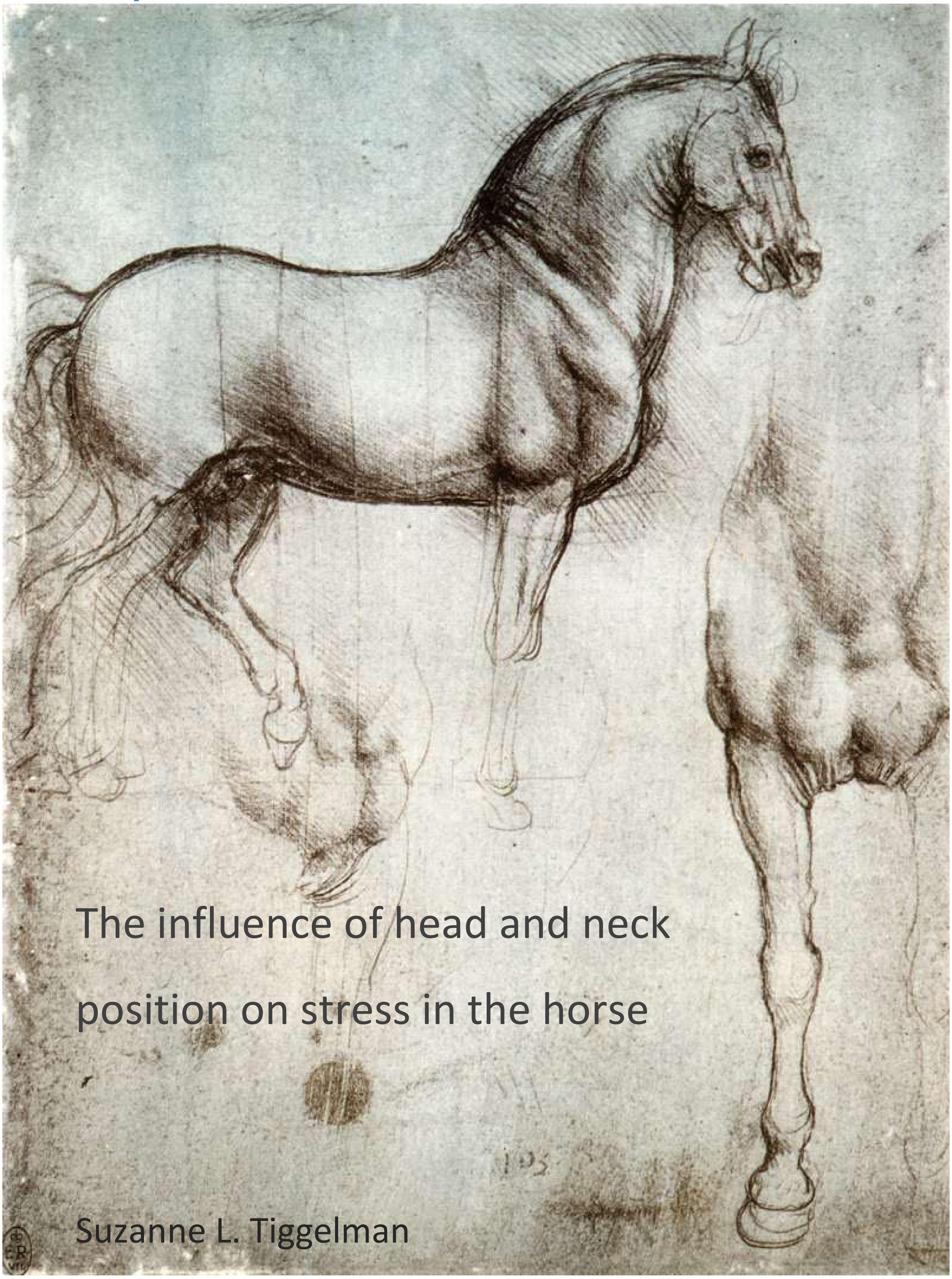

The influence of head and neck position on stress in the horse

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Introduction

There has been considerable controversy in the media about the method used for training horses, called "*Rollkur*", *"Low deep and round"* or "*over-bending*". Some riders, members of the public and knowledgeable experts in equestrian sports, including dressage, considered the technique unnecessary, offensive to the viewer and - most important - a potential welfare issue to the horse (FEI-Workshop 2006). Therefore a workshop was organized by the FEI in 2006 about the use of over-bending in FEI competition to investigate the issues surrounding the technique. According to the general principal of the FEI the object of dressage is "the development of every horse into a happy athlete through harmonious education". As a result the horse is calm, supple, loose and flexible. But also confident, keen and attentive thus creating a perfect understanding between rider and horse (FEI-Dressage-rules 2009). The FEI code of conduct implies that the welfare of a horse should be a paramount and under no circumstance be compromised. This code prohibits training methods that are abusive or cause fear in the horse and training methods that are used in horses that are not properly trained (FEI-Dressage-rules 2009).

There hasn't been a formal definition to describe "over-bending". The German press introduced the term "Rollkur" and the English press introduced the term "bite-the-chest". During the FEI workshop in 2006 the term "*hyperflexion*" was introduced and the following definition of hyperflexion was suggested: *"Hyperflexion of the neck is a technique of working or training to provide a degree of longitudinal flexion in the midregion of the neck that cannot be obtained by the horse itself for prolonged period of time without welfare implications."*
But also: *"There must be an understanding that hyperflexion as a training aid must be used correctly, as the technique can be an abuse when attempted by an inexperienced or unskilled rider or trainer"* (FEI-Workshop 2006).
This means that it can be a serious impairment to the welfare of the horse when hyperflexion is used the wrong way.

Behavior and the way horses are ridden.

In the last century the way horses are ridden and kept has changed significantly. In the 18th century riding changed from mainly functional to an art. The baroque philosophy was to render a horse so supple that its natural gaits and displays became even more beautiful and could be performed on request. The goal was to develop this through a slow process without the use of violent means (Ödberg and Bouissou 1999). The modern way of schooling is more coercive compared to the baroque philosophy and high results are to be reached quicker, which means that there are high expectations to be met by the horse. Ödberg (1999) suggests that a high percentage of horses are slaughtered at a relatively young age, because the way they are ridden is leading to problem behaviors and consequently to “difficult horses” or “problem horses”. It is suggested that when the horses become too difficult to handle and go from owner to owner they finally end up in the abattoir (Ödberg and Bouissou 1999). In this article no scientific proof was presented whether the main reason for the high numbers of young horses being slaughtered, was because they were badly schooled or ridden and therefore became too difficult to handle. Nevertheless the modern way of horsemanship has several consequences for the horse that has to cope with the way it is kept and ridden and the expectations of the owner it has to fulfill in its performance. Therefore, thought should be given to the best way of horsemanship and training so trainers and owners can handle in best interest of the welfare of the horse.

Equitation science is a hot issue and the first equitation science symposium was held at the Equine Behaviour Centre in Melbourne Australia (First Equine Equitation Science Symposium 2005). The aim was to offer a symposium to inform attendants about the science behind equitation and by that way improve horsemanship by implementing behavioral science in equitation. Hereby the welfare of the horse can be improved and undesirable behaviors caused by flaws in the horse-human relation can be reduced. By means of equitation science more understanding of the horse’s behavior can help to improve the human-horse relationship (McGreevy 2007). When it’s possible to better understand equine behavior and therefore are capable to measure variables, it enables us to compare techniques that are used to train and ride horses and measure the consequences on the horses’ welfare. With the background mentioned above we can more easily identify the way we handle horses and improve them, so the welfare of the horse is impaired as little as possible. To measure validated ethological or physiological stress parameters, for example, is one way of determining if the horse’s welfare is impaired. One possibility of doing this is by measuring behavioral signs that are associated with the horse experiencing stress.

Horses have different ways of coping with stressful circumstances, depending on the individual they will react differently. Both acute and chronic stress in a horse's live can be caused by the surroundings, the circumstances in which the horse is kept and the way in which the horse has to perform every day. Horses, as well as other animals, can react actively or passively to stress. If horses act passively to stress they don't show it as much as horses that react actively to stress, but this doesn't mean they don't suffer from stress. These horses have learned that they are unable to help themselves since the responses, that normally relieve them from pain or discomfort, don't work. By a process of habituation these horses often become unresponsive to the stimuli given by the rider (McGreevy 2004). In a way they are trapped in inescapable stress, pain or discomfort, to which they react passively and when a possibility is given to escape the stress, pain or discomfort they don't, as if they have learned that no other form of life exists (Ödberg 1987). These horses have seemed to accept the situation, but there is no evidence that they do not suffer from stress although giving the impression of not suffering (Ödberg and Bouissou 1999). Some horses fight back or tend to develop active abnormal behaviors such as stereotypes, aggression, rushing, etcetera. The horses that actively react to stress and fight back are often referred to as problem horses, because they simply refuse to obey. Often they are sold repetitively up till the point it isn't possible to ride the horse anymore and eventually in the worst case they get slaughtered at a young age as is mentioned earlier (Ödberg and Bouissou 1999). A lot of these problems can be prevented or solved by good training.

There are several ways that can be used to train a horse. All training methods use operant conditioning and classical conditioning. One part of operant conditioning is negative reinforcement; this is when the action of the horse leads to the subtraction of something aversive. This way the desired response is rewarded and lowers the motivational drive for that reinforcer (Skinner 1953). Horses are highly motivated to avoid physical or psychological pressure, therefore negative reinforcement is an effective way of training. Negative reinforcement takes the form of pressure release of for instance the bit, (thus, negative meaning the removal something aversive in this case the pressure performed on the mouth by the rider via the bit) and so horses in work learn to offer responses that results in pressure release (McGreevy 2007). Another part of operant conditioning is positive reinforcement; in this case the behavior of the horse evokes a reward, which is received or given when the desired response is given (Skinner 1953). Positive reinforcement is not often used in training. The reason negative reinforcement is used by humans on ridden horses is because it gives them a sense of control and security and because it feels natural to apply. By using pressure on the bit for example the horse can be controlled relatively easy, because the bit can be a source of tremendous discomfort, this is especially the case when used in the wrong way (Mills and McDonnell 2005).

The goal that the rider should strive after is to ride the horse with light contact, so a state of "self-carriage" can be obtained, this means light rein- and leg contact and not riding the horse hard handed so that the horse leans on the riders hand as if it is "the fifth leg", as it is sometimes called (McGreevy and McLean 2007). The horse has to habituate to the 'normal' level of rein contact and learn to differentiate this from the 'stop-signal' (McGreevy 2004). For riders this is sometimes difficult to understand because they aren't familiar with equine learning processes and use much stronger pressure on the mouth. Pressure on the mouth is sometimes used to let the horse walk with its head and neck in poll flexion. To avoid the pressure the horse bends its neck but the real purpose isn't reached, and that is to get the horse collected. The aim of the collection of the horse is as described by the FEI:

1. To further develop and improve the balance and equilibrium of the horse, which has been more or less displaced by the additional weight of the rider.
2. To develop and increase the horse's ability to lower and engage its hindquarters for the benefit of the lightness and mobility of its forehand.
3. To add to the "ease and carriage" of the horse and to make it more pleasurable to ride.

(FEI-Dressage-rules 2009)

Dressage or "dressed" meant trained or put into alignment in England in the 18th century, but also conveyed the raising of the neck and head of the horse which cannot naturally take place if the horse isn't trained to collection. In England it was thought that the horse was not fully "dressed" unless able to carry out full collection (Loch 1990). Riders ride their horses being "on the bit" with a poll flexion in an attempt to get their horses to collect, because collection is necessary to execute dressage routines. To achieve collection often the power of pressure on the bit is used to let the horse walk with its head in poll flexion, but the horse isn't really collected. If the simultaneously given 'go' and 'stop' cues become confusing to the horse a state of conflict can occur, which can lead to problem behaviors or agonistic behaviors (McGreevy 2004; McGreevy and McLean 2007).

Conflict behaviors typically occur when performance horses are continually pressed to perform movements that they simply cannot physically express, or when they don't understand what the rider wants them to do, when they are in pain when performing the movement or when it brings them to a situation that the horse cannot control (for instance cannot see in the direction in which they are going). Trainers should be aware of the difference between movements the horse cannot express physically and unwillingness to comply. In dressage at higher levels the horses are taught very complex movements which demand a shift in gravity towards its hindquarters, which is why the horse has to be fully collected to be able to perform such movements (Mills and McDonnell 2005).

Studies performed researching hyperflexion

Since the workshop, organized by the FEI (FEI-Workshop 2006), a number of studies were performed which I will discuss in this paragraph.

In a study performed by van Breda (2006) stress levels were measured in elite dressage horses trained in the "Rollkur" posture and in recreational riding horses. Stress was measured using heart rate variability. The conclusion drawn was that the preliminary data obtained from this study weren't sufficient as evidence that the rollkur training method has detrimental effect on stress and pain in the horse. Although no behavioral measurements were taken and therefore no definitive conclusions could be made (Breda 2006). In this study it was suggested that behavior observation with a standardized pain scoring system for objectivity is always necessary to determine pain in horses (Rietmann, et al. 2004). The conclusion drawn by van Breda 2006 based on the heart rate variability outcome was that training dressage horses in rollkur posture was less stressful than recreational training for recreational riding horses. In conclusion to this van Breda (2006) writes "these results indicate that intense exercise with an extensive period of Rollkur training more closely fits the nature of the horse than recreational riding".

In 2006 a study was done considering the effects of riding in low deep and round posture on workload and stress in the horse and compared this to workload and stress in horses ridden in a natural posture (Sloet van Oldruitenborgh-Oosterbaan, et al. 2006). The workload was evaluated using heart rate and blood lactate concentrations, among other variables that are commonly used to evaluate the effects of exercise, namely blood glucose concentration, blood pH, packed cell volume, blood pCO_2, electrolytes and creatine kinase. Stress was measured using plasma cortisol concentration. It was noted by the author that cortisol is not a very reliable variable to measure stress, because many factors influence plasma cortisol concentrations. And also that the circadian rhythm can be easily disrupted by physical and psychological stress, this is also concluded by Irvine and Alexander (1994) and is discussed in the paragraph "cortisol". In this study no significant differences were found in cortisol samples taken immediately after the test or in the samples taken the evening of the test. Higher blood lactate concentrations and higher heart rates were found in horses ridden deep low and round compared to the horses ridden in natural posture (Sloet van Oldruitenborgh-Oosterbaan, et al. 2006).

The study by von Borstel et al. (2009) is the only study in which behavior was scored using a standardized ethogram to measure stress in horses. In this study almost all types of behavior in the ethogram were shown significantly more in rollkur posture compared to regular poll flexion. For example more tail swishing, head tossing and attempted bucks were seen in the rollkur posture compared to regular poll flexion. The conclusion was drawn that the horses showed more behavioral patterns suggestive of stress, discomfort or frustration when ridden in rollkur posture compared to poll flexion. Also the horses had to be encouraged more to move forwards in rollkur posture compared to regular poll flexion (Borstel, et al. 2009). As is suggested by the author, the horses were confused and trapped between the “stop signs” given by the reins and “go signs” given by the riders legs as is described by McGreevy (2004). Another possibility is that they were more reluctant to move forward, because of their limited eyesight in the direction in which they were going when ridden in rollkur posture.

Measuring stress in the horse using behavior, cortisol and β-endorphins

Stress in horses can be measured in different ways. In this study we chose to measure stress by behavioral observations, cortisol and β-endorphins measurement in plasma.

Behavior

Although there is a lot of research done about equine behavior in the wild, there is little information about equine behavior during training or exercise and thus little information about behaviors shown by horses caused by stress during training and exercise. In this study an ethogram is used, outlined in appendix 1, which is composed using several sources and our own observations during testing. In this study an overview was made that was as complete as possible. Behaviors displayed during stress aren't described very extensively and some can be interpreted in multiple ways. The behaviors most observed in this study are briefly discussed below.

Headtossing and headshaking are one of the most reviewed behaviors when it comes to behaviors shown attributable to stress. They're almost always attributable to physical pain, discomfort and irritation McDonnell 2003; Mills and McDonnell 2005). In wild conditions the horse shows different types of headshaking, one of them are rotary swinging head movements which occur as substitute activity in conflict situations or more generally in states of overexcitement (Zeitler-Feicht 2004). The syndrome headshaking can be described as a behavior during which the horse exhibits violent, rhythmic head movements in a vertical or, less commonly a horizontal direction without a detectable external stimulus (Zeitler-Feicht 2004). A lot of causes for headshaking have nothing to do with stress, but are pathological and thus of clinical significance, these will not be reviewed here, only mentioned shortly. The causes can be classified globally in the following categories according to Zeitler-Feicht (2004):

1. Symptomatic behavioral aberration which is caused by disease or photosensitivity
2. By management and handling induced behavioral aberration resulting in stereotypic headshaking, or
3. Unwanted behavior

Unwanted behavior can be caused by fear or pain due to equipment deficits or improper influence of the rider. Horses express this behavior, because they have no other way to communicate pain or fear they're suffering (Zeitler-Feicht 2004). Zeitler-Feicht (2004) also points out that if headshaking or head tossing is shown mainly under saddle, without the presence of pathological reasons, it can be referred to as a behavioral response.

Tail swishing is a form of communicating agitation (Weeks and Beck 1996). The tail can be indicative of mood and can be a sign of irritation. Tail swishing can be seen when behavioral conflicts arise. In dressage competition this is recognized as a sign of resistance (McGreevy 2004; FEI-dressage rules 2009). Forceful sideward motions of the tail, occasionally with vertical lashing are commonly shown when the horse is annoyed and can be preparatory for bucking or kicking (Waring 2003).

Snorting is seen in equestrian contexts in association with exercise and conflict during restraint (McGreevy 2004) and it's also associated with startle, fear and pain responses (McDonnell and Haviland 1995; Waring 2003). It can be a way to tell herd members there is something going on, beware! (Kiley-Worthington 1997)

Shying is defined by McDonnell (2003) as "a sudden veering to avoid novel or fear provoking animate or inanimate stimulus". Shying is a form of fear and is a normal response when a horse in startled for example. It can be due to impaired vision (Waring 2003), to bad experience in the past or lack of experience (Kiley-Worthington 1997; Zeitler-Feicht 2004). It can also be induced by the riders influence, riding with a hard hand, a harsh bit, or suppression of visual perception can evoke shying reactions. Shying can be rewarding if the rider gives up riding past the frightening stimulus or if the horse finds out that shying gives them release from rein or leg pressure. Shying can eventually evolve progressively into rearing if the horse receives sufficient practice, motivation (if pressure is not resolved) and reinforcement by dislodging the rider (McGreevy 2004).

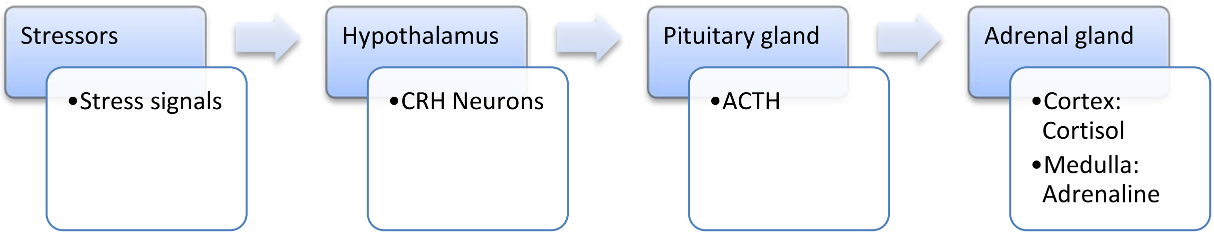
A more extreme behavior indicative of stress can be bucking (Kaiser, et al. 2006). Bucking can be a conflict response that arises from unclear cues given by the rider, e.g. when rein pressure is still high, as if a 'stop signal' is given, but at the same time leg pressure is also high and a 'go signal' is given (McGreevy 2004). Bucking can also be caused by pain or fear, but pain can also be the cause of fear (Zeitler-Feicht 2004).

Shying and bucking can be a form of evasion and are in fact behaviors accompanied by a raised flight response (Mills and McDonnell 2005). In his domestic environment the horse doesn't have the means to flee as in the wild. The horse tries to evade the pain, but isn't able to, from this a state of panic can evolve, which can lead to behaviors that will seem inappropriate to the rider and can be interpreted as problem behaviors.

Cortisol

Stress is a state that results in an increased activity of the Hypothalamo-Pituitary-Adrenal Axis (HPA axis). When an animal comes upon a stressful event a physiological response is activated. Corticotrophin-releasing hormone is released by the hypothalamus causing the release of adreno-corticotrophic hormone (ACTH) by the pituitary gland. ACTH stimulates the adrenal cortex to release corticosteroid hormones such as cortisol and corticosterone (Covalesky, et al. 1992). This is illustrated in figure 1. Cortisol has a negative feedback effect on the pituitary gland and the hypothalamus (Berne, et al. 1998). Increased plasma cortisol concentrations have been used as indicator of HPA axis activity. Because stressors consistently prompt cortisol production, plasma cortisol concentrations have frequently been used to characterize the stress responses of horses (Pell and McGreevy 1999). Cortisol accounts for almost 90% of the circulating corticosteroids in plasma (Covalesky, et al. 1992) next to cortisone, corticosterone and deoxycorticosterone which are present in plasma in much smaller amounts (Stull and Rodiek 1988). Unbound cortisol can be measured in both blood plasma and saliva of the horse.

Figure 1: Simplified scheme of the HPA axis. (Berne, et al. 1998)



Cortisol is transported in the blood primarily in association with binding proteins called cortisol binding globulins. The horse has relatively low concentrations of cortisol binding globulin, which consequently leads to a relatively large rise in bioactive "free" cortisol, when a small increase in total plasma cortisol occurs. Ten - fifteen percent of the cortisol remains unbound (Pell and McGreevy 1999) and can cross into the saliva. (Kolk, et al. 2001) However, in a study performed by Irvine and Alexander (1998), reference is made to a study by Gayrard et al., in which it is said that 67-87% of the cortisol is bound to cortisol binding globulin in several domestic species including the horse (Alexander and Irvine 1998). In a study by Lebelt et al. (1996) a close correlation (r = 0.83) was found between cortisol levels in saliva and plasma in four stallions. van der Kolk et al. showed a correlation (r = 0.93) between plasma and salivary cortisol concentrations at 6.00 hr. In a study by Pell and McGreevy (1999) also a significant correlation was found, but only in horses with an oral stereotypy (r = 0.65; P = 0.01). Social stress in the horse decreases the binding capacity of cortisol binding globulins which results in a higher concentration of free cortisol in plasma (Alexander and Irvine 1998).

Horses have a circadian rhythm in plasma glucocorticoid concentrations, with a peak between 6.00 and 9.00 hr and a trough between 19.00 and 23.00 hr. (Stull and Rodiek 1988) (Irvine and Alexander 1994). It has been described that this rhythm can easily be disturbed or changed by e.g. placing horses in a novel environment. In the study by Irvine and Alexander (1994) the total cortisol concentrations were elevated during the normal trough in the circadian rhythm, whilst the peak concentrations in the early morning were unaffected when the horses were placed in a novel environment (Irvine and Alexander 1994). The mean morning concentration of plasma cortisol was 193 nmol/l, and that of corticosterone 14 nmol/l (James, et al. 1970). In the study by Lebelt et al. (1996) the mean plasma cortisol concentration of four stallions in the morning was 120 nmol/l and salivary cortisol concentration was 110 nmol/l. In the afternoon the mean plasma cortisol concentration was 50 nmol/l and mean salivary cortisol concentration was 2.5 nmol/l. In the study by Irvine and Alexander (1998) the cortisol values rose quite quickly after social stress, which was measured 30 minutes after the stressor (Alexander and Irvine 1998). In a study by Fazio et al (2008) the concentration of cortisol, in Thoroughbreds, increased between 5 and 30 minutes after a competition which was the “stressor” in this study..

The cortisol half life lies between 60 and 90 minutes (Lassourd, et al. 1996). This explains why, after the stressor stops, the cortisol concentration drops to normal levels quite quick (Stull and Rodiek 2002). This was also seen in the study performed by Lebelt et al. (1996) in which cortisol concentrations were measured in 4 stallions after semen collection. In saliva, cortisol concentration peaked about 35-65 minutes after the stressor. Cortisol levels reached 190% of the mean basal value. After 80 minutes post semen collection the saliva cortisol levels were not different to pre-collection values. Knowing this we can conclude that the best time to measure cortisol concentrations in saliva is 30-45 minutes after the stressor has stopped. In blood plasma, cortisol concentrations peak around 30 minutes after the stressor has stopped (Fazio, et al. 2008).

β-endorphins

An alternative way to monitor stress is measuring β-endorphins. β-endorphin has the same precursor as the *adrenocorticotrophic hormone* (ACTH), namely *proopiomelanocortin* (POMC). In the anterior pituitary lobe, ACTH and β-*lipotropin* (β-LPH) are the major products of proopiomelanocortin. In the intermediate pituitary lobe and in the central nervous system ACTH is cleaved to produce *α-melanocyte stimulating hormone* (α-MSH) and *corticotrophin-like intermediate lobe peptide* (CLIP). β-LPH is cleaved in *β-endorphin* and γ-LPH. So, ACTH and β-endorphin have the same precursor and are both produced in the pituitary gland (Reed, et al. 2004). Therefore the release of ACTH and β-endorphins is probably often concomitant. (Bossut, et al. 1983) There is a considerable variation amongst individuals in basal β-endorphin concentrations and rise in β-endorphins as result of exercise. McCarthy (1993) suggests this is a result of differences in age and relative fitness of the horses studied. Also, Canali et al. (1996) found that older horses had higher levels of plasma β-endorphins and that there was great variability between individuals, although a relatively constant basal concentration within individuals (Canali, et al. 1996). Another study by McCarthy (1993) showed that the basal concentration and response to exercise of plasma β-endorphin concentrations decreased with increasing fitness (McCarthy, et al. 1993). Opioids are involved in many responses to stress (Canali, et al. 1996). β-endorphin concentrations rise in response to stressors and can be successfully used to monitor stressful situations in the horse (McCarthy, et al. 1993). β-endorphin levels rise immediately in response to a stressor and decrease rapidly after removal of the stressor in the case when a lip twitch is used as stressor (Canali, et al. 1996). In a study performed by Hydbring et al. (1996) there were different stressors that the horses had to endure and two of the stressors caused an elevation in β-endorphin concentration in blood plasma. In these two cases the plasma cortisol concentration tended to be more elevated for a longer period of time (Hydbring, et al. 1996). In this study nose twitching was one of the stressors.

Objective

This study was part of a greater study by Sleutjens (2009), which focused on the physiological, physical and anatomical effects of different head neck positions. The objective of this partial study was to determine whether horses experienced different head neck positions as stressful compared to a free head neck position. This was done by means of standardized behavioral observations and cortisol and β-endorphin measurement in blood plasma and saliva. The horses were trained on the lunge in the different head and neck positions intensively before testing began. During testing the horses were exercised on the lunge in 5 different head and neck positions while being filmed. Blood and saliva were taken at fixed times. The behavior was scored afterwards using an ethogram especially developed for this study based on publications and own observations.

Our hypothesis was that there would be no difference in behavior and plasma cortisol and β-endorphin concentrations indicating stress in the different head and neck positions compared to the free head neck position.

Animals, Materials and methods

Horses

For this study we used seven warmblood horses, 5 mares and 2 geldings, which were used as recreational riding horses and were base level trained. Their height was 161.2 ± 1.4 cm and their weight was 531 ± 47.3 kg. All the horses were clinically tested and had no history of respiratory, circulatory or neuromuscular disorders, no radiographic or echographic cervical abnormalities were found. Four of the horses were owned by Utrecht University, and three of the horses were privately owned and were leased by Utrecht University. Previous to testing the horses were intensively trained at the lunge every day for at least 3 weeks in all the different HNP's by the same handler that would be handling them during testing. The age of the horses varied between 4 and 14 with a mean of 10.3 ± 3.6 years. The horses were individually housed in boxes and their diet consisted of grass silage supplemented with concentrate feed and met the nutrient requirements for maintenance and performance. During training- and testing days they had access to ad libitum grass silage and were fed extra concentrate feeds. Water was available to them ad libitum.

Head neck positions

The following head and neck positions (HNP's) were tested based on the HNP's used in two studies performed by Weishaupt (2006) and Gómez Álvarez (2006). HNP 1, 2, 4 and 5 are identical to the HNP's used in the studies previously mentioned. HNP 7 was added based on a study by Back et al. (2010). The Head neck positions and their definitions are shown in the figures 2-6 below.

Figure 3: HNP 1
Unrestrained, free position. Reference position.

Figure 2: HNP 2
Neck raised with nose around the vertical. Position corresponding with the position determined as standard position during FEI competition. (FEI Dressage rules 2009)

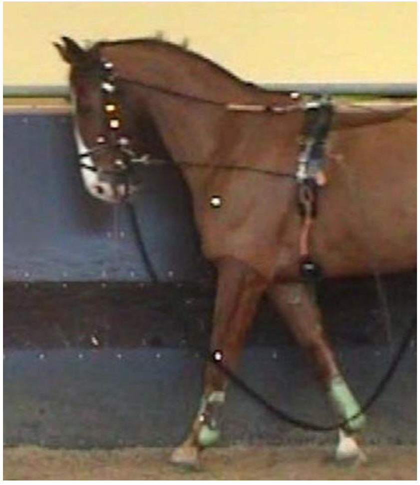


Figure 4: HNP 4
The neck low and flexed, with the nose towards the chest and neck extremely flexed.

Figure 4: HNP 5
The neck raised extremely high with the nose pointed forward considerably in front of the vertical.

Figure 6: HNP 7
The head and neck considerably flexed and low. The nose pointing towards the carpus.

Testing

Per horse, one HNP per day was tested, the HNP's were randomly chosen. On the first day every horse exercised in HNP 1, the following testing days each horse was tested in a different HNP.

There was a standardized testing protocol. 30 minutes prior to the test the horses were taken to a saddling area to which they were accustomed to. During saddling 2 Polar electrodes were attached underneath the girth, on the girth a Polar registration apparatus was attached to measure heart rate and heart rate variability (Polar® 810i, Polar electric B.V., Almere, the Netherlands). Next to the polar an electrocardiogram was continuously recorded during the test using a telemetric device (Televet 100 Rösch and Associates Information Engineering GmBH, Frankfurt am Main, Germany). Therefore 4 electrodes were positioned on the left chest just caudally of the elbow and on the left shoulder. As shown in figure 7. Before the bridle was put on blood and saliva were taken for cortisol measurements just before the test began. After blood and saliva were taken the bridle was put on and the horse was taken into the test arena by the handler. The arena measured 20 x 60 meters and has a standardized floor by Agterberg B.V.

Figure 5: Horse in saddling area during tacking up.

After entering the testing area the horse began the warming up, which consisted of 3 minute walk and a 5 minute trot on the lunge. After the warming up the HNP was applied, using side reins designed especially for each HNP, a set time span of 6 minutes was taken to do this. After this the exercise in the HNP started, which consisted of a 15 minute trot; 4 minute canter; 10 minute trot and a 5 minute walk respectively. During the training period we noticed that the horses had the most difficulty with walking in the different HNP's compared to trot and canter. During testing we could achieve full relaxation after the horse had completed trotting and canter. Therefore we chose to let the horses walk after they completed trotting and cantering during the test. After the exercise the side reins were removed during standstill and the horses were given the opportunity to relax and get used to the free position again for 2 minutes. After this the cooling down started which consisted of a 5 minute walk in free head and neck position. The warming up, exercise and cooling down were executed on the left hand side, because this was the preferred side by all the horses.

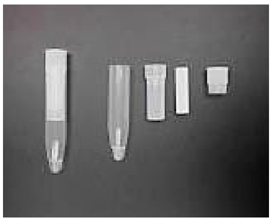
After the exercise the horses were taken to the saddling area and blood and saliva samples were taken immediately. After the horses were unsaddled they were taken to their stables and groomed accordingly.

Cortisol and β-Endorphin samples

Blood and saliva cortisol and β-endorphin samples were taken every testing day at 8 AM, 5 minutes prior to warming up, directly after cooling down, 30 and 60 minutes after the exercise ended and the side reins were removed and at 8 PM and the day after testing at 8 AM. The samples were put on ice immediately and transported on ice to the laboratory. Every horse was tested every testing day at exactly the same time, in order to get the cortisol and β-endorphin blood and saliva samples at exactly the same time every day.

The blood samples were collected with vacuum blood collection tubes with EDTA and Heparin so exposure to oxygen could be minimized, samples were taken from the jungular vein.

Figure 6: Salivette tube



Saliva samples were taken using Salivette tubes (Sarstedt B.V. Etten-Leur). The cotton roll inside the tube was especially prepared for use in horses, a nylon string was sowed through it, with which it was attached to a double broken shank bit. The cotton roll was left in the mouth during a minimum of two minutes.

The blood and saliva samples were centrifuged at 6000 G for 5 minutes and the supernatant plasma was put into Eppendorf tubes and put directly in the freezer of -20 °C. The sample taken 5 minutes prior to testing was put on ice during the test and was processed directly after the test. The cortisol and β-endorphin samples were processed and measured in a laboratory after validation using radioimmunoassay (RIA).

Observation

The horses were filmed with a handheld camcorder by the brand Panasonic using mini DV (Digital Video) Tapes. Every test was filmed by the same person. The horses were filmed from the moment they entered the arena until leaving. The observer stood next to the handler, who lunged the horses, and followed the horses in the circle in which they moved by turning on the spot. During the exercise external distractions were prevented as much as possible so the horses' behavior could be recorded in the most optimal way. The point of view is shown in figure 9 below.

Figure 7: The horse is being lunged by the handler in the exercise arena. As it is seen from the point of view of the observer.

The films were observed using The Observer 5.0, by the same person that filmed during testing. An ethogram was developed using literature and our own observations. This way we tried to make an overview that was as complete as possible for this study. All behaviors we observed in this study are listed in the ethogram in appendix 1. To allow detailed observations each horse was watched on tape two times in the same HNP to score each of two categories: head position, with mouth and ear gestures and locomotion with tail and nose gestures and movements.

Every behavior displayed by the horse was scored. Behaviors were scored in frequency or duration in time. The observations were split in warming-up, applying HNP, trot 1, canter, trot 2, walk and cooling down. The observations "trot 1, canter, trot 2 and walk" are listed in the results as *exercise*.

Besides the video recording observations, a general subjective of resistance or stress level the horses showed during different parts of the test was created. We called this the independent scoring possibility. We scored them during different events besides the exercise itself. These events were: warming up, transition to trot during warming up, applying HNP, detaching HNP and cooling down. The scoring possibilities used are listed in appendix 2.

Statistics

The different HNP's were compared to HNP 1 as the reference position. For quantitative purposes the data were analyzed in 4 different periods: namely "Applying the HNP" during which we applied the reins and put the head and neck of the horse into the different positions during 6 minutes. "Exercise" which consisted of respectively trot, canter, trot and walk. "Detaching" during which we removed the side reins and let the horse get used to the 'free' position during two minutes and the "Cooling down" period of five minutes in which the horse could move its head and neck freely. The Poisson distribution was used for the behaviors scored in frequencies and the Gaussian distribution was used for the behaviors scored in duration. The hypothesis we postulated was that no difference in behavior could be found when comparing HNP 2, 4, 5 and 7 to HNP 1. Data were compared in a linear mixed model corrected with a post-hoc Bonferroni correction by multiplying the P-value by four, being the number of comparisons made; which were: HNP 2, 4, 5 and 7. The outcome we used to assess significance in relation to $P = 0.05$.

Statistical results behavior tests

Below the results of significant importance are listed in tables divided according to different parts of the test, namely "*Applying the HNP*" during which we applied the reins and put the head and neck of the horse into the different positions during 6 minutes. "*Exercise*" which consisted of respectively trot, canter, trot and walk. "*Detaching*" during which we removed the side reins and let the horse get used to the 'free' position during two minutes and the *"Cooling down"* period of five minutes in which the horse could move its head and neck freely.

In appendix 1 the definitions for the different behaviors used here are listed in the ethogram.

Table 1: Statistical results of behavior observation during applying of the HNP.

Applying HNP	In combination with	D/F	T-Value	Z-Value	P-Value
HNP 2					
Tail swishing	-	F		0.001	<0.01
Helper slow down	-	F		0.004	0.02
HNP 4					
Neutral head position	Playing with bit	D	3.6		<0.01
HNP 7					
Head held low	Neutral mouth	D	3.2		0.01
Head held low	-	D	3.4		0.01

(D: Duration, F: frequency)

Table 2: Statistical results of the behavior observation, during the Exercise.

Exercise	In combination with	D/F	T-Value	Z-Value	P-Value
HNP 2					
Head neutral position	-	D	-5.9		<0.0001
Head neutral position	Mouth neutral with foam	D	3.5		<0.01
Head neutral position	Tense mouth with foam	D	2.8		0.03
Head pulling	-	D	6.9		<0.0001
Head pulling	Tense mouth	D	2.8		0.02
Head pulling	Earplay	D	4.0		<0.001
Head pulling	Neutral ears	D	4.8		<0.0001
Head held low	Playing with bit	D	-3.1		<0.01
Head tossing	-	F		8.4	<0.0001
Head shaking	-	F		4.3	<0.0001
Tail swishing	-	F		5.9	<0.0001
Velocity neutral	Nostrils wide open	D	3.4		<0.01
Nose blowing	-	F		3.7	<0.001
Helper Encouragement	-	F		14.7	<0.0001
Helper slow down	-	F		8	<0.0001
HNP 4					
Head neutral position	Playing with bit	D	4.0		<0.001
Head neutral position	Neutral mouth with foam	D	3.2		<0.01
Head held low	-	D	-3.4		<0.01
Head held low	Earplay	D	-3.5		<0.01
Neutral velocity	Nostrils wide open	D	4.4		<0.0001
Neutral velocity	Nasal discharge	D	2.9		<0.02
Halting	-				0.03
Nose blowing	-	F		3.5	<0.01
Helper encouragement	-	F		14.1	<0.0001
HNP 5					
Head neutral position	Tense mouth	D	4.7		<0.0001
Head held low	-	D	-3.4		<0.01
Head held low	Playing with bit	D	-3.1		<0.0001
Head held low	Earplay	D	-3.5		<0.01
Head shaking	-	F		3.5	<0.01
Head tossing	-	F		12.8	<0.0001
Neutral velocity	Nostrils open	D	3.4		<0.01
Helper slow down	-	F		8.1	<0.0001

(D: Duration, F: frequency)

Table 3 (continuation): Statistical results of the behavior observation, during the Exercise.

Exercise	In combination with	D/F	T-Value	Z-Value	P-Value
HNP 7					
Head neutral position	Playing with bit	D	4.0		<0.01
Head neutral position	Playing with bit with foam	D	2.6		0.04
Head neutral position	Neutral mouth with foam	D	3.4		<0.01
Head neutral position	Earplay	D	-14.9		<0.0001
Head held low	Earplay	D	3.5		<0.01
Head held low	-	D	-3.4		<0.01
Head tossing	-	F		-4.2	<0.0001
Snorting	-	F		3.7	<0.001
Shying	-	F		-2.8	0.02
Velocity neutral	Nasal discharge	D	2.7		0.03
Helper encouragement	-	F		6.4	<0.0001

(D: Duration, F: frequency)

Table 4: Statistical results of the behavior observation, during detaching of the HNP.

Detaching	In combination with	D/F	T-Value	Z-Value	P-Value
HNP 2					
Head neutral position	Earplay	D	-2.9		0.03
Head neutral position	Neutral mouth	D	-3.6		<0.01
Head shaking	-	F		4.4	<0.0001
Tail swishing	-	F		3.4	<0.001
HNP 4					
Head neutral position	Ears neutral	D	4.2		0.001
Head neutral position	Earplay	D	-3.2		0.02
Head neutral position	Neutral mouth	D	-5.3		<0.0001
Head shaking	-	F		3.5	<0.001
HNP 5					
Head held low	-	D	2.9		0.03
Head shaking	-				<0.001
HNP 7					
Head neutral position	Neutral mouth	D	-2.9		0.03
Head neutral position	Neutral ears	D	4.1		<0.01
Head shaking	-	F		3.5	<0.001
Tail swishing	-	F		2.6	0.03

(D: Duration, F: frequency)

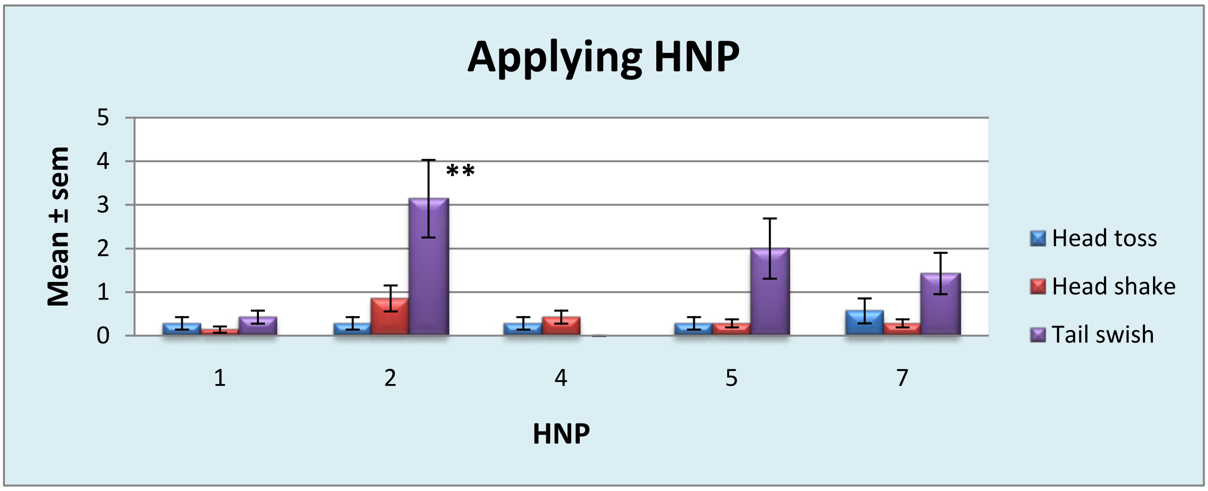
Table 5: Statistical results of the behavior observation, during cooling down.

Cool down	In combination with	D/F	T-Value	Z-Value	P-Value
HNP 2					
Head neutral position	Neutral mouth	D	-4.4		<0.001
Head held low	Earplay	D	3.3		0.01
Head held low	-	D	3.1		0.02
HNP 4					
Head neutral position	Neutral mouth	D	-3.4		0.01
Head held low	-	D	2.9		0.03
Halting	Neutral tail	D	2.9		0.03
Halting	Neutral nostrils	D	2.7		0.05
HNP 5					
Head held low	Neutral mouth	D	4.1		<0.01
Head held low	Neutral ears	D	2.99		0.02
Head held low	-	D	3.97		<0.01

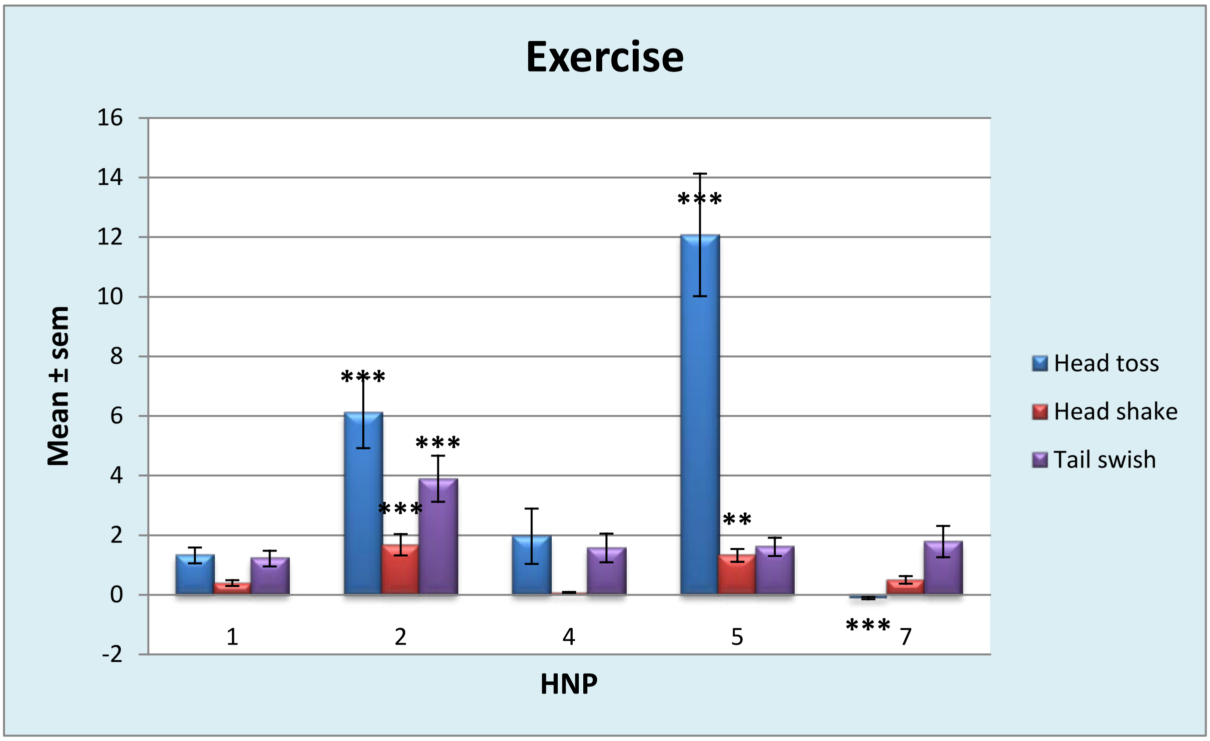
(D: Duration, F: frequency)

Looking at the tables above it can be concluded that certain behaviors occur more often than others during the different parts of the test, namely applying HNP, exercise, detaching HNP and cooling down. The most observed behaviors, tail swishing, head tossing and -shaking, were outlined in subsequent graphs.

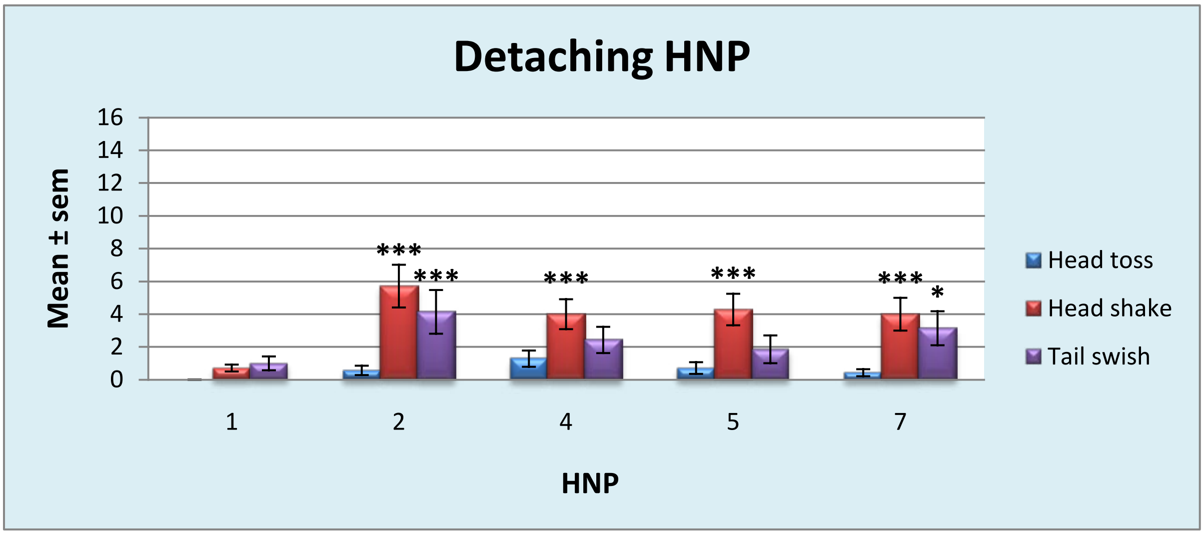
Graph 1: Mean of the most displayed conflict behaviors during the applying of the HNP ± standard error of the mean. (*: p<0.05, **: P<0.01, ***: p<0.001)



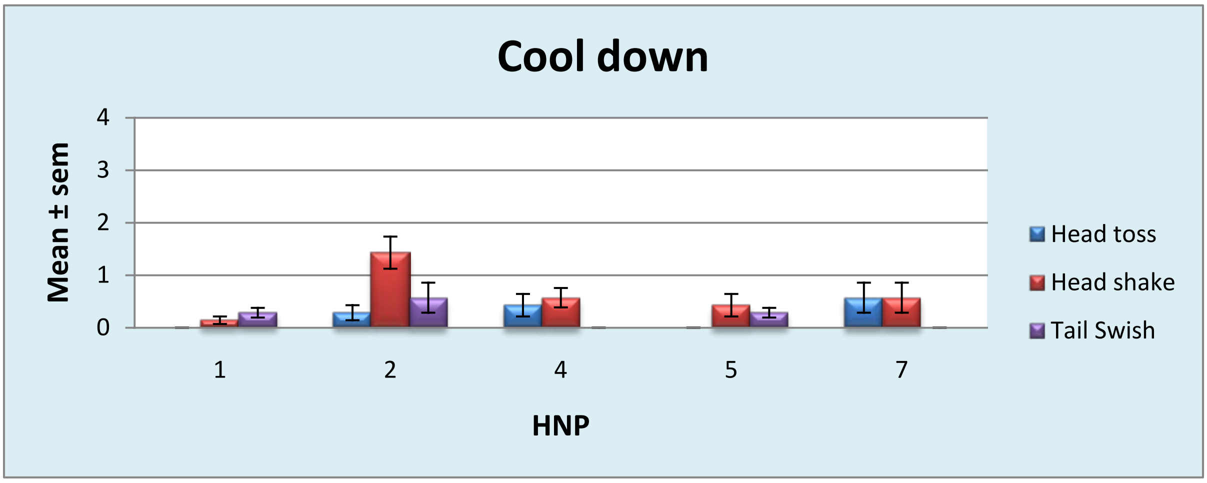
Graph 2: Mean of the most displayed conflict behaviors during exercise ± standard error of the mean. (*: p<0.05, **: P<0.01, ***: p<0.001).



Graph 3: Mean of the most displayed conflict behaviors during detaching of the HNP ± standard error of the mean. (*: p<0.05, **: P<0.01, ***: p<0.001).

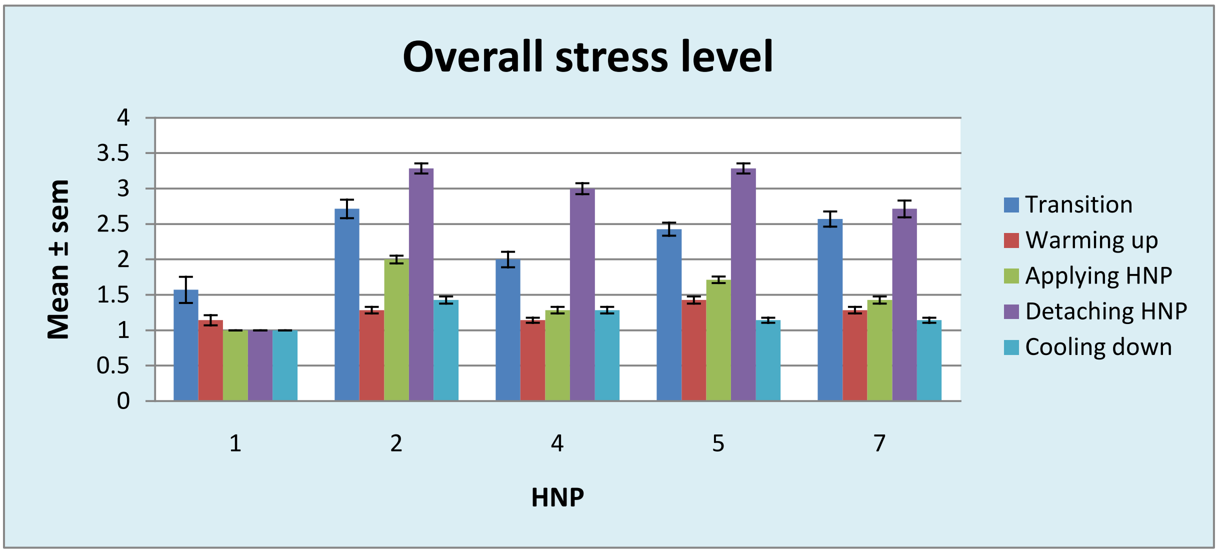


Graph 4: Mean of the most displayed conflict behaviors during cooling down ± standard error of the mean.



Results of overall stress level

Graph 5: Mean of level of resistance or stress level in the horses. (For scoring possibilities see appendix 2)



Outlined in the graph 5 is the mean of stress level and the general resistance per HNP with standard error of the mean (sem). The scoring possibilities used for determining the stress level are outlined in appendix 2. It must be noted that the mean score in HNP 2 during "applying HNP" was 2, which means that horses showed one conflict behavior during the applying of the side reins. Remarkable was that a high score was seen during the detaching of the reins in HNP 2, 4, 5 and 7. The mean for all HNP's, except HNP 1, was around 3. This means that the horse stretched its neck and showed at least one conflict behavior.

Results cortisol and β-endorphin measurements

Results are still to be awaited.

Discussion

Behavior results

With the behavior analysis in this study we can conclude that several behaviors were seen that indicate stress or resistance in the horse in the different head neck positions. I will discuss the behavior shows in different parts of the test below.

Applying HNP

In HNP 2 the horse had to be slowed down more often, because the horses started walking during the application of the reins. We interpreted this as avoidance of the pressure of the reins when they were applied. Also the increased swishing of the tail implicated irritation (McGreevy 2004). When HNP 4 was applied the horses held their head neutral when playing with the bit as if they were getting accustomed to the position. Besides this we noticed the horses' eyes almost closed as if in a trance. Although this was not in the ethogram it is worth mentioning. It is suggested that head lowering has a calming effect on horses, although in a study done no evidence was found that head lowering influenced cardiac responses (Warren-Smith, et al. 2007). The horses played with the bit significantly more after the head was put into position 4 compared to HNP 1.

Exercise

In HNP 2 and 5 both headtossing and headshaking were seen significantly more, this indicates irritation, pain and discomfort (McDonnell 2003; Mills and McDonnell 2005). The number of times headtossing or headshaking were seen wasn't significantly more when HNP 4 and HNP 7 were compared to HNP 1, whereas headtossing and headshaking were seen significantly more in HNP 2 and 5 when compared to HNP 1. There can be several reasons for this. In HNP 4 the horse's head was restrained in a way that the range of movement was diminished, therefore head shaking and head tossing was more difficult compared to HNP 1. Another reason could be that HNP 4 and 7 irritated the horses less than HNP 2 and 5 when compared to HNP 1. In HNP 7 the number of times headtossing occurred was significantly lower when compared to HNP 1.

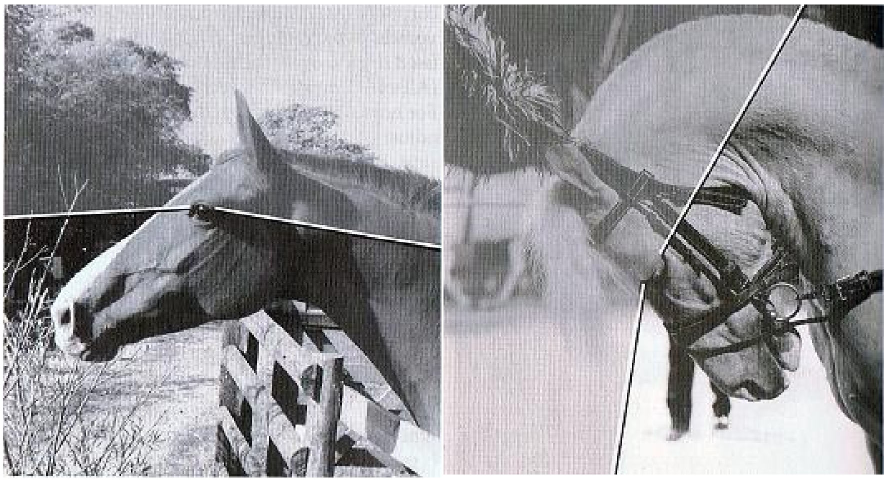
A significant increase of head pulling was seen in HNP 2, this we interpreted as a form of resistance against the side reins and bit. The head pulling was as severe at times that the mouth was opened wide.

Tail swishing was seen more in HNP 2, which can be a sign of irritation or behavioral conflict (McGreevy 2004). Helper encouragement was raised in HNP 2, 4 and 7. Whilst helper's slow down was increased in HNP 2 and 5, this could mean that the horses were in conflict and didn't know which cues to follow, 'stop' or 'go' (McGreevy 2004).

In HNP 4 the nostrils were seen wide open while exercising in normal velocity and discharge from the nose was seen significantly more compared to HNP 1, this could indicate that breathing is more difficult in this position due to the head neck position.

S*hying* was seen less in HNP 7 compared to HNP 1. Because of the lack of good vision in the direction the horses were going it is to be expected that the horse shies more easily. The horses had to concentrate to keep their equilibrium, because of the posture. We thought that therefore the horses were more careful to shy away. In HNP 4 the horses' vision in the direction they were going was also limited this is illustrated in figure 10.The difference is shown between the vision the horse has in HNP 1 and when ridden with its nose to the chest. The posture shown in the figure isn't exactly the same as HNP 4, but the vision the horse is comparable in both postures.

Figure 8: The eyesight of the horse in free head and neck position (left) and a posture comparable to HNP 4 (right) (McGreevy 2004).



Detaching HNP

After detaching of the side reins more headshaking was seen in all the positions compared to HNP 1, probably indicating relieve after the stressor, which in our case was the exercise in the head and neck position. It has to be noted that one horse showed a body shake after the exercise. This is remarkable because horses usually show this behavior after rolling (McDonnell 2003; Zeitler-Feicht 2004) or after a period of recumbency (Waring 2003). It is also seen after untacking (McGreevy 2004), which was in a certain degree the case here.

In HNP 2 and 7 tail swishing was increased which can indicate irritation even after the stressor was relieved. After the detachment of the reins in HNP 5 the head was held lower for an increased amount of time. This could be interpreted this as a compensation for the position in which the head had to be carried very high for a long period of time.

Cool down

During the cool down in HNP 2, 4 and 5 the head was carried low significantly longer and the time the head was in neutral position significantly decreased in HNP 2 and 4. This is probably attributable to relaxation of the neck muscles and stretching of the neck. Remarkable was that the horses halted more often in the cool down after exercise in HNP 4, this could be attributable to exhaustion. Since the horses weren't made to walk in the position we didn't think of it as protest, also no behavioral signs of protest were seen during the cool down.

Overall discussion

Due to the discussion surrounding the hyperflexion posture several studies were done in the last few years to evaluate stress levels in horses caused by this head and neck position (Sloet van Oldruitenborgh-Oosterbaan et al. 2006; Breda 2006; Borstel et al. 2006). Different parameters to determine stress levels were used in these studies, therefore the results can be interpreted in different ways. This study is part of a greater study done by Sleutjens (2009) in which physiological, anatomical and physical measurements were done to compare the different HNP's to the natural position. In combination with the results obtained from this study a conclusion can be drawn determining if the welfare of this population of horses is impaired, and why the horses showed more resistance when exercised in different HNP's.

In this study four different postures were compared to the natural head and neck position. Although hyperflexion is the posture most under discussion at the moment, other postures also occur regularly in riding. The postures that were used in this study were based on previous studies (Weishaupt 2006; Gómez Álvarez 2006). HNP 7 was a different interpretation of the long-deep-and-round or rollkur posture, because two different interpretations seem to exist in the equine community (Back, et al. 2010). In this study a way was designed to test stress levels in the horse in the different HNP's. Bias caused by external influences was diminished as much as possible by reducing these influences.

In this study the horses were lunged in the different head neck positions. It was chosen to do so based on the outcome of the pilot study done preceding this study in which the horses were trained on the lunge, on the treadmill and while being ridden. Stress levels caused by these different training methods were compared (Denderen 2009). The chance of bias was smaller when the horses were lunged by one person, because the horses weren't influenced by the rider, also lunging caused less stress compared to exercising the horses on the treadmill. We used side reins to let the horses walk in the desired HNP's. The side reins assured that the different horses walk in the same HNP the whole time during testing, which makes it easier to standardize the different HNP's. In daily practice side reins are used quite often by riders. It is shown that collection achieved by the application of side reins has demonstrated a decrease of the movement of the back (Rhodin, et al. 2005) which isn't preferable when riding dressage in which the goal is to render a supple horse by exercise.

The horses used in this study were not elite dressage horses and therefore not used to training regimes to which high level dressage horses are used to. The horses used in this study were intensively trained beforehand and therefore habituated to training in the different head neck positions. In daily practice less skilled riders ride their base level trained horses in head and neck positions similar to the HNP's used in this study, sometimes for longer periods of time with or without side reins so there is a resemblance when this study Is compared to the daily practice. It would be interesting to perform a study to compare stress levels caused by training in the different head-neck positions in elite dressage horses and compare this to the levels of stress in the base level trained horses that were used in this study.

Different studies were done previous to this study discussed in the paragraph "Studies performed researching hyperflexion". In the study done by Sloet van Oldruitenborgh-Oosterbaan (2006) cortisol samples were taken immediately after the test and the evening following the test. In our study it was chosen to collect samples for the plasma cortisol concentrations just before exercise and 30 minutes after the exercise had stopped and side reins were detached. We based this decision on the outcomes of studies by Lebelt et al. (1996) and Fazio (2008)in which was shown that plasma cortisol concentrations are highest 30-45 minutes after the stressor stops and concentrations fall relatively quick after that (Stull and Rodiek 2002). Several studies pointed out that cortisol was a good parameter to determine stress in the horse (Lebelt et al. 1996; Alexander and Irvine 1998; Fazio et al. 2008). Together with β-endorphin, which can also be an indicator of stress (McCarthy et al. 1993; Canali et al. 1996; Hydbring et al. 1996), these measurements can support our conclusion based on our behavior observation findings.

van Breda (2006) used both elite dressage horses and recreational horses. They were ridden without side reins, which made standardization of the HNP's more difficult. The riders were not blind to the treatments. This wasn't experienced using lunging as a training technique as was done in this study. van Breda used the physiological parameter heart rate variability for stress measurements and no behavioral observations were done to determine stress levels in the horses. Therefore the conclusion drawn by van Breda, that "intense exercise with an extensive period of Rollkur training more closely fits the nature of the horse than recreational riding", cannot be regarded as a definite one. Behavioral observation is a crucial element of studies done to determine stress, but stress is best determined when observation of behavior is combined with heart rate variability, cortisol and endorphin plasma concentrations.

In the study done by von Borstel (2009) the stress levels experienced by the horses ridden in HNP 4 is compared to stress levels experienced by horses ridden in HNP 2. A disadvantage in the study by von Borstel (2009) was that the horses were not used to riding in rollkur posture. Therefore the horses could have reacted differently if they were trained in rollkur beforehand and were therefore habituated, so stress caused by novelty of the posture could have been ruled out, as was done in this study. The results in the study by von Borstel are different from the results obtained in this study. The reason for this could be the novelty experienced by the horses, when exercised in HNP 4.

Recently the FEI ruled, after a special “round-table meeting” in Lausanne, “that the rollkur posture is no longer accepted as a training method, but that the low-deep-round (LDR) posture is still accepted as a training method, LDR wouldn’t be detrimental for the horse, provided that it is done by crafted horsemen” (Hendrix 2010). The rollkur and LDR method mentioned above are two training methods comparable with respectively HNP 4 and 7 used in this study. In this study there isn’t a significant difference in stress level in the horses based on behavioral observations when they were exercised in HNP 4 and 7.

Questions could be raised about the use of the standardized poll flexion (HNP 2), described by the FEI, in FEI competition and training. The question is if HNP 4 is an impairment to the welfare of the horse according to the ruling of the FEI mentioned earlier, isn’t this the same for HNP 2 or 5, when looking at the outcomes of the behavior observation in this study? The most important thing is that, when practiced by unskilled riders, every posture the horses’ head and neck are put in, the welfare of the horse could be impaired when force is used. The solution underlying this problem is training riders how to ride or train their horse the best way. Equestrianism has an old history and it isn’t going to change overnight, but an effort can be made to change parts of the traditions in riding by reconsidering them. This could be done using research and by educating horse owners the best way to keep, handle and train their horses. Horse behavior is well studied in the field, but there is more that we can find out and learn about the horse and its behavior in its domestic environment.

Conclusion

Behavioral findings in this study suggest that the HNP's compared to the natural position caused different levels of stress in the horse. The behavioral observations in this study according to our ethogram point out that the horses got frustrated, irritated and that they resisted against the different head neck positions they were exercised in.

HNP 2 and 5 have in common that the neck was raised and HNP 4 and 7 have in common that the neck was lowered. It can be concluded that, based on the results of the behavioral observations, less resistance was shown in HNP 4 and 7 compared to HNP 2 and 5. The reason why the horses show more stress in HNP 2 and 5 has to be assessed by measurements done in other studies.

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Appendix 1:

Ethogram used for behavior observation.

Ethogram during the exercise protocol:

State: Behavior that lasts for a longer period (in seconds)
Event: Behavior that happens in a shorter time (in frequency)

Head position:

States:		
Head neutral	hn	Head and neck are held reasonably stable in the desired position. In HNP 1 the head and neck are held in a less or more horizontal position.
Head flexed	hf	The horse flexes the head more than required in the desired position so it "comes loose" from the bit. The ropes are possibly dangling loosely. The muzzle is drawn toward the chest. (McDonnell 2003) (Waring 2003)
Head pulling	hp	The horse lets its head hang in the ropes and pulls the ropes forward. (Own observations)
Head low	hl	In position 1: head and neck are held lower than in neutral position, between the shoulder joint and the carpus. (Own observations)
Head to ground	hg	In position 1: head and neck are lowered so the nose is close to touching the ground. The head is lower than the carpus. (Own observations)
Head high	hh	In position 1: head and neck are held higher than in neutral position. (Own observations)
Head tilting	hk	The horse tilts its nose to one side. (Borstel, et al. 2009) (P. McGreevy, A. McLean, et al. 2005)
Head turned	ht	The horse moves its head lateral towards the left or right and leaves it there for a longer (>5s) period. (own observations)
Rope pulling	rp	Helper applies pressure on the ropes during the applying of the Head neck position. (Own observations)
Head not visible	hz	Head position is not visible.
Events:		
Head toss	ht	The horse attempts to move or moves the head in a quick forward-upward motion. This is in most positions restricted by the ropes. (Borstel, et al. 2009) (Kaiser, et al. 2006)(Own observations)
Head shake	hs	The horse attempts to shake or shakes its head in a quick left to right motion. This is in most positions restricted by the ropes. (Kaiser, et al. 2006)

Head stretch	hd	The horse attempts to stretch or stretches its head in a slow forward-downward motion. This is in most positions restricted by the ropes. (Waring 2003)(Own observations)
Head scratch	hc	The horse scratches its leg with its head, this is usually done during halt. (Own observations)
Walking forward	rf	The horse walks forward as reaction to applying the head neck position.
Walking backward	rb	The horse walks forward as reaction to applying the HNP.
Mouth gestures:		
States:		
Neutral mouth	mn	Cheeks are loose, the bit hangs loosely in the mouth without any movement of the jaw.
Tense mouth	mt	Teeth occluded, tense jaw, no bit movement. (Dierendonck, et al. 2007)
Play with bit	mp	Cheeks are loose; the horse lets the bit move inside its mouth. (Dierendonck, et al. 2007)
Abnormal oral behavior	ma	The horse opens the mouth for extended periods and/or opens and closes the mouth repetitively and/or grinds its teeth. (Borstel, et al. 2009) (P. McGreevy, A. McLean, et al. 2005)
Chewing on bit	mc	Horse chews on the bit and puts lots of pressure on it.
Tongue lolly	tl	Extraneous moving of the tongue in and out of the mouth. (McDonnell 2003)
Tongue out	to	Tongue hanging far out of the mouth. (McDonnell 2003)
Flehmen	mf	Flehmen
Mouth neutral + foam	m1	Cheeks are loose, the bit hangs loosely in the mouth without any movement of the jaw. Foam is seen on the lips.
Play with bit + foam	m2	Cheeks are loose; the horse lets the bit move inside its mouth. Foam is seen on the lips.
Mouth tense + foam	m3	Teeth occluded, tense jaw, no bit movement. (Dierendonck, et al. 2007) Foam is seen on the lips.
Mouth not visible	jz	Mouth gestures aren't visible
Ear Gestures:		
States:		
Ears neutral	en	Ears pointing sideward with little or rhythmic movement in line with the movement of the horse.
Earplay	ep	Ear movement from pointing forward to pointing backward; may be unilateral or bilateral. (Kaiser, et al. 2006)
Ears forward	ef	Ears pointed forward for an extended period (>5s). (McDonnell 2003)
Ears backward	eb	Ears turned backward (but not entirely flattened) for an extended period (>5s). (Borstel, et al. 2009) (Kaiser, et al. 2006)

Ears pinned back	ec	Ears pressed caudally against the head and neck. (Borstel, et al. 2009) (Kaiser, et al. 2006) (McDonnell 2003)
Ears not visible	ez	Ear gestures aren't visible

Locomotor behaviour:		
States:		
Neutral velocity	vn	The horse moves in desired velocity and desired gait.
Velocity faster	vf	The horse stays in the desired gait but moves faster.
Velocity slower	vs	The horse stays in the desired gait but moves slower.
Halt	vh	Cessation of movement of all four feet. (Kaiser, et al. 2006)
Backwards	vb	Backward movement of the horse. (Kaiser, et al. 2006) (P. McGreevy, A. McLean, et al. 2005)
Fast backwards	fb	Fast backward movement of the horse, usually followed after abrupt halt. (Kaiser, et al. 2006)(Own observations)
Abrupt halt	fh	Abrupt cessation of movement and reluctance of going forward. (Kaiser, et al. 2006)(Own observations)
Change in gait	vg	The horse moves in a different gait than desired, for example cantering during trot or walk or trotting during canter. (Borstel, et al. 2009) (P. McGreevy, A. McLean, et al. 2005)(Own observations)
Abnormal canter	va	Only during canter: horse moves in an unbalanced canter in which the front limbs are moving in a different gait than the hind limbs or in the different canter, or the horse travels in the wrong canter altogether: the horse is in the right canter while travelling on the left hand side. (P. McGreevy, A. McLean, et al. 2005)(own observations)
Locom. beh. not visible	vz	Locomoter behavior is not visible.
Events:		
Helper encouragement	up	Helper has to encourage the horse to move faster. (Own observations)
Helper slowing down	sl	Helper asks the horse to move slower. (Own observations)
Jumping/rearing/bucking	rb	Any form of movement in which the two front- and/or hind legs are detached from the ground. For example jumping, rearing or bucking. Or attempt of such a movement. (Borstel, et al. 2009) (Dierendonck, et al. 2007)(Own observations)
Change Canter	cc	The horse changes from left to right canter or right to left during canter with either the front limbs, hind limbs or both. (Own observations)
Crabbing	vc	The horse moves sideward-forward. The hind legs of the horse travel on a line beside the front legs. (Borstel, et al. 2009) (P. McGreevy, A. McLean, et al. 2005)

Stumbling	st	An interruption of the gait-specific, rhythmic footfall with loss of Balance. (Borstel, et al. 2009) (Own observations)
Falling	sf	The horse falls. The shoulder and hipbone of the horse touch the Ground. (FEI-Dressage-rules 2009) (Own observations)
Turning	tu	The horse suddenly stops moving and turns around. (Own observations)
Shying	sh	The horse shies away from an object or side of the arena. (Own observations)
Defecation	df	Expelling of feces. (Dierendonck, et al. 2007) (Kaiser, et al. 2006) (McDonnell 2003) (Waring 2003)
Blowing	ab	Non-pulsated sound produced by forceful expulsion of air through the nostrils
Snorting	as	Snorting sound of forceful exhalation through the nostrils with an audible flutter pulsation while the horse attempts to lower its head.
Groaning	ag	The horse makes a grunting sound. (Borstel, et al. 2009) (Waring 2003)
Sighing	ah	The horse makes a sighing sound. (Own observations)
Coughing	ac	The horse makes a coughing sound.
Tail-swishing	ts	Any exaggerated movement of the tail, usually more of a wringing motion than a rhythmic or directed swishing. (Borstel, et al. 2009) (Kaiser, et al. 2006) (P. McGreevy, A. McLean, et al. 2005)
Stridor	ms	A high pitched sound resulting from turbulent air flow in the upper airway. (Own observations)
Visibility of eye-white	me	The horse shows the white of the eye for an extended (>5s) period. (Borstel, et al. 2009)
Teeth grinding	tg	The horse grinds its teeth.
Whinnying	wh	The horse whinnies.
Body shake	bs	The horse shakes its whole body starting at the head and ending at the tail.
Air sucking	l4	The horse sucks air while walking.
Break out	l5	The horse breaks out of the circle in which it is supposed to walk.
Pawing	l6	The horse makes a kicking movement with its legs.

Tail Gestures:		
States:		
Neutral tail	tn	Tail is held in neutral position. Tail elevation is correspondingly with the velocity of the horse. With elevation increasing at a faster pace. (Waring 2003) Fleshy portion of tail stiffens with slight elevation at the posterior
Tense tail	tt	Fleshy part of the tail. Tail might also be held against body. (Waring 2003)
High tail	th	Tail is carried higher than could be expected at the present velocity. (Waring 2003)(Own observations)
Tail not visible	tz	Tail gestures are not visible.
Nose:		
States:		
Nostrils neutral	nn	Nostrils are in a neutral position.
Nostrils open	no	The nostrils are opened more than in neutral position.
Nostrils wide open	nw	The nostrils are opened wide.
Nose discharge	nd	Discharge is coming out of the nose.
Nose/eye not visible	nz	Nose not visible

Appendix 2:

Independent scoring possibilities.

Warming up
How does the horse behave during the warming up:
Possibilities of scoring:

1. The horse stays calm and shows no conflict behavior like for example: snorting, shying, jumping running, and stays in the desired gait and velocity.
2. The horse shows 1 stress behavior for no longer than ¼ of the warming up period or moves at a faster gait or faster velocity for no longer than ¼ of the period.
3. The horse shows conflict behavior or moves at a faster gait or velocity for more than ¼ and less than ¾ of the warming up period.
4. The horse shows conflict behavior or moves at a faster gait or velocity for more than ¾ of the warming up period.

Transition to trot during warming up:
How does the horse behave during the transition to trot inflicted by the lunger:
Possibilities of scoring:

1. The horse shows no conflict behavior and transists to trot without hesitation.
2. The horse has to be encouraged several times before transition to trot occurs.
3. The horse shows 1 conflict behavior.
4. The horse needs extra encouragement and shows 1 conflict behavior during transition.

Applying head-neck position
How does the horse react to the manipulation of the head and neck in the desired position while using rigid ropes?
Possibilities of scoring:

1. The horse shows no conflict behavior like for example: moving backwards, rearing, headshaking and pawing. The horse bends its head and neck without hesitation in the desired position.
2. The horse bends its head and neck without hesitation the first 30 seconds and shows 1 conflict behavior like for example stretching the ropes, scraping and/or moving backwards slowly for no more than ¼ of the period.
3. The horse shows resistance in bending its head and neck and shows severe conflict behavior like rearing and/or moving backwards fast.
4. The horse shows violent resistance, it’s impossible to apply the desired head neck position.

Detaching head-neck position
How does the horse react the two minutes following detaching the HNP?
Possibilities of scoring:

1. The horse doesn’t or hardly reacts at all
2. The horse seems relieved, stretches its neck for a short period of time, but doesn’t shake its head.
3. The horse shows a little compensation behavior like headshaking and/or some stretching with the neck.
4. The horse shakes its head vigorously and/or stretches its neck for a longer period of time.

Cooling down
How does the horse behave during the cooling down period in which it walks in a free head-neck position?
Possibilities of scoring:

1. The horse shows no conflict or stress behavior and exercises calmly with its head and neck in a low and long position.
2. The horse shows some conflict and/or stress behavior like head shaking and/or exercising with its head and neck held in a high(er) position then is the case in 1 during less than ½ of the cooling down time.
3. The horse shows conflict and/or stress behavior during ¾ of the cooling down period, like head shaking and/or keeping the head and neck in a high position, and may possibly move faster and/or in a faster gait than asked.

The horse shows stress behavior during the whole period, like headshaking, keeping the head and neck in a high position, bucking/jumping/rearing etc. and moves faster or in a faster gait than asked with occasional bursts of high speed.