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Stress Response of Three-year-old Horse Mares to Changes in Husbandry System During Initial Equestrian Training

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ABSTRACT

For initial training, horses are often transferred from group housing to individual boxes, which is a potential stressor. In this study, salivary cortisol concentrations, locomotion activity, and heart rate (HR) were analyzed and the HR variability (HRV) variables standard deviation of beat-to-beat interval (SDRR) and root mean square of successive RR differences (RMSSD) were calculated in 3-year-old mares ($n = 8$). Mares were transferred abruptly from a group stable with access to a paddock to individual boxes without a paddock and were studied from 4 days before to 5 days after changing the stable. Mares underwent routine equestrian training for young horses. On the days before mares were moved to individual boxes, cortisol concentrations showed a diurnal rhythm with values approximately 0.6 ng/ml in the morning and a decrease throughout the day. When horses were moved to individual boxes, cortisol concentrations increased to 1.8 ± 0.2 ng/ml within 30 minutes and did not return to baseline values within 6 hours (0.7 ± 0.1 ng/ml, $P < .05$ over time). On the following days, a diurnal rhythm was re-established but at a higher level than before the change of stable. Locomotion activity was higher when mares had access to a paddock than when kept in individual boxes. Heart rate increased for approximately 60 minutes when mares were separated from their group. In conclusion, separating young horses from their group and individual stabling are perceived as stressful.

1. Introduction

Species differ in their social organization and thus the nature of interactions between conspecifics differs. Many mammals, such as tigers, hamsters, or orangutans, live a solitary life with adults meeting primarily for mating and the offspring leaving their mothers at around puberty. Other species form stable social units of permanently bonded adult individuals [1]. Most equid species, under natural conditions, live in family bands or in bachelor groups but are not solitary [2,3]. In socially organized animals, the presence of appropriate conspecifics reduces the response to stressful challenges [1]. Adolescent domestic horses are usually kept in herds and thus have ample opportunity for free exercise and social interactions with conspecifics. In continental Europe, horses under human care are often separated from their group (eg, for equestrian training) and are stabled individually. Separation from group mates may be perceived

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as stressful as it involves novelty of situation and location, social isolation, and reduced space, restricting locomotion. The response of an animal to adverse stimuli involves the release of cortisol and an immediate reaction of the sympathetic-adrenomedullary system. During short-term stress, cortisol may improve fitness by energy mobilization [4] and changes in behavior [5]. Because cortisol rapidly diffuses into saliva, salivary cortisol concentrations reliably mirror cortisol concentrations in blood [6]. The most immediate stress response is an increase in sympathetic-adrenomedullary activity with an acute release of epinephrine, leading to an increase in heart rate (HR) [7]. In addition to HR, HR variability (HRV), that is, short-term fluctuations in heart rate, is an indicator of the stress response of the autonomic nervous system. HRV reflects the antagonistic influences of the sympathetic and parasympathetic branches of the autonomic nervous system on the sinus node of the heart. In general, a decrease in HRV reflects a shift toward sympathetic dominance, indicative of a stress response, while increased values indicate parasympathetic dominance [8]. Although changes in HRV have not been analyzed in response to standardized stressors, decreases in the HRV variables standard deviation of the beat-to-beat interval (SDRR) and root mean square of successive beat-to-beat intervals (RMSSD) have been found consistently during potentially stressful situations such as when young horses were mounted for the first time by a rider [9] or during training and performances of dressage at an advanced level [10].

Although individual stabling of horses usually allows visual and olfactory contact between animals in adjacent boxes and thus does not represent total isolation, stabling of horses previously kept in groups elicits behavioral responses indicative of stress [11,12]. While increases in cortisol release following initial confinement have been reported [13,14], in other studies, behavioral changes were not reflected by increases in HR or cortisol release [12,15].

Horses kept in individual boxes are restricted in their ability to move freely. Although daily equestrian exercise reduces the animals' need for additional physical activity [11], it does not completely replace the need for free exercise [17]. In addition, horses kept in social groups are easier to handle [18] and require less time to reach a defined training level than horses housed singly [19]. In addition, singly stabled stallions showed a higher level of aggression than did group-stabled stallions [20], indicating positive effects exerted through the presence of conspecifics.

In this study, we analyzed salivary cortisol concentration, HR and HRV in 3-year-old mares in an equestrian training program. The mares were transferred abruptly from a group stable to individual boxes. Changes in locomotion activity were determined by pedometers. We hypothesized that the change from group housing to individual boxes is a stressor and thus leads to increased cortisol release, increased HR, and decreases in HRV of horses. Our study did not allow us to identify the individual effects of specific stressors (eg, partial isolation, novel stable, and changes in physical activity). Nevertheless, this complex situation is valid in relationship to what is happening in the housing and training of horses.

2. Materials and Methods

2.1. Animals

A total of 8 three-year-old Warmblood sport horses of the Brandenburg State Stud in Neustadt (Dosse), Germany, were available for the study. They were either born and raised at the stud or brought there at 1 year of age. All horses included in the study were female. As yearlings and 2 year olds, all mares were kept in one group on pasture during summer and in a group stable (12×30 m) on straw with daily access to a paddock (100×100 m) during winter. As 3 year olds, the mares remained in the group stable and paddock. Initial training was performed as described [9] and commenced 4 months before the experiment with lunging of the mares, progressing to first mounting of a rider after 4 weeks. Mares were then ridden 4-5 times per week until 4 weeks before the experiment. No changes in the husbandry system were made before the experiment, that is, mares remained as a group with daily access to their paddock for 3-4 hours. They were fed concentrates and hay twice daily (6:00 AM and 5:00 PM), and water was available at all times.

2.2. Experimental Design

For the experiment, mares were followed from 4 days (days -4 to -1) before to 5 days after transfer from the group stable and paddock housing to individual loose boxes (days 0-5). On day 0, mares were brought from the group stable into the paddock at 7:00 and were transferred from the paddock to individual boxes at 8:30. No further activity was requested from them on that day. Individual boxes measured 3.0×3.7 m, with solid partitions between horses. A visual contact between horses in adjacent boxes was possible through a small window with metal bars in the sidewalls of the boxes (60×50 cm). The upper half of the front wall consisted of metal bars at 5-cm intervals, allowing visual contact to the horse in the opposite box. Width of the aisle between opposite boxes was 2 m. On the morning of day 1, mares were individually given access to an indoor riding arena (20×50 m) for 10 minutes and requested to freely jump (ie, without a rider) a combination of three fences. No activity was requested on day 2, while on the mornings of days 3, 4, and 5, horses were ridden for 30 minutes in the indoor arena (approximately 10 minutes' walk, 15 minutes' trot, and 5 minutes' canter). On each day, saliva was collected for cortisol analysis. Cardiac beat-to-beat intervals for analysis of HR and HRV were determined continuously for 11 hours, and locomotion activity was recorded with activity, lying, temperature (ALT) pedometers (Ingenieurbüro Holz, Falkenhagen, Germany) for two 5-hour periods per day. Competent authority for animal experimentation in Brandenburg State, Germany, approved the experiment.

2.3. Experimental Procedures

2.3.1. Cortisol

Saliva was collected for analysis of cortisol concentration by using a cotton swab (Salivette; Sarstedt, Nümbrecht-Rommelsdorf, Germany) as described previously

[21]. The swab was held with an arterial clamp, inserted at the angle of the lips into the mouth of the horse, and placed gently onto the tongue for 1 minute and afterwards returned to a polypropylene tube. After centrifugation for 10 minutes at 1000 × *g*, at least 1 mL of saliva was aspirated from each sample and frozen at −20 °C until analysis. The sampling procedure was well accepted by all mares and conducted by a single person without restraining the horse. Saliva samples were obtained daily at half-hour intervals in the morning (6:30-7:00 AM), at noon (12:00-12:30 PM), and in the evening (6:00-6:30 PM). On the day of transfer from group stable to individual boxes, saliva was collected at 6:30 and 7:00 AM. The mares were brought into individual boxes at 8:30 AM, and further saliva samples were collected at 0, 5, 15, 30, 60, 90, 120, 150, 180, 240, 300, and 360 minutes thereafter and at 6:00 and 6:30 PM.

Salivary cortisol concentration was determined with a direct enzyme immunoassay without extraction validated for equine saliva [22]. The antiserum cross-reacts with cortisone and several corticosterone metabolites, and the measured values must be interpreted as cortisol immunoreactivity (IR). The intra-assay coefficient of variation was 5.0%, the interassay variation was 6.7%, and the minimal detectable concentration was 0.3 pg per well.

2.3.2. Heart Rate and Heart Rate Variability

Cardiac beat-to-beat (RR) interval was recorded as described previously [21,23] with a portable recording system (S 810i; Polar, Kempele, Finland) attached to a girth around the thorax of the horse. The positive electrode was positioned on the right side of the withers and the negative electrode on the left side next to the heart base. Water and exploratory gel were used to optimize contact between electrode and skin. A second girth with a pocket for the recording watch was fixed upon the chest belt. During riding, the watch was kept in a pocket fixed in front of the saddle. The RR intervals were recorded continuously from 7:00 AM to 6:00 PM. At the end of each recording period, data from the watches were retrieved via infrared transmission. For statistical analysis and presentation of data, on all days, the first 5 minutes from each hour were taken. In addition, on the day of transfer to individual boxes, 5-minute intervals were analyzed starting at 0, 5, 10, and 30 minutes after mares were brought into individual boxes. On the days horses were exercised, in addition, the total time of exercise was analyzed, divided into 5 minute intervals.

From the RR intervals, HR was calculated. HRV was analyzed with HRV software (Kubios; Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). To remove trend components, data were detrended, and, in addition, an artifact correction was made following established procedures [21,23,24]. The RR interval was recorded, and the HRV variables SDRR interval and RMSSD were calculated. The RMSSD is used to estimate high frequency beat-to-beat variations that represent mainly vagal regulatory activity [8]. The SDRR and RMSSD are expressed in milliseconds (ms).

2.3.3. Locomotion

Locomotion activity and lying time of the mares were recorded with ALT pedometers [25] as described [26]. Pedometers were fixed on a tendon boot placed on one hind leg of the mare. The hind leg was chosen to prevent interaction with front leg activities like pawing and rearing. Recordings were made continuously from 7:00 AM to 6:00 PM. The pedometers count and store times of locomotion and lying for subsequent 15-minute intervals. To check pedometer function, secure recorded data, and remove sand accumulating underneath the tendon boots holding the pedometers, we removed pedometers shortly at 12:15, and data were transferred twice daily to a computer by radio transmission and stored in a database (Access; Microsoft). For analysis, the time windows 7:00-12:00 AM and 1:00-6:00 PM were chosen. Data for locomotion time and lying time are given as percentages of the total 10-hour recording time per day.

2.3.4. Statistical Analysis

Statistical analysis was performed with PASW software (version 17.0; SPSS, Chicago, IL). All data were normally distributed (Kolmogorov-Smirnov test). For comparisons between days, the area under the curve for the respective parameters was calculated, taking into account the sampling or recording intervals, and changes over time were analyzed with a general linear model for repeated measures. In case of overall significant effects, pairwise comparisons between times were performed with Bonferroni correction for multiple comparisons. In addition, correlations among locomotion activity and cortisol concentration and HR and HRV variables were analyzed. A P value of $<.05$ was considered significant. All data are means ± SEM.

3. Results

During the 4 days before mares were moved from group stabling to individual boxes, cortisol concentrations showed a diurnal rhythm with values of approximately 0.6 ng/ml in the morning and a decrease throughout the day. On the day of transfer, cortisol concentrations increased to 1.8 ± 0.2 ng/ml within 30 minutes after mares arrived in individual boxes. In the evening of day 0, cortisol concentrations were still higher than those at same time on any other day of the study. When daily cortisol release was calculated as the area under the curve for the time period from 6:30 AM to 6:30 PM, cortisol release differed significantly between days ($P < .001$), and the most pronounced release occurred on the day of transfer to individual stalls (day 0) and no significant decrease on the days thereafter (Fig. 1).

When mares were kept in group housing with access to a paddock for several hours per day, HR showed slight variations throughout the day, with highest values at the time mares spent in the paddock (Fig. 2). On day 0, HR increased to 103 ± 8 beats/min when the mares were put in individual boxes. Heart rate returned to near baseline values (66 ± 5 beats/min) within approximately 60 minutes (see Fig. 5). On the days after initial stabling in individual boxes, mares' HR showed no changes during the time spent in the stable, that is, on day 2, when mares were not exercised; also no changes in HR occurred (Fig. 2). When the area under the HR curve was calculated for each day, values differed significantly between days ($P < .001$). Individual post hoc comparisons revealed the lowest HR on

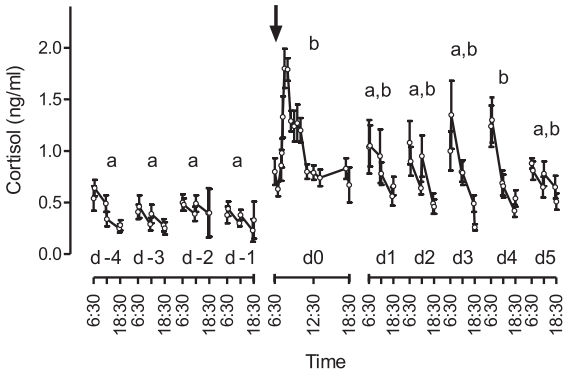

Fig. 1. Salivary cortisol concentrations in mares (n = 8) 4 days before (day 4-day 1), 5 days after (day 1-day 5), and on the day of transfer (day 0) from a group stable with access to a paddock to individual loose boxes (arrow indicates change of stable on day 0). Cortisol release was calculated as area under the curve for the 12-hour time period from 6:30 AM to 6:30 PM and differed significantly between days ($P < .001$). Letters a and b indicate individual differences between days with different letters.

day 2 in individual boxes without any further activity requested from the mares versus all other days ($P < .05$) (Fig. 2).

The HRV variable SDRR tended to change more on the days horses were kept in a group stable with access to a paddock than when mares were stabled in individual boxes. Short-term decreases in SDRR and RMSSD were found immediately after individual stabling (see Fig. 6). SDRR also decreased in association with riding on days 3, 4, and 5 (Fig. 3A). When SDRR and RMSSD, calculated as area under the curve for each day, were compared, no significant differences between days could be demonstrated (Fig. 3).

Locomotion was clearly affected by the transfer of mares from group housing to individual boxes, and analysis over the total study period showed significant changes over time ($P < .001$). Locomotion activity was highest when mares had access to a paddock in the morning, which included the day when they were transferred to individual boxes (day 0). On days 1-5 in individual boxes, total time of locomotion activity was significantly lower than during the

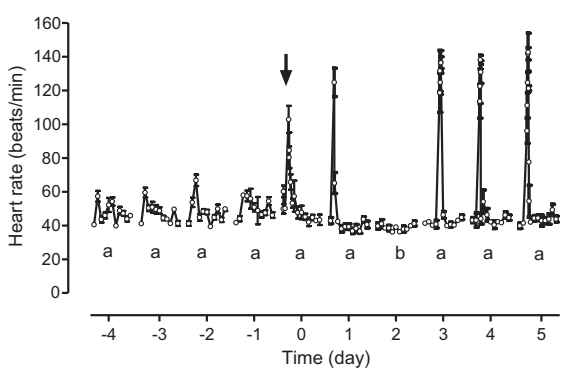

Fig. 2. Heart rate in mares (n = 8) 4 days before, 5 days after, and on the day of transfer (day 0) from a group stable with access to a paddock to individual loose boxes (arrow indicates change of stable on day 0). The area under the curve calculated for the 11-hour time period from 7:00 AM to 6:00 PM of each day differed significantly between days ($P < .001$). Letters a and b indicate individual differences between days with different letters.

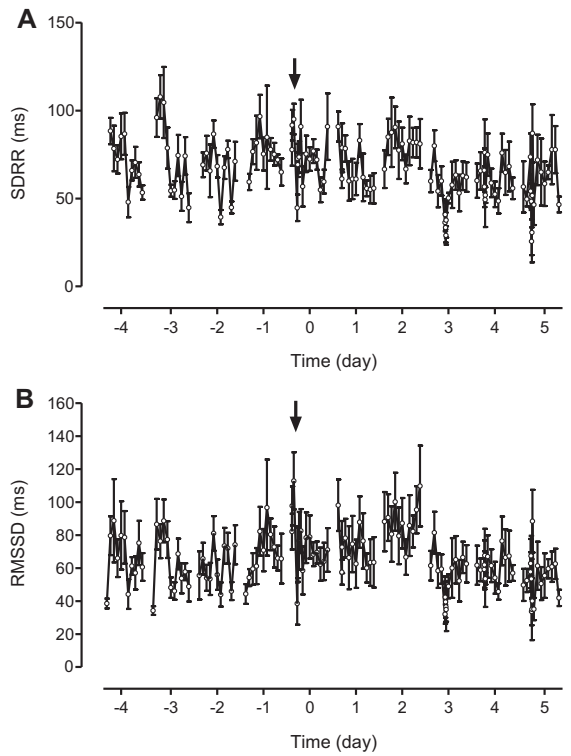

Fig. 3. The HR variables SDRR (standard deviation of RR interval) (**A**) and RMSSD (root mean square of successive RR differences) (**B**) are shown for mares (n = 8) 4 days before, 5 days after, and on the day of transfer (day 0) from a group stable with access to a paddock to individual loose boxes (arrow indicates change of stable on day 0). Neither the SDRR nor RMSSD differed significantly between days in the area under the curve, calculated for the 11-hour time period from 7:00 AM to 6:00 PM of each day.

days mares were kept in a group with access to a paddock ($P < .001$) (Fig. 4). The time mares spent lying down differed significantly for total time per day ($P < .05$) (Fig. 4), but post hoc pairwise comparisons were not significant.

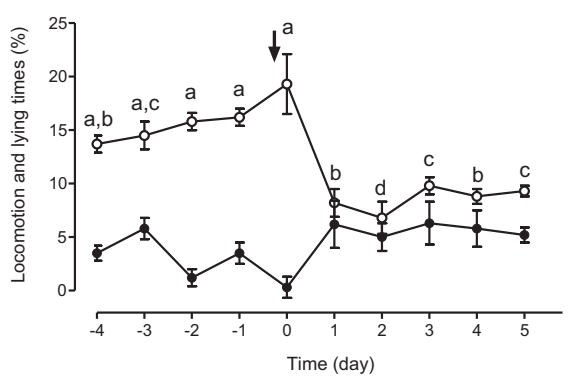

Fig. 4. The time (shown as percentage of a 10-hour daily recording) mares (n = 8) spent moving (○, locomotion) and lying down (●) before and after transfer from a group stable with access to a paddock to individual loose boxes (arrow indicates change of stable on d 0), where day 1 is free movement and jumping and days 3-5 is riding is shown. Times for locomotion and lying down were calculated as areas under the curve for each day that differed significantly between days (locomotion, $P < .001$; lying down, $P < .05$). Letters a–d indicate individual differences between days with different letters.

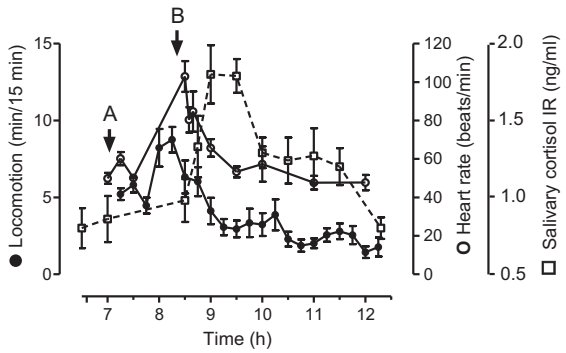

Fig. 5. Changes in locomotion activity (●), heart rate (○), and salivary cortisol IR concentration (□) in mares (n = 8) on the morning of transfer from group housing to individual boxes (A, mares are released from their group stable into a paddock; B, mares are placed into individual boxes after being led from the paddock over a distance of 600 m).

Regardless of the day, significant correlations existed between HR and locomotion activity ($r = 0.296$, $P < .05$) and between the HRV variables SDRR and RMSSD ($r = 0.724$; $P < .001$).

4. Discussion

Separating 3-year-old horses raised in a group until that age and stabling them individually was perceived as stressful by the animals. The stress response involved mainly the pituitary-adrenocortical system but also the sympathetic-adrenomedullary system, leading to prolonged increases in cortisol release and a transient increase in HR.

Immediately after the mares were transferred from the group into individual boxes, salivary cortisol concentrations increased and remained elevated for approximately 6 hours. Although mares had visual contact with horses in adjacent boxes and were thus not totally isolated, the increase in cortisol concentration persisted for longer than in pigs subjected to total isolation [27]. In the mares, until the evening of that day, cortisol concentrations did not decrease to values as in the evenings before, indicating that the diurnal rhythm in cortisol concentrations was transiently disturbed but was re-established within 1 day. An obliteration of the rhythm in cortisol release for several days by even minor perturbations in the horses' environment has been reported previously [28]. Our data are in agreement with behavior observations, indicating less stress-related behavior in stabled horses with access to a paddock than in horses only ridden, without paddock access [29].

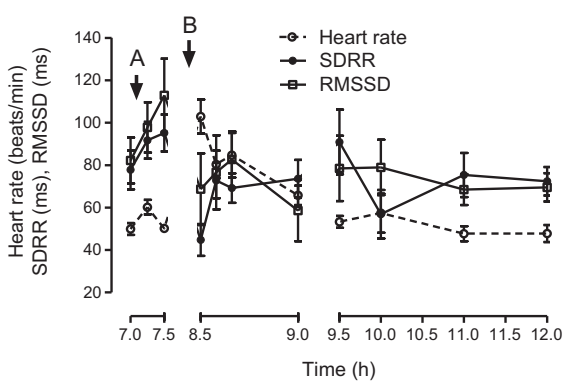

Fig. 6. Changes in heart rate (○) and the HR variables SDRR (●) and RMSSD (□) in mares (n = 8) on the morning of transfer from group housing to individual boxes (A, mares are released from their group stable into a paddock; B, mares are placed into individual boxes after being led from the paddock over a distance of 600 m).

Our results are in agreement with cortisol release in foals at weaning (ie, in another situation, horses were separated from familiar conspecifics) [26]. Using the same analytical method as in the present study, peak salivary cortisol concentrations in foals in response to abrupt weaning [26] approximately doubled the values measured in mares stabled individually for the first time. Peak cortisol concentrations in the current study were also lower than in horses during road transport [21,23,30] but were in the same range as those in ridden horses [9,10,31].

As in weaned foals [26], in mares stabled individually for the first time, a diurnal rhythm in cortisol release was re-established on the day following separation but at a higher level than before separation from the group. This indicates effects beyond the actual day of separation. It is extremely unlikely that the upward shift in cortisol release was caused by equestrian exercise. We recently showed in other young and older horses that cortisol release in response to equestrian training is short lasting, and baseline values are reached again within an hour after dismounting of the rider and return of the horses to their stables [9,10,31]. In addition, mares in the current study had been ridden regularly before the experiment and thus had habituated to training.

In contrast to our results, the study by Harewood and McGowan [12] did not find a diurnal cortisol rhythm in saliva, either before or during isolated stabling in young female horses. However, because of high basal cortisol concentrations, the authors suggested that horses in their study were already stressed in the group stable. Mares in the present study had been together for at least 2 years and formed a stable social group.

Although our data do not show how long the diurnal cortisol release was shifted to a higher level, results are in agreement with previous studies, demonstrating an increase in cortisol concentrations when horses were changed from group housing to individual stalls [13,14,32]. Recently, we were able to show a gradual decrease in cortisol response of young horses to road transport with repeated transportation [30]. After individual stabling of horses coming from a group, it might be beneficial to give the animals time for habituation to their new stable.

The sympathoadrenomedullary stress response of mares to separation into individual boxes was of shorter duration than the increase in cortisol release. Heart rate increased transiently in association with increased locomotion during the time mares spent in the paddock immediately before mares were separated and transferred to individual boxes. An increase in HR in response to individual stabling was not found by Harewood and McGowan [12] but has been reported earlier [33]. No long-term changes in the HRV variables SDDR and RMSSD could be demonstrated. It might be argued that HRV is not a reliable stress indicator. On the other hand, we found consistently

reduced SDRR and RMSSD values in ridden or lunged horses [10,31,34]. Thus, we propose that the lack of changes in HRV can be interpreted as the absence of a major sympathoadrenal stress response.

As expected, HR increased with physical activity when the horses were exercised, and HR and locomotion activity were positively correlated. Increases in HR in association with equestrian exercise were on the same order of magnitude as reported for other young horses [9]. On day 2 after separation, when horses remained in the stable, no increase in HR occurred.

Locomotion activity was higher when mares were kept as a group than when they were stabled individually. In agreement with findings in foals weaned either in single boxes or in groups [35], and also in the 3-year old mares, lying time was increased in individual boxes compared to that in the group stable. Thus, individual stables, although they reduce the opportunity for social interactions, may leave horses undisturbed so that they feel safer to lie down than in a group stable. However, it has also been hypothesized that stalled horses do lie more than horses kept in a group due to boredom and lack of opportunity to carry out other behaviors [35].

The ALT pedometers measured locomotion activity independent from the gait of the horse. They allow a reliable comparison of the number of steps a horse is taking but do not differentiate between walk, trot, and canter. Pedometers thus underestimate the activity of horses that move at higher gaits, either in free exercise or when being ridden [25]. As expected, when horses have access to a paddock in a group, they move more frequently than when kept in individual boxes. With regard to the number of locomotion events, the reduced locomotion activity in the boxes was not fully compensated by equestrian training over 30 minutes. However, as demonstrated by the increases in HR during equestrian training, exercise was a more demanding physical activity. Under natural conditions, horses move mainly in walk [36]. Thus, in agreement with other studies [16], our data suggest that equestrian training may reduce but does not totally fulfill the horses' need for free movement and activity. Moreover, free exercise in addition to equestrian training may help to increase physical fitness in sport horses.

In conclusion, based on physiological parameters, individual stabling of horses previously kept as a group in individual boxes is perceived as a transient stressor. Both separation from familiar conspecifics and reduced space are potentially stressful. Mares kept in individual boxes move less than when kept in a group.

Acknowledgements

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