Heart rate and heart rate variability during a novel object test and a handling test in young horses

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Abstract

Forty-one Dutch Warmblood immature horses were used in a study to quantify temperamental traits on the basis of heart rate (HR) and heart rate variability (HRV) measures. Half of the horses received additional training from the age of 5 months onwards; the other half did not. Horses were tested at 9, 10, 21 and 22 months of age in a novel object and a handling test. During the tests, mean HR and two heart variability indices, e.g. standard deviation of beat-to-beat intervals (SDRR) and root mean square of successive beat-to-beat differences (rMSSD), were calculated and expressed as response values to baseline measures. In both tests, horses showed at all ages a significant increase in mean HR and decrease in HRV measures, which suggests a marked shift of the balance of the autonomic nervous system towards a sympathetic dominance. In the novel object test, this shift was more pronounced in horses that had not been trained. Furthermore, statistical analysis showed that the increase in mean HR could not be entirely explained by the physical activity. The additional increase in HR, the nonmotor HR, was more pronounced in the untrained horses compared to the trained. Hence, it is suggested that this nonmotor HR might be due to the level of emotionality. HR variables showed consistency between years, as well as within the second year. These tests bring about a HR response in horses, part of which may indicate a higher level of emotionality; and horses show individual consistency of these HR variables over ages. Therefore, it is concluded that mean HR and HRV measures used with these tests quantify certain aspects of a horse’s temperament. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Heart rate; Heart rate variability; Consistency; Behavioral tests; Temperament; Emotionality; Horses

1. Introduction

Knowledge about temperament of horses may have very important implications for the breeding, housing and management of the horse. It may contribute to breeding or selecting the winning athlete in sports or to select the right horse to match with a recreational rider. These applications emphasize the need for quantification of the horse’s temperamental characteristics. Temperament has been defined as the individual’s basic stance towards continuing changes and challenges in its environment [1]. To select the horse with the right temperament for a specific goal, one has to review several aspects underlying the overall temperament. We presume that most of these temperamental aspects might be exhibited in situations where the individual shows emotional responses towards novel stimuli and in handling situations where the animal shows reactivity to the handler.

Over the last decade, a growing number of studies in many different species have focused on individual differences in animals in responses to challenges in behavioral tests (e.g., Refs. [2–7]). These individual differences have been related to different coping strategies or to differences in temperamental traits. The most commonly used variables to quantify temperamental traits are behavioral variables. However, recently, it was shown that heart rate (HR) and heart rate variability (HRV) in rats also are useful to differentiate between individuals [8]. Hence, they seem
relevant additional variables to quantify individual differences in temperamental aspects.

HR represents the net effect of the parasympathetic nerves that slow it down and the sympathetic nerves that accelerate it. In resting conditions, both parts of the autonomic nervous system are thought to be tonically active. Different stressors can induce a shift of the autonomic balance towards either a sympathetic or a parasympathetic dominance [9–12]. A decreased HRV reflects a shift of the autonomic balance towards a more sympathetic dominance [8,13,14]. Several studies on rodents have shown that stressful conditions, depending on their nature, can result in a decrease in HRV and hence low levels of parasympathetic nerve activity [8]. For example, social defeat in rats produces lower HRV measures compared to baseline recordings, whereas other stressors including restraint, swimming and shock-probe test do not [15]. In a human study by Friedman and Thayer [16], it was shown that patients with chronic anxiety (panic disorder) exhibit lower levels of HRV and higher levels of mean HR compared to their controls. The parasympathetic branch of the autonomic nervous system seems to be associated with adaptive responsiveness to the environment [17]. Individuals with a higher parasympathetic activity would be more exploratory and adaptive to environmental demands. In another study, Porges [18] showed that parasympathetic activity is suppressed during autonomic and behavioral responses to stress. Thus, responses of the autonomic nervous system on challenging situations differentiate between individuals. These differences could therefore reflect differences in temperament. It was shown that horses, when confronted with a challenging situation, exhibited individually different behavioral responses, and this was suggested to be linked with differences in temperament [7]. We hypothesize that responses of the autonomic nervous system may also be useful in differentiating between individual horses in terms of temperament.

In the present study, we aimed to investigate whether responses of HR and HRV to challenging situations could differentiate between individual horses’ temperament. It is hypothesized that a confrontation with a novel object causes an emotional state, possibly ‘fear’, which is reflected in an additional increase in mean HR and a decrease in HRV. Since HR (and HRV) is also affected by physical activity [8], physical activity was explicitly considered in the statistical analysis in an attempt to distinguish between variation in HR due to physical activity and variation in nonmotor HR [19]. A second test involving human handling was used to investigate whether individual horses responded differently to situations of challenge with or without the support of a human.

2. Materials and methods

All procedures involving animal handling and testing were approved by the Animal Care and Use Committee of the Institute for Animal Science and Health (ID-Lelystad) in Lelystad, the Netherlands.

2.1. Animals, housing, handling and management

A total of 41 Dutch warmblood horses, 26 colts and 15 fillies were used in this study. Experiments were carried out at the Research Station for Animal Husbandry in Lelystad. Horses were simultaneously used in a project aiming at the development of scientific criteria for the selection and effective and injury-free training of show jumpers. To evaluate the effects of training at a young age, half of the horses were trained from the age of 6 months, and the other half were not. Horses were blocked by pedigree, date of birth, sex and free-jumping performance at 5 months of age, and they were randomly allocated to one of the two treatment groups: training (n = 21: 13 colts, 8 fillies) and nontraining (n = 20: 13 colts and 7 fillies). Horses were held in groups in half-open, straw-bedded stables in winter and on pasture in summer. At stable, horses were fed concentrates in the morning and in the afternoon. Before noon, horses received additional straw and grass silage. Water was available ad libitum. (For more details, see Ref. [7].) Due to health problems, three horses were excluded from this study before the start of the second winter period.

2.2. Testing procedures

During two winter periods, a novel object test and a handling test were conducted. Both tests were carried out four times, i.e. when the horses were approximately 9, 10, 21 and 22 months old. The experimental design used in this study is described in detail by Visser et al. [7].

Briefly, the novel object test was carried out in an indoor arena of 18 × 21 m. After a 2-min waiting period in a so-called starting box, the horse was released into the arena. Two minutes after the horse entered the arena, the novel object (an open blue and white umbrella) was lowered from the ceiling, from approximately 6 m height. The umbrella stood straight up for another 5 min allowing the horse to investigate it. Then, the horse was caught by the handlers and led back to its stable. The handling test comprised of a familiar handler leading the horse to a so-called ‘bridge’. This bridge consisted of four concrete plates (four plates of \(2 \times 1\) m), creating a bridge 2 m wide and 4 m long. The top level of which was elevated 15 cm above the ground. While approaching the bridge, the handler did not pull, touch or talk to the horse. If the horse stopped walking, the handler walked on until the rope was slightly tensioned. If the horse continued the approach, the handler moved on. Tough resistance, like pulling backwards, rearing or walking along the sides, was followed by another trial. Each horse had a maximum of three trials per test to cross the bridge. The starting point of every trial was approximately 12 m in front of the bridge, the horse facing the bridge. After the horse had crossed the bridge or reached the maximum number of trials, it was led back to
Mean HR and HRV measures were performed after system-activity of the parasympathetic nervous system [13,14,20]. Activities of the heart. rMSSD focuses on high-frequency, state of balance between sympathetic and parasympathetic autonomic nervous system to HR variations: It measures the therefore includes the contribution of both branches of the parasympathetic dominance. The SDRR estimates the overall HRV and these indices indicate a shift towards a more parasympathetic dominance, while increased values of rMSSD) reflect a shift of the autonomic balance towards a more sympathetic dominance, and reductions in the values of HRV indices (SDRR and (3) the root mean square of successive beat-to-beat differences (rMSSD, ms) [13].

Generally speaking, beat-to-beat HR (bpm) (2) the standard deviation of beat-to-beat intervals (SDRR) and (3) the root mean square of successive beat-to-beat differences (rMSSD, ms) [13]. Generally speaking, reductions in the values of HRV indices (SDRR and rMSSD) reflect a shift of the autonomic balance towards a more sympathetic dominance, while increased values of these indices indicate a shift towards a more parasympathetic dominance. The SDRR estimates the overall HRV and therefore includes the contribution of both branches of the autonomic nervous system to HR variations: It measures the state of balance between sympathetic and parasympathetic activities of the heart. rMSSD focuses on high-frequency, short-term variations of HR, which are mainly due to the activity of the parasympathetic nervous system [13,14,20]. Mean HR and HRV measures were performed after systematic removal of artefacts on the basis of visual inspection. Approximately 8% of all HR recordings was not used because of excessive artefacts.

Behavior during both tests was videotaped with a camcorder and was analyzed using the Observer software program (Noldus Information Technology, Wageningen, The Netherlands). Several behavioral variables were scored (see Ref. [7]). In the present study, we used the percentage of physical activity of the total test time as a measure for physical activity. Since we were able to control the level of physical activity in the handling test, horses were led to the bridge at a low speed; we did not use a physical activity variable in the handling test to correct mean HR for activity.

2.4. Statistical analysis

To obtain homogeneous variances, observations for SDDR and rMSSD were log-transformed prior to analysis. All HR variables considered in this present paper were expressed as response values to baseline, i.e. for each variable, the difference was calculated between the value during the test and the value during baseline. In view of differential durations of HR recordings in the handling test, we used regression analyses within ages to examine whether HR variables were affected by the length of the test. The regression model used measures of HR and HRV (response value relative to baseline) as dependent variables and length of the test as explanatory variable. Factors for housing groups, sex and training were introduced as fixed effects. None of the HR variables was significantly affected by the length of the test, which was therefore not considered as a source of variation in subsequent analyses.

The estimation of main effects of sex, training and age involved two steps. First, variables (response value relative to baseline) were analyzed per age with an analysis of variance model with factors for housing groups (five levels), sex (two levels) and training (two levels). Tests

<table>
<thead>
<tr>
<th>HR (bpm)</th>
<th>9 Months</th>
<th>10 Months</th>
<th>21 Months</th>
<th>22 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>53.7±1.4 a</td>
<td>54.0±2.1 a</td>
<td>47.3±1.1 a</td>
<td>47.6±1.1 a</td>
</tr>
<tr>
<td>Test</td>
<td>159.5±4.4 b</td>
<td>142.6±4.6 b</td>
<td>129.4±5.6 b</td>
<td>113.9±5.4 b</td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>131.1±9.5 a</td>
<td>133.8±7.0 a</td>
<td>286.9±23.0 a</td>
<td>208.8±15.5 a</td>
</tr>
<tr>
<td>Test</td>
<td>91.4±7.0 b</td>
<td>106.6±7.1 b</td>
<td>131.2±12.0 b</td>
<td>152.3±9.1 b</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>46.3±4.8 a</td>
<td>42.0±2.8 a</td>
<td>61.3±4.7 a</td>
<td>56.1±6.1 a</td>
</tr>
<tr>
<td>Test</td>
<td>15.8±1.9 b</td>
<td>18.6±1.7 b</td>
<td>22.8±2.9 b</td>
<td>28.2±2.8 b</td>
</tr>
</tbody>
</table>

Means of baseline and corresponding test value with different letter per HR variable per age differ significantly (P<.05).

Table 2

Response values (means ± S.E.M.) for the HR and HRV indices (difference between test values and corresponding baseline values) for the trained (n=21) and untrained horses (n=20) in the novel object test at 9, 10, 21 and 22 months of age (n=41, 41, 38 and 38, respectively).

<table>
<thead>
<tr>
<th>HR (bpm)</th>
<th>9 Months</th>
<th>10 Months</th>
<th>21 Months</th>
<th>22 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td>95.8±7.4 a</td>
<td>78.9±5.3 a</td>
<td>64.8±6.4 a</td>
<td>44.5±5.3 a</td>
</tr>
<tr>
<td>Nontraining</td>
<td>116.3±6.4 b</td>
<td>99.8±5.4 b</td>
<td>100.2±6.6 b</td>
<td>90.5±6.3 b</td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>29.3±19.8 a</td>
<td>8.2±13.3 a</td>
<td>78.1±34.4 a</td>
<td>17.9±25.2 a</td>
</tr>
<tr>
<td>Nontraining</td>
<td>59.5±17.3 b</td>
<td>54.3±13.9 b</td>
<td>248.9±35.4 b</td>
<td>162.1±29.8 b</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>33.4±8.3 a</td>
<td>16.2±4.4 a</td>
<td>34.2±6.7 a</td>
<td>5.5±9.1 a</td>
</tr>
<tr>
<td>Nontraining</td>
<td>26.3±7.2 b</td>
<td>32.3±4.5 b</td>
<td>43.3±7.2 b</td>
<td>49.3±10.5 b</td>
</tr>
</tbody>
</table>

Means of trained and untrained horses with different letter per HR variable per age differ significantly (P<.05).
for main effects and interactions were based on the common $F$ test. Residuals $e = y - m$, where $y$ denotes an observation and $m$ the corresponding estimated mean were saved and used as variables corrected for fixed effects. Second, differences between all ages were also analyzed as repeated measurements with a mixed analysis of variance model with a general covariance matrix for the observations at the different ages. Dispersion parameters were estimated by restricted maximum likelihood (REML) [21]. Tests for main effects for housing groups and main effects, and interactions between sex, training and time, were performed with the Wald test [22].

To assess the amount of variance in mean HR as explained by physical activity, at each age, two models for mean HR were compared: the model with factors sex and training (Model A) and the model with an additional covariate for the physical activity, including interaction terms (Model B). The percentage of variance explained by physical activity was defined as the reduction of the residual variance, i.e. the difference between the residual variances of Models A and B, expressed as a percentage of the residual variance of Model A. The residuals of Model B were saved as a new variable that effectively represents HR corrected for physical activity.

Consistency of variables was assessed by calculating Spearman correlations between residuals $e = y - m$ from the aforementioned analysis for each pair of ages. These residuals can be regarded as observations corrected for possible effects of housing group, sex and training. Hence, correlations can be interpreted as pooled correlations within combinations of housing group, sex and training. All calculations were performed with Genstat [23]. Statistical significance was set at $P < .05$.

### 3. Results

#### 3.1. Effects of the novel object test on HR variables

Mean HR and the HRV indices SDRR and rMSSD all differed significantly between baseline and test values at all ages (see Table 1). All HR variables showed a more pronounced response for the untrained horses, apart from rMSSD at 9 months of age (see Table 2). There was no significant effect of sex on any of the HR variables at any age. Fig. 1 shows the response values, i.e. differences between baseline and test values, for the different HR variables per age. The HR response decreased over ages, the HRV indices did not exhibit an apparent decrease over ages.

#### 3.2. Effect of physical activity on HR in the novel object test

The percentage of HR, expressed as a response value, explained by physical activity decreased within years (9 months: 41.1; 10 months: 23.3; 21 months: 42.3; and 22 months: 29.9). The trained and untrained horses in the novel object test tended to differ in their physical activity at 9 months [$F(1,38) = 3.3$, $P < .1$], differed significantly at 10 months of age [$F(1,38) = 7.2$, $P < .05$] but did not differ at 21 and 22 months (Table 3). Meanwhile, the untrained horses displayed a significantly higher mean HR than the trained horses at all ages. The predicted nonmotor HR

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**Table 3**

Means ($\pm$ S.E.M) of physical activity, response values for mean HR and nonmotor HR for the trained ($n = 21$) and untrained horses ($n = 20$) in the novel object test at 9, 10, 21 and 22 months of age ($n = 41, 41, 38$ and 38, respectively)

<table>
<thead>
<tr>
<th>Model</th>
<th>Dependent variable</th>
<th>9 Months</th>
<th>10 Months</th>
<th>21 Months</th>
<th>22 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Physical activity</td>
<td>Training</td>
<td>69.6 ± 3.3$^a$</td>
<td>63.8 ± 3.3$^a$</td>
<td>55.7 ± 4.4$^a$</td>
</tr>
<tr>
<td>A</td>
<td>Physical activity</td>
<td>Nontraining</td>
<td>78.4 ± 3.4$^a$</td>
<td>75.6 ± 3.5$^a$</td>
<td>62.7 ± 2.7$^a$</td>
</tr>
<tr>
<td>A</td>
<td>HR</td>
<td>Training</td>
<td>95.8 ± 7.4$^a$</td>
<td>78.9 ± 5.3$^a$</td>
<td>64.8 ± 6.4$^a$</td>
</tr>
<tr>
<td>A</td>
<td>HR</td>
<td>Nontraining</td>
<td>116.3 ± 6.4$^a$</td>
<td>99.8 ± 5.4$^a$</td>
<td>100.2 ± 6.6$^a$</td>
</tr>
<tr>
<td>B</td>
<td>Nonmotor HR</td>
<td>Training</td>
<td>97.6 ± 5.8$^a$</td>
<td>82.9 ± 4.9$^a$</td>
<td>70.5 ± 5.3$^a$</td>
</tr>
<tr>
<td>B</td>
<td>Nonmotor HR</td>
<td>Nontraining</td>
<td>107.7 ± 6.1$^{ab}$</td>
<td>97.5 ± 6.0$^a$</td>
<td>95.7 ± 8.1$^a$</td>
</tr>
</tbody>
</table>

Means of physical activity and HR were predicted from Model A (model with factors housing group, sex and training), means of nonmotor HR were predicted from Model B (same as Model A with an additional covariate for the physical activity, see Section 2.4).

$^a,b,c$Means of trained and untrained horses with different letter per variable per age differ significantly ($^{a,c}P < .05$) or tend to differ significantly ($^{a,b}P < .1$).

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**Fig. 1.** Response values (means ± S.E.M.) for the HR and HRV indices (difference between test values and corresponding baseline values) in the novel object test at 9, 10, 21 and 22 months of age ($n = 41, 41, 38$ and 38, respectively). The HRV indices SDRR and rMSSD have been log-transformed. Means of a single HR variable with different letters differ significantly between ages ($P < .05$).
(Model B) was significantly higher for the untrained horses than for the trained horses at 10 [\( F(1,36) = 4.1, P < .05 \)], 21 [\( F(1,30) = 15.1, P < .01 \)] and 22 [\( F(1,31) = 39.5, P < .001 \)] months of age and tended to be higher at 9 months of age [\( F(1,32) = 3.4, P < .1 \)].

### 3.3. Effects of the handling test on HR variables

Table 4 shows that the baseline values of the mean HR (HR) and the HRV indices (SDRR and rMSSD) all differed between baseline and test values for all ages. Although the untrained horses had more pronounced HR and HRV responses, these were not, except one, significantly different from the trained horses (see Table 5). When horses got older, the mean HR response decreased significantly (\( P < .05 \)). The response of SDRR differed between years (9 and 10 months vs. 21 and 22 months of age) and the response of rMSSD was not significantly different between ages (see Fig. 2). There were no interaction effects among the sexes, training and ages.

**Table 4**

<table>
<thead>
<tr>
<th>HR (bpm)</th>
<th>9 Months</th>
<th>10 Months</th>
<th>21 Months</th>
<th>22 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>54.1 ± 1.7(^a)</td>
<td>51.0 ± 1.0(^a)</td>
<td>48.5 ± 1.7(^a)</td>
<td>47.5 ± 0.9(^a)</td>
</tr>
<tr>
<td>Test</td>
<td>112.5 ± 2.8(^b)</td>
<td>105.5 ± 2.9(^b)</td>
<td>96.2 ± 3.0(^b)</td>
<td>90.5 ± 3.2(^b)</td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>143.2 ± 11.1(^a)</td>
<td>131.5 ± 8.9(^a)</td>
<td>239.9 ± 16.8(^a)</td>
<td>193.1 ± 9.4(^a)</td>
</tr>
<tr>
<td>Test</td>
<td>90.9 ± 6.4(^b)</td>
<td>102.3 ± 4.7(^b)</td>
<td>100.5 ± 6.0(^b)</td>
<td>89.2 ± 9.0(^b)</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>49.2 ± 8.9(^a)</td>
<td>48.9 ± 5.1(^a)</td>
<td>57.8 ± 4.7(^a)</td>
<td>50.6 ± 2.7(^a)</td>
</tr>
<tr>
<td>Test</td>
<td>24.6 ± 2.1(^b)</td>
<td>24.2 ± 1.8(^b)</td>
<td>24.4 ± 2.2(^b)</td>
<td>26.6 ± 3.0(^b)</td>
</tr>
</tbody>
</table>

\(^a\)Mean of baseline and corresponding test value with different letter per HR variable per age differ significantly (\( P < .05 \)).

### 3.4. Consistency over ages in the novel object test and handling test

Table 6 shows the Spearman rank correlations between ages for the responses of the HR variables in the novel object test. All responses of HR variables and the residual of HR (after correction for physical activity) were consistent between years (9 and 21 months of age) and within the second year. In addition, in the handling test, horses showed significantly consistent responses between years (9 and 21 months of age).

**Table 5**

<table>
<thead>
<tr>
<th>HR (bpm)</th>
<th>9 Months</th>
<th>10 Months</th>
<th>21 Months</th>
<th>22 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td>57.3 ± 4.6(^a)</td>
<td>49.7 ± 3.9(^a)</td>
<td>43.0 ± 4.4(^a)</td>
<td>39.1 ± 4.0(^a)</td>
</tr>
<tr>
<td>Nontraining</td>
<td>60.4 ± 4.9(^a)</td>
<td>59.0 ± 4.1(^a)</td>
<td>54.5 ± 4.6(^a)</td>
<td>46.6 ± 5.0(^a)</td>
</tr>
<tr>
<td>SDRR (ms)</td>
<td>28.8 ± 18.8(^a)</td>
<td>27.9 ± 13.0(^a)</td>
<td>121.9 ± 24.0(^a)</td>
<td>91.7 ± 16.4(^a)</td>
</tr>
<tr>
<td>Nontraining</td>
<td>75.2 ± 19.9(^a)</td>
<td>29.9 ± 13.8(^a)</td>
<td>169.5 ± 25.4(^a)</td>
<td>122.2 ± 20.6(^a)</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>26.1 ± 12.6(^a)</td>
<td>24.9 ± 7.6(^a)</td>
<td>29.0 ± 8.3(^a)</td>
<td>22.4 ± 4.8(^a)</td>
</tr>
<tr>
<td>Nontraining</td>
<td>18.8 ± 13.3(^a)</td>
<td>25.5 ± 8.0(^a)</td>
<td>37.2 ± 8.8(^a)</td>
<td>28.2 ± 6.0(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Means of trained and untrained horses with different letter per HR variable per age differ significantly (\( P < .05 \)).
months or 10 and 22 months of age) and within the second year (see Table 7).

4. Discussion

The results show that the challenge of the novel object test induced a physiological state characterized by an increase in mean HR and a decrease in HRV. In addition, for the handling test, in which a challenge was faced with the support of a familiar handler, a similar pattern was found. HR variables showed significant positive correlations within tests both between years and within the second year.

4.1. The response of the autonomic nervous system to challenge tests

At all ages, in both tests, a significant increase in mean HR was found together with a significant decrease in the HRV indices SDRR and rMSSD. This indicates a shift of the autonomic balance towards a sympathetic dominance [8]. Because both the SDRR and the rMSSD decreased significantly, it is suggested that the shift towards the sympathetic dominance results from the lack of a sufficient parasympathetic counteraction to sympathetic activation [12]. A similar response was found for rats facing a social defeat challenge [15]. Furthermore, studies showed that fast alteration in mean HR due to sudden excitement or fear, in horses up to 110 bpm, are almost entirely attributed to decreases in parasympathetic nerve activity, while slow HR alterations can be mediated by both sympathetic and parasympathetic nerve activity [18,24–26]. This also seems the case in this study. A high degree of parasympathetic control allows for enhanced responsivity of the HR to environmental demands [18] and a low degree of parasympathetic tone is found with panic [16,17]. In the present study, individual horses differed in their HR responses to both challenge tests and therefore may also have differed in their adaptiveness to environmental demands. Horses with a low degree of parasympathetic nerve activity are thought to be more emotionally stressed compared to individuals with a higher degree of parasympathetic nerve activity. Hence, it is suggested that HR responses may relate to aspects of temperament, especially emotionality. Aspects of emotionality, like fear and frustration, emerging during isolation or other stressors, may be relevant for horse owners and trainers to be able to predict horses’ responses to environmental demands. Subsequently, a horse trainer can adjust training methods accordingly and a horse owner can select a specific horse for a specific goal.

4.2. Effect of physical activity on mean HR

Behavioral efforts of coping with the challenge influence the magnitude of the cardiovascular responses [27]. For example, the physical activity displayed by the individual might influence its HR [8]. The percentage of variation in mean HR in the novel object test that was accounted for by physical activity was 41%, 23%, 42% and 30% at 9, 10, 21 and 22 months of age, respectively. This implies that the remainder, or the nonmotor HR [19], was explained by variation in a factor other than physical activity, e.g. emotional reactivity.

4.3. Effect of training

Several horse studies showed that a mean HR was significantly higher for individuals that were characterized as more emotional [28,29]. In the novel object test of the present study, the response of the untrained horses was more pronounced compared to the response of the trained horses. Thus, there was a larger increase in HR and a larger decrease in the HRV indices. Hence, the shift in the balance of the autonomic nervous system towards a sympathetic dominance, presumably related to a poor vagal antagonism to sympathetic activation, was stronger for the untrained horses compared to the trained horses. The more pronounced increase in HR in the untrained horses could not be explained by a higher level of physical activity: While the level of physical activity hardly differed, the overall HR and the nonmotor HR were significantly higher for the untrained horses than for the trained horses, suggesting a higher level of emotional activation. This difference in responsivity between trained and untrained horses may be explained by the fact that the trained horses were exposed more often to changing environmental demands during their training sessions. The difference in emotionality between the trained and untrained horses was evident in the handling test, as suggested by similar values of HR variables in the two groups. We speculate that the social support of the handler has buffered possible differences between these groups in the handling test [30,31].
4.4. Consistency of variables within tests

Quantification of potential temperamental traits implies developing tests in which animals show consistent responses [2,7,32–36]. Whether the use of variables indicating the response of the autonomic nervous system would fulfil this requirement depends on whether these HR variables show consistency over time within individuals. The principal findings of the present study were that mean HR and the HRV indices SDRR and rMSSD showed significant positive correlations within individuals across ages. Although correlations were most robust within the second year, all HR variables showed a significant correlation between the first and the second year. These findings suggest that mean HR and HRV are reliable measures and can be used in the quantification of temperamental traits of horses. It was shown that behavioral responses of these horses to these tests were consistent both within the first and within the second year, but not between years [7]. It was suggested that the lack of consistencies in behavioral variables between years could be explained by maturational effects including puberty [7,29]. Since in the present study, we did find significant correlations between years for the HR variables, we suggest that the effect of maturation on the variables measured is different for the HR and for the behavioral responses of the horses.

In a parallel study with adult horses, it was found that HR variables measured in a novel object test and in a handling test showed significant correlations with temperamental traits assessed by riders [37]. In this latter study, it was shown for example that horses with a low HRV (rMSSD) in the handling test were characterized by the riders as highly responsive to changes in the environment. The reliability of the HR variables in the present study and the correlation of the HR variables with temperament assessed by riders in the study of Visser et al. [37] suggest that HR variables are highly suitable in objectively quantifying aspects of temperament.

5. Conclusion

Horses showed pronounced responses in HR and HRV measures when confronted with changes in the environment. The additional increase in mean HR when exposed to a novel object was related to emotionality rather than physical activity. This nonmotor HR showed to be higher for the untrained horses compared to the trained horses. All HR variables used in this study were consistent over years and within the second year. Our results strongly suggest that HR variables are useful in differentiating between individuals and quantifying aspects of temperament.

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