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Social Learning and innovative behaviour in horses

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The evaluation of important parameters for measuring the horses' cognitive capacities is one of the central topics of the equine behaviour team at Nürtingen-Geislingen University. Social complexity has been said to be one of the settings in which needs for cognitive capacities arise in animals. A variety of studies throughout the last two decades proved the horses' social complexity to be far more elaborate than previously assumed. Horses form social bonds for the protection of offspring, intervene in encounters of others, identify group mates individually and easily orientate in a fission fusion society.

In such socially complex societies, animals will benefit from learning socially. In many bird and primate species the degree of social complexity correlates nicely with the species abilities for social learning. Social learning was, therefore, argued to be an indicator for elaborate mental capacities in animals. We were delighted to prove that horses actually copy social behaviour and techniques for operating a feeding apparatus from older and higher ranking group members. In a recent study we found young horses, at the age of 3 to 12, to copy the operation of a feeding apparatus from a human demonstrator. Social learning seems to work nicely in horses when the social background of the animals is considered.

The degree to which individual animals adapt to changes in their social or physical environment by finding innovative solution appears to be the other side of the coin, of whether animals adjust to challenges by social learning. It is not very astonishing, that along with the animals' social

complexity and their ability to learn socially also the degree to which they show innovative behaviour was claimed to be one of the most important demonstrations of advanced cognitive capacities. In a recent approach, we started to ask horse owners and horse keepers in many countries to tell us about unusual behaviour of their horses via a web site (http://innovativebehaviour.org). To date, we received 204 cases of innovative behaviour descriptions from which six cases were clear examples of tool use or borderline tool use. We categorized the innovative behaviours into the classes, a) innovations to gain food, b) innovations to gain freedom, c) social innovations, d) innovations to increase maintenance, and e) innovations that could not be clearly assigned to a category. About 20% of the innovative horses showed more than one innovation. These animals could be termed "true innovators". Again, young horses were more innovative than older ones with the age group 5 – 9 showing the highest number of innovative behaviour descriptions.

In a nutshell, the horses' cognitive capacities appear to be underestimated throughout the last decades. The horses' social complexity is far more elaborate than previously assumed, horses learn socially from conspecific and humans, some of them demonstrate innovative behaviour adaptations to their environment and even simple forms of tool use.

Keywords: social learning, innovative behaviour, Equus caballus, cognitive capacities

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Social Networks: Linking Form with Function in Equid Societies

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Animal societies develop from interactions and relationships that occur among individuals within populations. The fundamental tenet of behavioral ecology is that ecological factors shape behavior and determine the distribution and associations of individuals on landscapes. As a result, different social systems emerge in different habitats and under different environmental conditions. Since characterizing social systems depends on time and motion studies of individual actions and interactions that are often bilateral, such characterizations are often coarse-grained. If social relationships can be characterized using social networks, however, seemingly similar social organizations often reveal informative differences in terms of deep structure. Thus social network theory should be able to provide insights in to the connections between social form and function. This talk will explore how the network structures of horses, zebras and asses can provide novel insights into the functioning of animal societies with respect to the spread of memes, genes and diseases.



Non-invasive monitoring of stress hormones for welfare assessment in domestic and wild equids

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Stress responses play an important role in allowing animals to cope with challenges. Glucocorticoids, key elements in the neuroendocrine stress axis, are traditionally measured as a parameter for welfare assessment. As blood sample collection itself disturbs an animal, non-invasive or minimal invasive methods have gained importance for assessing stress. In horses saliva and faeces are most frequently used. Faecal samples offer the advantage that they can be collected easily and stress-free. In faecal samples circulating hormone levels are integrated over a certain period of time. As a consequence faecal glucocorticoid metabolites represent the cumulative secretion and they are less affected by short episodic fluctuations of hormone secretion.

However, in order to gain reliable information about an animal's adrenocortical activity, certain criteria have to be met: Depending whether the impact of acute or chronic stressors is assessed, frequent sampling might be necessary whereas in other cases, single samples will suffice. Background knowledge regarding the metabolism and excretion of glucocorticoids is essential and a careful validation is obligatory. In addition, this presentation will address analytical issues regarding sample storage, extraction procedures, and immunoassays and various examples of a successful application in equids will be given. Applied properly, non-invasive techniques to monitor stress hormones are a useful tool for animal welfare assessment.



Facial expressions of the Caspian pony to its own picture, mirror and a combination of these two

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Abnormal behaviors in horses are among the most important problems in the riding clubs. Digestive and somatic abnormal behaviors are the two most important abnormal behaviors in the horses, with the loneliness and boredom as the two most important causes of these abnormal behaviors. Many studies showed that spending time (increasing the exercise and training time) would stop such abnormal behaviors. The man power scarcity is the important reason many riding clubs face and this is the reason why the abnormal behavior are mostly observed in such clubs.

Current study is the first report regarding facial expressions of the Caspian Pony to different objects. Totally 10 Caspian ponies were used in this study. The pictures were taken both in the calm and in the furious (nervous) situation. The pony's pictures were the alternatives we used in this research to combat the man power scarcity. We also used a mirror to compare the expressions of the ponies to the pictures and the mirror. The results of this study showed that the ponies showed more attention to the picture in calm position when compared with the picture in nervous position. In the box with the mirror and the picture (in calm position) in it, the ponies paid much more attention to the mirror than to the picture.

We conclude that despite of resistant of ponies for leaving outdoor and entering to indoor (paddock to box), installing mirror can prevent (almost completely) the horse's boredom and loneliness, a very cheap (but not wise) alternative for manpower). The results of this research were applicable and were suggested to many riding clubs with horses with stereotypic behaviors, receiving almost completely positive results.

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COMPARISON OF BEHAVIORAL AND PHYSIOLOGICAL STATE IN CASPIAN PONY BEFORE AND AFTER STRESS

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Behavioral scores (BS) offer an non-invasive, objective and easy to use way of assessing welfare in horses. Their development has, however, largely focused on behavioral reactions to stressful events (often induced), and so far no use of physiological measures has been made to underpin and validate the behavioral measures in the Caspian ponies. This study aimed to develop a physiologically validated scale of behavioral indicators of stress for the purpose of welfare logically validated scale of behavioral and physiological data assessment in the stabled Caspian ponies. To achieve this, behavioral and physiological data were collected from 16 Caspian ponies that underwent routine husbandry procedures. The ponies were divided into two groups. a control and a treatment group (8 each). The ponies in the treatment group took part in a 700 meter race. Analysis of the behavioral data were undertaken by a panel of equestrian industry professionals. Physiological measures (salivary and serum cortisol level) were significantly correlated with the behavioral scores confirming that the scores were meaningful and reflected the physiological stress. The scores offer an easy to use tool for rapid, reliable non-invasive welfare assessment in Caspian ponies, and reduce the need for potentially invasive physiological measures.

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Effect of varying dimensions of the littered lying area on the lying behaviour of group-housed horses (*Equus ferus caballus*)

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Although horses can sleep while standing, recumbency is required for REM sleep and since all sleep stages must be completed for an entire sleep cycle, the opportunity for recumbency is essential for animal welfare. Observations on feral horses indicate a minimal lying duration of 30 min, preferably on a deformable and dry ground. In contrast to feral horses, lying behaviour in stabled horses is often affected by the dimensions of the provided lying area and rank.

In Switzerland, minimum requirements (MR) for the littered lying area are established by law to ensure animal welfare (BLV, 2008) (A/N: approximately match German recommendations (BMEL, 2009)). The aim of this study was to assess the adequacy of the dimensions of the minimum requirements for group-housed horses by investigating 38 horses in 8 groups. Further, hard rubber mats were provided supplementary in order to assess their suitability as an alternative to litter. Four treatments were each applied in randomised order:

0x MR: no litter + 1.5x MR with rubber mats 0.5x MR: 0.5x MR with litter + 1x MR with rubber mats

1x MR: 1x MR with litter; 0.5x MR with rubber mats

1.5x MR: 1.5x MR with litter + no rubber mats For each treatment, after a habituation period of 8 days, lying behaviour was recorded (video, accelerometers) continuously for 72 hrs. Statistical analysis was performed using mixed effects models.

Regardless of the ground chosen, the duration of recumbency per 24 hrs was increasing with increasing dimensions of the littered area ($F_{1,93}$ = 12.9, p = 0.0005; Fig. 1). Whereas the effect flattened from 1x to 1.5x MR, the duration spent on litter – a deformable ground – was increasing continuously ($F_{1,62}$ = 23.1, p < 0.0001). Further, the proportion of lateral recumbency

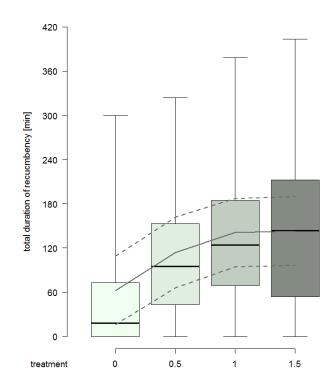


Figure 1: Total duration of recumbency [min] per individual per 24 hours (summarising recumbency on litter, rubber mats and other firm ground) in treatment with 0x, 0.5x, 1x and 1.5x minimal requirements of the littered lying area. Boxplots show medians, interquartile and absolute ranges of data. In addition, model predictions with estimated means (solid lines) and 0.95 confidence intervals (dashed lines) are shown.

was increased with increased dimensions of the littered area ($F_{1,79}$ = 12.3, p = 0.0007). Regarding the number of lying bouts, no differences were apparent between treatments providing litter, but recumbency occurred very seldom if only rubber mats were provided ($F_{1,93}$ = 14.7, p = 0.0002). Further, low-ranking horses spent more lying bouts on rubber mats than high-ranking horses ($F_{1,29}$ = 4.4, p = 0.04). Additionally, the larger the dimensions of the littered area the more horses were present in the lying area at the moment

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of lying down ($F_{1,79}$ = 6.6, p = 0.01). Moreover, low-ranking horses showed considerably higher percentages of involuntarily terminated lying bouts than high-ranking horses if 0.5x and 1x MR were littered ($F_{1,76}$ = 8.43, p = 0.005).

Although the indicated minimal lying duration was averagely performed, large individual differences occurred and at least 8% were lying down less than 30 min per 24 hrs in every treatment. Further, the inclusion of social parameters indicated a beneficial effect of an exceedance of the minimum requirements especially for low-ranking horses. Therefore, the minimum requirements established by Swiss law can be stated as adequate but should be perceived as minimum and not optimum dimensions.

Key words: group housing, lying behaviour, littered lying area, rubber mats

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BLV Federal Food Safety and Veterinary Office, 2008. Tierschutzverordnung (TSchV 455.1).

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Mirror on the wall, who is the horsest of our all? Self-recognition in *Equus caballus*

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Mirror Self-Recognition (MSR) is an extremely rare capacity in the animal kingdom that reveals the emergence of complex cognitive capacities (de Waal 2008). So far, MSR has been reported only in humans, chimpanzees (Gallup, 1970), bottlenose dolphins (Reiss and Marino, 2001) and Asian elephants (Plotnik et al, 2006), all species characterized by a highly developed cognition. There is growing evidence that domestic horses posses high cognitive abilities, such as crossmodal individual recognition (Proops et al, 2009), triadic post-conflict reunion to maintain social homeostasis (Cozzi et al, 2010), complex communicative systems (Whatan and McComb, 2014), flexibility in problem-solving (Lovrovich et al. 2015), and long-term memory (Hanggi and Ingersoll, 2009). All these capacities make horses a good candidate to test the ability of MSR in a domestic species. Through a classical MSR experimental paradigm (de Waal 2008) we tested eight horses living in social groups under semi-natural conditions (from the Italian Horse Protection rescue centre). Animals showing MSR typically go through four stages (Plotnik et al, 2006): (i) social response, (ii) physical mirror inspection (e.g., looking behind the mirror), (iii) repetitive mirror-testing behaviour (i.e., the beginning of mirror understanding), and (iv) selfdirected behaviour (i.e., recognition of the mirror image as self). The final stage, known as the "mark-test", is verified when a subject spontaneously uses the mirror to check for a coloured artificial mark on its own body which it cannot perceive otherwise. The horses underwent a three-phase "mark-test": 1) with sham mark (transparent ultrasound water gel) positioned on both side at jaw level, 2) mark (yellow eye shadow mixed with ultrasound water gel) positioned on left side of jaw (with sham mark on the right), 3) mark (yellow eye shadow mixed with ultrasound water gel) positioned on right side of jaw (with sham mark on the left)



Figure 1: Shape, dimension and position of the coloured mark. Shape, dimension and position are identical with the sham mark on the opposite site.

The mirror was one 0.5-cm-thick piece of 140x220-cm plexiglass glue on wood. Each test lasted one hour, horses were tested once a day, in consecutive days and at the same time. Our preliminary result on 1 horse shows some changes in self-directed behaviours which can be attributed to presence of the coloured mark. Firstly, the presence of the coloured mark significantly increased the frequency of scratching on both sides of the muzzle (p < 0.0001). The most intriguing result (p < 0.0001) comes from the comparison of the scratching rates directed towards the coloured mark side (N = 41) and the sham mark side (N = 23). Under the control condition (i.e. sham mark on both sides) no statistical difference was found for the scratching rates directed to the muzzle sides (dx N = 8; sxN = 5). Although further analyses are needed to confirm these preliminary results, our finding opens new scenarios about the evolution of Mirror Self-Recognition. The capacity of horses to recognize themselves in a mirror may be the outcome of an evolutionary convergence process driven by the cognitive pressures imposed by a complex social system and maintained despite thousands years of domestication.



Keywords:

Domestic horse · Mark test · Socio-cognitive skills · Self-awareness

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Feasible animal-based indicators for assessing equine welfare

Current status of the development of an animal welfare assessment system for horse husbandries

as a part of a sustainability management system

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Introduction

Are horses doing well in their husbandries? For the first time the answer shall be given objectively by an integral on-farm welfare assessment system for horse husbandries. A current research project at the Technical University Munich evaluates indicators for well-being, pain and suffering in horses in order to develop a welfare assessment system (Baumgartner and Zeitler-Feicht 2013, 2014a, 2014b, 2015, Zeitler-Feicht et al. 2015). The research project is professionally supported by horse husbandry experts from academics, industries as well as leading organizations for horse owners and veterinarians.

The aim of the project is to develop a welfare assessment system for all horse husbandry systems which can be applied both for sport horses and for leisure horses. It is based on national animal welfare standards (BMELV 2009) and does take sustainability into consideration. Animal-based indicators are completed with resource-based indicators if necessary. It is stipulated that indicators are valid, reliable and feasible.

Welfare criteria were formulated for the two principles "good health" and "behavioural demands" (see figure 1 and 2). Each has to be represented by at least one indicator.



Fig. 1: Welfare Criteria for "good health"



Fig. 2: Welfare Criteria for "behavioural demands"

The principle "behavioural demands" aims at the possibility for horses to practise species-specific behaviour. It is gathered to what extent the housing conditions allow the horses to live out and show species-specific behaviour. Furthermore in the present studies the frequency of selected behaviours including abnormal behaviour were collected in precise timeframes.

Potential indicators for assessing equine welfare on-farm were selected by study of literature and field tests. The field tests included direct observations on free-ranged horses, horses in group-housing systems and single-stabled horses. The following section presents selected indicators that are feasible for assessing equine behaviour on-farm.

Feasible behavioural indicators for wellbeing

The literature research revealed that "being together" is linked with affiliative behaviour. It includes "resting together", "foraging together" and additionally "walking together". Horses do have a strong need for social bonds. "Beingtogether" amongst horses must be voluntary and not caused by bad weather conditions or lack of space. Therefore the context must be



considered. For temporary direct observations the frequency in group-housing systems is sufficient Key words $(0.57 \pm 0.67 \text{ per horse per 20 minutes})$. That's why in our study "being-together" is considered as a feasible indicator for well-being for horses in permanent or temporary groups. It is intended to conduct further studies on its validity.

Other behaviours such as "social play" is not only linked with positive emotional states in adult horses. Several studies showed that horses use "social play" as a stress relief. However, it is too seldom to collect in an on-farm assessment system. Because of the lack of feasibility and validity we excluded "social play" as an indicator for well-being.

Feasible behavioural indicators for suffering

Horses show "abnormal behaviour" in distress, frustration, deprivation or conflict situations. The present studies showed a relatively high frequency in single-stabled horses (3.3 ± 6.45 per horse per 20 minutes). Hence "abnormal behaviour" is a feasible and valid indicator for suffering. However, established stereotypes need to be excluded, because they may indicate a previous welfare status rather than the current welfare status.

Horses use "agonistic behaviour" to regulate social relations, to defend themselves or to defend resources. If husbandry or management is inadequate, "agonistic behaviour" increases and thereby the frequency of injuries caused by social conflicts. A high frequency of "agonistic behaviour" indicates a high aggression level in group-housed horses and therefore distress and suffering. The mean frequency of group-housed horses is sufficient for temporary observations (2.6 ± 2.26 per horse per 20 minutes). As a result "agonistic behaviour" is a feasible and vaild indicator for suffering. Further studies need to be done on the scoring and severity.

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Animal-based indicators Equine welfare Being-together Abnormal behaviour Agonistic behaviour

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Evaluation of living conditions in free running animals by chronobiological analysis of continuously recorded behavioural data

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We developed a biorhythmical method to assess behaviour patterns and to evaluate living conditions of animals. All kinds of continuous and equidistant long-term recordings of behaviour are suitable for this method. As simple behavioural parameters, such as motor activity, can be conveniently recorded by telemetry from wild animals now, it is possible to investigate stressors by analysing its biorhythmic structure. It is the purpose of this report to describe the basic idea, and the procedure, and to give some examples of application measured on Przewalski horses in an Semireserve.

Keywords:

non-invasive, stress detection, chronobiology, activity

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Sex, but not relatedness nor age, affect the social network of horses in a seminatural reserve

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For time now, social network analysis provides tools to describe concisely the social structure of Nevertheless, the factors that shape the social network and determine the frequency of different types of affiliations are often unclear. For example, the effects of relatedness on social networks have been only studied on relatively few species. Both among social or sub-social species the effects ranged from no effect to a substantial effect. Here we test the hypothesis that relatedness has an effect on the social network of horses (Equus caballus) that live freely in semi-natural conditions, and specifically, that individuals with high values of relatedness to their neighbors in the network will have fewer links Wolf et al, 2011). This hypothesis is based on the notion that related individuals have common interests and are likely to assist each other, and may need less links to other, unrelated individuals. In addition, individuals of similar age and sex are likely to have common needs, and thus are more likely to be associated. We thus tested a second hypothesis: homophily to individuals with similar age and sex will have a role in determining the associations within the social network. The field study was conducted on 27 horses in the Blauwe Kamer reserve in the Netherlands (1.1 sq km). We videotaped horses and their groups and used the information from 22 horses, after excluding the five foals from the analysis. Relatedness was calculated from the pedigree, which was based on parentage, determined by DNA analysis. The social network was constructed based on spatial proximity data. We assessed the influence of relatedness. age-homophily and sex-homophily on the network structure with Multiple Regression Quadratic Assignment Procedure (MRQAP) (Krackhardt 1988), with the R package sna. The results show that there was no significant effect of relatedness on the network, nor an effect of age-homophily. Nevertheless, we found a significant effect of sex-homophily, the tendency of individuals to associate with individuals of the same sex. We argue that the lack of a relatedness effect is not

likely to have been caused due to the inability to detect who is kin. The structured social system in horses includes strong associations between often unrelated individuals, alongside with young individuals leaving their natal harem as part of the inherent inbreeding avoidance (Linklater & Cameron 2009; Boyd et al. in press). The significant effect of sex-homophily could stem from the protection females get from associations with other females, e.g., in the reduction of harassment from males. Previous studies on feral horses showed that mares that were better connected with other females in their harem benefited from higher survival rates to their foals (Cameron et The associations among bachelor al. 2009). males could also contribute to the strength of the sex-homophily effect.

In order to generalize from our results, one needs to examine additional populations of horses, because the conditions in the Blauwe Kamer reserve may not be representative, mainly due to the limited opportunity for dispersal in a restricted area.

Keywords:

Long-term affiliation; spatial proximity, kin detection References:

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Crib-biting behaviour of horses: stress and learning capacity

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Crib-biting is a stereotypy in horses that is potentially linked to both chronic stress and genetic predisposition. Chronic stress can cause neurobiological changes such as alteration of the dopaminergic modulation of the basal ganglia [1]. These neurobiological changes could alter and modify the learning profile of the horses [2,3]. We tested 19 crib-biters and 18 non-crib-biting horses (controls) in five challenging spatial tasks, in order to test if differences in dopaminergic modulation impair learning capacities. The tests were performed in two time periods, in a small arena (8 x 10 m) that was familiar to the horses. For each trials, the horses were led to the start zone in front of a four-meter-long solid fence and were then left alone in the arena. Their task was then to find a bucket containing food, which was situated in different positions around the fence, depending on the tests. The time to reach the food bucket, the trajectory taken by the horse (left or right side of the fence) and the ECG trace were recorded continuously. Additionally, salivary cortisol was collected before the tests (baseline), after the first time period, and after the second We found that crib-biters and time period. controls behaved similarly during the learning tasks. However crib-biters that did crib-bite on the solid fence during the task (group A; 10 horses) behaved differently than crib-biters that did not crib-bite (group B; 9 horses) and controls (group C; 18 horses) for some tests, in their trajectory or time to reach the bucket. These differences are more likely explained by the time taken to crib-bite, than by differences in learning capacity. We did not find any difference between groups

in their heart-rate variability (RMSSD). Yet, we found a difference in salivary cortisol after the first time period between groups A, B and C. Indeed, the crib-biters that did not crib-bite had higher salivary cortisol values than all the other horses (mean±SE: A, 0.51±0.16ng/ml, B, 0.78±0.17ng/ ml, C, 0.59±0.20ng/ml; Linear mixed model (LMM), p<0.05). Our results suggest that cribbiting horses that did not crib-bite during the learning tasks were more stressed than all other horses. This difference could be due to higher stress sensitivity in crib-biters, which could be reduced by the opportunity to crib-bite. These results replicate our previous findings testing differences in cortisol levels between crib-biters and control horses during an ACTH challenge test. Therefore, crib-biting behaviour might be a coping strategy helping stereotypic horses to reduce their stress during frustrating situations [4].

Keyword:

stereotypy, chronic stress, learning task

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Narcolepsy – or REM-deficient?

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Narcolepsy is a neurological sleep disorder characterized by excessive daytime sleepiness, cataplexy (loss of muscle tone), sleep paralysis and hypnagogic hallucinations, also called the "tetrad of narcolepsy". Although the pathogenesis is not completely understood, the disorder is well described in humans and it has been shown that a lack of the hormone hypocretin (orexin) synthesized in the hypothalamus is crucial. Narcolepsy with cataplectic attacks has also been reported in dogs, horses, cattle (STRAIN et al., 1984) and a lamb (WHITE und DE LAHUNTA, 2001).

In dogs up to 17 breeds have been shown to be affected sporadically, while familial forms occur in dobermans, labrador retrievers and dachshounds (TONOKURA et al., 2007). In horses there appear to be two syndroms (HINES, 2005), the first in which animals are affected within a few days after birth (possibly a familial form, reported in Suffolk, Shetland ponies, Fell ponies, Warmbloods, Miniature Horse foals (MAYHEW, 2011), Lipizzaner (LUDVIKOVA et al., 2012) and Icelandic horses (BATHEN NÖTHEN et al., 2009)) and the second in which animals are affected as adults (adult-onset narcolepsy).

It has been shown that both forms of canine narcolepsy are associated with a deficit in hypocretin/orexin neurotransmission (LIN et al., 1999). In the horse a similar etiology is suspected, but so far there are no studies to support this hypothesis.

The cataplectic attacks in humans and dogs occur during excitement or emotional stimulation such as laughing in humans or eating and playing in dogs. In contrast, the cataplectic or sleep attacks in adult horses happen almost exclusively while resting. The collapses observed in equines vary from drowsiness with hanging of the head, swaying, buckling at the knees or total collapse (see fig.1). Affected horses often show injuries and scars at the dorsal fetlocks, dorsal knees or at the face and the lips. ALEMAN et al. (2008) describe some of the suspected adult-

onset narcolepsy cases as possible examples of sporadic idiopathic hypersomnia instead of true narcolepsy.

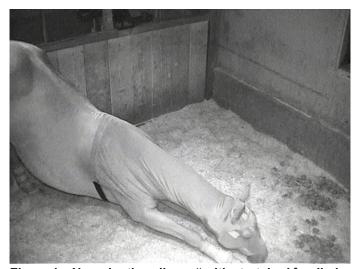


Figure 1: "Narcoleptic collapse" with stretched forelimbs

Since there are not many studies about adultonset narcolepsy in horses, the authors set themselves the challenge to examine horses with supposed narcolepsy, evaluate associated husbandry conditions and to create sleep profiles by polysomnographic examinations of the subjects.

For this purpose a call to owners who's horses were affected by narcolepsy in the German horse magazine Cavallo® was made, followed by the completion of a detailed online survey. 177 owners of horses with diagnosed narcolepsy completed the questionnaire. After a personal selection, 39 of these horses were visited. The horses were clinically examined and blood samples were taken. Video surveillance with infrared cameras and direct observation took place for a period of 24 hours and polysomnographic measurements were performed. Furthermore a closer look was taken at the stabling and management conditions and the medical history of the horses was documented.

So far the results of the study show that the affected horses suffer from up to more than 150



collapses a day. Collapses mainly took place during the night (see fig.2) and most of the horses refused to lie down during sleep time. Shortly before the collapses many horses showed typical REM (rapid eye movement) patterns in the polysomnography.

In a previous study about the sleeping behaviour of horses (KALUS, 2014), it has been shown that REM-sleep occurs every night and only while the equine is in a recumbent position, which can be explained by the characteristically decreased muscle tone during this sleep stage.

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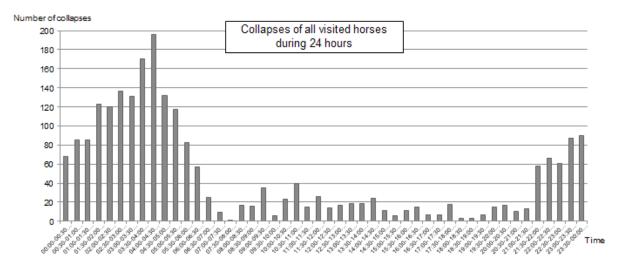


Figure 2: Total number of the "narcoleptic collapses" per 30 min in a 24-hour-time period (n=37, 2 horses did not show any collapses); remarkable is the cumulative occurence of the collapses in the early morning hours

The authors presume that the "narcolepsy" of most adult horses is not a neurological disorder but a REM-sleep deficiency because of recumbent sleep deprivation caused by illness, ethological deficits or non animal-friendly husbandry.

Key words: narcolepsy, cataplexy, polysomnography, REM-sleep deficiency

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In your eyes... What eye wrinkles in horses tell us about their emotional state

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Wrinkles above the eye ball are common in domestic horses but may differ in number and shape both between and within individuals. They are caused by contraction of the inner eye brow raiser, and some people working with horses call them "worry wrinkles", considering them to reflect emotional states. However, as yet no study has formally investigated the relationship between eye wrinkles and emotional state in horses.

The aim of the present study was to induce states of different emotional valence and to assess whether positive emotional states would reduce the expression of eye wrinkles while negative emotional states would increase it. Sixteen horses were confronted in a balanced order with two presumably positively and two negatively valenced situations each. Positive situations included anticipation of a food reward (FA) and petting (P), negative situations included food competition (FC) and waving a plastic bag (PB). Each situation lasted for 60s (TRT) and was preceded by a 60s control phase (CON). Throughout CON and TRT pictures of the eyes were taken, and for each horse four pictures per situation (FA, P, FC, PB) and phase (CON and TRT) were randomly selected (n = 512) and scored in random order and blind to treatment for six outcome variables: overall impression (qualitative), number, angle and markedness of eye wrinkles, presence of eye white, and shape of eye lid.

Data were analysed separately for the right and left eye using linear mixed effects models (angle, number), generalised linear mixed models (eye white, markedness), and ordered logistic regression (qualitative, shape of eye lid), with "situation" (FA, P, FC, PB), "phase" (CON, TRT) and their two-way interaction as fixed effects.

Expression of eye wrinkles did not vary consistently across "situation" and "phase". Independent of phase, eve white appeared less frequently during P than during FA (z=-3.15, p=0.009), FC (z=-2.94, p=0.02), and PB (z=4.17, p<0.001) in the left eye and during PB (z=4.10, p 0.001) in the right eye. Similarly, wrinkles were less marked during P compared to the other situations in the left eye (FA: z=3.15, p=0.009; FC: z=-2.94, p=0.017; PB: z=4.17, p<0.001) and compared to PB in the right eye (z=4.10, p=0.001), while no differences between situations occurred in number of wrinkles, overall impression and shape of eye lid for both eyes. Consistent with our hypothesis, P induced relaxation of the underlying muscle in the right eye resulting in a wider angle compared to its control phase (interaction situation*phase: $F_{3,10} = 3.71$, p=0.055; post-hoc comparison: z=-3.57, p=0.009), while FC induced muscle contraction, resulting in a sharper angle in the left eye (interaction situation*phase: F_{3,11}=6.57, p=0.011; z=3.73, p=0.005).

We conclude that emotional valence may affect characteristics of eye winkle expression in horses which might therefore be a promising indicator of horses' emotional states, but further research is needed to validate the relevant outcome variables.

Keywords: eye wrinkles, emotional valence, positive and negative emotions, welfare assessment



Hyperflexing the horse's neck: a cost-benefit and meta-analysis

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In ethical discussions, a cost-benefit analysis requires that welfare costs associated with an activity can be reliably estimated and balanced against the potential benefits of the activity to both humans and animals. The current study applies a meta-analysis to the peer-reviewed evidence for costs and benefits of hyperflexion of the neck in horses; a practice that has attracted enormous public and scientific scrutiny over the past 15 years. A literature review identified 55 studies dealing with horses' head and neck postures. Fourty-two of these studies examined the impact of various postures on equine welfare, for example, by assessing behavior, physiological stress parameters, health or rider-horse interaction. Thirty-five studies examined the impact of various postures on gymnastics (e.g. kinematics, shifts in weight distribution, muscle activity, airway functioning or overall workload). For the meta-analysis a dataset containing information from each of the individual studies was created. Data included information such as type. degree, duration and circumstances of hyperflexion applied in that particular study as well as information on the horses (e.g., sport discipline, level of training, breed) and on the study design (e.g., size of study and experimental or epidemiological research design). The results of the study regarding the impact of hyperflexion on a) welfare and b) gymnastics were coded as positive (1), insignificant or contradictory (0) or negative (-1). The significant majority of studies (88%) concluded that a hyperflexed head and neck posture negatively impacts welfare. Just one study suggested welfare advantages of training in a hyperflexed head and neck posture. An analysis using a generalized linear mixed model to assess the influence of the above factors collated in the dataset revealed that none of these factors significantly influenced the probability of a study to detect negative welfare implications. Thus hyperflexing the neck appears to impair horses' welfare regardless of, for example, the duration or the way of achieving hyperflexion. A concurrent assessment of the evidence for gymnastic benefits showed that approximately one quarter of studies conclude that there may be benefits, while another quarter of the studies conclude that hyperflexion has detrimental effects on gymnastics. Thus, on the costs-side there is a clear reduction in equine welfare and some undesirable gymnastic effects, as well as likely a compromised profile of the equestrian sports in public. Benefits, on the other hand, include some desirable gymnastic effects, and potentially increased control of the horse for the rider. On balance, it appears that the costs associated with hyperflexion exceed the potential benefits of the activity to both humans and horses.

Keywords horse, head-and-neck posture, hyperflexion, welfare, gymnastics

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Referential communication in the domestic horse (*Equus caballus*): first exploration in an ungulate species

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An important question in the study of animal communication is whether non-human animals are able to produce communicative gestures, i.e. nonvocal bodily actions directed to a recipient. physically ineffective but with a meaning shared in the social group [1]. Passive gestures are instrumental, tuned to the mere presence/absence of others, whereas active informers recognize receivers as communicative agents and activate shared-attention mechanisms for identifying their attentional state (SAM [2]; e.g. Schwab and Huber [3]). Six operational criteria must be evaluated to classify a signal as referential and intentional [4]: (1) alternative gazes between the partner and the target; (2) apparent attentiongetting behaviours are deployed; (3) an audience is required to exhibit the behaviour; (4) the attentional status of an observer influences the propensity to exhibit behaviours; (5) communication is persistent and (6) there is elaboration of communicative behaviour when apparent attempts to manipulate the partner fail. Dogs [5] and non-human primates (reviewed in Liebal and Call [6]) can tune a human receiver's attention to the object of interest by combining directional and attention-getting signals, such as turning the head or body, gazing to the receiver, and/ or establishing eye contact. Research on other species is scarce.

Horses rely on humans to survive in domestic settings and may have evolved skills for communicating flexibly with them [7]. Horses understand human attentional cues (such as body and head orientation, eyes opened/closed) [8], permanent pointing [9] and, to some extent, gazing [10]. Here we tested the ability of 14 outdoor, herd-living domestic horses to communicate referentially with a human partner about the location of a desired target, a bucket of food out of reach. After the baiting of two buckets placed in opposite, unreachable locations were shown by the experimenter, the subject would

walk to one of the two buckets. Because approaching a bucket would reveal that the food is out of reach, we expected the horse to look back to the experimenter, then to the bucket, and alternate this gazing several times to indicate its intention. To test whether our prediction is correct and alternate gazing is indeed the result of the horse's referential communication, we video-recorded the behaviour of the subjects in the test (FORWARD) and three control conditions: (1) FORWARD: experimenter oriented to the center of the arena, (2) BACK: experimenter backward oriented in respect to the arena, (3) ALONE: experimenter absent, (4) MANY: as FORWARD plus a familiar human oriented to the subject behind the bucket (Figure 1).

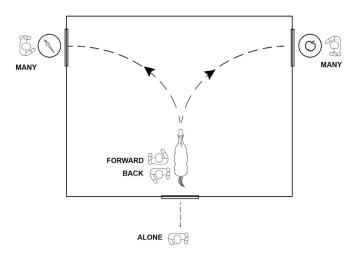


Figure 1: Sketch of the experimental set-up from above. MANY, BACK, FORWARD, ALONE: position of the experimenter and/or the familiar humans during test FORWARD and its three controls. The dotted line represents the direction headed by the experimenter while distancing from the test arena.

We used a conservative criterion of back gazing by considering only turning the head back more than 90 degrees. The results confirmed our prediction. The horses alternated gazes between the partner and the buck significantly more often in the FORWARD than in all the other



FREQUENCIES	FWD vs ALONE	FWD vs MANY	FWD vs BACK
Gazes to Partner	AND LIGHT CONTRACTOR		
N positive differences	3	1	2
N negative differences	8	13	10
Smaller total of ranks	20	5.5	7.5
Two-tailed probability	P = 0.278	P = 0.001***	P = 0.009**
Head Nods			
N positive differences	14	3	2
N negative differences	0	9	12
Smaller total of ranks	0	18	9
Two-tailed probability	P = 0.001***	P = 0.110	P = 0.004**
Head pointed to buck			
N positive differences	3	8	2
N negative differences	10	5	9
Smaller total of ranks	13.5	37	8
Two-tailed probability	P = 0.021*	P = 0.588	P = 0.000***
ALTERNATIONS			
Gazes to partner / buck			
N positive differences	3	3	1
N negative differences	11	11	12
Smaller total of ranks	21	10	8.5
Two-tailed probability	P = 0.049*	P = 0.005**	P = 0.006**
Gazes to partner / head no	ds		
N positive differences	3	2	3
N negative differences	10	10	10
Smaller total of ranks	14	5	9
Two-tailed probability	P = 0.026*	P = 0.005**	P = 0.008**

Table 1:

Comparisons of Frequencies and Alternations Between the Test Forward and All Other Conditions. Head Nods: extension of muzzle left/right or up/down; Head pointed to buck: neck stretched towards the buck. All given results are from Wilcoxon's tests and are two-tailed. Mean and standard deviation are indicated for each condition.

conditions (Table 1), thus satisfying operational criteria #1, #3 and #4. They also alternated head nods with gazes to the partner significantly more often during the FORWARD condition. We thus considered head nods not an instrumental signal of arousal, but an attention-getting behaviour with communicative function. Subjects used both head nods and neck stretched toward the buck more often in the FORWARD than in the BACK and the ALONE conditions, thus satisfying criteria #2, #3 and #4. In condition MANY, the frequency of head nods did not differ from condition FORWARD, probably because nods were directed to the additional partner behind the buck. This also satisfies criteria #4. The horses gazed to the partner most often in the FORWARD than in the BACK and the MANY conditions, but not in the ALONE. In this condition, subjects could

observe the partner walking further from the test arena. To test for the different functions of gazes in presence and in absence of the partner, we compared their average duration between the two conditions: the significantly longer duration of gazes when the subject was alone suggests the instrumental monitoring function of gazes in this experimental condition.

Altogether, the findings suggest that domestic horses possess the ability to use referential communication in an interspecific context, but additional analyses are needed to test for operational criteria #5 and #6. Flexible and voluntary use of communicative signals reveal sophisticated cognitive processes involved in the strategic emission of these signals, and the finding of referential communication skills in an ungulate species forces us to reconsider the evolutionary path of intelligence. Furthermore, ungulates are used intensively by humans (transportation, meat, agriculture, leisure activities), and their welfare is often compromised. Determining whether ungulates can communicate their needs and preferences is paramount to a proper ethical management.

Key words: domestic horse, referential communication, human-horse communication, intentionality

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Hair testosterone and cortisol concentrations and their relationships to physiological and social status in feral horses (*Equus ferus caballus*)

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Determining steroid hormone concentrations in hair has been frequently performed in humans, and increasingly in wildlife and domestic animals. Hair hormone concentrations may provide insight on how individuals are responding to their physiological condition or social situation. Cortisol is most frequently measured in hair as a biomarker of long-term stress, while testosterone may be linked with reproductive status in males. These hormones are commonly measured in substances that reflect either current (e.g. blood) or very recent (e.g. saliva, urine, feces) circulating levels. However, these hormones are also incorporated into hair during hair growth and provide a chronological record of circulating hormone levels. Thus, analysis of steroid hormones in hair provides a much longer representation of an animal's endocrine status than other tissues frequently targeted for non-invasive monitoring.

The feral horse (Equus ferus caballus) population on Sable Island, Nova Scotia, Canada, has been annually censused during the mid-late summer since 2008 to track individual life histories and population dynamics. We collected tail hair (n = 144 females, n = 162 males) from known individuals either opportunistically or from natural or artificial snags to investigate how hair cortisol and testosterone might be associated with physiological state (e.g. lactating vs. non-lactating, body condition, age), as well as their social situation (e.g. dominant band stallion, subordinate band stallion, or bachelor) and measures of sociality. The proximal 5 cm of hair (excluding first 4mm or root region) were ground to a fine powder and hormones extracted with methanol and analyzed by using enzymelinked immunoassay.

Preliminary analyses of the data showed a general sex based difference in hair cortisol

concentrations (females lower than males; t = 3.16, df = 317, P=0.002). Among females, the presence of nursing foals was accompanied by an increase in hair cortisol (z = 2.93, df =140, P = 0.003); however, no significant difference was found in hair cortisol concentrations based on sex of the foal (t = -0.06, df = 82, P =0.95). Horses in poor body condition tended to have higher hair cortisol than those in good or excellent condition (slope= -0.203, df = 312, P = 0.003). We also observed an increased concentration of hair cortisol as horses increased in age from 3-6 or entered into reproductive maturity. Adult male dominant band stallions did not have significantly less cortisol than bachelors or subordinate stallions but these three groups were significantly greater than young males (aged 3 and 4) who generally do not challenge the older males for reproductive opportunities.

Additionally, we looked at hair testosterone concentrations for n=46 males. Testosterone is known to influence traits and behaviours that enhance sexual selection. Often there is an inverse relationship between cortisol levels and testosterone; in particular, being able to maintain high testosterone and not have elevated cortisol related to the metabolic costs of sexual trait production ensures that traits or behaviours honestly signal the quality of the individual. For this reason we'd expect to see band stallions (those males in a position to mate) have a lower value in the ratio Cortisol: Testosterone. Early indications suggest we see this phenomenon in feral horses.

As a relatively new approach in wildlife research, the use of hair hormone analysis shows promise in contributing to our understanding of physiological aspects of sexual selection and other processes. Additionally, hair hormone analysis may have applications in advancing knowledge of animal husbandry and in particular, welfare.



A horsekeeping system for the 21st century

Jane Myers, Stuart Myers

The Equicentral System is a horse management system that incorporates equine grazing behaviour, equine welfare, good land management practices (in order to maximise the production of safer and more biodiverse pasture plants), reduced human labour input, reduced costs and better wider environmental management. A true win - win situation all round. We have been teaching horse owners about this system for many years now and it has been implemented in every type of climate. Most horse properties can implement this system with very little or no outlay.

Keywords group housing, sustainability, environment, management, land

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The analysis of social bonds in feral horses

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In many social mammals, individuals preferentially affiliate with a small subset of available partners instead of distributing their social behaviors equally among all group members. The resulting social bonds have been investigated in several mammalian taxa, especially in primate societies, but also in other taxa such as birds, dolphins, rodents and ungulates. In feral horses, a great number of studies on social bonds can be found, but with a huge variety between methods for the analysis. There seems to be a lack of a clear and common definition of social bonds in horses and of comparable analyses. For example, there are irregularities between the studies regarding the research designs, the selection of recording methods and the interpretation of the measurements. Mutual grooming is used most often for the analysis of social relationships in many species. As mutual grooming is rare in horses, especially measurements of spatial proximity are commonly used for the analysis of social bonds in addition to other behavioral patterns. However, the combination of mutual grooming and nearest neighborhood analyses for the analysis of social bond is debatable, as in contrast to mutual grooming. which must occur deliberately by both grooming partners, the spatial distribution can be influenced by one partner alone, which may even force the other horse to keep a certain distance or to stay in close proximity.

In this study, we investigated the comparability of mutual grooming and nearest neighborhood data for social bond analyses in feral horses. Therefore, we observed five groups of semi-wild

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living Przewalski's horses and six groups of feral horses.

We analysed the horses' social ranks by

We analysed the horses' social ranks by applying an Average Dominance Index, we recorded the distances between the animals and observed the number of mutual grooming events as well as friendly approaches.

Our results show that there was only a weak correlation between the frequency of staying in nearest neighborhood and mutual grooming in all observed horse groups. In contrast to this, the correlation between the number of friendly approaches and mutual grooming events was higher in most groups.

Hierarchies did not affect social bonds, as mutual grooming was similarly induced by higher and lower ranking animals and the social rank did not affect the choice of the grooming partner. Similarly, likelihoods of staying in the neighborhood of particular animals were not affected by the animals' social rank.

The grooming frequencies differed between the different horse groups and between the individual horses living in the particular groups. They seem to be effected by individual predisposition.

Altogether we suggest that the ratio of mutual grooming seems to be a better indicator for social bonds in feral horses than the frequency of staying in the nearest neighborhood. Mutual grooming occurs deliberately and is bidirectional, whereas nearest neighborhoods could be enforced and unidirectional. For the calculation of social bonds in horses, we consider it to be more reliable to combine the frequency of mutual grooming with the frequency of friendly approaches.

Keywords:

social bonds, grooming, nearest neighborhood, rank



Does housing in a "social box" change faecal cortisol metabolites concentration in stallions?

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In order to improve the housing conditions of stallions in individual boxes by offering a possibility to have more social contact, the Swiss national stud farm tested a new box system for horses, allowing increased physical contact with the neighbouring stallion. The aim of this part of the study was to investigate whether this type of housing system (named "social box") potentially induces a change in stress reactions in stallions compared to conventional boxes. Therefore faecal cortisol metabolite (FCM) concentration was measured as a non-invasive parameter to assess endocrine responses related to this new environment.

Four groups each consisting of eight adult Freiberger breeding stallions were included

in the test design. Every stallion spent three weeks in a conventional box and in a social box respectively (cross-over design). The conventional box consisted of a separation wall with a lower opaque part and an upper part with vertical barriers (5 cm between barriers), allowing visual and olfactory contact but strongly limiting

tactile contact. The separating wall of the social box consisted of two lateral sections, one part being opaque to the ceiling and the second part consisting of vertical barriers (30 cm between barriers), allowing the horse to have physical contact with its neighbour or to avoid it.

In horses, FCM concentration reflects an average level of circulating cortisol over a period of approximatively 24h. Faecal samples were collected the day following integration in social / conventional boxes, reflecting the potential stress induced by increased social interactions

during the integration. In order to asses potential chronical stress, faeces samples were also collected in week one, two and three after the integration into the social / conventional box (in total: 4 samples per horse and housing system). The samples were immediately stored at -20°C until they were analysed. The samples were not analysed in the laboratory until the end of the experiment, therefore the duration of conservation in the freezer varied from 40 to 429 days.

A considerable percentage of data from groups 1 and 2 was below the detection limit (<0.8 ng/g) (Tab. 1). Thus the statistical analysis was conducted with the FCM concentration from groups 3 and 4 (n horses = 16) which contained no values below the detection limit.

Tab. 1: Details about FCM values and storage time for the 4 groups of stallions

Group	Storage duration [d]	Proportion of data below the detection limit (<0.8 ng/g)	Mean [ng/g]	Median [ng/g]
Group 1	384-429	55.6 %	2.2	0
Group 2	315-360	25.5 %	5.8	6.3
Group 3	41-79	0.0 %	8.7	8.0
Group 4	40-85	0.0 %	5.8	5.4

Despite the impressive social interactions observed between the stallions directly after being introduced into the social boxes, we did not find any differences in FCM concentration between the stallions being introduced into the conventional box and the social box on the day of integration (social box: n samples = 16, mean±SD: 6.9±4.7 ng/g; conventional box: n samples = 16, mean±SD: 9.0±11.2 ng/g; Wilcoxon signed rank test V = 70, p = 0.94).

Overall the samples taken during integration and in week one, two and three did not show



evidence of changes in FCM concentration in either housing system over a longer period of time (social box: n samples = 64, mean±SD: 7.9±6.2 ng/g; conventional box: n samples = 64, mean±SD: 6.6±3.4 ng/g; Linear mixed model (LMM), p = 0.56).

Our results suggest that the possibility of having physical contact with a conspecific does not induce changes in FCM concentration in breeding stallions. The considerable percentage of values below the detection limit in groups 1 and 2 seemed to correlate with the increasing duration of storage before analysis. During the IESM Network Meeting 2015, we would like to discuss possible methodological issues and the possibilities to correctly integrate these low values in the statistical analysis.

Keywords:

housing system, stallions, social interaction, stress, faecal cortisol metabolites



Poster Presentations



Verbal expressions of the horses to the pain

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For communicating with the environment (other horses, foals, owner, etc.), horses have to use different methods. In contrast with the human, for whom talking is the most important way of communication, the horses can't talk. In the years 1990s and before, the imagination was such that the infants do not express verbally to the pain either (like horses).

To communicate, horses use their body language. Vocalization (if not the body language), seems to be the most important way of communication in horses, though it seems they use the same tone when exposing to different events.

In this study which was performed in collaboration with the electronic institute of Sharif technical university (the top most technical university of the country), totally 25 horses were used. The horses were exposed to different events (hunger, pain, loneliness, mating, parturition and separation of the 1-2 weeks old foals from their dams). The verbal expressions of the horses were studied using spectrogram.

The results of this study showed that there were significant differences between the spectrograph of the voices of the horses, exposed to hunger, and the separation of the foals from dams. This was the same (no significant differences) when horses were exposed to loneliness, separation and hunger. There were no significant differences between the verbal expressions of the horses while exposed to mating, parturition.

One of the most important reasons why the horses do not have verbal expressions when exposing to the pain, might be the absence of the part of the brain, responsible for the pain interpretation. Morse research has to be performed to prove this.

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Effect of mud treatment from Heviz Spa Lake on the joints and locomotion activities of horses

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Medical research on the effects of thermal mud in the human medical field already retrospect for many years (Gyarmati és Kulisch 2008). As a result, the thermal sludge product is successfully applied more widely, especially in the rehabilitation of rheumatic diseases (Gyarmati és mtsai., 2012). Research results related to the management of horses for the time being, however, are not available.

The aim of our study was to investigate how a mud treatment from Heviz Spa Lake affects the movement quality and flexibility of certain joints in horses. An experiment was carried out with 10 male and female school and sport horses. All of the horses had been ridden longer period than 3 years and had correct and healthy movement. Horses were treated with mud ten times, respectively, daily in the evenings. Wet sludge was blamed on the knee, hock, elbow, shoulder, back, stifle, front and hind cannons and fetlock joints. The sludge used for treatments was washed off in the morning. At the beginning of the experiment, after the treatment and 8 weeks following the average stride length and the longest distance between the print of hind and front foot during walking and trotting, maximal flexibility of knee, hock and fetlock joints were measured. To calculate the number of steps horses were lead straight during walking and trotting on 30 m flat distance. Following this the stride length was determined. To determine the longest distance between the print of hind and front foot on flat, sandy soil, the distance between hind and front prints was measured three times. The maximal flexibility of each joint was measured with a joint protractor. Statistical analysis was carried out with one way analysis of variance (ANOVA) with SPSS 7.0 program.

According to the results (table 1.), the horses responded positively to the treatments. The most positive results were detected by the average stride length during walking, maximal flexibility

of the front fetlock, knee and hock. This is partly explicable with the beneficial effects of sulphur on the joints, which is well-known in human field (Kovács és mtsai., 2012). The stride length and longest distance between the print of hind and front foot were lower but positively influenced by the mud treatment. Eight weeks after the treatments, most of the parameters similar to human therapeutic results (Kulisch és mtsai., 2012), compared to directly after the mud baths completion values were further improved, a slight negative effect was observed only for a few test values, but the results obtained here were more favourable, as at the beginning of the experiment. The results seem to confirm that the treatment effects can be considered long term. This is also explained by the slurry preparation from which absorbed elemental sulphur and sulphur oxidizing hydrogen sulfide absorbed in the body may be another source of hydrogen sulphide formation at the skin (Gyarmati, 1982).

Our results show, that the mud treatment from Heviz Spa Lake may have benifical effects on the joints, playing an important role in the locomotion of horses.

The results are remarkable as well, also because of the evidence of the chemical impact of mud also can help. Such modes of action are still under research and only partly demonstrated in human medicine (Odabasi és mtsai., 2008). Further veterinarian research has to be carried out to confirm the results. The results of the present experiment and the prospect of further research could be pioneer, as the Heviz mud, as well as the thermal effect of water even before in the equine medicine has not been demonstrated experimentally, only individual observations are aviable. So the veterinary use of Heviz mud, which has been proven many times in human medicine, seems to be a new research field.

Key words: mud treatment, Heviz Spa Lake, maximal flexibility of joints, locomotion activities



Table 1.: The effect of Heviz mud treatments on the examined parameters

	before the treatments	<u>after the last</u> treatment	8 weeks after the last treatment
average stride lenght during walking (m)	1,80±0,07 a *	1,86±0,12 ab	1,93±0,11 b
average stride lenght during trotting (m)	2,45±0,22	2,63±0,24	2,67±0,20
the longest distance between the print of hind and front foot during walking (cm)	30,80±10,17	31,25±10,26	33,06±12,85
the longest distance between the print of hind and front foot during trotting (cm)	17,65±9,04	22,80±10,10	19,13±12,94
maximal flexibility of knee (degree)	39,5±2,8 a	36,2±3,5 ab	33,8±5,3 b
maximal flexibility of hock (degree)	49,2±6,21	47,8±9,45	46,6±8,35
maximal flexibility of front fetlock joint (degree)	121,5±7,47 a	112,7±9,41 b	115,6±2,39 ab
maximal flexibility of hind fetlock joint (degree)	92,0±9,49 a	83,6±6,72 ab	81,9±6,51 b

*ab: Averages with different letter marks differ siginficantly (p<0,05)

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Integration of zoo-kept Przewalski horses into a herd of Przewalski horses living in a semireserve

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Naturally, horses live in groups in which all individuals are long-term acquainted with each other and a stable hierarchical system is established. In conservation management, introduction of horses into foreign groups is often required but will lead to fights, stress and increased health risks for the animals. We investigated the integration process of four Przewalski mares from the Zoo Leipzig into the herd of five Przewalski horses of the semireserve Liebenthal (Brandenburg, Germany). We observed changes in social hierarchy as well as a higher stress level (expressed by disturbed activity pattern and lower synchronization with environmental conditions) especially in the introduced horses (from Zoo Leipzig). We investigated the animals continuously over two years to detect what time is needed for a successful integration. Finally, we give some advice for the integration of Przewalski horses into a new herd to reduce the stress of the animals substantially.

Keywords: Przewalski horse, introduction, stress



Energetic adaptations of Shetland pony mares

Brinkmann, Lea, Gerken, Martina, Riek, Alexander

Recent results suggest that wild Northern herbivores exhibit signs of a hypometabolism during times of low ambient temperature and food shortage in order to reduce their energetic needs. However, there are speculations that domestic animals lost the ability to reduce energy expenditure. To examine energetic and behavioural responses 10 Shetland pony mares were exposed to different environmental conditions (summer and winter). During winter ponies were allocated into two groups receiving two different food quantities (60% and 100% of maintenance energy requirement). We measured the field metabolic rate, water turn over, body temperature, locomotor activity, lying time, resting heart rate, body mass and body condition score.

In summer, the field metabolic rate of all ponies (FMR; 63.4±15.0 MJ/day) was considerably higher compared with food restricted and control animals in winter (24.6±7.8 and 15.0±1.1 MJ/day, respectively). Furthermore, during summer, locomotor activity, resting heart rate and total water turnover were significantly elevated (P<0.001) compared with winter. Animals receiving a reduced amount of food (N=5) reduced their FMR by 26% compared with control animals (N=5) to compensate for the decreased energy supply. Furthermore, resting heart rate, body mass and body condition score were lower(29.2±2.7 beats/min, 140±22 kg and 3.0±1.0 points, respectively) than in control animals (36.8±41 beats/min, 165±31 kg, 4.4±0.7 points; P<0.05). While no difference could be found in the observed behaviour, nocturnal hypothermia was elevated in restrictively fed

animals. Our results indicate that ponies adapt to different climatic conditions by changing their metabolic rate, behaviour and some physiological parameters. When exposed to energy shortage, ponies, like wild herbivores, exhibited hypometabolism and nocturnal hypothermia.

Keywords:

Body temperature, Energy expenditure, Food restriction, Hypometabolism, Locomotor activity, Shetland pony



Shetland ponies (Equus caballus) show quantity discrimination

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The complex housing environment and the close contact between humans and horses in equine sports place high demands on the learning capacity of horses. To date only limited information is available on the learning ability of the horse including higher order cognition. A type of higher order cognition is to perceive and discriminate quantities. Several mammals and birds have shown to be capable of discriminating objects due to their quantity (Brannon and Roitman, 2003). With regard to horses, there are only few studies available concerning their numerosity judgment (Uller and Lewis, 2009) and this ability is discussed controversially (Henselek et al, 2012). Possibly, the legacy of "Clever Hans" overshadowed further research on numerical capacity in horses, a horse to whom several psychologists incorrectly attributed the capacity of symbolic calculation (Pfungst, 1907; Rosenthal, 1965). In the present study we wanted to show whether Shetland ponies are able to transfer a previously learned concept of sameness to a numerosity judgment. The base of the test design was a "matching to sample" task, where the ponies had learned to relate abstract symbols to another which were presented on a LCD screen. Three Shetland ponies, which previously solved the matching to sample task, were tested in two test phases. In the first test phase different quantities of dots were presented (1 vs. 2; 2 vs. 3; 3 vs. 4; 4 vs. 5). To exclude discrimination due to the shape of the stimuli, the dots were varied in size and arrangement. The stimuli were presented in a triangular arrangement on the LCD screen; the sample stimulus was presented in the middle above and the discrimination stimuli in the two lower corners (S+ and S-). The pony received a

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Tel.: +49-(0)551-396680 Fax: 49-(0)551-395587 Email: vgabor@gwdg.de (S+). When the negative stimulus (S-) was chosen, the pony entered the next trial. Each learning session consisted of 20 decision trials. To investigate whether the numerosity judgment was transferable to mixed geometrical symbols (tri-, rectangle, rhomb, dot and cross) a second test phase was designed. All of the three Shetland ponies met the learning criterion of the first test phase (80% correct responses in two consecutive sessions) within the first eight sessions. One pony could transfer all judgments to the mixed symbols (up to 4 vs. 5), another pony up to 3 vs. 5 and the third on the level 2 vs. 3. These are the first reported findings that ponies are able to discriminate up to five objects. The numerosity judgment seemed to be easier for the ponies when homogenous objects were presented, than in the case of heterogeneous symbols. The reaction of the ponies occurred within few seconds, suggesting that the animals used subitizing for their numerosity judgment.

food reward, by choosing the positive stimulus

Keywords:

Cognition, numerical capacity, numerosity judgment, Shetland ponys

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The impact of paddock design on the behaviour of the domestic horse (Equus caballus)

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The design of a captive environment should facilitate the expression of the natural behavioural repertoire of the species concerned. The domestic horse (Equus caballus) is a social, herd dwelling species that is often housed in isolation from con-specifics or kept in groups in paddock enclosures. Although the latter allows for social interaction and does not restrict movement per se, it may not promote natural activity patterns or group cohesion. The aim of the current study was to assess the impact of two different paddock designs on the behaviour of a stable group of horses (n=6: 2 mares, 4 geldings).

The paddock designs tested were adjacent and grassed similarly, but configured differently. A central paddock (75 x 75m) designated NT, was surrounded by a track 1.6-5.5m wide, designated T. The horses were turned out in their group into T or NT for 3 consecutive days. Their behaviour was recorded for one hour three times each day (10.00, 13.00, 16.00 hrs). Within each observation period of one hour focal sampling was used, each individual horse being observed for a 10 minute period. They were then moved to the other enclosure type for a further 3 consecutive days, followed by a repeat of each condition. When turned out the horses were fitted with a global positioning system device (Garmin Forerunner 305) to monitor distance travelled and speed for the period 10.00-17.00 hrs. The mean percentage of time spent in each behavioural state (standing alert, standing resting, walk/trot, grazing, lying, social interaction) during the periods observed was calculated. Behaviour during social interactions was classified as either affiliative (approach, follow, friendly contacts, mutual grooming) or agonistic (approach and retreat, bite, chase, head threat).

The horses travelled significantly further in

T than in NT (paired samples t-test: t (5) = 11.74, p<0.001) and moved significantly faster (Wilcoxon signed rank test: z = -2.21, p=0.03). See Table 1. When the percentage of time spent in each behavioural state in T and NT was compared some significant differences were found. A significantly higher percentage of time was spent active (walking /trotting) in T than in NT (paired samples t-test: t(5) = 5.74, p=0.002). Standing alert was only recorded in T (paired samples t-test: t(5) = 3.48, p=0.02). A significantly higher percentage of time was spent grazing in NT than in T (paired samples t-test: t(5) = -3.58, p=0.016). Significantly more social interaction occurred in T than in NT (paired samples t-test: t(5) = 5.93, p=0.002). See Figure 1. In T, 91% of social interactions were affiliative and 9% agonistic, whereas in NT 29% were affiliative and 71% agonistic. No difference was found in the percentage of time spent standing resting or lying down in T and NT.

The benefits of housing horses in groups as opposed to individually have been demonstrated in previous studies. In addition to better satisfying the behavioural needs of the horse it has been found that group housed horses adapt more easily to training and display less undesirable behaviour than those housed individually (Rivera et al. 2002; Søndergaard and Ladewig 2004; Visser et al. 2008). However, individual housing is frequently selected by horse owners in preference to group housing to avoid the risk of injury during agonistic encounters. Fureix et al. (2012) suggest that management practices may well contribute to aggressiveness in horses and that the conditions under which we keep horses should be reviewed. The findings of the present study indicate that the design of the enclosure in which groups of horses are kept affects the nature of social interactions. The



T paddock design resulted in reduced intragroup aggression. However, this paddock design also reduced the time spent grazing and increased vigilant behaviour. Although the results demonstrate that a paddock system including tracks may facilitate group cohesion and more natural movement patterns, the long-term impact on behaviour and welfare requires further investigation.

Key words: Horses, social behaviour, management, housing, paddock

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Innovative behavior in horses (Equus caballus)

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Contrary to the widely-spread assumption that horses just have restricted cognitive capacities and are not very flexible in their behaviors, we showed that horses display innovative behavior and even make use of tools (Krueger 2015, Krueger et al. 2015). These findings derive from a database (http://innovative-behaviour. org/) the Equine behavior team managed in the past two years. Some horses did not only show single innovations, but several different innovations. The number of innovations per individual varied from 1 to 10. 20 % of all innovative horses in the database showed more than one innovation. These individuals can be called the 'true innovators'. Moreover innovations were dependent on age. Young horses were more innovative than older ones, whereby horses at the age of five to nine years were the most innovative. When considering the housing system innovative horses in a single housing (inside box, outside box, paddock box) had a slight majority towards horses in group housing (open stable, active stable, pasture day and night). But given the fact that ratings on housing system frequencies state 95% of the horses to be kept in individual housing, innovations in individual housing are rare. Nevertheless, horses kept in inside boxes without a window, opened doors more often than all other horses. Aside from this effect, housing systems did not trigger the frequency of innovative behavior. Innovations for gaining freedom and innovations in general were widespread among horses with daily access to pasture and daily contact with conspecifics. Innovations for gaining food were not more likely

to occur in horses that were fed little amounts of roughage. In conclusion, the housing of horses does not seem to be the primary catalyst for developing innovative behavior in horses. What makes a "true innovator" in horses, in addition to age, remains to be seen.

Keywords: innovative behavior, age, housing system, horse, *Equus caballus*

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Effects of a walk phase at the warm up onset on physiological and behavioural parameters of ridden horses (*Equus caballus*)

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For human athletes, any physical performance classically begins with a warm-up including a phase of cardio-respiratory activation as well as a phase of neuro-muscular mobilization. For the equine athletes similar routines are widely followed despite limited scientific studies addressing its effectiveness. Although physiological bases are of a similar nature in humans and horses, the latter are showing a different "telos" related to their status of prey. Indeed, their survival depends on their ability to detect any predator or potential danger in their environment and to estimate the need to run away. Therefore warm-up techniques should be adapted to this specificity and we added a walk phase preceding the usual warm-up during which the horse was allowed to visually assess its environment without constraint imposed by its rider, reins being long. We assumed that this walk phase would allow a more relaxed mental state and then a decrease in cognitive resources involved in environmental monitoring. Consequently this would release cognitive resources then available for the communication between the horse and the rider.

The autonomous nervous system (ANS) regulates the heart rate via the sympathetic and the parasympathetic nervous systems. The sympathovagal balance and the heart rate variability are considered as good indicators of an acute stress state as well as emotional states. These measures are frequently used to estimate well-being in animals. During the walk phase, we observed a significant decrease of the heart rate (HR), a significant increase of its variability and

a significant modification of the sympathovagal balance in favour of a higher contribution of the parasympathetic control. We also measured the non-linear correlation dimension which reflects the degree of freedom of a system. To the best of our knowledge, this method was used until now only in humans to whom it was shown that its decrease is related to stressful events and is associated with a bad prognosis for survival. During the walk phase in horses, the dimension of correlation of cardiac activity was increased.

From a behavioural point of view, we observed a significant decrease of side movements related to head orientation as well as significant changes in ears position, the latter being preferentially directed forwards at the beginning of the walk phase and laterally at the end. As previous research suggests that ears position and head orientation can be involved in attentional mechanisms in horses, our results suggest a decrease of the attention focused on the environment. Furthermore, a significant lowering of the neck was observed as well as a significant decrease of behaviours related to stress.

To conclude our results suggest that a walk phase preceding the usual warm-up of horses would contribute to improve their mental state in favour of more relaxation and of a decrease of the attentional resources invested in environmental monitoring.



A pilot study on horses' behaviour and distance travelled in a "Paddock Trail" husbandry system

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With most modern horse husbandry systems, horses' locomotory behavior is quantitatively as well as qualitatively considerably altered, compared to the slow and continuous movement shown by horses living under conditions similar to those they have evolved under. This lack of locomotion as well as the change in quality of locomotion is thought to be responsible for a number of health issues seen in present day horses. The aim of the present study was to assess behaviour and particularly locomotion in horses kept in a husbandry system specifically designed to stimulate locomotory behaviour in horses. This type of husbandry system is named "Paddock Trail", (PT) but is also known as "Paddock Paradise", and the key concept of this husbandry system involves strategic placement of small portions of feed along a track which is an integral part of this husbandry system. For the present study, 11 horses, not used for riding or other activities and kept in one Paddock Trail husbandry system were available. Seven horses used in equine assisted therapy lessons and housed individually in conventional paddockboxes (IB) served as a control group. Both groups of horses were composed of a similar mixture of horses with regard to age and breeds. Using timesampling, behaviour of the horses was observed in 8 bouts (4 morning and 4 afternoon sessions) of 5 hours each. In addition, the average speed and distance covered was assessed in 3 (PT) and 5 (IB) horses, respectively, using a GPS system. Since the GPS signal is blocked by buildings and reliable recording would not be possible indoors, the IB horses were recorded

only during their work in the therapy sessions. The behavioural observations revealed that the PT horses allocated a larger proportion of time to locomotory behaviour compared to the IB horses (on average 12 vs 3% of the 5 h observation periods; P<0,05), and in turn resting behaviour was reduced in PT horses compared to IB horses (30 vs. 46%; P<0,05). Time spent grazing (10 vs. 8%) and feeding other than grazing (47 vs. 44%) did not differ significantly between the two groups of horses in the two different husbandry systems (P>0,1). In addition, resting and feeding behaviour was influenced by social rank, such that higher ranking horses spent more time feeding and less time resting compared to horses of lower ranks (both P<0,05). Within the 5 hour observation periods, horses of the PT system covered on average a distance of 2,7 km at an average speed of 0,5 km/h. In comparison, IB horses covered during their work in the therapy sessions on average a distance of 2,1 km at an average speed of 3,6 km/h for a duration of 35 minutes. Although the confounding of groups of horses with husbandry system and although the GPS data does not allow for a direct comparison of the husbandry systems and does not cover the entire 24 hours of a day, these data indicate along with the behavioural observations that the PT system stimulates the horses to increase their locomotory behaviour.

Keywords horse, husbandry, paddock trail, locomotion, behaviour



Questionnaire survey personality assessment of horses of different use

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We collected data from 248 horses of different breeds, age, sex and use, forming four groups: 74 trotters, 70 gallop horses, 60 horseback archery horses and 44 police horses. All horses were trained and ridden/driven in a regular base. Caretakers or owners who were familiar with the target animals were asked to assess their horses' temperament. The temperament scores were obtained with the 7-point scale questionnaire according to the Horse Personality Questionnaire which has 25 items and has previously been shown to be reliable for the assessment of personality in horses. It measures six personality components in horses: Dominance, Anxiousness, Excitability, Protection, Sociability and Inquisitiveness.

Component scores were calculated according to Lloyd et al. (2008). The component scores were compared across the four examined groups using the Kruskal–Wallis test. Post hoc multiple comparisons tests were then carried out to explore specific breed differences on each component. The value of alpha was set at 0.05 for all statistical tests.

differed significantly regarding Groups Anxiousness and Excitability, but no significant differences were found regarding Dominance, Sociability Inquisitiveness Protection, or among groups. This finding is in line with the findings of Lloyd et al. (2008) who showed that Anxiousness and Excitability components have the highest level of variation between breeds. In our study, gallop horses had the highest rank regarding Excitability and they differed signifi-

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cantly from police horses which had the lowest rank for this personality component. Interestingly gallop horses had the lowest rank regarding Anxiousness, and trotters got the highest rank in in this component.

According to our results gallop horses are the most extreme in their personality. It is conceivable that being excitable is a more favourable trait for a race horse than for a working police horse. However, it is interesting that trotters are more anxious than gallop horses since they also have a high thoroughbred ancestry. The effect of work and training on these horses needs further surveys.

Keywords: horse, personality, questionnaire,

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The Familiarity Heuristic in the Horse (Equus caballus)

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This study replicated an unreported finding observed in a color perception experiment (Pick. Lovell, Brown, & Dail, 1994) where, after using the method of successive approximations to train a blue-gray discrimination, red-gray trials were initiated without further training. Although a gray choice had never been reinforced, the subject chose gray on the first 20 trials (p < .000001). In the study reported here, a horse was trained to approach a red feed bucket and not a green feed bucket. After the subject mastered the discrimination, a blue bucket was substituted for the previously reinforced red bucket. With double-blind controls in place, the subject chose the unreinforced green bucket on 15 out of the first 20 blue-green trials yielding a binomial p = 0.0148 that this outcome could be due to chance alone. These results are contrary to all behavioristic psychological learning theories, but consistent with prospect theory (Kahneman & Tversky, 1979). Prospect theory predicts that given a choice between two previously unreinforced stimuli, one familiar and the other novel, humans will choose the familiar. It is argued that the bias toward the familiar is the basis to a heuristic that has a genetic origin and should exist in other animals on the phylogenetic scale. The results of this study indicate that the heuristic is available at least as far down the scale as the horse. Conceptual replications using shape stimuli and sound stimuli are in progress.

Key words: color perception, learning theory, prospect theory

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Characterisation of the splenius muscle's activity (*Splenius cervicis*) during a walk phase at the warm up onset of ridden horses (*Equus caballus*)

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For human athletes, any physical performance usually begins with a warm-up including a phase of cardiorespiratory activation as well as a phase of neuro-muscular mobilization, including stretching patterns. To be efficient, the stretching has to be performed on relaxed muscles. In order to be able to apply this on ridden horses, these lasts have to be relaxed. However, the horse is a prey whose survival depends on its capacity to detect any potential danger in its environment and to flee if needed. As a result the warm up would be improved if adapted to this equine specificity. Therefore we added a walk phase preceding the usual warm-up during which the horses were allowed to visually assess their environment without constraint from their rider, who hold the reins loose. In a previous study, we showed that a walk phase at the warm up onset helps to mentally relax the horses and so decrease attentional resources involved in environmental monitoring. In this study, we have analysed the electromyographic activity of the neck supporting muscle (splenius cervicis) associated with the biomechanics of the strides to assess if this walk phase contributes to muscular relaxation, which would lead to an efficient stretching.

We assumed that when the horse displays a high neck as if monitoring its environment, the neck supporting muscles would be highly activated. In contrast, when the horse is more relaxed, the neck would be lowered, supported mostly by the nuchal ligament, a structure composed by elastin and collagen fibrils, running from the occipital skull to the thoracic dorsal spinous processes through the cervical

vertebrae. Indeed, thanks to its composition and the anatomical organization, the nuchal ligament supports passively the neck and head of the horse against gravity and contributes to locomotion by storing and returning elastic strain energy during the swings of the head

For each walk stride, two bursts of electromyographic activity were observed when the neck oscillatory cycle is in its lowest position. The duration, the amplitude, the integral, the root mean square along with the power of the electromyographic activity of the bursts have been significantly decreased. The duration of the EMG bursts added to the interval inter-bursts has not changed, which is consistent with the biomechanical measures showing that the duration of the strides has not changed while their length along with the members movement speed has significantly increased. A significant lowering of the neck has also been observed.

In conclusion, our results suggest that a walk phase at the warm up onset would help to improve muscular relaxation. The contribution of the elastic structures of the nuchal ligament to the neck swings is increased while the energy expenditures are decreased. Remarkably, the beat of the strides is not changed but more ground was covered.



Laterality and emotions: behavioural response to an approach of a novel object by young ridden horses.

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Laterality is now known to be an ubiquitous phenomenon among vertebrates. In most studies, horses' left eye is associated with fear eliciting situations associated with conspecific aggression (Austin 2014), with inanimate objects (Austin 2007, De Boyer des Roches 2008) or with people (Farmer 2010, Sankey 2011). In this study, we have evaluated the fear reaction induced by a novel object when the ridden horse turned around the object either in a clockwise or in a counterclockwise direction.

During breeding shows, 70 jumping horses (3 years old) were tested for their emotivity to a novel object: they were ridden at walk toward a novel object then around it 2 times. Their usual rider were said how to ride: no aids during the approach and the 1st circle, more active aids if necessary during the 2nd circle, direction (clockwise (object at right) or counterclockwise (object at left), the direction was changed at every other horse)). The novel object consisted in

a red and black pop up tent vertically supported by an obstacle stand making a 1 m x 1.7m x 0.4 m object. The evasion distance from the object (1:0 to 2 m, 2: 2 to 4 m, 3: > 4 m) for each 1/8 circle was measured. Time to perform the test was also noted. This was a part of a personality assessment.

The evasion distance (for each 1/8 circle or globally) was similar for each direction (P= 0.7) (Fig.1A) as the total time to perform the test (P =0.48) (Fig. 1B).

Results show variability among horses. Only a reduced and similar proportion of horses succeeded to walk near the object (< 2 m) (10/35 (29%) with object at right vs 6/35 horses (17%) with object at left: (P=0.25)). Similar proportion of horses made bouts of trot and fast movements (8/35 (23%) with object at right vs 9/35 (26%)

with object at left) (P=0.78)).

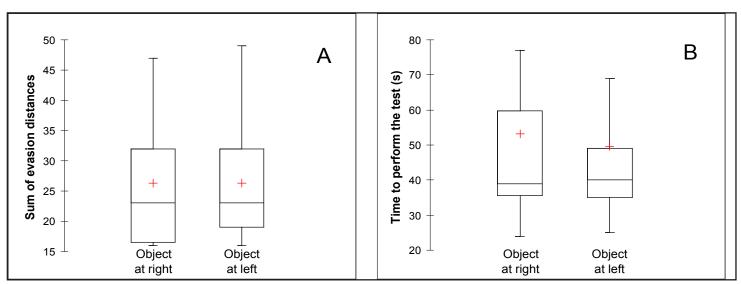


Fig. 1A and 1B. Box plots (Q1, Q2, Q3), + : mean.
According to the direction, clockwise (object at right) (n = 35 horses) or counterclockwise (object at left) (n= 35 horses) :

1A. Evasion distance from the object : sum of distances along the successive 1/8 circles (2 circles)

1B. Time to perform the test (s).

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In conclusion, horses showed no asymetry in their reactivity when turning around the novel object in one direction or in the opposite direction. Here, the object was first seen in binocular vision (straight approach from 20 m), then seen by one eye only (during the two circles) and horses could not interact freely with the object. This test could be performed similarly in the two directions.

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Equine welfare assessment and feedback to owners

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To assess horse welfare and develop a system for feedback to horse owners is the aim of the present study. A protocol developed in line with the Welfare Quality® project was used to assess 26 stables and 497 horses. Questionnaires were used to investigate what kind of feedback horse owners wanted and where they currently got their questions about horse welfare answered. The questionnaire was sent to participating stables and made available to the public via websites and social media.

Questionnaires revealed that 38% retrieved information from popular science articles, 77% from discussions with peers while 8% generally perceived their peers to lack knowledge on horse welfare. Factors affecting decision making were horse health (85%) and economy (38%). 85% wanted exhaustive information and advice on improvements, 69% preferred to get feedback as a digital document and 92% were interested in benchmarking.

Answers from participating stables lead to the development of a feedback consisting of results, scientific background of used measures, copies of assessment protocols for each horse, supportive telephone calls regarding decision making and benchmarking from all participating stables.

Questionnaires to the public had 688 respondents of which 54% were amateur riders/drivers. Main questions respondents regarding horse welfare were within feeding regimes (62%), housing (57%) and field size (54%). Main motivational factors in decision making were horse health (83%) and behavioural problems (71%).

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81% got information about horse welfare and support for changes from discussions with peers and 63% based decisions on their own personal opinion. 91% were interested in benchmarking scores to compare themselves with other stables.

The results highlight the need for independent assessment and feedback with a scientific base to horse owners. This will enable horse owners to make informed decisions with a scientific background that will result in increased horse welfare.

Lay persons message

A developed protocol was used to assess horse welfare and horse owners were questioned regarding how they wanted the results presented. This resulted in the development of a feedback system that will aid horse owners to make informed decisions about horse welfare.

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