Assessing Equine Anxiety-Related Parameters Using an Isolation Test in Combination with a Questionnaire Survey

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ABSTRACT. The aim of the study was to determine parameters reflecting equine anxiety trait by comparing results obtained in a behavior test and an anxiety score assessed by familiar caretakers in response to a questionnaire. In the behavior test, horses were individually led into a novel room by their caretakers and loosely tethered to decrease excessive movement using the common cross-tying technique with two leads and breakable plastic cords. The horses initially remained with their caretaker for 2 min; the caretaker then left and the subject animal was left alone for 2 min. The latency to break the plastic cords, heart rate, the number of steps and pawings were recorded. When the horses were left alone, most parameters changed significantly and some showed sexual differences. A correlation analysis revealed that anxiety score assessed by caretakers showed a negative correlation with the latency to break the plastic cord and a positive correlation with heart rate only when horses were isolated. These two were suggested feasible parameters for assessing the anxiety trait of unfamiliar horses. Both the behavioral results and the anxiety scores also indicated that females were more anxious than males. Our results suggest that it would be a useful strategy for assessing other temperament traits as well to combine behavior tests with a questionnaire survey.

KEY WORDS: Anxiety, behavior tests, heart rate, questionnaires, sexual differences.

Anxiety is defined as an emotional state induced by the perception of potential danger that threatens the individual [1]. A moderate level of anxiety leads to functional reactions, which enables the individual to cope with and adapt to the environment. However, excessive level of the anxiety can cause unexpected and undesired reactions, which may result in injuring themselves or endangering people such as veterinarians, riders, and handlers [4, 6]. Therefore, it is important to understand the anxiety trait of individual horses. One method to assess equine temperament is to administer a questionnaire to persons familiar with the horses being assessed. We have previously designed two questionnaires and investigated their reliability and validity [12, 13]. Among various temperament traits, factor analysis indicated that the factor designated anxiety trait had the highest internal consistency in two separate surveys (Cronbach’s α reliability coefficients > 0.9) [12]. Morris et al. [18] also reported highly successful ranking for the anxiety trait of horses by using the modified Neuroticism-Extversion-Openness (NEO) Personality Inventory. This questionnaire has been one of the most popularly employed self-response questionnaires to evaluate human personality in the human psychiatric and psychological fields. In a separate study, Morris et al. [19] reported that anxiety trait was rated with the highest confidence by the respondents. Taken together, these studies suggest that anxiety trait can be assessed more accurately than other traits in horses.

However, it is difficult to use the questionnaire to assess unfamiliar horses, because the respondents are required to have sufficient experience with the test subjects through care and daily training. In addition, since reliability is highly dependent on the respondents’ ability to assess equine temperament based on extensive experience in handling horses, the questionnaire survey cannot always be used.

Behavioral tests are therefore often used as an alternative. Some researchers have assessed equine anxiety based on changes in behavior, as well as autonomic and endocrine functions, in response to various stimuli [e.g., 10, 26]. Heart rate [25] as well as behavioral parameters such as exploring novel object and snorting [23] were shown to be significantly consistent within individual horses when the results were compared among repeatedly conducted novel environment tests. These results suggest that reliable assessment can be obtained even with a single test. However, their validity remains untested in most cases. One reason for the difficulty in validating the parameters from behavioral tests is that these parameters, including heart rate and endocrine responses, are largely influenced by physical activity. It is therefore considered almost impossible to determine whether observed changes are caused by anxiety or simply by physical activity in behavior tests of freely-moving behavioral horses, although the heart rate variability could be a potential measure free from the effect of physical activity [25]. Additional difficulties arise from having few indices with which to examine the validity of equine behavioral...
tests. Researchers have had to determine which parameters obtained from behavioral tests reflect equine anxiety based on experimental conditions alone.

The present study was therefore conducted in an attempt to overcome these difficulties; we carried out an isolation test under a loosely restrained condition to prevent physical activity and compared the results obtained from the behavior test with the anxiety scores of subjects, which had been previously assessed by familiar caretakers in response to a questionnaire [12], as outer index. Since the factor designated anxiety trait showed high internal consistency and this assessment was considered reliable, some parameters in the behavior test, which had significant association with the anxiety scores, were regarded appropriate for assessing the equine anxiety.

MATERIALS AND METHODS

Subjects: The subjects in this study were 2-year-old thoroughbred horses (34 males and 27 females) at the Hidaka Yearling Training Farm (Japan Racing Association).

Test procedure: Isolation tests were performed in a 100-m² indoor experiment building, which had a normal treatment place that was unfamiliar to the subject and from which no other horse could be seen. Before starting the test, each subject was equipped with a heart rate monitor (Vantage XL New, POLAR, Kempele, Finland) in its home stable. It was then led into the treatment place by its familiar caretaker and was loosely tethered using the common crossing technique with two leads to prevent excessive movement, as shown in Fig. 1. The subject was initially placed in the treatment place facing a mirror. Breakable plastic cords (PS5-300, Yutaka Make, Osaka, Japan) were used to connect the leads and the wall to avoid physical injury to the horse in the event of a sudden struggle (Fig. 1). The plastic cord breaks under tension of 856.2 ± 9.8 N (mean ± SE). The ease of breakability of the plastic cords prevented physical injury, and no adverse effects due to this test were observed.

In the first 2 min (Phase I), each subject was accompanied by its caretaker and kept in front of the mirror with its reflection visible. Even if the subject moved and tried to break the plastic cords, the caretaker was asked not to reassure the subject either by moving the leads or by voice. Some subjects broke the plastic cords during the 2 min of Phase I; in that case, they were tethered again before Phase II was started. At the end of Phase I, the caretaker went away, and the subject’s image that had been reflected in the mirror was erased by turning on a weak backlight with a remote switch as a Phase II. This was done so that the subject would feel as if it was suddenly left alone in the treatment place. Results obtained from a single trial of the behavioral test were postulated to reflect the individual behavior characteristics of each horse according to the findings by Visser et al. [23, 25].

Data collection: The latency to break the plastic cords, the change in heart rate, the number of steps in place (hereafter, “steps”), and pawing motions (hereafter, “pawings”) were recorded. The latency for horses that had broken the cords immediately after being tethered was assigned to 0 second and the latency for those that did not break was assigned to 120 seconds, respectively. Steps and pawings were recorded by an outside VTR camera and analyzed after the experiment ended. The number of defecation events in both phases was also recorded but was not used for later analysis due to its rare occurrence. Data obtained from the subjects that had broken the plastic cords within 2 min, in either phase, were adjusted to per minute rates.

Anxiety score: The anxiety trait of individual horses had been already assessed using a questionnaire in our previous study [12]. In brief, for each horse, three caretakers completed 20 questions each of which was graded using a 9-point scale at the same time as the behavioral tests; ‘Nervousness’, ‘Concentration’, ‘Self-reliance’, ‘Trainability’, ‘Excitability’, ‘Friendliness toward people’, ‘Curiosity’, ‘Memory’, ‘Panic’, ‘Cooperation’, ‘Inconsistent emotionality’, ‘Stubbornness’, ‘Docility’, ‘Vigilance’, ‘Perseverance’, ‘Friendliness toward horses’, ‘Competitiveness’, ‘Skittishness’, ‘Timidity’, and ‘Gate entrance’. The sum of scores for seven question items (‘Nervousness’, ‘Excitability’, ‘Panic’, ‘Inconsistent emotionality’, ‘Vigilance’, ‘Skittishness’, and ‘Timidity’) were used as anxiety scores in this study, because factor analysis in the previous study showed that factor 1, designated anxiety trait, was composed of these seven items. The anxiety scores obtained from 61 horses were 36.9 ± 0.9 (21.7–51.3) points and seemed to display a normal distribution. The details are described in our previous paper [12].

Statistical analyses: All analyses were carried out using statistical software (StatView 5.0J, Abacus Concepts, Berkeley, CA). The data except for the latency were expressed as mean ± SE, and the significance level was set at P≤0.05. For the effects of phase and sex, all results except for the latency to break were analyzed by two-way repeated measures analysis of variance (ANOVA) followed by a paired t-test.
test and Student’s t-test, respectively, as post-hoc tests with Bonferroni corrections; the level of significance was set at $P \leq 0.05/4=0.0125$. Since the distribution of the latency to break was skewed, a Wilcoxon signed-rank test and a Mann-Whitney U-test were used to investigate the influence of phase and sex, respectively, with Bonferroni corrections. In addition, to investigate whether results of each horse was consistent between two phases, we calculated Spearman’s coefficient of rank correlation or Pearson product-moment correlation coefficient. To determine which parameter reflected equine anxiety trait, associations between behavior, heart rate, and anxiety scores obtained from the questionnaire were calculated using Spearman’s coefficient of rank correlation for the latency to break or Pearson product-moment correlation coefficient for the others. To examine whether the heart rate was influenced by physical activity even under a loosely restrained condition, we calculated partial correlation coefficients between the anxiety score, and the heart rate, the number of steps or the number of pawings in both Phase I and II. Latency to break plastic cords was excluded due to its skewed distribution.

RESULTS

Behavior test: Of 34 males and 27 females, eight males and two females in Phase I and seven males and 12 females in Phase II broke the plastic cords within 2 min. While the latency of males did not seem to change when they were isolated (Fig. 2A), females broke the cord significantly earlier in Phase II (Wilcoxon signed-ranks test: $P=0.0033$). In Phase II, females tended to break the cord earlier than males, but the difference was not significant after Bonferroni correction ($P=0.0360$). Significant correlation between the latency to break in two phases was observed ($r_f=0.327$, $P<0.05$). Four horses that broke the plastic cord immediately after being tethered were excluded from the following analysis.

Two horses were excluded from the heart rate analysis due to technical problems in monitoring. While there was no significant difference between sexes in heart rate in their home stable (males: $37.4 \pm 0.9$ bpm, females: $36.5 \pm 0.9$ bpm), significant differences were found between phases ($F_{1,53}=8.658$, $P<0.01$; Fig. 2B) and between sexes ($F_{1,53}=7.796$, $P<0.01$). The interaction between phase and sex was also significant ($F_{1,53}=5.615$, $P<0.05$). In post-hoc tests, only females had a significantly increased heart rate when isolated ($t_{25}=3.039$, $P<0.01$), and there was a difference between the sexes in Phase II ($t_{8}=3.421$, $P<0.01$). In addition, significant correlation between heart rate in two phases was observed ($r=0.522$, $P<0.0001$).

The number of steps also increased significantly ($F_{1,55}=39.194$, $P<0.0001$) when horses were isolated, and an interaction was found between phase and sex ($F_{1,55}=7.236$, $P<0.01$; Fig. 2C). Both males and females significantly increased the number of steps (males: $t_{26}=3.232$, $P<0.01$, females: $t_{25}=5.134$, $P<0.0001$). The number of steps in Phase II, females tended to be more than those in males in Phase II, but the difference was not significant after Bonferroni correction ($t_{26}=2.241$, $P=0.0291$). Significant correlation between the number of steps in two phases was also observed ($r=0.461$, $P>0.001$). The number of pawings also increased significantly when subjects were left alone ($F_{1,55}=10.450$, $P<0.01$; Fig. 2D). In post-hoc tests, only males significantly increased the number of pawings ($t_{30}=3.507$, $P<0.01$). In addition, significant correlation between results in two phases was observed ($r=0.764$, $P<0.0001$).

Correlation analyses: We examined associations between the behavioral results and the anxiety scores assessed by the caretakers. Anxiety scores assessed by caretakers showed a negative correlation with the latency to break the plastic cord in Phase II ($r_f=-0.310$, $P<0.05$), and a positive correlation with heart rate in Phase II ($r=0.453$, $P<0.01$; Table 1). Table 2 shows partial correlation coefficients between the anxiety score and three parameters in Phase I and II. Significant associations were observed between the anxiety score and the heart rate only in Phase II ($r_f=0.404$, $P<0.01$), while there were no association between the anxiety score and the number of steps ($r_f=0.005$, $P=0.972$) or between the anxiety score and the number of pawings ($t_{26}=0.017$, $P=0.904$) in Phase II. The heart rate was also significantly associated with the number of steps in both Phase I ($r_f=0.491$, $P<0.001$) and Phase II ($r_f=0.411$, $P<0.01$).

DISCUSSION

Most parameters showed significant changes when the subjects were isolated. Comparisons between the behavioral results and anxiety scores determined by caretakers in the questionnaire revealed two significant correlations (Table 1). The heart rate and the latency to break the cords under isolated condition were found to be feasible parameters for assessing the anxiety trait of unfamiliar horses.

Heart rate as an indicator for anxiety trait: We previously reported that horses assessed as highly anxious animals by their caretakers showed a great increase in heart rate and defecated often during exposure to a balloon stimulus [13]. Visser et al. [24] reported that heart rate during exposure to a novel object was correlated with riders’ rating scores on items such as ‘spooky’ and ‘sensitive to disturbance’. McCall et al. [10] carried out a novel object test and an isolation test with or without the administration of a tranquilizer. They concluded that heart rate reflected the reactivity of the horse, but behavior parameters were not consistently ranked in the two behavior tests. Correlation coefficients for the latency to break ($r=0.310$) and for the heart rate ($r=0.453$) obtained in this study were as high as those of canine studies (0.33–0.55) and higher than those of human studies (0.20–0.30) [5], which suggested that equine anxiety trait could be assessed with sufficient validity by using the methods herein described. In addition, when the association between the anxiety score and the heart rate in
Phase II is separately analyzed in each sex. Correlation coefficients for males and females are 0.274 and 0.543, respectively. This appears to indicate that the anxiety predisposition is more clearly reflected in the autonomic response (i.e., heart rate) to the isolation stress in female horses than males, but further study is required to clarify this point.

All results of each horse were respectively consistent between phases, which suggested that each behavior trait might be stably expressed regardless of difference in phases. However, poor correlations between heart rate, the latency to break, and anxiety scores were found in Phase I. This could indicate that assessing equine anxiety trait in the presence of a human handler may result in misleading outcomes, as suggested by McCall et al. [10]. Partial correlation coefficients shown in Table 2 support this view. However, the heart rate was also associated with the number of steps in both phases, suggesting that the heart rate might have been influenced by physical activity even under a loosely restrained condition. It therefore seems likely that, if behavior tests were conducted in freely-moving horses, the association between the anxiety score and the heart rates would be more masked by physical activities.

Sexual difference: Only a few studies have assessed sexual differences in equine temperament so far. Wolff et al. [26] found no sexual differences when horses were examined using a novel object test. However, as shown in Fig. 2, sexual differences in some parameters were observed. Since horses assessed as more anxious animals broke the plastic cord significantly earlier and had higher heart rates in Phase II (Table 1), the results suggest that females may be more anxious animals than males. The anxiety scores obtained from the questionnaires in our study (males: 34.5 ± 1.2, females: 39.4 ± 1.2, Student’s t-test: t59=2.771, P<0.01) lower side of square and each circle below the bar indicate 25 percentile limit and the extreme date less than 10 percentile, respectively. Solid and open triangles indicate median values of males and females, respectively. ** P<0.01 and **** P<0.0001 between phases in either sex and †† P<0.01 between sexes in Phase II. The number of subjects was 61 (34 males and 27 females) for the latency to break, 55 (31 males and 24 females) for heart rate, and 57 (31 males and 26 females) for the number of steps and pawings.

Table 1. Correlation coefficients between behavioral and heart rate in both phases together with anxiety scores assessed by caretakers

<table>
<thead>
<tr>
<th>Phase</th>
<th>Parameters</th>
<th>Correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Latency to break</td>
<td>-0.021</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>Step</td>
<td>-0.047</td>
</tr>
<tr>
<td></td>
<td>Pawing</td>
<td>-0.219</td>
</tr>
<tr>
<td>II</td>
<td>Latency to break</td>
<td>-0.310*</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>0.453***</td>
</tr>
<tr>
<td></td>
<td>Step</td>
<td>0.219</td>
</tr>
<tr>
<td></td>
<td>Pawing</td>
<td>-0.094</td>
</tr>
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</table>

* P<0.05 and *** P<0.001.
also suggested that horse caretakers perceived sexual differences in anxiety trait. In females, the estrous stage may also influence reaction to isolation, but that was not taken into consideration in this study. One study [3] found that estrous cycle stage did not significantly affect the reaction to a novel object or to isolation, but the test order influenced the outcome of the tests. To assess female anxiety trait more accurately, the effect of estrous cycle on reactions should be analyzed in further studies.

**Importance of examining validity:** Recently, an increasing number of equine behavioral tests have been developed. To measure the reliability and validity of these tests, researchers have performed repetitive tests using the same horses [23, 25], subjected the same horses to different behavioral tests [8, 10, 22, 26], and in some cases measured differences when subjects were or were not tranquilized [10]. In this study, we examined the associations between results obtained from behavioral tests and anxiety scores assessed by caretakers to determine which parameters were most closely associated with equine anxiety trait. We found that heart rate and the latency to break the plastic cords when isolated could be accurate determinants of anxiety trait. Although the number of steps and pawings increased significantly when the horses were isolated, these are not thought to represent equine anxiety trait because of their rather poor association with the anxiety scores (Table 1).

Had we only searched for parameters that changed significantly when the horses were isolated, without using the questionnaires, we would have interpreted the number of steps and pawings as being associated with equine anxiety trait. In conclusion, two parameters (the latency to break the plastic cords and heart rate when isolated) are likely to be useful for assessing equine anxiety trait based on their correlations with anxiety scores assessed by caretakers, while the number of steps and pawings may not reflect equine anxiety trait. In addition, females were estimated to be more anxious animals than males based on the behavioral results and anxiety scores. Our results also suggest that appropriate parameters for other temperament traits may be determined by other behavioral tests in combination with temperament questionnaires. This kind of approach appears indispensable for improving equine management from practical point of view but also for understanding the molecular background of temperament from basic viewpoint in conjunction with genetical survey of temperament-associated genes such as what we are currently conducting [14–17].

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Table 2. Partial correlation coefficient between anxiety score and three parameters

<table>
<thead>
<tr>
<th>Phase</th>
<th>Anxiety score</th>
<th>Heart rate</th>
<th>Step</th>
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<tbody>
<tr>
<td></td>
<td>Anxiety score</td>
<td>Heart rate</td>
<td>Step</td>
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<tr>
<td>I</td>
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<tr>
<td></td>
<td>0.114</td>
<td>0.491***</td>
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<td></td>
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<td>-0.077</td>
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<tr>
<td></td>
<td>-0.208</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.404**</td>
<td>0.411**</td>
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</tr>
<tr>
<td></td>
<td>0.005</td>
<td>-0.162</td>
<td>-0.092</td>
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**P<0.01 and *** P<0.001.

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