during habituation in horses. Appl. Anim. Behav. Sci. 129, 83–91.

Cooper, J.J., 2007. Equine learning behaviour: common knowledge and systematic research. Behav. Proc. 76, 24–26.

Creel, S., 2001. Social dominance and stress hormones. Trends Ecol. Evol. 16, 491–497.

Creel, S., Creel, N.M., Monfort, S.L., 1996. Social stress and dominance. Nature 379, 212.

Gorgasser, I., Tichy, A., Palme, R., 2007. Faecal cortisol metabolites in Quarter horses during initial training under field conditions. Wien. Tierärztl. Mschr./Vet. Med. Austria 94, 226–230.

Haag, E.L., Rudman, R., Houpt, K.A., 1980. Avoidance maze learning and social dominance in ponies. J. Anim. Sci. 50, 329–335.

Heird, J.C., Lokey, C.E., Cogan, D.C., 1986. Repeatability and comparison of two maze tests to measure learning ability in horses. Appl. Anim. Behav. Sci. 16, 103–119.

Hellhammer, D.H., Buchtal, J., Gutberlet, I., Kirschbaum, C., 1997. Social hierarchy and adrenocortical stress reactivity in men. Psychoneuroendocrinology 22, 643–650.

Houpt, K.A., Parsons, M.S., Hintz, H.F., 1982. Learning ability of orphan foals, of normal foals and of their mothers. J. Anim. Sci. 55, 1027–1032.

Lansade, L., Simon, F., 2010. Horses' learning performances are under the influence of several temperamental dimensions. Appl. Anim. Behav. Sci. 125, 30–37.

Mader, D.R., Price, E.O., 1980. Discrimination learning in horses: effects of breed, age and social dominance. J. Anim. Sci. 50, 962–965.

McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205–216.

Mendl, M., 1999. Performing under pressure: stress and cognitive function. Appl. Anim. Behav. Sci. 65, 221–244.

Merl, S., Scherzer, S., Palme, R., Möstl, E., 2000. Pain causes increased concentrations of glucocorticoid metabolites in horse feces. J. Equine Vet. Sci. 20, 586–590.

Mills, D.S., Marchant-Forde, J.N., McGreevy, P.D., Morton, D.B., Nicol, C.J., Phillips, C.J.C., Sandøe, P., Swaisgood, R.R., 2010. The Encyclopedia of Applied Animal Behaviour and Welfare. CAB International, UK, pp. 124–125, 512–513.

Mooring, M.S., Patton, M.L., Lance, V.A., Hall, B.M., Schaad, E.W., Fetter, G.A., Fortin, S.S., McPeak, K.M., 2006. Glucocorticoids of bison bulls in relation to social status. Horm. Behav. 49, 369–375.

Morris, R., 2007. Stress and the hippocampus. In: Andersen, P., Morris, R., Amaral, D., Bliss, T., O'Keefe, J. (Eds.), The Hippocampus Book. Oxford University Press, New York, pp. 751–768.

Murphy, J., Arkins, S., 2007. Equine learning behaviour. Behav. Process. 76, 1–13.

Möstl, E., Messmann, S., Bagu, E., Robia, C., Palme, R., 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. J. Vet. Med. A 46, 621–631.

Nicol, C.J., 2002. Equine learning: progress and suggestions for future research. Appl. Anim. Behav. Sci. 78, 193–208.

Palme, R., Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Z. Saugetierkd.: Int. J. Mammal. Biol. 62 (Suppl. 2), 192–197.

Poulsen, J.M., Søndergaard, E., Luthersson, N., Malmkvist, J., 2011. Behaviour and stress responses in horses with gastric ulceration. In: Proc. 7th Int. Equitation Sci. Conf., Hooge Mierde, The Netherlands, October 27–29, 2011, www.equitationscience.com.

Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. Science 308, 648–652.

Sapolsky, R.M., Alberts, S.C., Altmann, J., 1997. Hypercortisolism associated with social subordinance or social isolation among wild baboons. Arch. Gen. Psychiatry 54, 1137–1143.

Shively, C.A., 1998. Social subordination stress, behaviour, and central monoaminergic function in female cynomolgus monkeys. Biol. Psychiatry 44, 882–891.

Thorpe, W.H., 1963. Learning and Instinct in Animals, 2nd ed. Methuen, UK, p. 55.

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N.Y. Acad. Sci. 1046, 54–74.

Visser, E.K., van Reenen, C.G., Schilder, M.B.H., Barneveld, A., Blokhuis, H.J., 2003. Learning performance in young horses using two different learning tests. Appl. Anim. Behav. Sci. 80, 311–326.

Wolff, A., Hausberger, M., 1996. Learning and memorisation of two different tasks in horses: the effects of age, sex and sire. Appl. Anim. Behav. Sci. 46, 137–143.