Do horses show more stress during riding, lunging or working on a treadmill?

A Pilot to determine Possibilities and Threats using different exercise methods in future Hyperflexion Research.

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Chapter 1: Introduction

Anything forced and misunderstood can never be beautiful.
And to quote the words of Simon: If a dancer was forced to dance by whip and spikes, he would be no more beautiful than a horse trained under similar conditions. – Xenophon

The use of horses provided our ancestors with a decisive competitive advantage, but horses have also been valued companions through the ages. Already in 1200 BC, Anatolian horses were reported to live in better quarters than their grooms. This meaning that from early in our history, people have been concerned about equine welfare (Derksen and Clayton 2007).
When agility in the military horse became important in the 16th century, suppleness and obedience of the horse was optimised and military men started to compare their horseman skills with each in competitions (Ödberg and Bouissou 1999). These competitions evolved eventually into the modern equestrian sports that have become very popular today. Horses competing in international competitions nowadays are expected to perform at a very high level and must be trained and managed so they can achieve these high standards (Derksen and Clayton 2007).

The international Equestrian Federation (Fédération Equestre Internationale; FEI) understands that safeguarding the welfare of the horse is a key factor in maintaining the general acceptability of a sport that ‘uses’ animals. The federation has made a special ‘Code of Conduct’ in which respectful behaviour towards the horse is underlined. This ‘Code’ states that: “*At all stages during the preparation and training of competition horses, welfare must take precedence over all other demands. This includes good horse management, training methods, farriery and tack, and transportation*.” (FEI, KNHS 2008)

Discussions about equine welfare in sports are very common and do not restrict themselves to the horse community, resulting in huge debates in which not only the sport governing bodies are involved but also politics and the general public. In the 1970’s the debate revolved around the practice of rapping, which was then outlawed from show-jumping by the FEI. Nowadays the current focus is on dressage and particular on the topic of hyperflexion of the neck, more commonly known as Rollkür (McGreevy 2007). This practice is used as part of warming-up prior to dressage tests and draws criticism on the basis of its tendency to restrict vision, respiration and head movement (McGreevy 2007). Some riders, knowledgeable experts in equestrian sport and members of the public consider this technique unnecessary, offensive to the viewer and, importantly, a potential welfare issue for the horse (FEI 2006).

Not only social, but also financial interests are important in the modern equestrian world. So, prudence is necessary when talking about the disapproval or restriction of the hyperflexion technique. Little scientific research is known considering this matter, especially concerning the welfare issue, which is rather difficult to research. It is hard to set up a test in which the possible, sometimes subtle visible, reduction of well-being of the horse trained in hyperflexion is measured, and in which this welfare reduction can only be related to the used hyperflexion technique.
In this report the possibilities and threats of hyperflexion research will be outlined and the published articles regarding the associated welfare issue will be reviewed. This report will try to provide some guidelines for decent research about the welfare issue concerning the use of the ‘hyperflexion of the neck’-technique in the training of horses for dressage competitions.

Chapter 1.1: Hyperflexion in modern dressage training

The FEI rules describe the object of dressage as: "*The development of the horse into a happy athlete through harmonious education. As a result the horse is supple, loose, and flexible, but also confident, attentive, and keen, thus achieving perfect understanding with its rider*."
To gain this, the neck should be raised and the head should remain in a steady position, slightly in front of the vertical. No resistance should be offered to the athlete (FEI 2009).

Figure 1.1.1 Coby van Baalen riding Olympic-Ferro (from Ulft) in the desired head/neck position by the FEI dressage.

In this posture, which is considerably more upright than the position the horse will assume by nature, competitors are required to show their horses in the arena. However, in the early 1970s several showjumpers started using a technique in which the neck was positioned in a hyperflexed way with a lower and deeper position of the head, which was to a certain extent rolled up against the chest. Usually this was achieved by using special draw reins. This position has later been baptised 'Rollkur' in the German literature. The technique was copied by a number of dressage riders, of whom some became extremely successful. (Van Weeren 2007). These riders made the technique well known by a great number of professional and amateur dressage riders and therefore this training method a commonly used tool in the education of the modern dressage horse.

Riders and trainers that use the technique experience that their horses are very loose and manoeuvrable, extremely focused on the rider and that it is a useful aid in their gymnastic training (Hector (date unknown); Janssen 2003). The main focus is to acquire maximum development and maintenance of looseness in movement, and – according to the people in favour of using the method – particularly conditions the abdominal muscles, if it is used in short intervals and varied with other and less extreme postures (Schrijer 2001).
These statements have understandably been studied and in scientific literature several articles can be found regarding the kinetics and kinematics of the horse's movement in different head/neck positions.

Rhodin *et al.* (2005) compared three different postures, namely a free-, reasonably lower- and high position, in which the third corresponded with regular poll flexion. Their results showed that the stride length and flexion and extension of the caudal back were significantly reduced in the high position, but the movement of the back in the low position decreased as well. Gómez Álvarez *et al.* (2006) and Weishaupt *et al.* (2006) compared six postures differing in more or less extreme head extension and flexion. They found that elevating the head and neck led to extension in the cranial part of the spine and flexion in the caudal part, and that lowering the head and neck had the opposite effect (Gómez Álvarez *et al.* 2006). These changes were larger with more extreme positions, in which neck extension restricted the spinal range of motion more than flexion (Gómez Álvarez *et al.* 2006). They concluded that an extremely high head posture affected the functionality of the horse's movements much more than an extremely low posture. No impressive shifts in load distribution between forehand and hindquarters were observed by changing the head neck position though (Weishaupt *et al.* 2006).

The 'Rollkur'-technique has led to many debates, especially in the German lay press (Balkenhol *et al.* 2003; Janssen 2003, Pochhammer 2005) These debates led to several complaints towards the FEI regarding the technique, where after the federation decided to arrange a workshop to investigate the issues surrounding the use of the technique. This workshop consisted of a meeting of fifty invited representatives from all aspects of international dressage and equestrian sport. These representatives considered the term 'hyperflexion' a suitable form of words to describe the technique and defined hyperflexion as followed:

> *"Hyperflexion of the neck is a technique of working/training to provide a degree of longitudinal flexion of the mid-region of the neck that cannot be self-maintained by the horse for a prolonged time without welfare implications."*

Figure 1.2.2. A horse in hyperflexion. On the internet photo's of more exaggerated flexion of the neck, in which the horses mouth thouches it's chest. can be found.

Evidence presented at the workshop indicated that in experienced hands there was no apparent abuse, improper welfare or clinical side effects associated from the use of hyperflexion. However, an important remark was made: when the technique was not practiced correctly, serious concerns for welfare and possible clinical injury that will affect a horse's well-being and performance could occur (FEI 2006).

Another conclusion made during the workshop was that in order to be able to confirm in the future whether of not a welfare issue is involved in the use of hyperflexion, more research regarding this training method was necessary (FEI 2006).

Chapter 1.2: Hyperflexion and its possible concerns for horse welfare

As mentioned earlier the hyperflexion debate mainly focuses on the alleged decrease in horse welfare. Several aspects that may lead to this decrease have been pointed out in scientific and non-scientific literature, of which a number are listed below.

Enormous tension on the neck:
Heuschmann (2006) claims that the hyperflexion method puts an enormous tension on the upper neck muscles and on the back via the supraspinous ligament. The horse will often end up leaning on the forehand, rather than shifting its weight bearing to the hind limbs as is asked in (modern) dressage. However, Heuschmann has never published about his statements in a peer reviewed (veterinary) journal.

The horse is forced into the position by hand:
Horses may be brought into the hyperflexed position by force. This tension causes the rider to resort to even stronger hand influence, and into a rigid, ‘pushing’ seat, often with the upper body inclined backward (Heuschmann 2006 not reviewed). A cervical flexion as a result of sustained bit pressure has however more to do with compliance and pain avoidance than suppleness (McGreevy 2007).
Further do horses forcefully ridden by hand usually produce quite a lot of ‘foam’ or drool at the mouth because of the stiffening of the jaw and tongue due to the tension of the bit(s) and reins according to a website claim (Sandin 2005).

Welfare and safety issues:
A horse with a tense back and an excessively bent neck often tries to escape from the riders weight by running away (Heuschmann 2006, not reviewed). The already strong tension on the mouth can ‘deaden the brakes’ and lead to bolting and other unwelcome behaviour as a manifestation of habituation (McGreevy 2007). This ‘lack of control’ over the horse can lead to dangerous situations for the rider, other competitors and/or spectators.
Otherwise the technique can compromise welfare since horses learn that there is nothing they can do to remove the pressure. If the horse’s head is on its chest it has nowhere else to go and the animal may develop a state of learned helplessness (McGreevy 2007).

Hyperflexion limits the horse’s vision:
The horse’s eyes allow it to visualize a wide panorama of the horizon and also the area ahead where feet will be placed. The animal achieves the best frontal vision of the ground when it flexes slightly at the poll, so it can improve focus and enhance images of the ground ahead. However, when overflexed the horse cannot see the space directly in front of its nosebridge and thus probably is completely reliable on its rider (figure 1.2.1) (McGreevy 2004).

Hyperflexion limits breathing:
The trachea of the horse runs from the jaw along the bottom of the neck into the body cavity. When the neck is curled up, the trachea is pressed against the neck vertebrae at the base of the neck and bent at its top. This compression of the trachea may cause a mild form of airway obstruction resulting in the occasionally heard stridor horses make while being exercised in this position (Website of Sandin 2005).

Considering all the above, some may say that it can be concluded that some forms of hyperflexion may be extreme, dangerous and capable of compromising welfare (McGreevy 2007).

(a)

(b)

(c)

Figure 1.2.1. (a) The visual field in front of a horse when allowed to carry its head naturally. (b) The same field in poll-flexion. (c) The blind area in front of a hyperflexed horse. (Reproduced from Paul McGreevy 2004)

Chapter 1.3: Welfare of horses in hyperflexion in scientific research

It is plausible that at least some of these above mentioned welfare issues do in fact decrease horse welfare, but it is still not sure how and to what extent this decrease in welfare comes to expression in the normal riding horse. In the scientific literature to date, only three studies attempted to investigate the effects of hyperflexion on horse welfare.

Van Breda (2006) compared seven recreational trained horses ridden in normal poll flexion with five international Grand Prix level dressage horses ridden with a hyperflexed neck. In this study stress was measured by using heart rate variability analysis. The elite dressage horses showed a less sympathetic and increased parasympathetic dominance compared to the recreational horses and the conclusion was made that these findings suggested that the elite dressage horses tended to have less acute stress post exercise than the recreational horses. It was stated that the outcome of this study proved credibility to hyperflexion as a training method.

Figure 1.3.1. Horse in poll flexion

Nevertheless, it is not sure whether the differences found in heart rate variability were either due to the different training methods or due to the differences between the groups of horses. These variations could also be due to differences in horse personality, conformation, level of training (eg. muscle ache), way of riding and/or stable management.

Figure 1.3.2. Horse ridden a fraction deeper and rounder than poll flexion

Sloet van Oldruitenborgh-Oosterbaan et al. (2006) compared the workload of eight riding-school horses when being ridden in a natural frame with only light rein contact and when being ridden in what the authors claimed to be the 'Rollkur', but what was, according to Von Borstel et al. (2008) in fact just a fraction rounder and deeper than normal poll flexion.
They measured that heart rate and blood lactate were slightly higher when horses were ridden in their so-called 'Rollkur'. Furthermore, they found no differences in cortisol concentration between the two positions, but reported that subjective observations suggested improvement of movement. They concluded that higher heart rates were a sign of higher workload during 'Rollkur', since there were no signs of uneasiness in the horses. A note is that the horses used were never ridden in hyperflexion before and were physically not used to serious dressage training, this could probably alternatively explain the higher HR.
Von Borstel *et al.* (2008) included behavioural observations and a motivational and preference test to their hyperflexion study. They used fifteen horses without any previous

experience with hyperflexion. These horses were ridden through a Y-maze in which one arm was always followed in a round of hyperflexion and the other arm with a round in normal poll flexion.
Their results showed that horses moved slower when being ridden in hyperflexion, and that they more often showed behavioural signs of discomfort, like tail-swishing, head-tossing or attempted bucks.
Hereafter, horses were again repeatedly ridden into the maze, but the riders left it to the horse to decide which arm of the maze, and its associated training method, to enter. Most horses chose significantly more often the maze-arm associated with normal poll flexion rather than hyperflexion. The rider was however not blinded and a possible influence of the rider cannot be excluded.
Subsequently, eight of the horses were also subjected to two fear tests following a short ride in normal poll flexion or hyperflexion. Horses tended to react stronger to the fear stimuli and to take longer to approach them while ridden in hyperflexion. They concluded that a coercively obtained hyperflexion position may be uncomfortable for horses and that it made them more fearful and therefore potentially more dangerous to ride.

Most of the used horses in these studies were not accustomed to being ridden in hyperflexion and so were physically not prepared to accomplish such a position. An analogy is the same as asking an untrained person to perform a split and than conclude that this posture is actually to stressful to perform, when in fact trained gymnastic athletes have no difficulties of achieving such a position at all.
To acquire a decent opinion regarding this matter research is necessary in which psychological, behavioural and physiological parameters are measured. It is also very important that all horses used are familiar with the studied head/neck positions and are physically able to perform these positions.

Chapter 1.4: Horse behaviour

Although not systematically investigated and validated, scientists use a range of evasive behaviours to show stress in horses while being ridden (Visser *et al.* 2008). Some of the most useable evasive behaviours and social gestures linked to aggression, fear or evasion are stated below.

Locomotor patterns:
In agonistic situations horses can show a number of flight responses like balk, bolt and shy. Balk is the refusal to move to something suspicious, a sudden dash is known as bolt and a horse shies when body parts quickly withdraw from the suspicious object but the feet only shift slightly and typically to one side (Waring 2003) Bucking occurs in play and as a resistance to forward movement in horses under saddle or on the lunge. A rearing horse balances on its hindlegs and while ridden usually does that to avoid rein pressure Fear responses can be reduced through habituation and horses with prior experience of restraint often yield sooner than naïve individuals (McGreevy 2004). .
A horse that aggressively thumps the ground with a foot with its ears laid back, while being prepared for a ride seems to signal objection or protest. This knocking can also be frequent when insects and other irritations occur at the belly and the flanks (Waring 2003).

Head position and facial expressions:
Neck posture changes significantly during agonistic encounters between horses, being flexed during approach responses, arched in response to threats and lowered when signalling aggression (sometimes) or submission. Authors agree that it is hardly possible to obtain an emotional status from a head position alone. Several facial expressions existing of ear, mouth, nostril and eye gestures are necessary to evaluate stress in horses (McGreevy 2004). The ears are the most important body part in the non-vocal communication of horses. All concurrent interactions are agonistic when the ears are flattened. McGreevy 2004).
The eyes and ears are usually oriented to the side in a horse in a relaxed way while being ridden. Also in general relaxation, with the eyes and ears usually oriented to the side. If the eyes and ears are rotated backwards a horse is stressed, uncomfortable, or seems apprehensive about its rider (Waring 2003). Alarm is often indicated by switching the direction of the ears and by a tense mouth, dilated nostrils (McGreevy 2004) and widely opened eyes.
Although not recorded in scientific literature, head lowering and lip-licking have been identified as signs of submission in round-pen exercises and tend to precede movement of the horse towards the trainer (McGreevy 2004). During riding, abnormal oral behaviour, like teeth grinding, displacing the tongue out of the mouth or opening the mouth for longer periods, are signs of discomfort and are considered faults in the education of the dressage horse (Van der Heijden *et al.* 2007). Head tossing and shaking may indicate the presence of something unwelcome (Kaiser 2006).

Tail position:
Swishing movements of the tail are a sign of irritation and riders often report this as a response when a horse resists or enters behavioural conflict (McGreevy 2004). Defecation can occur as a result of stimulations or arousal and therefore could indicate a stressful situation (Kaiser *et al.* 2006).

Sounds:
Sounds can be useful in the assessment of the emotional status of a horse. Some sounds associated with agonistic or conflict behaviour are groan, blow and snort.
The groan is a monotone vocalization and seems to be an expression of mental conflict, suffering or physical effort (Waring 2003). The blow is a non-pulsated sound produced by forceful expulsion of air through the nostrils. It is usually an expression of alarm in which the nostrils dilate completely during the brief blow. A snort is a forceful exhalation through the nostrils and characterized by an audible flutter pulsation (Waring 2003). It is used defensively and aggressively and is in equestrian contexts associated with exercise and conflict during restraint (McGreevy 2004). Snorts appear to be a displacement activity and seem to express the horse's restlessness (Waring 2003).

Several studies about stress in ridden horses have used specially designed ethograms, listing mainly agonistic and arousal behaviours as those described above, shown by horses in the studied situations (Kaiser *et al.* 2006, Von Borstel *et al.* 2008).

Chapter 1.5: Cortisol

It is recognised that the amount of psychological stress that an animal encounters determines the degree of response of the hypothalamic-pituitary-adrenal (HPA) axis (Cayado *et al.* 2006). One of the hormones produced by the HPA-axis is cortisol, commonly known as the 'stress-hormone', as psychological and physical stress increase its presence in bloodplasma and saliva (Berne and Levy 1998).

In normal horses, cortisol secretion, like that of ACTH, exhibits distinct diurnal variation with a peak in the morning and a nadir in the evening (Berne and Levy 1998, Van der Kolk *et al.* 2001, Irvine and Alexander 1994). Cortisol pulses follow ACTH bursts ten minutes later and tend to be present in plasma fifteen to thirty minutes after the actual stressor, that triggered the original ACTH burst, has taken place. Cortisol binds to a specific cortisolbinding globulin called transcortin in plasma (Berne and Levy 1998). Only unbound, free, cortisol is biologically active. The horse has relatively low concentrations of this cortisol binding globulin, so consequently a small increase in total plasma cortisol leads to a large rise in free cortisol. Only the unbound cortisol can cross into saliva (Van der Kolk *et al.* 2001).
The plasma cortisol concentration will peak approximately thirty minutes after the stressor has stopped (Fazio *et al.* 2008), and the saliva cortisol concentration peaks fifteen minutes after the plasma peak (Van Sommeren 2008 (personal communication dr. E.K. Visser)).

Chapter 1.6: Aim of the study

The aim of the overall study, to which the presented study is a part of, was to examine whether ethological and physiological (cortisol) stress indicators in the horse were related to exercise in different head/neck positions (HNP).

The aim of this study was to develop an ethogram and protocol for the overall study. Before this protocol could be developed it was necessary to know what kind of training technique would cause the least stress to horses, so the measured stress could only be attributed to the applied HNP.

In most scientific studies the treadmill is used when horses are exercised in a standard protocol, tThis is because most environmental influences, like wind, rain or footing, will not affect the performance and speed can easily be controlled. Moreover it is possible to monitor the exact movement and posture of the horse and even more invasive techniques like endoscopy and oxygen measurements are easily carried out when a treadmill is used (Seeherman 1991, Sloet and Clayton 1999, Weishaupt *et al.* 2006, Rhodin *et al.* 2005).
While habituating the horses to training and the different HNP's on the treadmill it was experienced that the technique was most likely too stressful for the horses to move in some of the desired HNP's. They subjectively showed more stress when worked in certain postures on the treadmill compared to when they were exercised in the same positions under the saddle or on the lunge, even when they were completely habituated to working on the treadmill. This made it clear that scientific evidence should be presented regarding the stress experience of horses during different training techniques. In this study in which stress is measured by using behavioural observations, any other stressor than the actual 'test-stressor' has to be avoided because this may possibly cloud the ethological scoring. In this study the most accepted exercise technique in scientific research, namely the treadmill (T), is compared with two techniques commonly used in equestrianism, riding (R) and lunging (L). The study aimed at assessing whether the treadmill could be an acceptable and useful aid in the exercise part of the research of horses in which behavioural stress is measured as well. Stress was measured both using cortisol saliva concentrations and behavioural scoring. The heart rate variability will be discussed in an other report.
The hypothesis was that horses would indeed suffer more stress on the treadmill than while being lunged or ridden.

Chapter 2: Material and Methods

Horses:
Six horses, five warmblood mares and one Frysian gelding were used. All horses were housed at the horse clinic of the faculty of veterinary medicine at Utrecht state university. During the first months (treadmill test) of the study, the horses were held on pasture in a large group for 24 hours a day, but during the riding and lunging tests they were housed inside single boxes because of weather conditions. They had no physical contact with other horses while staying in the stable, but were able to see them.
While being used for this study, the horses were also used for veterinary education and in a riding school where they were ridden on a Dutch beginner or light level. They were all used to being lunged and ridden and were familiar with the used indoor arena. They had been on the treadmill in all three gaits and in the treadmill area for at least three times before testing began. The ethical committee of Utrecht University approved the study.

Horse No.	Breed	Sex	Age	Level *(Dutch dressage level)*
1	Frysian	Gelding	8	Beginner
2	Dutch Warmblood	Mare	8	Light
3	Dutch Warmblood	Mare	12	Beginner
4	Dutch Warmblood	Mare	13	Light-Medium
5	Dutch Warmblood	Mare	12	Light-Medium
6	Dutch Warmblood	Mare	13	Light

Figure 2.1. Classification of horses used in dressage-training level. The Dutch system is used which switches from beginner to light, medium, heavy, very-heavy, light-professional and heavy-professional.

Tests:
The horses were prepared and tacked up for the exercise test in the same room as where the test was to be performed. This was an indoor riding area for the riding and lunging test and the equine exercise laboratory during the treadmill test. The indoor riding arena used was the same one as where the horses were commonly exercised by the riding school students. In the treadmill laboratory a second horse (small familiar pony) was present so the horses would not be stressed because of social isolation.
With the treadmill (Mustang 2000, Switzerland) three animal handlers were present, two at the front of the horse to make sure that the horse stayed on the treadmill and one at the back that was in control of the horse and the speed. This handler had a whip to encourage the horse to go faster if necessary. During the lunging test the horses were exercised by a single person standing in the middle of a large (20m diameter) circle, holding a rope and a whip to get the horse to move around her. During the riding test a person rode the horses in the same large circle around the camera. The person performing the lunging and riding tests was the same person for all tests, for the treadmill different personnel were used.
The horses were exercised in a natural frame with little or no rein contact and in a speed that was comfortable for the individual. They wore a normal bridle and snaffle bit and a commercially available lunging-girth, except for the riding test in which they wore a general purpose saddle.

The exercise test consisted of five (5) minutes walk, ten (10) minutes trot, three (3) minutes canter and five (5) minutes cooling down in walk.
Due to organizational issues the treadmill test was performed three months earlier than the other two tests, so the tests could not be randomised between the horses. There were also no

saliva samples taken during the treadmill test. During the other two tests, the individual horses were exercised at exactly the same time of day during all tests, this for the cortisol measurements. To make sure the results could not get completely altered by one horse that actually behaved differently than normal on a test day, all horses were tested twice.

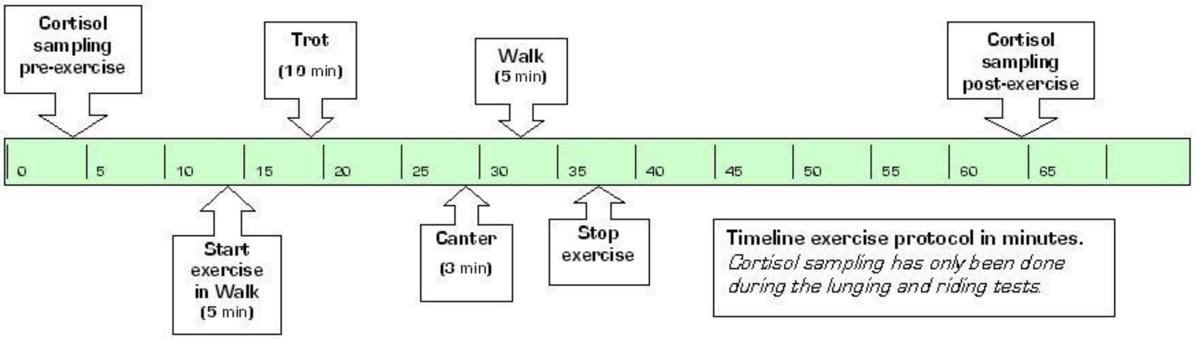

Figure 2.2. Timeline exercise protocol in minutes.

Observations:
The horses were extensively monitored while entering the research area and while tacking up. For the treadmill test two additional scores were given: while entering and leaving the treadmill. Behaviour was scored on a scale from one (1) to four (4) in which one stood for showing no conflict behaviour at all and four for showing conflict behaviour for almost the whole period (figure 2.3.). The actual description and protocol for this scaling can be found in figure 6.1. During the exercise test horses were filmed using a commercially available handheld camera (Sony). After all exersises were performed the behaviour was scored with the focal animal technique using The Observer 5.0 (Lehner 1985). For the scoring an specially designed ethogram was used The ethogram can be found in Appendix I.

For the actual analyses the behaviours were categorized in categories namely:

- Locomotion behaviour: in which behavioural elements regarding speed and movement were categorized,
- Head/neck position (HN): scoring all movements of the head and neck,
- Ear gestures: all movement of the ears
- Mouth gestures: all oral behaviour
- Tail behaviour: all movement with the tail
- Sounds.

The behavioural elements either were part of the horses time budget (and called in general a state) and their duration was recorded by Observer or the behaviour was very short and only its frequency was scored (so called events).

Later these categories were transformed into three classifications for the data processing, namely 1) locomotion states: calculating duration locomotion- and tail behaviours, 2) duration of HN states: consisting of HN-, ear- and mouth gestures and 3) all frequencies of the "events".

How does the horse enter the research area/ enter the treadmill/ leave the treadmill?
(Circle what applies)

1. At ease with no signs of conflict behaviour.
2. The horse hesitates for no longer than ten (30) seconds and enters the area with showing none or one (1) sign(s) of conflict behaviour.
3. The horse has to be encouraged to enter the area and shows one (1) or more signs of conflict behaviour.
4. The horse has to be forced to get in the research area and shows several signs of conflict behaviour.

How does the horse behave during tacking up? *(Circle what applies)*

1. At ease with no signs of conflict behaviour.
2. With one (1) sign of conflict behaviour: for example scraping or headshaking.
3. The horse shows conflict behaviour during most or the entire period: for example scraping, headshaking, bite threads or avoidance.
4. It is (almost) not possible to tack up because the horse shows violent resistance.

Figure 2.3. The 1 to 4 scale used to score behaviour during entering the research area and tacking up period.

Cortisol:
Cortisol samples were taken on the same time of day on every exercise day for each horse, so the relative changes in cortisol concentration of each horse could be compared later on without interference of the circadian rhythm (Van Sommeren 2008). Saliva was collected using Salivette tubes (Sarstedt B.V. Etten-Leur). The Salivette container has an opening in the bottom and is placed inside another tube. The cotton roll was attached to a double-jointed snaffle bit attached to a bridle (figure 2.4.).
Two samples per exercise test were taken and the horses wore the bridle with one cotton roll for 3,04 ± 0,96 minutes, fifteen (15) minutes before the start of the tests and thirty (30) minutes after the test was finished. After obtaining saliva, the Salivettes were stored on ice during the exercise day and later centrifuged for fifteen (15) minutes at 4000 G. The supernatant was frozen at –20 ºC (Van Sommeren 2008) and later analysed with the YY kit..

Figure 2.4. Double-jointed snaffle bit with Salivette attached.

Data processing and statistical evaluation:

The total time of time-consuming behaviours (states) per exercise test were recorded in seconds. The time budget was calculated as percentages of the observed time. These data were Ln transformed to acquire a normal distribution. In order to avoid zero's all percentages were increased by 0,00001. The data were analysed statistically using SPSS 15.0 (SPSS Inc., Chicago). The statistical test used was a linear mixed model and the statistical significance was set at $P < 0,05$. In the model thesubject variable the horse was entered and the fixed effects were training technique and if the horse did the test for the first or the second time (to get an idea about the possible learning effect). The three training techniques were later pair wise compared with each other, same as the first or second time of performing the test.

The one to four scores for tacking up and entering the area were statistically analysed using a One-way ANOVA in which the results of the three training techniques were compared with each other. Statistical significance was set at $P < 0,05$.

Chapter 3: Results

Behavioural observations

During the treadmill tests horses travelled on a confined speed of 6,08 ± 0,42 km/h in walk, of 13,36 ± 2,64 km/u in trot and of 25,82 ± 9,18 km/u in canter. The speed during the lunging and riding tests was not recorded and horses were allowed to travel in their own comfortable speed. This may have differed from the confined speed asked on the treadmill. The constant movement of the treadmill stimulated the horses to keep working only in the gait needed, while especially during lunging the horses were quite free to travel in their own preferred speed. Occasionally they changed gait accidentally. During riding and on the treadmill the horses were more in control compared to lunging, because during lunging horses could have sudden bursts of speed that could not be controlled easily.

Ears neutral, head low and neutral jaws (table 3.1.) had significant shorter times on the treadmill compared to during lunging and riding. The horses significantly had their ears backward for less time during lunging compared to treadmill and riding. Ears forward was significantly shorter on treadmill compared to riding.
The horses played with their bit significantly more often during riding than on the treadmill or during lunging. While lunging, horses also statistically showed more play with bit compared to the treadmill. There were significant differences in the time the mouth was not visible for all three techniques. This was especially noticeable on the treadmill where 84,08% of the time the mouth was not visible. This indicates that it may not be possible to draw conclusions from mouth behaviour because it could simply not be scored.
Head high and tense tail were significantly higher on the treadmill compared to lunging and riding. Horses travelled statistically longer time in neutral velocity during riding compared to lunging and statistically longer in velocity faster during lunging compared to riding and treadmill. Head to ground and abnormal canter were significantly seen more often during lunging compared to treadmill.
The visibility of the ears, head and tail were all significantly higher during lunging compared to riding and treadmill.
Helper slow down and turning was significantly seen more during lunging compared to treadmill and riding. Defecation was statistically seen more often on the treadmill compared to lunging. Snorting was significantly seen more during riding compared to the treadmill and head stretch was seen more often during riding compared to treadmill and lunging.
Not statistically different but with the p-value coming very close to 0,05 were ear play, neutral tail and change in pace. Ear play was seen more during riding compared to treadmill. Neutral tail more during riding compared to lunging and change in pace more during lunging compared to riding.

No significant differences were found between the first and second test with the same training technique. This indicates that if a possible learning effect had taken place it was of no statistic influence.

Cortisol:

Only during the lunging and riding tests cortisol was measured. Only three from the fourty-eight samples had cortisol levels in them higher than the detectable amount of 2,7 nmol/l.

Ethogram and protocols for HNP study:

The ethogram in this study was based on the observations tested and adjusted so it can be used during the HNP study. Several protocols, including an exercise protocol and the scaling for applying the HNP can be found in Appendices II – V.

Table 3.1. Mean frequencies and percentages ± SD of different behaviours per training technique (T = treadmill, L = lunging, R = riding), and significance of difference.

Behaviour	T: mean ± SD	L: mean ± SD	R: mean ± SD	*P*-value T-L	*P*-value T-R	*P*-value L-R
Tail Swishing	6,0 ± 34,5	1,5 ± 4,2	5,2 ± 10,9	n.s.	n.s.	n.s.
Tense Tail	26,1 ± 53,9	0,1 ± 0,6	0,000	0,001***	0,001***	1,000
Head Low	3,5 ± 9,2	70,0 ± 75,2	80,7 ± 73,5	0,001***	0,001***	n.s.
Head High	69,2 ± 121,1	9,3 ± 26,9	6,9 ± 12,8	0,014*	0,049*	n.s.
Ears Neutral	3,8 ± 8,2	135,0 ± 107,4	40,4 ± 43,2	0,001***	0,001***	n.s.
Ears Backward	204,4 ± 131,3	24,4 ± 35,7	59,6 ± 60,0	0,001***	n.s.	0,006**
Neutral Jaw	50,2 ± 88,2	321,7 ± 153,5	310,4 ± 155,2	0,001***	0,001***	n.s.
Play with Bit	1,1 ± 2,8	13,7 ± 24,1	32,3 ± 43,0	0,008**	0,001***	0,026*
Defecation	0,8 ± 1,2	0,2 ± 0,4	0,4 ± 0,8	0,004**	n.s.	n.s.
Head Stretch	1,5 ± 2,5	3,0 ± 4,7	6,7 ± 7,8	n.s.	0,001***	0,003**
Helper Slow Down	0,5 ± 0,6	2,0 ± 2,7	0,6 ± 0,6	0,001***	n.s.	0,001***

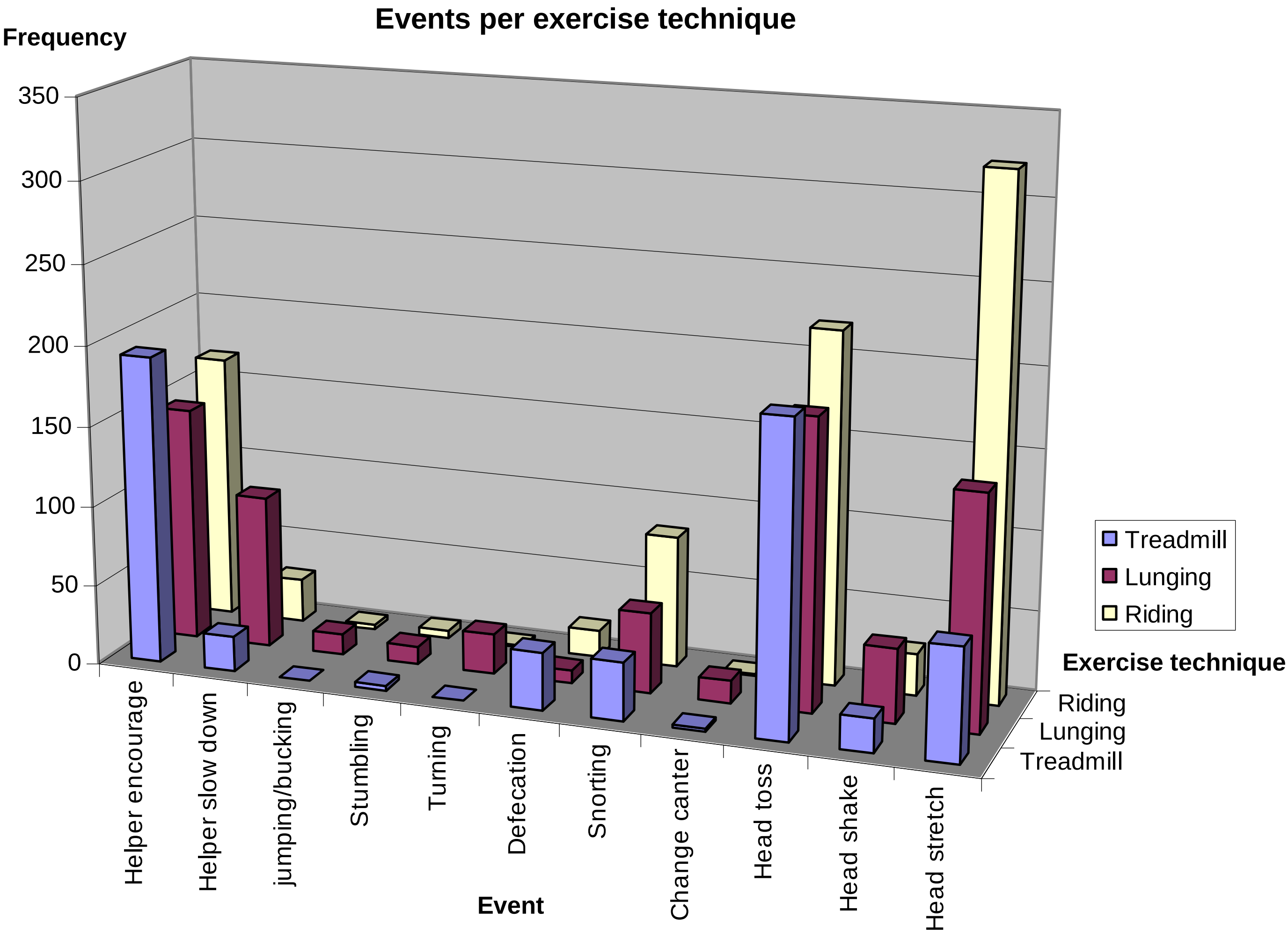

Figure 3.1. Total frequencies of events per exercise technique (blue = treadmill, purple = lunging, yellow = riding).

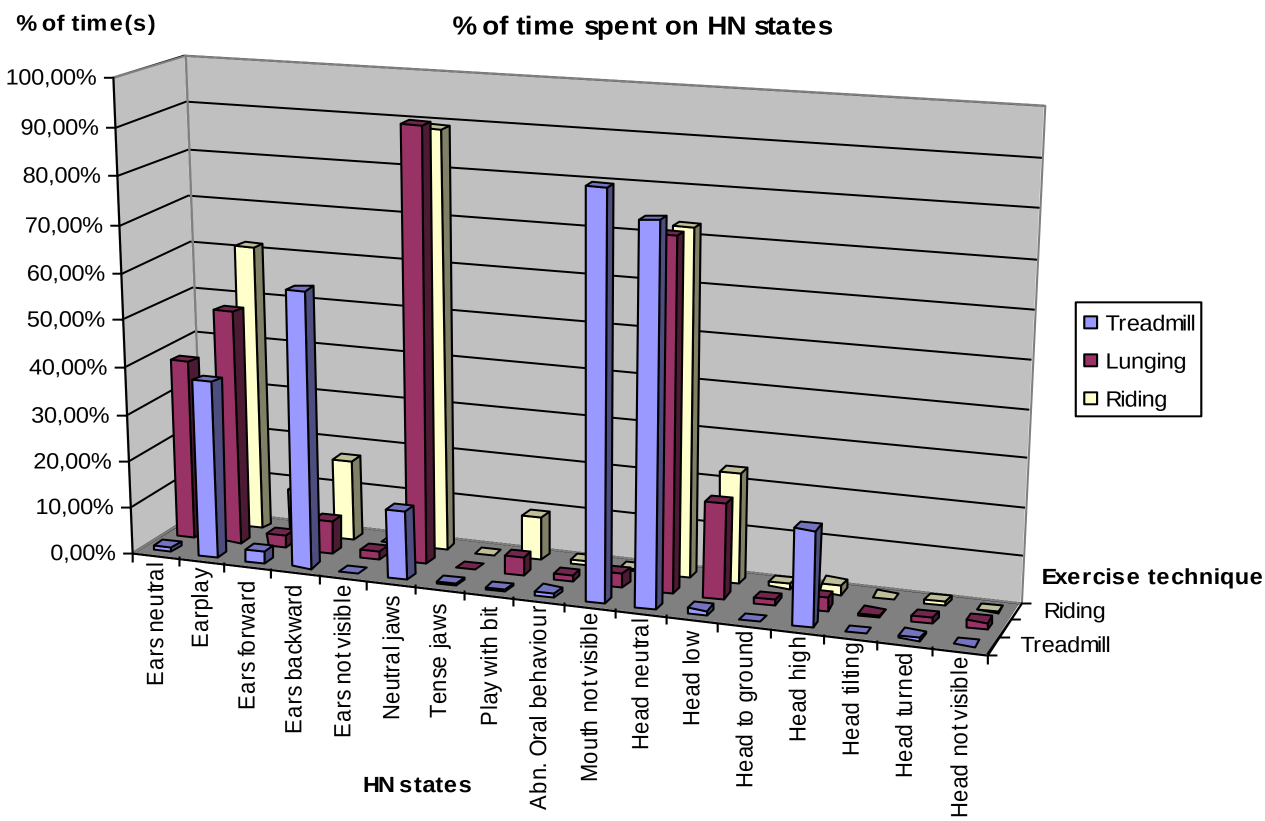

Figure 3.2. Total percentage of time spent on HN states per training technique (blue = treadmill, purple = lunging, yellow = riding).

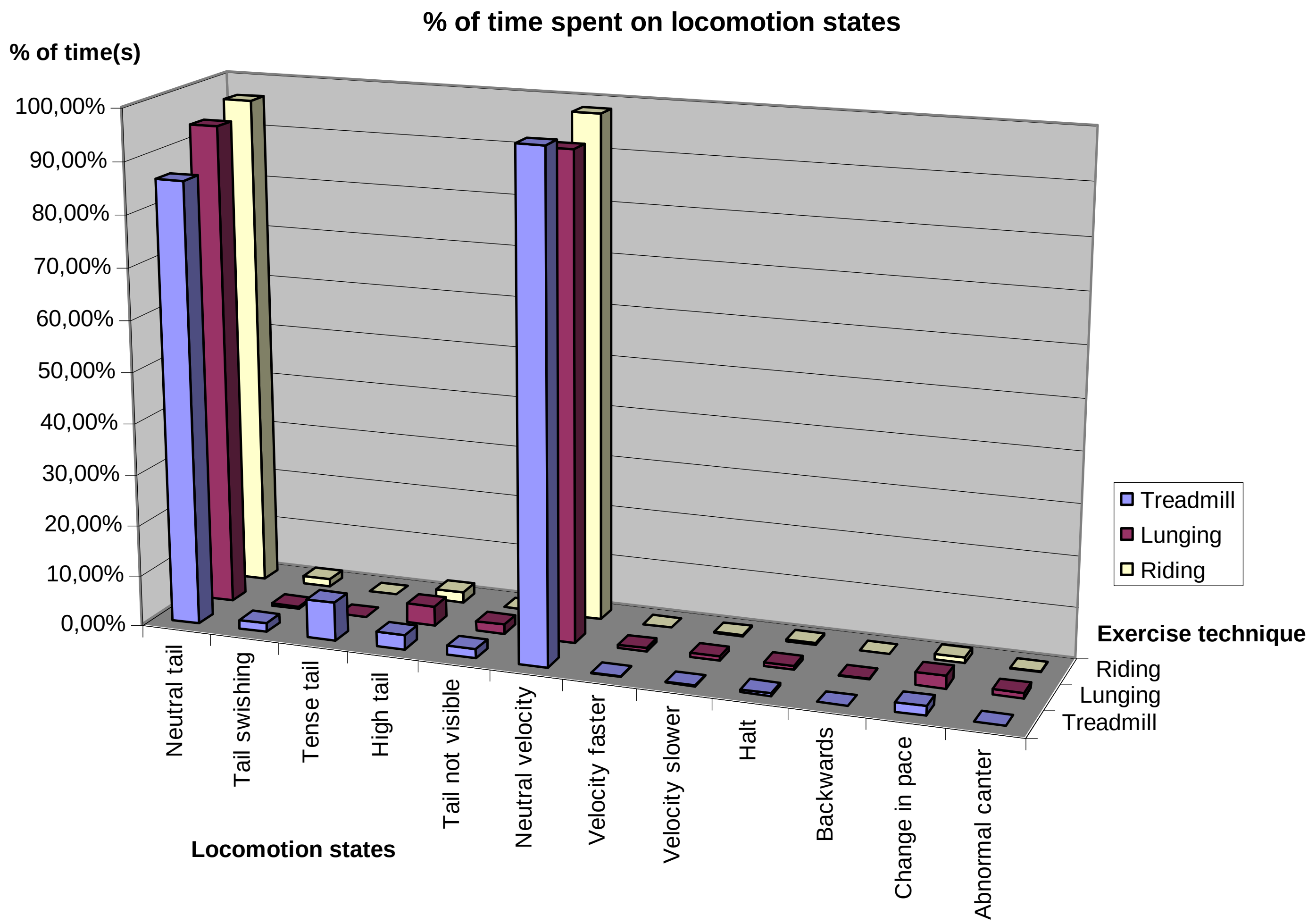

Figure 3.3. Percentage of time spent on locomotion states per training technique (blue = treadmill, purple = lunging, yellow = riding).

Chapter 4: Discussion

In this study the behaviour of six individual horses was studied. Like in human behaviour it is probably justified to claim that each individual horse has an individual approach to cope with stress and so each individual horse will behave in a different manner while being exposed to the same stressor. This may indicate the enormous wide standard deviations in the results. But every horse was exposed to all three techniques and was scored in the same way and for the same length of time. So although an individual horse may have reacted in it's own individual way, he or she has behaved in this individual way during all exercise tests. This means that every horse has served as it's own control group.

No abnormalities were seen in behaviour during tacking up and entering or leaving the research area. All horses could be categorised into category 1 or 2 (out of the 4 categories mentioned earlier), and horses scored as a 2 stayed in category 2 during the whole study and did not show more or less abnormal or agonistic behaviour during the research, and vice versa. This indicates that the horses were not already stressed because of other surrounding elements outside of the test.

In the ethogram used, all neutral behaviours can be regarded as relaxed behaviours. A horse relaxed while being ridden has the ears usually oriented to the side (here seen as neutral) (Waring 2003). The horses had their ears significantly more of the time in neutral position during riding and lunging compared to the treadmill. Head lowering can be acknowledged to (sometimes) aggression or submission, but is in the riding community general accepted as a submission (McGreevy 2004) or relaxation sign. In this research "head stretch", "head low" and "head to ground" were all three less seen on the treadmill indicating that the treadmill caused most likely more stress and less submission. The horses needed actually to be held by two helpers at the halter on the treadmill to prevent the horse from jumping off. So it was possible that the horses had no chance of head lowering on the treadmill because the helpers lifted them. This indicated that these elements probably could lead to multi interpretable results.

A high head on the other hand can indicate stress because the whole body usually stiffens when the head is held excessively high during locomotion. The same can be said for a "tense tail" which either is correlated either with anxiety or stress. Those two parameters were both significantly higher on the treadmill than riding and lunging. Also defecation, indicating a stressful situation (Kaiser *et al.* 2006) was statistically seen more often on the treadmill compared to lunging. These three parameters indicated that the horses did in fact show more stressful behaviour on the treadmill than during riding or lunging. However "tail swishing", which is regarded as a strong agonistic behaviour did not significantly differ between the three techniques. This may indicate that neither of the three gave that much stress, so that the horses did not need one of their strongest agonistic behaviours to show their discomfort.

Mouth not visible was significantly higher, and with a very high percentage, on the treadmill compared to the other two techniques. However, the mouth could not be seen and scored on the treadmill for most of the time, no conclusion can be drawn using mouth behaviour. There were no obvious differences in mouth behaviour between riding and lunging.

During lunging the helper had less control over the horse and some horses had sudden dashes forward, indicating the lower level of controllability during lunging. Helper slow down, turning, velocity faster and abnormal canter were all significantly seen more often during lunging, compared to riding and treadmill. Neutral velocity was actually higher during riding compared to lunging, which also indicates this lower level of controllability. Except from the sudden locomoter dashes in which above parameters were scored, no other significant differences in stress behaviour were found between riding and lunging.

Snorting gave interesting results. This sound was expressed statistically more often during riding compared to the treadmill and usually this sound was accompanied by a head stretch. There is still a lot of controversy about the meaning of this sound (Van Dierendonck 2009). In most scientific literature the snort is linked to conflict during restraint (McGreevy 2004), but in the equestrian world snorting is thought to be a relaxation sound during riding. This makes it not possible to draw a straight conclusion from the results of this parameter. But because in this study it was accompanied by the relaxing gesture of head stretch or head low, and because all other stress parameters indicate that the treadmill was the most stressful training technique of the three, it may indicate that also snorting in this study could be linked to relaxation.

Although there was a substantial variability between horses, the majority was habituated to treadmill exercise after one or two exposures (King *et al.* 1995). All horses had at least been on the treadmill for three times prior to testing. This will probably be enough, but it can not even out the experience they had with lunging and riding. After all, they were trained using those techniques for several years. It is probable that horses travelled on a higher (because fixed) speed on the treadmill than when they were allowed to choose their own velocity. But Sloet van Oldruitenborgh-Oosterbaan and Barneveld (1995) found that on basis of heart rate and plasma lactate concentrations, the workload of horses was significantly greater in normal exercise than on a horizontal treadmill. It is therefore reasonable to state that the differences in speed can be crossed off against the difference in workload.

Making an ethogram and protocols for a hyperflexion study had some major pitfalls. The horses used in this study were normal lower level riding horses, not used to work in different HNP's. While the hyperflexion technique (most closely related to NHP4) is used by many dressage riders, of whom some are extremely successful (Van Weeren 2007). Therefore experienced dressage riders and/or animal handlers and of course extensively trained healthy horses should be used.
By using sound observation techniques in which all behaviour is scored together with cortisol samples and heart rate variability (done in a separate study) it is possible to come up with a reliable impression regarding the stress levels during exercise.
The best way to acquire a stable research environment is by using a treadmill. Conditions like speed and workload can be monitored and kept the same for all tests (Sloet van Oldruitenborgh-Oosterbaan and Clayton 1999). But the treadmill apparently gave more stress to the horses, so the possible differences in stress in the future study with different positions may be clouded by the stress given from the treadmill.
The reason why the treadmill gives more stress is not quite known, but several speculations can be made. For instance were the horses used in this study trained for years in a sand footed arena. Although they were habituated to the treadmill prior to the study, this may not have been sufficient enough so that the horses felt completely comfortable to working on a treadmill. Thereby were the horses confined into a narrow walkway in which they had to move and in which there were no other possible manoeuvres than the treadmill exercise. This lack of freedom or controllability of their own movements may have given the extra stress, as well as being in a noisy room with a lot of humans and only one other horse.

Lunging and riding are training techniques that gave the horses less stress and on one side riding should be preferred: mainly because the hyperflexion posture is mostly used during riding and during the lunging technique horses were less controllable. But on the other side it is almost impossible to standardise the influence of the rider. Having the same rider does usually take some time for the horses to adjust, while using the horses own rider makes that less comparable. So finally this pilot has lead to the conclusion that lunging the horses in future studies, especially with highly trained horses and thus also these lower ranked study horses is the best option, provided that they are trained considerably on the lunge. Also during the observations disturbance from outside should be kept to a minimum.

It is advisable to include the measurement of rein pressure, used to get the horses in the desired positions, in future studies, for instance by using rein tension meters (Warren-Smith 2006, Clayton *et al.* 2005). This makes it probably possible to distinguish whether differences in stress behaviour are caused by the inflicted postures or of the diversity in rein tension.

Conclusion

Horses in this study showed significantly more stress behaviours and significantly less relaxation behaviours on the treadmill compared to riding and lunging. Lunging and riding were not much different, however, the horses were better under control during riding than lunging. However, using a rider (either one rider for all horses or the horse's own rider) for highly trained horses also introduces several scientific difficulties. So for the future study in which stress is measured and for what most conditions have to stay the same, lunging should be the recommended technique.

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Appendix I: Ethogram

Equid Ethogram to be used during the observation of horses exercising in different head-neck positions.

Ethogram during the exercise protocol:		
State: Behaviour that lasts for a longer period (in seconds) **Event**: Behaviour that happens in a shorter time (in frequencies)		
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.
Head low	hl	In position 1: head and neck are held lower than in neutral position, between the shoulder joint and the carpus.
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.
Head high	hu	In position 1: head and neck are held higher than in neutral position.
Head tilting	hk	The horse tilts its nose to one side (Von Borstel *et al.* 2008, McGreevy *et al.* 2005).
Head turned	hb	The horse moves its head lateral towards the left or right and leaves it there for a longer (>5s) period.
State 1	s1	
State 2	s2	
State 3	s3	
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 (Von Borstel *et al.* 2008, Kaiser *et al.* 2002).
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.* 2002)
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).
Head scratch	hc	The horse scratches its leg with its head, this is usually done during halt.
Event 1	e1	
Event 2	e2	
Event 3	e3	

Mouth gestures:

States:		
Neutral jaws	jn	Cheeks are loose, the bit hangs loosely in the mouth without any movement of the jaw.
Tense jaws	jt	Teeth occluded, tense jaw, no bit movement (Van Dierendonck *et al.* 2005).
Play with bit	jp	Cheeks are loose, the horse lets the bit move inside its mouth (Van Dierendonck *et al.* 2005).
Abnormal oral behaviour	ja	The horse opens the mouth for extended periods and/or opens and closes the mouth repetitively and/or grinds its teeth (Von Borstel *et al.* 2008, McGreevy *et al.* 2005).
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).
State 4	s4	
State 5	s5	
State 6	s6	
Mouth not visible	jz	Mouth gestures are not 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.[3]
Ears forward	ef	Ears pointed forward for an extended period (>5s).[5]
Ears backward	eb	Ears turned backward (but not entirely flattened) for an extended period (>5s).[1,3]
Ears pinned back	ec	Ears pressed caudally against the head and neck.[1,3,5]
State 7	s7	
State 8	s8	
State 9	s9	
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.* 2002).
Backwards	vb	Backward movement of the horse (Kaiser *et al.* 2002, McGreevy *et al.* 2005).
Fast backwards	fb	Fast backward movement of the horse, usually followed after abrupt halt (Kaiser *et al.* 2002).
Abrupt halt	fh	Abrupt cessation of movement and reluctance of going forward (Kaiser *et al.* 2002).
Change in pace	vp	The horse moves in a different gait than desired, for example cantering during trot or walk or trotting during canter (Von Borstel *et al.* 2008, McGreevy *et al.* 2005).
Crabbing	vs	The horse moves sideward-forward. The hind legs of the horse travel on a line beside the front legs (Von Borstel *et al.* 2008, McGreevy *et al.* 2005).
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 (McGreevy *et al.* 2005).
State 1	s1	
State 2	s2	
State 3	s3	
Locom. beh. not visible	vz	Locomotor behaviour is not visible.
Events:		
Helper encouragement	up	Helper has to encourage the horse to move faster.
Helper slowing down	sl	Helper asks the horse to move slower.
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 (Von Borstel *et al.*2008, Van Dierendonck *et al.* 2005).
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.
Stumbling	st	An interruption of the gait-specific, rhythmic footfall with loss of Balance (Von Borstel *et al.* 2008).
Falling	sf	The horse falls. The shoulder and hipbone of the horse touch the Ground (KNHS 2007).
Turning	tu	The horse suddenly stops moving and turns around.
Shying	sh	The horse shies away from an object or side of the arena (Waring 2003).
Defecation	df	Expelling of feces (Van Dierendonck *et al.* 2005, Kaiser *et al.* 2002, McDonnell 2003, Waring 2003).
Blowing	ab	Non-pulsated sound produced by forceful expulsion of air through the nostrils (Waring 2003).
Snorting	as	Snorting sound of forceful exhalation through the nostrils with an audible flutter pulsation, while the horse attempts to lower the head (Von Borstel *et al.* 2008, Waring 2003).
Groaning	ag	The horse makes a grunting sound (von Borstel *et al.* 2008, Waring 2003).
Sighing	ah	The horse makes a sighing sound.
Coughing	ac	The horse makes a coughing sound.
Event 1	e1	
Event 2	e2	
Event 3	e3	

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).
Tail-swishing	ts	Any exaggerated movement of the tail, usually more of a wringing motion than a rhythmic or directed swishing (Von Borstel *et al.* 2008, Kaiser *et al.* 2002, McGreevy *et al.* 2005).
Tense tail	tt	Fleshy portion of tail stiffens with slight elevation at the posterior 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).
State 4	s4	
State 5	s5	
State 6	s6	
Tail not visible	tz	Tail gestures are not visible.
Miscellaneous:		
States:		
Stridor	ms	A high pitched sound resulting from turbulent air flow in the upper airway.
Visibility of eye-white	me	The horse shows the white of the eye for an extended (>5s) period (Von Borstel *et al.* 2008)
State 7	s7	
State 8	s8	
State 9	s9	
Nose/eye not visible	mz	There are no miscellaneous behaviours or miscellaneous behaviour is not visible/audible

Appendix II. Exercise protocol and one to four behavioural scale to be used in the hyperflexion test.

Equid (*Equus caballus*) **behavioural protocol during an exercise test in different head-neck positions.**

Independent variables	
Horse:	Date:
Sex: Mare / Gelding / Stallion	Time:
Age:	H/N position: 1 / 2 / 4 / 5 / 7
Breed:	Helper/rider
Use:	Researcher:
Comments:	

Entering research area
How does the horse enter the research area?
Possibilities of scoring:

1. The horse enters the area without hesitation and without any 'stress' signs like for example snorting, shying, running or jumping.
2. The horse hesitates for no longer than ten (10) seconds before entering the area without showing any 'stress' signs, or enters the area without hesitation but shows one (1) stress sign for no longer than a third (⅓) of the entering period.
3. The horse needs fair encouragement to enter the area or enters the area without hesitation but shows one (1) or two (2) stress signs during almost the complete entering period.
4. The horse shows violent resistance before entering the area and/or severe stress signs during the complete entering period.

Tacking up
How does the horse react to tacking up?
Possibilities of scoring:

1. The horse stays at calm and shows no conflict behaviour.
2. The horse shows one (1) conflict sign like scraping and/or headshaking for no longer than half (½) of the tacking up period.
3. The horse shows stress/conflict behaviour like: scraping, headshaking, avoidance and/or bite threats during half (½) or more of the tacking up period.
4. The horse shows violent resistance.

Warming up protocol: Warming up on the <u>right hand side</u> in a free head neck position.		
10 min. walk		Comments:
3 min. trot		Comments:
1 min. canter		Comments:

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

1. The horse stays calm and shows no stress behaviour like for example: snorting, shying, jumping running, and stays in the desired gait and velocity.
2. The horse shows one (1) stress sign for no longer than a quarter (¼) of the warming up period or travels in a faster gait or faster velocity for no longer than a quarter (¼) of the period.
3. The horse shows stress behaviour or travels in a faster gait or velocity for more than a quarter (¼) and less than three quarters (¾) of the warming up period.
4. The horse shows stress behaviour or travels in a faster gait or velocity for more than three quarters (¾) of the warming up period.

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

1. The horse stays calm and shows no conflict behaviour 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 or at least within thirty (30) seconds and shows one (1) conflict sign like for example stretching the ropes, scraping and/or moving backwards slowly for no more than a quarter (¼) of the period.
3. The horse shows resistance in bending its head and neck and shows a lot of or severe conflict behaviour like rearing and/or moving backwards fast.
4. The horse shows violent resistance, it is (almost) impossible to apply the desired head-neck position.

Exercise protocol:* Exercising on the <u>left hand</u> side in the desired head-neck position.		
Position: 1 / 2 / 4 / 5 / 7		
10 min. trot		Comments:
4 min. canter		Comments:
5 min. trot		Comments:
5 min. walk		Comments:

* For scoring possibilities see ethogram.

Detaching head-neck position
How does the horse react to detaching of the ropes so it can move its head and neck freely again?
Possibilities of scoring:

1. The horse does not or hardly reacts at all
2. The horse shakes its head no more than four (4) times
3. The horse shows little compensation behaviour 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.

<u>➔ Time from detaching head-neck position until start cooling down protocol has to be exactly **two (2) minutes**!!!</u>

Cooling down protocol: Cooling down on the right hand side in a free head neck position.		
5 min. walk		Comments:

Cooling down
How does the horse behave during the cooling down period in which the animal is worked in trot and walk in a free head-neck position?
Possibilities of scoring:

1. The horse shows no conflict or stress behaviour and exercises calmly with its head and neck in a low and long position.
2. The horse shows one (1) or two (2) conflict and/or stress behaviours like head shaking and/or exercising with its head and neck held in a high(er) position.
3. The horse shows conflict and/or stress behaviour during more than half (½) 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.
4. The horse shows stress behaviour 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.

Appendix III. Helper/rider questionnaire for the hyperflexion test.

For the subjective measurement of the horse's behaviour and its (possibly altered) 'workability' in the different head/neck positions there is a short questionnaire which the helper or rider needs to fill out at the end of the test. It is important that the helper/rider will work the particular horse during all tests, so he or she can get a good impression of the subtle differences in behaviour during the different positions.
To gain a better vision about the normal training of the horse at home a question is added in which is asked if the particular head/neck position is used in the day-to-day training of the horse.

An example of this questionnaire in English is provided below.

Dear helper / rider,

For the subjective measurement of the "workability" and stress of the horse we would like to have your opinion about the behaviour of the horse during the workload in the asked head-neck position. The questions have a special relation to the degree in which the horse was showing signs of stress or tension.

Horse	Date:
Sex: Mare / Gelding / Stallion	Time:
Age:	H/N position: 1 / 2 / 4 / 5 / 7
Breed:	Helper/rider:
Use:	Researcher:

Is this particular Head/neck position applied to this horse during daily training?
1. Yes, I use this position on this horse for three (3) or more days a week.
2. Yes, I use this position on this horse approximately one (1) or two (2) day(s) a week.
3. Sporadically, I use this position on this horse only a few times a month.
4. Hardly ever or never at all.
5. This position is only applied to this horse on competition days.
6. Other, namely:…………………………………………………………………………….

How could you describe the behaviour of the horse during the workload in the desired head-neck position?
1. The horse was very relaxed and reasonably at ease.
2. The horse was attentive but showed no or little signs of stress.
3. The horse was tense but the tension stayed under control.
4. The horse was very tense and showed a lot of signs of stress.

How could you describe the workability of the horse during the workload in the desired head-neck position?
1. The horse was very attentive and responded to small aids.
2. The horse was reasonably attentive and responded to small or medium sized aids and responded after the first or second time the aid was given.
3. The horse was reasonably tense and responded exaggerated or hardly at all to the aids.
4. The horse was very tense and did not respond to the aids at all.

Did the horse react, behave, move etc. any different than it usually does? If the answer is yes, please explain what was different and if possible why.
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Other comments:……………………………………………………………………………….
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Thank you for filling in this questionnaire.

Appendix IV. Exact protocol of plasma and saliva cortisol samples in the hyperflexion test.

Plasma cortisol samples:

Sample time	**Exact time**	**Code**	**Type**	**Comments**
1: 0800 h			EDTA	
1: 0800 h			Heparin	
2: Prior exercise			EDTA	
2: Prior exercise			Heparin	
3: Straight after exercise			EDTA	
3: Straight after exercise			Heparin	
4: 30 min. post exercise			EDTA	
4: 30 min. post exercise			Heparin	
5: 60 min. post exercise			EDTA	
5: 60 min. post exercise			Heparin	
6: 2000 h			EDTA	
6: 2000 h			Heparin	
7: 0800 h			EDTA	
7: 0800 h			Heparin	

Salivary cortisol samples:

Sample time	**Exact time**	**Code**	**Time sampling (s)**	**Comments**
1: 0800 h				
2: Prior exercise				
3: Straight after exercise				
4: 30 min. post exercise				
5: 60 min. post exercise				
6: 2000 h				
7: 0800 h				

Time sampling: is the time in seconds in which the bit with Salivette is present in the mouth.

Appendix V. Exercise protocol during "stress-test" HNP-test

Independent variables	
Horse:	Date:
Sex: Mare / Gelding / Stallion	Time:
Age:	H/N position: 1 / 2 / 4 / 5 / 7
Breed:	Helper/rider
Use:	Researcher:
Comments:	

Heart rate monitor: **Exact time on:** **Exact time off:**

Cortisol: samples blood/saliva taken at: 08:00, after entering research area, after exercise protocol, 30 min. after exercise protocol, 60 min. after exercise protocol, 20:00, 08:00 the next day

	Exact time:	**Code:**		**Exact time:**	**Time of taking sample (s):**	**Code:**
Plasma 1			**Saliva 1**			
Plasma 2			**Saliva 2**			
Plasma 3			**Saliva 3**			
Plasma 4			**Saliva 4**			
Plasma 5			**Saliva 5**			
Plasma 6			**Saliva 6**			
Plasma 7			**Saliva 7**			

Entering research area: **1 2 3 4**
Tacking up: **1 2 3 4**

Warming up protocol: Warming up on the <u>right hand side</u> in a free head neck position.		
10 min. walk		Comments:
5 min. trot		Comments:
2 min. canter		Comments:

Warming up: **1 2 3 4**
Applying head-neck position: **1 2 3 4**

Exercise protocol:* Exercising on the <u>left hand</u> side in the desired head-neck position.		
Position: 1 / 2 / 4 / 5 / 7		
1 min. trot		Comments: transition period: horse can get used to position
5 min. walk		Comments:
5 min. trot		Comments:
5 min. canter		Comments:

Detaching head-neck position: **1 2 3 4**

➔ <u>Time from detaching head-neck position until start cooling down protocol has to be exactly **2 minutes**!!!</u>

Cooling down protocol: Cooling down on the <u>right hand side</u> in a free head neck position.		
2 min. detaching		Comments:
1 min. trot		Comments:
10 min. walk		Comments:

Cooling down: **1 2 3 4**