Assessment of mental stress in warmblood horses: heart rate variability in comparison to heart rate and selected behavioural parameters

T.R. Rietmann, A.E.A. Stuart, P. Bernasconi, M. Stauffacher, J.A. Auer, M.A. Weishaupt

Equine Hospital, Faculty of Veterinary Medicine, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland
Via Retica 26, CH-7053 Samedan, Switzerland
Physiology and Animal Husbandry, Institute of Animal Sciences, Swiss Federal Institute of Technology (ETH), Zurich CH-8092, Switzerland

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Abstract

The aim of the study was to investigate whether heart rate variability (HRV) could assess alterations of the autonomic nervous system (ANS) at different levels of excitement. The behavioural and physiological responses of 20 warmblood horses to a challenging ground exercise task were studied. Prior to the experiment, the horses were evaluated at rest and during forward walking (FW). The horses were then forced to move backwards continuously during 3 min according to a standardised protocol (BW1). Subsequently, the horses were exposed to two training sessions, after which the backward walking (BW2) was re-evaluated. Heart rate (HR) and HRV-parameters such as the standard deviation of the beat-to-beat intervals (SDRR), the low (LF; sympathetic tone) and high frequency (HF) component of HRV (HF; parasympathetic tone) and their ratio (LF/HF; index representing the sympa-tho-vagal balance) were sampled at rest, and during FW, BW1 and BW2. Stress-related behaviour during BW1 and BW2 was determined from video recordings. The results of the different evaluations were compared to each other.

Compared to rest and FW, the first backward experiment induced a significant rise in HR, LF and LF/HF and a significant decrease of HF. SDRR decreased from both FW and rest with only the latter reaching significance. In BW2 after the training sessions, HR and the parameters of the sympathetic branch of the ANS (LF, LF/HF) were decreased and the vagal tone (HF) increased compared to BW1; all changes were significant. The duration of stress indicating behavioural patterns revealed...
also a significant decrease of excitement after the training, when backward walking did not differ from forward walking in any parameter. Correlations between HRV-parameters and stress indicating behaviour as well as HR were found.

We conclude that the HRV-parameters LF and HF are valuable measures to quantify sympatho-vagal balance, which allows a more precise assessment of the responses of HR and SDRR to mental stress during low intensity exercise.

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1. Introduction

Animal welfare and stress-related disorders are being recognised more and more by horse owners and breeders. The lack of knowledge on the psychology of the horse is often coupled to stress-related disorders (Miller, 2001). Deficiencies may be found regarding housing, feeding, training, including preparation for trailer loading and hauling, veterinary and farriery procedures, which compromise thereby the performance capacity and health of horses.

Currently, the assessment of stress responses is difficult, as different physiological systems are involved (e.g. neuroendocrine and cardiovascular), which are manifested in the somatomotor (behavioural) stress response (Herd, 1991). The specificity of a single parameter for a certain stimulus is poor. Because of the need of recording different parameters simultaneously, new and easily applicable methods are necessary to increase stimulus specificity and obtain more knowledge on stress responses in the horse.

Generally, stress may be defined as a threat, real or implied, to the psychological or physiological integrity of an individual (McEwen, 1999). The stressor disturbs homeostasis and the body’s stress response tries to re-establish the internal balance (Seyle, 1936) in the so-called coping intent. Thus, the stress response is adaptive, a “normal feature of life” (Seyle, 1936), and necessary for learning and developing effective behaviour (Dawkins, 1980; Levine, 1985; Committee on Pain and Distress in Laboratory Animals, 1992). Facing the potential damage of stress, Keeling and Jensen (2002) pointed out that whether a stressor has adverse effects or not does not depend so much on the physical characteristics of the stressor (intensity, duration, frequency, etc.), but whether or not the animal can predict and control the stressor. The control is normally exerted by means of performing a (coping) behaviour, which is relevant for the stimulus. Thus, behavioural problems and stress-related disorders may be found when successful coping is not possible (Stauffacher, 1992). The standard model of stress, developed in the middle of the 20th century, postulates the activation of the two neuroendocrinological axes: the sympatho–adrenomedullary axis and the hypothalamo–pituitary–adrenocortical axis. Long-term activation of the sympathetic nervous system as well as chronically elevated or depressed adrenocortical functions were found to be harmful to the organism (Keeling and Jensen, 2002). Short time activation of the sympatho–adrenomedullary axis is widely used for monitoring acute stress responses, but the impact of the parasympathetic part of the autonomic nervous system (ANS) in modulating the stress response of both acute and chronic stress was largely neglected. Porges (1995)
postulated that a reduced activity of the vagus, one of the efferent parasympathetic nerves, would limit physiological and behavioural capacity to cope with stressful events. Therefore, methods measuring the vagal tone would provide more knowledge of both individual stress vulnerability and the magnitude of an actual stress response (Porges, 1995). Heart rate (HR) is the net effect of the vagus that slows it down thereby increasing the variability between the consecutive beats and the sympathetic nerves that accelerate it by producing a more metronome-like heart beating. Hence the variability of consecutive heart beats (heart rate variability; HRV), measured non-invasively using an electrocardiogram (ECG), would quantify the vagal as well as sympathetic activity.

In this study, we present a method of HRV-analysis described in humans (Bernasconi et al., 1998). An autoregressive (AR) model of power spectral analysis (PSA) is used which estimates the spectral distribution of intervals between biological signals, as e.g. heart beats (Bernasconi et al., 1998). The power spectrum of the horse resembles that derived from humans, cats and dogs (Kuwahara et al., 1996; Thayer et al., 1997a) showing two major peaks; one is mainly attributable to the sympathetic activity (low frequency component, LF) and the other reflects the vagal activity (high frequency component, HF) (Akselrod et al., 1981; Pagani et al., 1986; Bernasconi et al., 1998). Thus, the ratio LF/HF is an indicator of the sympatho-vagal balance (Pagani et al., 1986; Bernasconi et al., 1998).

In horses, HRV was used to characterise different temperamental traits in young individuals (Visser et al., 2002). Clement and Barrey (1995b) and Thayer et al. (1997b) described a reduced HRV for individuals with higher emotionality, young horses, mares and a hot-blooded breed. During exercise on a treadmill HRV tended to decrease (Thayer et al., 1997a; Physick-Sheard et al., 2000; Voss et al., 2002). A study which compared crib-biting horses to controls, indicated that stereotyping individuals might be more stress sensible because of their lower basal parasympathetic and higher sympathetic activity, though HR did not differ between the groups (Bachmann et al., 2003). In humans, HRV is currently used to assess shifts of the parasympathetic and sympathetic cardiac control of individuals predisposed to or suffering from different diseases associated with psychological disorders and stress (Rechlin et al., 1994; Sloan et al., 1994; McCraty et al., 1995; Thayer et al., 1996; Sloan et al., 1997; Bernasconi et al., 1998; Sato et al., 1998; Bernardi et al., 2000; Hanson et al., 2001; Papousek et al., 2002). Children showed a reduced HRV when they could not perform a successful coping response facing psychological challenges (Fabies et al., 1993). Adults suffered from symptoms of stress-related diseases with reduced HRV when living in an imbalance of effort and reward (Hanson et al., 2001).

To our knowledge, there is no study evaluating the response of the ANS by means of HRV during sustained mental distress and elucidating its disappearance by providing the horse predictability and control over the stressor. The aim of this study was to test the sensitivity of the autoregressive model of power spectral analysis of HRV to detect mental stress and to investigate the correlation of LF and HF with behavioural patterns indicative for stress and with other HRV-parameters, such as mean heart rate. Therefore, in a first part of the study HRV-parameters from a first group of adult warmblood horses were recorded at rest to verify the settings of the software, programmed for the use in humans. In the second part, another group of horses underwent recordings at rest and during different low intensity ground exercises: They contained the mental stress test which was repeated after the horses were trained twice following a specific training system, where the animals were proposed.
to learn the predictability of the aids and how to maintain control during the challenge. We hypothesise that the stress response would be reduced in the repetition of the test.

2. Materials and methods

2.1. Animals and housing

2.1.1. Group A

Eighteen physically sound warmblood horses were used to establish the basal HRV data at rest and to verify the threshold settings for HF and LF bands. The 11 geldings and 7 mares varied in age between 6 and 22 years (mean ± S.D.: 12.39 ± 4.58). All of them were used for recreational or low-grade competitive purposes. All horses were housed under comparable conditions in individual box stalls, where the measurements took place between October and December 2001.

2.1.2. Group B

To raise the validity of the data, factors that possibly would influence the results, such as breed, age, housing conditions, and amount of exercise, were standardised. Thirty eight Swiss warmblood horses destined for the study underwent a thorough physical examination by the same veterinarian with special emphasis on the cardiovascular system. Eighteen horses had to be excluded because of intermittent (physiological) atrio-ventricular (AV) blocks. The animals included in the study were nine mares, aged from 5 to 13 years (mean ± S.D.: 10.33 ± 2.15) and 11 geldings, aged from 6 to 14 years (mean ± S.D.: 10.18 ± 2.52). All horses were owned by the Swiss National Equestrian Centre, Berne, Switzerland.

During the experiments, the horses were used by the National Equestrian Centre in their normal daily work (riding school and training for dressage, show jumping and cavalry-mounted-band). They were not ridden more than once a day. On non-riding days they were exercised on a horse-walker for an hour. All horses were broken and started training at the age of 3 years. The horses were housed in conventional single straw-bedded boxes or kept in stands and were not allowed to pasture or paddocks. The feeding consisted of hay and concentrates three times daily. The investigations were conducted at the Swiss National Equestrian Centre. For the experiment, the horses were worked in groups during 1 month, with the last group’s final evaluation taking place in mid May of 2002.

2.2. Experimental design

2.2.1. Group A

The horses were tied in their boxes between 11:00 and 13:00 at least 30 min after feeding was finished. Subsequently, the two electrodes of the Polar RR Recorder© system used (Polar Electro Q, Kempele, Fl) were placed on wet skin to the left lateral thorax wall and fixed with a stable girth. The electrodes were directly connected to the RR Recorder© which was attached onto the girth. After giving the horse 10 min to get accustomed to the equipment, the electrocardiogram was started. ECG recordings were performed continuously over 20 min.
During the ECG recording session, the respiratory frequency was counted five times during a full minute by a person from outside the box. The mean respiratory frequencies were used to determine the baseline settings of the PSA used in this study as suggested by Bernasconi (personal communication) and explained by Porges (1995) and Sahar et al. (2001).

2.2.2. Group B

During the experimental period, lasting three consecutive days, the horses received their daily routine training, and no changes in the management were provided. The standardised test protocol is described in detail in Fig. 1. On day 1 and 3 the horses were not ridden in the morning because of the experimental procedure.

Each horse was recorded at rest on day 1 between 08:00 and 10:00 when tied at the familiar grooming place. The ECG was recorded continuously over 20 min as described for group A.

Recording of the forward walking (FW) and the backward walking tests (BW1 and BW2) always took place between 10:00 and 12:00 to minimise the effects of the diurnal variation of heart rate variability (Kuwahara et al., 1999).

For data collection of FW, the horse was brought to the familiar arena on a rope halter and a lead rope. In a fenced area (22 × 15 m) within the arena (22 × 90 m) the horse was led forward by the handler in a calm and regular pace during 5 min.

Following the FW period, the handler introduced the horse to the standardised aids used to obtain the backward movement of the first test (BW1). The horse was positioned face to face with the handler. Subsequently, the handler started the aids, first by mimicking a tall posture, looking straight forward at the horse and walking towards the horse. When the horse responded by placing a leg back, the stimulating aids were immediately stopped and the horse was rewarded by stroking. If it did not respond, the intensity of the aids was increased. The lead rope was wiggled up and down, then a thick whip was used to hit the rope and finally to touch the horse’s breast or the legs smoothly and increasingly until the horse initiated the backward movement. This introductory procedure was
repeated three times. BW1 started as soon as the horse was again standing calm facing the handler.

To record the behaviour of the horse, a video camera (Panasonic S–VHS) was used, fixed on a tripod standing outside the fence. The criterion of BW1 was a continuous and rhythmic backward movement during 3 min. Every attempt of the horse to interrupt the backing-up movement was anticipated by the handler by increasing the intensity of the aids. Moreover, every movement away from the proposed direction along the fence of the experimental area was corrected.

On day 3, the backing-up test procedure was repeated (BW2) as soon as the horse paid attention to the handler. The horses had to complete exactly the same procedure as in BW1. The handler used the same aids as on day 1. The handler, the person operating the video camera, the equipment and the surroundings (stalls, arena) stayed unchanged during the 3 days.

After BW1 on each day 1 and day 2, a training session of 30–60 min was conducted. It consisted of playing the seven games of PNH® (Parelli Natural Horsemanship, Pagosa Springs CO, USA). The PNH®-training is a thoroughly explained system based on species-specific communication (Miller, 2001). In our opinion, it is highly suited for teaching predictability of handling and riding aids and ensuring successful coping responses for the horse. Throughout this training the horses were not forced to move more than 5–10 strides backwards, and per session the backing-up was only demanded a maximum of four times. The aim of these training sessions was to eliminate fear and create acceptance by desensitisation with the entire equipment and the handler’s hands (e.g. Miller, 1993; Parelli, 1993; Roberts, 2002). Thereafter, several manoeuvres of moving the horse in various directions and stopping it served to establish leadership following the hypothesis of the above mentioned authors, that a leading horse is able to direct the movement of the others. During the two sessions all the demanded manoeuvres had to become predictable for the horse and whenever it responded adequately to the aids it was immediately rewarded (Parelli, 1993, 2000).

2.3. Data analysis

2.3.1. Heart rate and heart rate variability

Heart rate and heart rate variability were analysed using ProBeat® (ProBeat, Bellinzona, CH) and ProFit® (Quantum Soft, Zurich, CH) software. ECG data recorded at rest were analysed over 256 RR intervals (=beats). In the FW recordings 130±39 beats were analysed, in BW1 165±50 beats and in BW2 263±52 beats (mean ± S.D.). LF and HF components were calculated by a non-parametric autoregressive model of PSA (Fig. 2) and expressed in normalised units (n.u.). LF n.u. and HF n.u. represent the relative value of each power component in proportion to the total power of the spectrum. The changes of total power are minimised, allowing the comparison of the LF and HF results even with changing total power (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Since the total power of the HRV-spectrum is equal to the variance of the signal (RR intervals) and the square root of variance is the standard deviation (S.D.) of the analysed intervals, the SDRR (SD of RR intervals) is an often used parameter for quantifying HRV (Task Force of the European Society of Cardiology and
Fig. 2. Examples of the power spectral analysis and spectral components determined for backward walking 1 (BW1) (above) and backward walking 2 (BW2) (below).

the North American Society of Pacing and Electrophysiology, 1996), though lacking the advantages of the PSA. The analysed ECG segments of BW1 and BW2 were time-matched with the 3 min of video analysis of the behaviour. The threshold between LF and HF was verified with

\[ Hz_{\text{threshold}} = 5\% \text{ confidence interval of mean } \left( \frac{RF}{60} \right) \]

where Hz-threshold represents the lower limit of the HF range and RF the respiratory frequency.
Table 1
Definition of behavioural patterns

<table>
<thead>
<tr>
<th>Behavioural parameter</th>
<th>Pattern</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head position during movement</td>
<td>Head low</td>
<td>Nose between the abdominal line and carpus</td>
</tr>
<tr>
<td></td>
<td>Head normal</td>
<td>Nose between the withers and abdominal line</td>
</tr>
<tr>
<td></td>
<td>Head high</td>
<td>Nose above the withers</td>
</tr>
<tr>
<td>Movement ^a</td>
<td>Straight backward on the track</td>
<td>Handler has the control over the movement of the horse (in direction as well as velocity)</td>
</tr>
<tr>
<td></td>
<td>Stop</td>
<td>Horse discontinues the backward movement, stops with all four feet on the ground</td>
</tr>
<tr>
<td></td>
<td>Deviation equal pace</td>
<td>Horse leaves the track by turning away in the same pace as straight backward</td>
</tr>
<tr>
<td></td>
<td>Deviation explosive</td>
<td>Horse turns quickly on the haunches. Horse rears Horse steps forward into the handler’s personal space Horse at the trot</td>
</tr>
<tr>
<td>Tail swishing ^b</td>
<td></td>
<td>Tail movement to the left or right.</td>
</tr>
<tr>
<td>Defecation ^b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Indicative for stress were: head high, deviation explosive, deviation equal pace, stop, tail swishing and defecation.

^a In duration.

^b As frequency.

2.3.2. Behaviour

Behaviour was analysed in BW1 and BW2 continuously during the 3 min of the test, using Behaviour 3.6 (Hammerschmidt et al., Scientific Software Products, Berlin) and Microsoft Excel 97 (Microsoft, Redmont, Washington, USA). Table 1 lists the definitions of the different behavioural patterns.

We assumed that the head position in movement would reflect the horse’s emotionality; the lower the head, the calmer the horse. As the head rises and musculature becomes tense, the sympathetic ANS gains influence (Parelli, 2000; Roberts, 2002), peaking in a panicky flight (Schäfer, 1993; Budd, 1996; Wolff et al., 1997). With respect to the movements we suggest that in this set-up the willing co-operation with the handler is in opposition with either a fight or flight response. Apparent escape tendencies or disrespect as manifested in rearing or stepping in the handler’s personal space, represent different degrees of flight (Visser et al., 2001) and fight behaviour, respectively. Tail swishing, especially when there are no insects present, can express uncertainty and discontent that may erupt as fear or aggression (Huff, 1988; Budd, 1996; Grandin, 1997; Roberts, 2002). Defecation is typically observed together with tenseness and fear (Schäfer, 1978). The two classical behavioural parameters, eye-expression and ear position, could not be analysed because of invisibility on the video recording (eyes) and multiple meanings (ears).

The behavioural patterns representing the different degrees of flight and fight were correlated to the HRV-parameters to reveal the activation of the sympathetic nervous system (Goldstein, 1987). Each variable was analysed twice by the same person to avoid discrepancies. To compare the physical activity of BW1 and BW2, strides of the backward movement...
during the 3 min challenge were counted for each horse. A stride was defined as an entire limb cycle, i.e. a specific limb takes up the same position in relation to the other limbs as it had at the beginning of the movement cycle (Muybridge, 1957).

2.4. Statistical analysis

Statistical analyses were carried out in SigmaStat 2.03® (SPSS Science, Chicago, USA). Descriptive statistics (mean, S.D.) were computed for each parameter. Differences between the two groups at rest were tested using a t-test (for HR, SDRR, LF and HF) or a Wilcoxon Rank Sum test (for LF/HF). Differences of HR and HRV-parameters between rest, FW, BW1 and BW2 were tested for significance with a one way repeated measure ANOVA (for HR, SDRR, LF and HF) followed by a Bonferroni t-test or a Friedman repeated measure ANOVA (for LF/HF) followed by a Dunn’s test. Differences of the behavioural parameters between the two challenges were tested with a paired t-test (for ‘Head High’, ‘Deviat. Explosive’, ‘Stop’, ‘Straight Backward’ and ‘No. of Strides’ or a Wilcoxon Signed Rank test (for ‘Deviat. Equal Pace’ and ‘Tail swishing’). Correlations between HRV and behavioural parameters were analysed using a Spearman (rank) correlation matrix; the data sets of BW1 and BW2 were therefore pooled. Data sets were tested for normality distribution using a Kolmogorov–Smirnov test. If not indicated otherwise, significance level was set at P < 0.05.

3. Results

3.1. Determination of the LF- and HF-thresholds of PSA of HRV

The respiratory frequencies of the group A horses ranged between 7.2 and 26.4 l/min (mean ± S.D.: 14.04 ± 4.37). The 5% confidence interval was found to be at nine breaths per minute. The lower limit of the HF (LF–HF threshold) was set at 0.15 Hz Therefore, all analyses were carried out with the threshold set at 0.05–0.15 Hz for LF and 0.15–0.5 for HF.

3.2. Heart rate and HRV at rest

Of group B, two horses were excluded from the rest analysis because of intermittent AV-blocks. Mean and S.D. of HR and HRV-parameters at rest are summarised in Table 2. The comparison of HRV-parameters (LF, HF, LF/HF, SDRR) between the two groups revealed no significant difference. Mean heart rates of the two groups differed by two beats per minute (bpm); however this difference was significant. Geldings did not differ in any parameter from mares.

3.3. HR and HRV during the stress experiment

One horse had to be excluded from the FW analysis because of intermittent AV-blocks. Means and S.D. of HR and HRV-parameters of FW, BW1 and BW2 are summarised in Table 2.
Table 2
Heart rate and heart rate variability as determined in groups A and B

<table>
<thead>
<tr>
<th></th>
<th>Rest group A (n = 18)</th>
<th>Rest group B (n = 18)</th>
<th>Forwards B (n = 19)</th>
<th>BW1 group B (n = 20)</th>
<th>BW2 group B (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>33 (3.33)</td>
<td>35 (2.67)</td>
<td>57 (8.46)</td>
<td>88 (22.00)</td>
<td>61 (9.00)</td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>111 (50.55)</td>
<td>132 (30.15)</td>
<td>63 (21.18)</td>
<td>51 (21.41)</td>
<td>62 (21.91)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.70 (1.69)</td>
<td>1.49 (1.11)</td>
<td>2.12 (2.37)</td>
<td>26.83 (21.92)</td>
<td>5.71 (7.77)</td>
</tr>
<tr>
<td>LF (n.u.)</td>
<td>53.3 (19.5)</td>
<td>54.9 (17.1)</td>
<td>51.7 (21.9)</td>
<td>87.4 (11.9)</td>
<td>58.3 (21.8)</td>
</tr>
<tr>
<td>HF (n.u.)</td>
<td>46.8 (19.5)</td>
<td>50.6 (18.8)</td>
<td>40.7 (20.7)</td>
<td>6.5 (5.4)</td>
<td>31.9 (21.8)</td>
</tr>
</tbody>
</table>

n.u.: Normalised units; SDRR is index for general beat to beat variability, LF/HF is the sympatho-vagal balance, LF is index for sympathetic modulation and HF for vagal modulation of the heart rate (means ± S.D.).

a Significant differences (P < 0.05) between: rest A and rest B.

b Rest B and forwards B.

c Forwards B and BW1 B.

d BW1 B and BW2 B.

Between rest and FW no difference was found for LF, HF and LF/HF, whereas changes in heart rate and SDRR were significant.

Between FW and BW1 all parameters except SDRR showed significant differences (Table 2).

When comparing BW2 to BW1 marked changes occurred in all parameters. HR, LF and LF/HF decreased significantly and HF increased significantly. Though SDRR increased from BW1 to BW2, this difference was statistically not significant.

Comparing BW2 mean values with those of FW no significant differences were found in any of the parameters.

3.4. Behaviour during the stress experiment

During BW1 the pattern head high was expressed by all 20 horses during 58% of the total 3 min. During BW2, 13 of the 20 horses showed at one point a head high position lasting 6% of the total 3 min in the average of all horses. Fifteen of the twenty horses showed explosive movement and all of them left the track in equal pace, the patterns lasting 5.2% and 12.5% respectively in BW1. These movement patterns during BW2 decreased to 0.5% for explosive movement and 1.2% of total time for equal pace. They were exhibited by 7 and 11 of the 20 horses, respectively. Head normal increased during BW2 as did the sum of straight backward strides. Table 3 gives the absolute mean values and S.D. of the behavioural parameters and the statistical significance between BW1 and BW2. All parameters except the reduction in tail swishing did reach significance. The pattern ‘Defecation’ was not analysed statistically because of the small number of observations; in only three horses a single observation during BW1 was made. The sum of backward strides during the 3 min of the test, representing the intensity of raw physical activity, were significantly higher in BW2 (93.38 ± 14.08 steps) than in BW1 (85.60 ± 17.91 steps).
Table 3

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>BW1 group B (n = 20)</th>
<th>BW2 group B (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head high (s)</td>
<td>104.80 (60.15)</td>
<td>11.35 (15.49)*</td>
</tr>
<tr>
<td>Head normal (s)</td>
<td>74.20 (60.15)</td>
<td>167.05 (15.51)*</td>
</tr>
<tr>
<td>Straight backward (s)</td>
<td>143.80 (23.49)</td>
<td>175.95 (3.33)*</td>
</tr>
<tr>
<td>Deviation explosive (s)</td>
<td>9.40 (11.91)</td>
<td>0.85 (1.42)*</td>
</tr>
<tr>
<td>Deviation equal pace (s)</td>
<td>22.55 (17.89)</td>
<td>2.20 (2.73)*</td>
</tr>
<tr>
<td>Stop (1/180s)</td>
<td>5.15 (4.11)</td>
<td>0.55 (1.23)*</td>
</tr>
<tr>
<td>Tail swishing (1/180s)</td>
<td>5.40 (10.10)</td>
<td>2.00 (5.34)</td>
</tr>
</tbody>
</table>

* Significant differences (P < 0.05) between: BW1 and BW2.

Table 4

<table>
<thead>
<tr>
<th>HRV parameter</th>
<th>HR</th>
<th>Head high</th>
<th>Deviation explosive</th>
<th>Deviation equal</th>
<th>Stops</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.79</td>
<td>0.65</td>
<td>0.71</td>
<td>0.59</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>SDRR</td>
<td>−0.51</td>
<td>−0.36</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>LF/HF</td>
<td>0.68</td>
<td>0.65</td>
<td>0.38</td>
<td>0.53</td>
<td>0.43</td>
<td>n.s.</td>
</tr>
<tr>
<td>LF</td>
<td>0.63</td>
<td>0.90</td>
<td>0.32</td>
<td>0.50</td>
<td>0.43</td>
<td>n.s.</td>
</tr>
<tr>
<td>HF</td>
<td>−0.46</td>
<td>−0.63</td>
<td>−0.48</td>
<td>−0.60</td>
<td>−0.49</td>
<td>−0.35</td>
</tr>
</tbody>
</table>

P < 0.05, n.s.: no significance.

3.5. Correlation of HRV-parameters with HR and behavioural patterns

Meaningful correlations were found between HRV-parameters, HR and selected behavioural parameters (Table 4). Tail swishing corresponded poorly with physiologic parameters.

4. Discussion

Compared to rest and FW, BW1 induced a significant stress response as depicted in all parameters, except for SDRR. In BW2, after the training sessions, the indicators of the ANS as well as HR decreased to the FW level. The duration of stress indicating behavioural patterns revealed also a significant decrease of excitement after the training.

4.1. Methodological aspects

The analysis of HRV is complex and analytical errors may produce misleading data (Perkins et al., 2000). Especially, the different analytic procedures used to analyse HRV may produce results, which are not comparable in terms of absolute values. Previous analyses of HRV in horses have used time domain analysis (Perkins et al., 2000) or frequency domain analysis based on a fast fourier transformation (FFT) of the RR intervals (Clement and
Barrey, 1995a,b; Kuwahara et al., 1996, 1999; Thayer et al., 1997a,b; Physick-Sheard et al., 2000; Ohmura et al., 2001). However, compared to the FFT, the autoregressive model for calculating the power spectrum presents many advantages. Using FFT implicitly assumes that data are cyclic, which is not strictly true for heart rate fluctuations. This difficulty can be bypassed by the use of the AR model. Further advantages are a more consistent and smoother spectral estimation, the possibility of avoiding windowing procedures and, most importantly, a spectral resolution that is independent on the number of samples, i.e. RR intervals (Bernasconi et al., 1998). Also in our study a low and a high frequency component could be observed as previous described for horses (Kuwahara et al., 1996). In contrast to other studies using fast fourier transforms (Kuwahara et al., 1996) we defined the HF range from 0.15 to 0.5 Hz of the frequency spectrum. Probably, this can be attributed to the different respiratory range found in our study (six breaths per minute in the study of Kuwahara et al. (1996) compared to nine breaths per minute), even though the difference might also be due to the different method of defining the HF range used. Nonetheless, calculating the vagal contribution to the heart, and hence the HF range upon the respiration is an appropriate approach (Porges, 1995; Sahar et al., 2001). Comparing the pharmacological vagal block used by Kuwahara et al. (1996) to our approach remains subject to further research.

The equipment was adequate for the purpose of this study. ECG recordings could be obtained from all horses and, unless artefacts were frequent, isolated peaks were corrected manually. Artefacts were mainly attributed to muscle twitching caused by insects. In this study a serious limitation for the HRV-analysis were the AV-blocks. They were observed in a third of the horses at rest. This supports the findings of Holmes, who also found approximately one third of the horses with arrhythmias (Evans, 1994).

4.2. Rest measurements

Between the two groups of warmblood horses, no statistical difference in HRV-parameters was found at rest. However, considerable standard deviations in all HRV-parameters were observed. The individual temperament and reactivity to environmental changes may be responsible for the different individual modulation of the ANS (Visser et al., 2001), which can be detected by the sensitivity of the HRV method.

Statistically, the resting heart rates differed between the two groups. However, the absolute mean values and their small standard deviations were barely dissimilar and corresponded to normal values reported for sound horses at rest (25–40 bpm, Evans, 1994).

In contrast to other authors (Clement and Barrey, 1995b) gender related differences in HRV-parameters were not observed in this study. In humans, greater vagal cardiac influences could be found in women, even though the HR did not differ between the two sexes (Rossy and Thayer, 1998).

4.3. The response of the ANS and behaviour during FW, BW1 and BW2

Based on HR data, FW is classified as a very low exercise intensity (Lekeux and Art, 1994; Scheffer and Sloet van Oldruitenborgh-Oosterbaan, 1996). Interestingly, the heart rates of BW2 did not differ in mean and standard deviation from FW, indicating a similar sum of physical and psychogenic influences. This seems surprising, since backward walking
is generally believed to be more strenuous, which might indicate a low or absent mental stress load. In comparison to FW and BW2, the HR of BW1 was significantly higher although the covered distance estimated from the number of strides was even significantly shorter than in BW2. A possible reason for increased sympathetic activity during BW1 could have been physical activity as a result of jumping or rearing, but this behaviour was executed in only 5% of the total experimental time. Thus, the higher HR together with the large SD of HR during BW1 might indicate that considerable mental stress was present. During BW1 mental stress was likely to arise because of the novelty of the exercise (horses were not trained to move backward many steps before this experiment) paired with a hierarchical confrontation (backwards moving on the handler’s or other horse’s demand implicates submission). Moreover, the horses were urged to keep the continuity of the backward movement over several minutes and were not rewarded during the test. Hence, the horses were given little opportunity to calmly learn the predictability of the aids and the controllability of the situation.

This hypothesis could be further supported by the HRV results: The sympathetic branch of the ANS was activated, as presented by an increased LF component. The vagal tone was reduced as expressed in a decrease of the HF component and consequently, the sympatho-vagal balance (LF/HF) was significantly elevated, reflecting the alteration of the ANS from rest to a highly stressed state. During BW2, not only did HR reach a lower level, comparable to the FW, but also the LF component indicated that the sympathetic drive to the heart was reduced. The increase of SDRR from BW1 to BW2 was only moderate in contrast to the increase from FW to rest. This may be an indication of a reduced sensitivity of the SDRR-parameter for higher mental stress loads. The response of all measured HRV-parameters indicates clearly that influences other than exercise are powerful activators of body functions. These results support the finding of Persson (1983) who described a non-linearity between workload and HR because of psychogenic factors influencing the HR below 120 bpm. He stated that exercise heart rate is affected by apprehension and anxiety, and Voss et al. (2002) could confirm that the psychogenic component of the heart rate response to exercise is proportionally larger on an aqua treadmill at the trot than at lower relative work loads, e.g. walking.

The HF component showed, as a function of HR, a linear \( R^2 = 0.9564 \) decrease from rest (50.6 n.u.) to FW (40.7 n.u.), BW2 (31.9 n.u.) and BW1 (6.5 n.u.), whereas the LF component only changed distinctly during BW1. This may be the consequence of the predominant vagal tonus at rest (Hamlin et al., 1972; Rugh et al., 1992; Kuwahara et al., 1996; Ohmura et al., 2001), which may be responsible for the primary adjustment to low intensity stress.

The Spearman correlation matrix revealed associations of HRV-parameters with HR and most of the behavioural patterns (Table 4). This may confirm that horses show characteristic behavioural patterns as described in scientific and lay literature when subjected to mental stress as during BW1. The presence of these stress indicating behaviours decreased clearly during BW2. Whether the reduction was mainly due to less novelty during BW2 or whether the reduction was due to increased willingness to calmly co-operate in the backward manoeuvre learnt during the training sessions between the two tests, remains subject to speculation. From our point of view rather the latter hypothesis is applicable to the set-up of the tests. However, the additional recording of another group of 20 horses that would
not have been trained according to the principles of predictability and control could have
clarified this question.

We conclude that PSA of HRV is a suitable method to assess more objectively the stress re-
sponse of horses at low exercise training situations. In addition, we may postulate that train-
ing methods considering the actual knowledge of species-specific communication rather
provide control and safety than uncertainty and fearfulness. As a result, the increased con-
fidence could lead to better performance and reduced distress, thereby increasing welfare.

This study did not address the question, whether dissociation of HR and HRV would be
characteristic for horses suffering from psychological disorders (e.g. hyper-excitability) or
combined psychosomatic disorders (e.g. refusal to riders demands). Sahar et al. (2001) asso-
ciated this phenomenon to a physiologic state with difficulties in behavioural self-regulation.
Another hypothesis, which merits further research is addressed in the recent literature, where
evidence grows that humans and horses with a lower vagal tone at rest may lack autonomic
flexibility when being exposed to stress (Friedman and Thayer, 1998; Bachmann et al.,
2003).

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