Hormones and Behavior 58 (2010) 628–636

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses

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ARTICLE INFO

Article history:
Received 15 March 2010
Revised 14 June 2010
Accepted 17 June 2010
Available online 23 June 2010

Keywords:
Horse
Initial training
Cortisol
Heart rate variability

ABSTRACT

Based on cortisol release, a variety of situations to which domestic horses are exposed have been classified as stressors but studies on the stress during equestrian training are limited. In the present study, Warmblood stallions ($n=9$) and mares ($n=7$) were followed through a 9 respective 12-week initial training program in order to determine potentially stressful training steps. Salivary cortisol concentrations, beat-to-beat (RR) interval and heart rate variability (HRV) were determined. The HRV variables standard deviation of the RR interval (SDRR), RMSSD (root mean square of successive RR differences) and the geometric means standard deviation 1 (SD1) and 2 (SD2) were calculated. Nearly each training unit was associated with an increase in salivary cortisol concentrations ($p<0.01$). Cortisol release varied between training units and occasionally was more pronounced in mares than in stallions ($p<0.05$). The RR interval decreased slightly in response to lunging before mounting of the rider. A pronounced decrease occurred when the rider was mounting, but before the horse showed physical activity ($p<0.001$). The HRV variables SDRR, RMSSD and SD1 decreased in response to training and lowest values were reached during mounting of a rider ($p<0.001$). Thereafter RR interval and HRV variables increased again. In contrast, SD2 increased with the beginning of lunging ($p<0.05$) and no changes in response to mounting were detectable. In conclusion, initial training is a stressor for horses. The most pronounced reaction occurred in response to mounting by a rider, a situation resembling a potentially lethal threat under natural conditions.

Introduction

Domestic animals are exposed to a variety of anthropogenic stressors. Interactions between humans and horses have developed over millennia. They are probably more intricate than with any animal species and go far beyond the animals' natural behavioral repertoire. Until the early 20th century, effective interactions between horse and rider have been considered both an art and a military necessity. Riding has always been also a leisure activity and today equestrian sports are a growing recreational activity in many countries. While research in equine exercise physiology has developed science-based programs to improve the physical fitness of equine athletes (Hinchcliff et al., 2008) with regard to the teaching of horses, the theories of classical equitation (e.g. De la Guérinière, 1733; Podhajsky, 1965) so far have not been supplemented to a larger extent by scientific studies. Modern equestrian sports have been criticized for training methods not acceptable under animal welfare aspects. However, scientific studies on the stress experienced by horses during initial equestrian training are limited.

Based on increases in cortisol release, a variety of situations to which domestic horses are regularly exposed have been classified as potential stressors. This includes physical training (Snow and Rose, 1981; Marc et al., 2000), equestrian competitions (Dybdal et al., 1980; Lange et al., 1997; Cayado et al., 2006), transport (Baucus et al., 1990; Clark et al., 1993; Schmidt et al., 2010a; Schmidt et al., 2010b), veterinary examinations (Berghold et al., 2007) and exposure to a new group (Alexander and Irvine, 1998). During short-term stress, glucocorticoids enhance energy mobilisation (Raynaert et al., 1976) and may change behavior (Korte, 2001). While in most studies, cortisol concentrations were determined in plasma, recently techniques to analyse cortisol in equine saliva have been established, avoiding the need of repeated venipuncture (Schmidt et al., 2010a, Schmidt et al., 2010b).

Additional parameters for stress determination are heart rate and heart rate variability. Heart rate variability (HRV), i.e. short-term fluctuations in beat-to-beat (RR) interval, reflects the balance of sympathetic and parasympathetic tone and provides information on the stress response of the autonomic nervous system. Increases in the values of the HRV variables standard deviation of RR interval (SDRR) and root mean square of successive RR differences (RMSSD) reflect a

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doi:10.1016/j.yhbeh.2010.06.011

shift towards parasympathetic dominance, while reduced values indicate a shift towards more sympathetic dominance (Mohr et al., 2002; von Borell et al., 2007).

The training of Warmblood sport horses usually starts when the animals are three years old. In the present study, 3-year-old Warmblood stallions and mares were followed through a 9- and 12-week classical equitation training program from lunging to first mounting by a rider and progressing to moderate work. It was the aim of the study to determine potentially stressful steps during this initial training. We tested the hypothesis that a careful and systematic training allows the horse to adapt to these stressors. Salivary cortisol concentrations, heart rate and HRV were determined repeatedly throughout the training program.

Materials and methods

Animals

For the study, 16 three year-old Warmblood horses of the German Sport Horse breed were available. Animals were mares ($n=7$) and stallions ($n=9$) of the Brandenburg State Stud. All horses had been at the stud either since birth or since weaning. The seven mares were kept in a group stable on straw and had access to an outdoor paddock 2 to 3 h per day. They were fed concentrates and hay twice daily, water was available at all times. Except for routine procedures such as feeding, grooming, hoof care, vaccinations or deworming, mares has not been handled before. The 9 stallions were kept in individual loose boxes on straw and were fed concentrates three times daily and hay twice daily. Water was available at all times. Stallions had no access to a paddock during the experimental period. The stallions had been prepared for stallion licensing during the past 4 months. This preparation included showing at hand, jumping of obstacles without a rider and lunging but not riding or any other equestrian activity.

Experimental procedures

Three-year-old horses were followed through a 12-week (mares) and 9-week (stallions) classical training program from lunging to first mounting of a rider and progressing to moderate work. Due to limited availability of riders it was not possible to synchronise training in the mares and stallions. Because the stallion group was dispersed in week 10 (beginning of the breeding season), the stallions could not be followed after week 9. During the observation period, in both groups training was scheduled from Mondays to Fridays and always during the morning. The training schedules are summarized in Tables 1 and 2. Saliva sampling, recording of beat-to- beat (RR) intervals and HRV analysis were always performed on 2 days per week. In stallions, there was a one-week training break before week 6 (Christmas week) with daily free movement in the riding arena only and no saliva sampling and heart rate recording. The study was approved by the Brandenburg State Ministry for Rural Development, Environment and Consumer Protection (license number 32-2347/5+21#87915/2007).

Salivary cortisol

Salivary samples for cortisol analysis were taken on Tuesday and Thursday of each weak. On these days, 2 samples were collected in the stable at 60 and 30 min before the training unit. Further samples were taken immediately after the training unit (time 0) and at 5, 15, 30, 60, 90, 120 and 180 min thereafter. Samples were collected as described (Schmidt et al. 2010a) with cotton swabs (Salivette, Sarstedt, Nümbrecht-Rommelsdorf, Germany) grasped by use of arterial forceps and placed loosely onto the tongue of the horse for at least 1 min until the swab was well soaked with saliva. All horses tolerated this procedure without resistance. The swab was then placed into the Salivette tube and centrifuged for 10 min at 1000*g*. At least 1 ml saliva per sample was collected and frozen at −20 °C until analysis. Concentrations of cortisol were determined with a direct enzyme immunoassay without extraction (Palme and Möstl, 1997) validated for equine saliva (Schmidt et al., 2009). The antiserum cross-reacts with cortisone and several corticosterone metabolites. Thus values have to be interpreted as immunoreactivity (IR). The intra-assay coefficient of variation was 5.0%, the inter-assay variation was 6.7% and the minimal detectable concentration was 0.3 pg/well.

Table 1
Training schedule for 3-year-old mares ($n=7$), approximate duration of steps is indicated.

Week	Training steps
1	Free movement with snaffle bit in indoor riding arena (10 min) Lunging with snaffle bit in indoor arena (10 min)
2	Free movement with a lunging girth in indoor arena (10 min) Lunging with a lunging girth in indoor arena (10 min)
3	Lunging with side rein and lunging girth in indoor arena (15 min) Free jumping (without rider) in indoor arena (10 min)
4	Lunging with side reins in indoor arena (15 min) Lunging with side rein and saddle in indoor arena (15 min)
5	Lunging with side rein and saddle in indoor arena (15 min) Rider lying over back of the horse after lunging in indoor arena (20 min)
6	After 10 min lunging mounting of a rider in indoor arena and riding for 5 min After 10 min lunging mounting of a rider in indoor arena and riding for 5 min
7	After 10 min lunging, rider mounting and riding for 10 min in indoor arena After free movement for 5 min, riding in group of three horses over 15 min in indoor arena
8	After free movement for 5 min, riding in group of three horses over 15 min in indoor arena After lunging for 5 min, riding in group of three horses over 15 min in indoor arena
9	Lunging with side rein and saddle in indoor arena (15 min) Lunging with side rein in indoor arena (15 min)
10	Lunging with side rein in indoor arena (15 min) Riding in a group in indoor arena (15 min)
11	Riding alone in outdoor arena (20 min) Riding alone in outdoor arena (20 min)
12	Riding alone in outdoor arena (20 min)

Table 2
Training schedule for 3-year old stallions ($n=9$), approximate duration of steps is indicated.

Weeks	Training steps
1	Lunging with side rein and saddle in indoor arena (15 min) Free movement with saddle in indoor arena (10 min)
2	Lunging with side rein and saddle in indoor arena (15 min) Lunging with side rein and saddle in indoor arena (15 min)
3	Rider lying over back of the horse after lunging in indoor arena (20 min) After lunging for 10 min in indoor arena mounting of a rider and riding for 10 min
4	After lunging for 10 min riding in group of three horses for 10 min in indoor arena After lunging for 10 min riding in group of three horses for 10 min in indoor arena
5	After lunging for 10 min riding in group of three horses for 10 min in indoor arena Lunging with side rein in indoor arena (15 min)
6	After lunging for 10 min riding in group of 4–5 horses for 15 min in indoor arena After lunging for 10 min riding in group of 4–5 horses for 15 min in indoor arena
7	Riding alone for 15 min in indoor arena Riding in group of 4–5 horses in indoor arena (20 min)
8	Riding in group of 4–5 horses in indoor arena (30 min) Riding in group of 4–5 horses in indoor arena (30 min)
9	Riding in group of 4–5 horses in indoor arena (40 min) First riding in outdoor arena (30 min)

Heart rate and heart rate variability

Beat-to-beat (RR) intervals and HRV were recorded on Tuesdays and Thursdays continuously from 30 min before to 30 min after the training unit on these days. Recordings were made as described (Schmidt et al., 2010a) with a mobile recording system (S810i, Polar, Kempele, Finland) attached to a girth around the thorax of the horse. The positive electrode was located at the right shoulder and the negative electrode at the mid of the left thorax. Data were recorded and transmitted to a computer for further analysis.

Heart rate variability was analyzed with the Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, and University of Kuopio, Finland). To remove trend components, data were detrended and, in addition, an artifact correction was made as in previous studies on horses (Schmidt et al., 2010a; Schmidt et al., 2010b) following established procedures (Tarvainen et al., 2002; Tarvainen and Niskanen, 2008). From the recorded RR intervals, the HRV variables standard deviation of the RR interval (SDRR), RMSSD (root mean square of successive RR differences) and the geometric means standard deviation 1 (SD1) and 2 (SD2) were calculated. For calculation of the geometric means, the duration of each RR interval is plotted against the duration of the proceeding RR interval (Poincaré plot). In order to parameterize the shape of the plot, the Kubios HRV Software fits an ellipse to the plot. The ellipse is oriented according to the line-of-identity (RRj = RRj + 1) at 45° to the *X*-axis. The standard deviation (SD) of the points perpendicular to the line-of-identity (SD1) describes short term HRV mainly caused by parasympathetic activity. The standard deviation along the line-of-identity (SD2) describes long-term variability (von Borell et al., 2007; Tarvainen and Niskanen, 2008).

HRV variables were analysed for periods of 5 min each: two periods for baseline determination beginning at 30 and 15 min before training, during lunging, 5 min around mounting of a rider on days when horses were ridden, during riding or at the end of lunging on days without riding and two times after training beginning at 5 and 15 min thereafter.

Statistical analysis

For statistical analysis, the SPSS 14.0 statistics program (SPSS, Chicago, Illinois, USA) was used. Changes in salivary cortisol concentrations, fecal cortisol metabolites and heart rate variables over time were analyzed for each day of training by ANOVA using a general linear model for repeated measures. In addition, the deviation from the mean pre-training baseline was calculated as the area under the curve (AUC) for the actual training time and AUC values between training units were compared by repeated measures ANOVA within groups (stallions and mares). In case of overall significant effects, training units differing from the day of first mounting of a rider were identified by testing for least significant differences. AUC values from comparable training units at different times throughout the training program were compared between mares and stallions by analysis of variance. For training units when the horses were ridden, correlations between salivary cortisol concentrations immediately after the training unit and RR interval and HRV variables during mounting of the horses and during the last (riding) part of the training unit were calculated. For all statistical comparisons, a p-value <0.05 was considered significant. Data are given as mean ± standard error of mean (SEM).

Results

Salivary cortisol

Cortisol concentrations in saliva were determined for two training units per week. Each training unit except the last training in week 12 in mares and one training unit in week 7 in stallions was associated with a significant ($p<0.05$) increase in salivary cortisol concentrations from baseline values below 1 ng/ml to highest values between 2 and 3 ng/ml. Highest cortisol concentrations were reached between 5 and 15 min after the end of training. Cortisol concentrations returned to baseline values within approximately 1 h after training. As an example, cortisol concentrations in response to first mounting of a rider are given in Fig. 1a and b for mares and stallions, respectively, and data for the whole study period are summarized in Figs. 2a and 4a. Baseline cortisol values did not change throughout the 12- and 9-week training program in mares and stallions, respectively. In mares, cortisol release in response to individual training units calculated as area under the curve was compared to the day of first mounting of a rider and cortisol AUC values were significantly ($p<0.05$) lower at two occasions before that day and at four occasions thereafter (Fig. 1a). Out of these four training units two were associated with lunging and not riding (Fig. 2a). In stallions, differences in cortisol release compared to the day of first mounting were less evident (Fig. 4a). Cortisol release calculated as AUC did not differ between mares and stallions at first mounting but was more pronounced in mares than in stallions for several training units thereafter ($p<0.05$; Table 3).

Heart rate and heart rate variability

The basal RR interval recorded 30 and 15 min before training for 5 min each was around 1500 ms, corresponding to a heart rate of approximately 40 beats/min and neither in mares nor in stallions changed significantly throughout the study. The RR interval decreased significantly during each training unit, corresponding to an increase in heart rate and this response was evident throughout the study period ($p<0.05$ except one training unit in week 7; Figs. 1c, 1d, 2a, 4a). When horses were ridden, the RR interval decreased already in response to lunging before mounting of the rider. A further decrease occurred at the time when the rider was mounting, i.e. with the horse not moving and not showing physical activity. When the young horses moved forward in walk and trot with the rider on their back, the RR interval at most times increased again, corresponding to a decrease in heart rate (Figs. 1c, 1d, 2a, 4a). The decrease in RR interval when the rider was mounting the standing horse was evident in mares and stallions but was more pronounced in mares than in stallions for 3 training units ($p<0.05$, decrease in RR interval calculated as AUC versus pre-training baseline; Table 3). After training, the RR interval returned to baseline values within approximately 10 min.

The HRV variable SDRR showed a significant decrease with each training unit. The SDRR started to decrease when the horses were lunged before riding but decreased further at the time when the riders were mounting the young horses. Changes over time in SDRR reached statistical significance ($p<0.05$) for mares in all training units except for 3 training days in weeks 1, 2 and 10. In these 3 training units horses were lunged and not ridden (Figs. 2c and 4c). In stallions, a significant decrease in SDRR was seen with every training unit ($p<0.05$; Fig. 4c). Only at one time the decrease in RR interval was significantly more pronounced in mares than in stallions ($p<0.01$; Table 3). The HRV variable RMSSD also decreased in response to each training unit in mares as well as in stallions. This decrease was significant at all times ($p<0.05$) except for one training unit in mares (week 10, lunging, Figs. 2d and 4d). Lowest values were reached either with continuous lunging or during the period of mounting by a rider. During riding itself, RMSSD showed no further decrease and on most days already increased again. After training units, RMSSD values rapidly returned to baseline values (Figs. 2d and 4d). The RMSSD response to mounting and subsequent riding differed between mares and stallions on two days ($p<0.05$; Table 3).

Geometric variables SD1 and SD2 showed a differential response to training. Changes in SD1 closely resembled RMSSD with a decrease during lunging, a further decrease during mounting by the rider and a

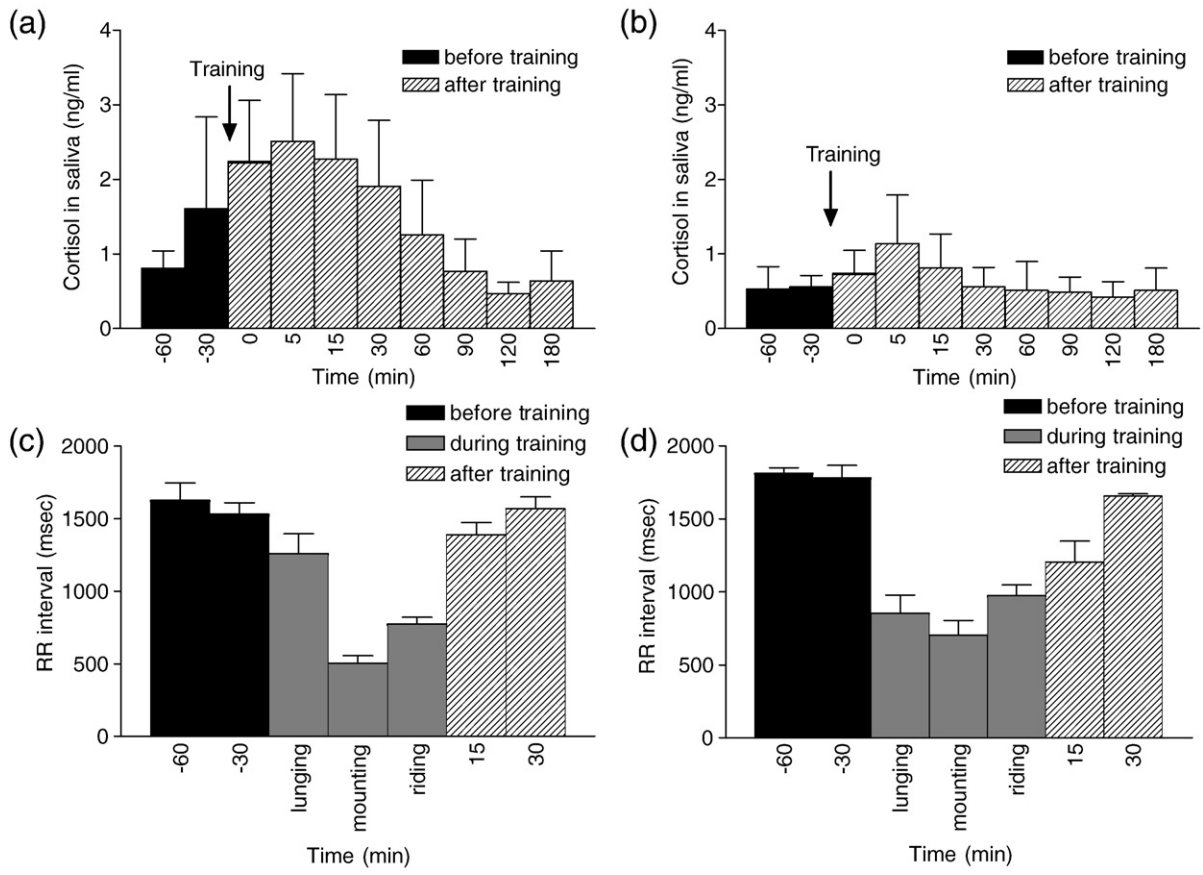

Fig. 1. Salivary cortisol concentrations in (a) mares and (b) stallions and RR interval in (c) mares and (d) stallions in response to training with first mounting of a rider. Post-training sampling of saliva began immediately after the riding period.

slight increase during the subsequent period of riding (Figs. 3a and 5a). The SD 1 increased further after training, but on most days, during the recovery period remained below the pre-training baseline. The decrease in SD1 in response to training calculated as area under the curve was more pronounced in mares than in stallions in response to 3 training units ($p<0.05$; Table 3). In contrast to SD1, SD2 increased markedly at the beginning of each training unit, i.e. with the beginning of lunging. Values of SD2 decreased rapidly thereafter and were at or below the pre-training baseline during the next recording periods. No changes in response to mounting by the rider were detectable. As for SD1, also SD2 values were below the pre-training baseline during the recovery period after training (Figs. 3b and 5b). The SD2 response was more pronounced in mares than in stallions only in response to one training unit ($p<0.01$; Table 3).

Salivary cortisol IR concentration immediately after training (time 0) was significantly correlated neither with the RR interval nor with HRV variables obtained during mounting of the horse by a rider. In contrast, salivary cortisol IR at time 0 was loosely but significantly correlated with the RR interval ($r=-0.209$, $p<0.01$) and the HRV variables RMSSD ($r=-0.151$, $p<0.05$), SD1 ($r=-0.153$, $p<0.05$) and SD2 ($r=-0.344$, $p<0.001$) but not SDRR obtained during the riding phase of the training unit, i.e. the phase directly preceding saliva collection immediately after riding.

Discussion

The initial training of young horses caused changes in cortisol release, RR interval and heart rate variability. Together these changes indicate stress in the animals (Kiley-Worthington, 1990). The decreased RR interval, i.e. increase in heart rate, and increased cortisol release are in part also caused by the physical activity of the animals when being lunged and ridden and our data do not allow differentiating at all times between stress and physical activity.

The lowest RR interval and lowest values for the HRV variables SDRR, RMSSD and SD1 were found when the riders were mounting the young horses. During these times, the horses did not show physical activity such as during lunging or when being ridden. Thus, reduced RR intervals and HRV in horses associated with mounting by a rider is indicative of stress. An increase in heart rate, i.e. a decrease in RR interval, indicates increased sympathetic activity, decreased parasympathetic activity or a combination of both. Stress in horses (Visser et al., 2002) or calves (Mohr et al., 2002) leads to a reduction in variability of the RR interval. RMSSD and SD1 are the primary HRV variables used to estimate high frequency beat-to-beat variations that represent parasympathetic activity (von Borell et al., 2007). The decrease in RMSSD and SD1 thus reflects a markedly reduced parasympathetic tone in reaction to mounting by the rider. Geometric analysis also showed significant changes for SD2, representing long-term changes in HRV that are predominantly caused by changes in sympathetic regulation (von Borell et al., 2007). In horses of all groups, the beginning of each training unit was associated with a short, but marked rise in SD2, i.e. a transient activation of the sympathetic branch of the autonomous systems. At rest, horses have a high parasympathetic (vagal) tone and sympathetic activity plays little role in determining heart rate (Hamlin et al., 1972; Kuwahara et al., 1996). The onset of each training unit might be associated with transient sympathetic activity which returns to normal while vagal activity was further reduced with mounting by a rider. Activation of the sympathetic branch of the autonomous nervous system and decreased parasympathetic tone are the body's immediate physiological reaction to perceived danger (Korte, 2001). Such physiological parameters have rarely been determined in studies on riding-associated stress in horses so far.

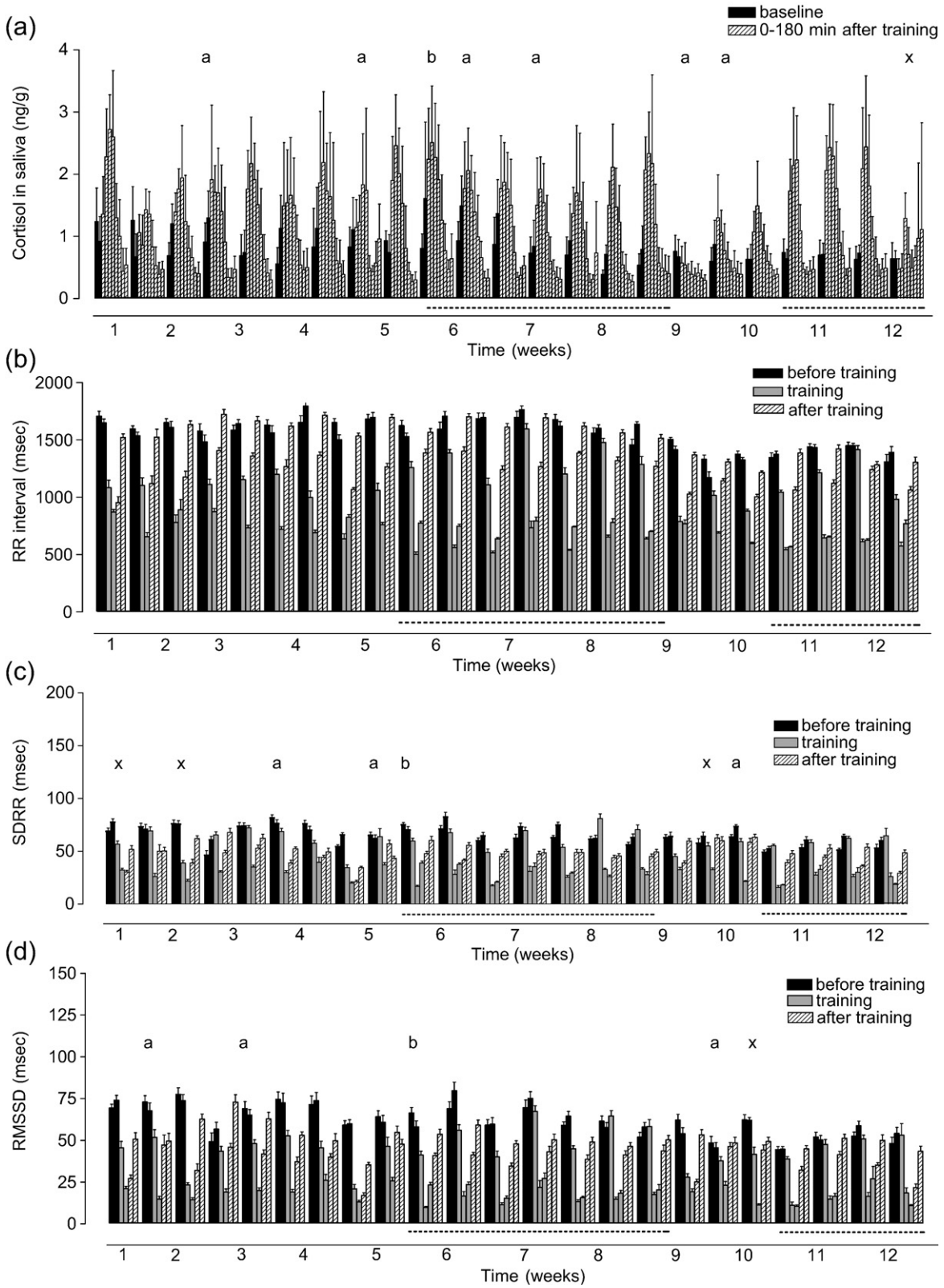

Fig. 2. (a) Salivary cortisol concentrations, (b) RR interval, (c) SDRR and (d) RMSSD in response to individual training units throughout a 12-week training program in mares ($n=7$). Blocks of columns represent individual training units and two training units per week were analysed. Dashed line indicates days when horses were ridden, on the other days training occurred without a rider. Significant ($p<0.05$) changes for all individual training units except those marked with x. a, b: AUC values marked with "a" differ significantly from the day of first mounting of a rider (marked "b"; $p<0.05$).

Mounting was made as carefully as possible with one person holding the head of the horse and another person assisting the rider first to lay over the back of the horse and then to move into an upright position in the saddle. All persons involved were professional riders experienced in the training of young horses. While most pronounced changes in RR interval and HRV variables were found when the rider

Table 3
Significant differences ($p<0.05$) in salivary cortisol concentrations, RR interval and HRV variables calculated as area under the curve for comparable training units between mares and stallions (1st and 2nd lunging and 1st to 11th riding with cortisol and HRV analysis; see also Figs. 2 to 5, riding includes training units in weeks 6–9, 11 and 12 in mares and in weeks 3–8 in stallions).

	Lunging		Riding										
	1	2	1	2	3	4	5	6	7	8	9	10	11
Cortisol	–	–	–	–	–	–	–	**	*	–	**	*	–
RR interval	–	–	–	–	–	–	–	–	–	*	*	***	–
SDRR	–	–	–	–	**	–	–	–	–	–	–	**	–
RMSSD	–	–	–	–	–	–	–	–	–	*	–	*	–
SD1	–	–	–	–	–	–	–	–	–	*	*	*	–
SD2	–	–	–	–	–	–	–	**	–	–	–	–	–

*$p<0.05$; **$p<0.01$; ***$p<0.001$; –, non significant.

was mounting the horse, these variables either changed or tended to change towards normal when horses were requested to move with the rider. If activity-induced changes were predominant at that time, a further reduction in RR interval and HRV should have occurred. Thus, after the initial stress of being mounted, the young horses adapted to the new situation. Moving with the rider on their back might contribute to a rapid decrease of the stress response. In men, acute physical exercise at moderate level reduced anxiety (Garvin et al., 1997) and in women was associated with an improvement in affect and feeling state (Gauvin et al., 1996). In a rodent model, stress-induced hypertension is attenuated by exercise (Mills and Ward, 1986) and comparable mechanisms might be activated in young horses. It would have been interesting to keep the animals motionless for a longer time with the rider mounted and determine HRV continuously during that period. However, this was not considered because it might have been perceived as an additional immobilisation stress (Forkman et al., 2007) provoking aversive behavior of the horses.

The presence of a rider on its back and outside the vision field of the horse (Saslow, 2002), resembles a predator in the same position and thus a situation extremely dangerous for horses under wildlife conditions. As domestic horses in response to appropriate stimuli express the same behavioral repertoire as wild horses (Christensen et al., 2008; Christensen and Rundgren, 2008) this can be expected to evoke a flight reaction which is suppressed in ridden horses. The requirement of tolerating a potential predator on its back is probably the most profound intervention undertaken in horses and has been considered an activity for which no naturally occurring analogue exists (McGreevy et al., 2009).

Once the young horse has adapted to the presence of the rider on its back, the next steps of training request predominantly natural behavior patterns. The horse is expected to regain its balance under the rider and to move in walk, trot and gallop. As long as this is trained in a systematic and stepwise program it will be more a physical demand than an anthropogenic stressor. Our data underline that mounting of the rider as one of the first steps in equestrian training should be performed with care. It is generally accepted that the horse is a species with good memory (reviewed by Nicol, 2002; Murphy and Arkins, 2007). Experience during handling and training affects the attitude of horses towards humans either positively or negatively (Henry et al., 2006) and a negative experience may erase previous positive ones (Fureix et al., 2009). Negative experience with the initial steps of riding may thus seriously endanger the future equestrian career of a young horse.

The reduction in RR interval and HRV variables associated with mounting of a rider was evident in mares and in stallions but especially after the first weeks of riding was most pronounced in mares. This might

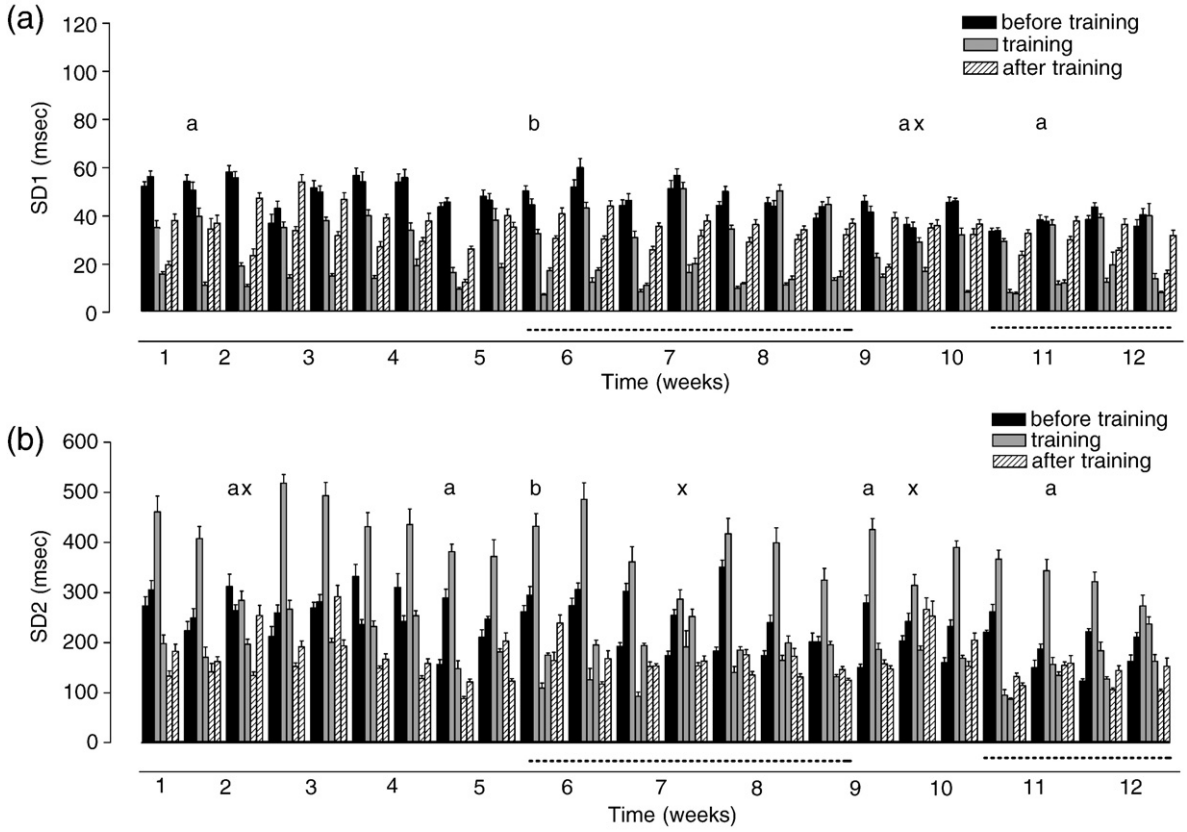

Fig. 3. Geometric mean HRV variables (a) SD1 and (b) SD2 in response to individual training units throughout a 12-week training program in mares ($n=7$). Blocks of columns represent individual training units and two training units per week were analysed. Dashed line indicates days when horses were ridden, on the other days training occurred without a rider. Significant ($p<0.05$) changes for all individual training units except those marked with x. a, b: AUC values marked with "a" differ significantly from the day of first mounting of a rider (marked "b"; $p<0.05$).

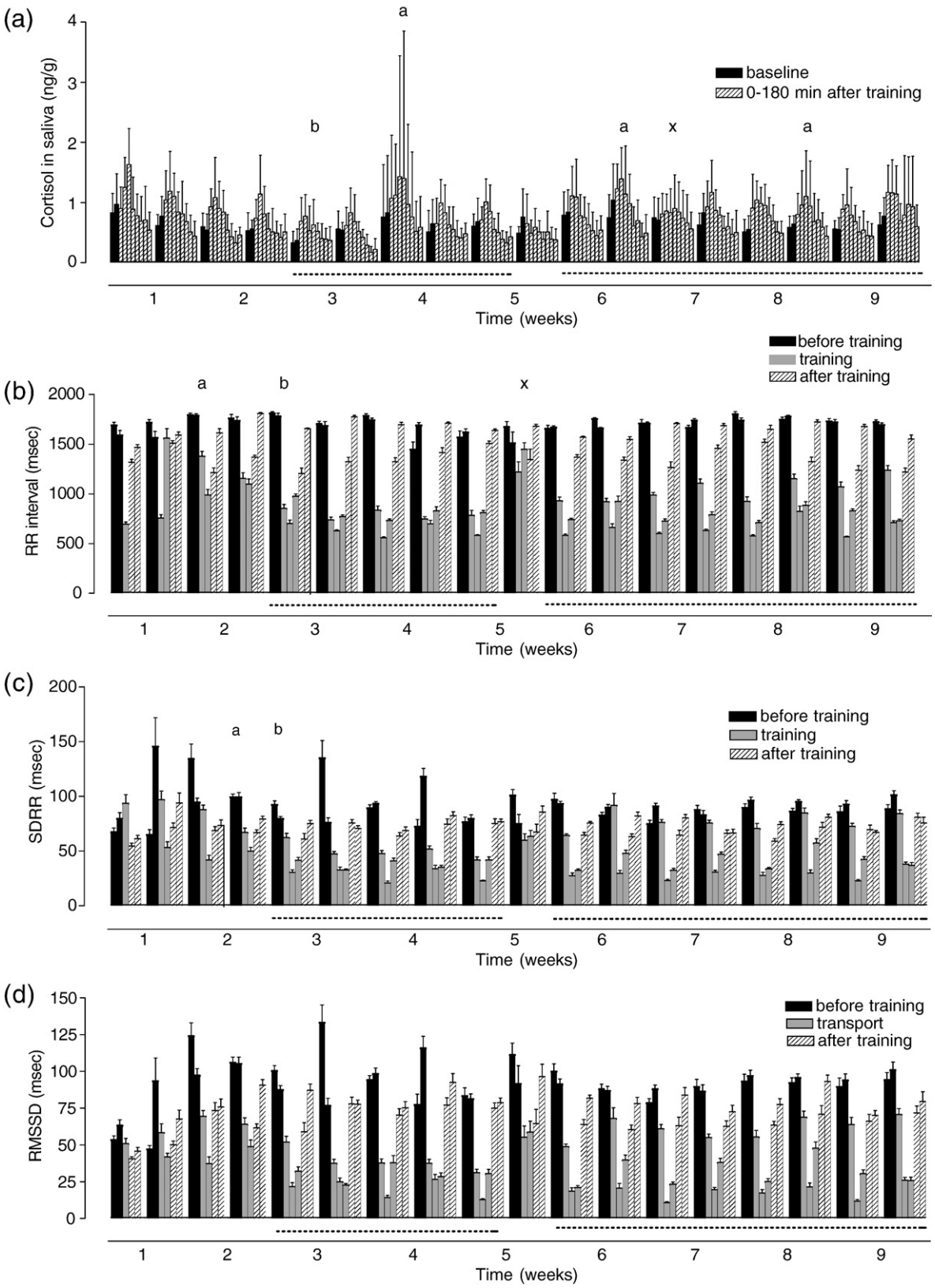

Fig. 4. (a) Salivary cortisol concentrations, (b) RR interval, (c) SDRR and (d) RMSSD in response to individual training units throughout a 9-week training program in stallions ($n=9$). Blocks of columns represent individual training units and two training units per week were analysed. Dashed line indicates days when horses were ridden, on the other days training occurred without a rider. Significant ($p<0.05$) changes for all individual training units except those marked with x. a, b: AUC values marked with "a" differ significantly from the day of first mounting of a rider (marked "b"; $p<0.05$).

either be caused by the different handling experience of mares and stallions or could be due to sex differences with a less pronounced stress response in stallions. Although stallions were not ridden before the experiment, they had been prepared for stallion licensing which included daily grooming and handling for a period of 4 months. Training horses in-hand, i.e. from the ground before riding has been suggested as

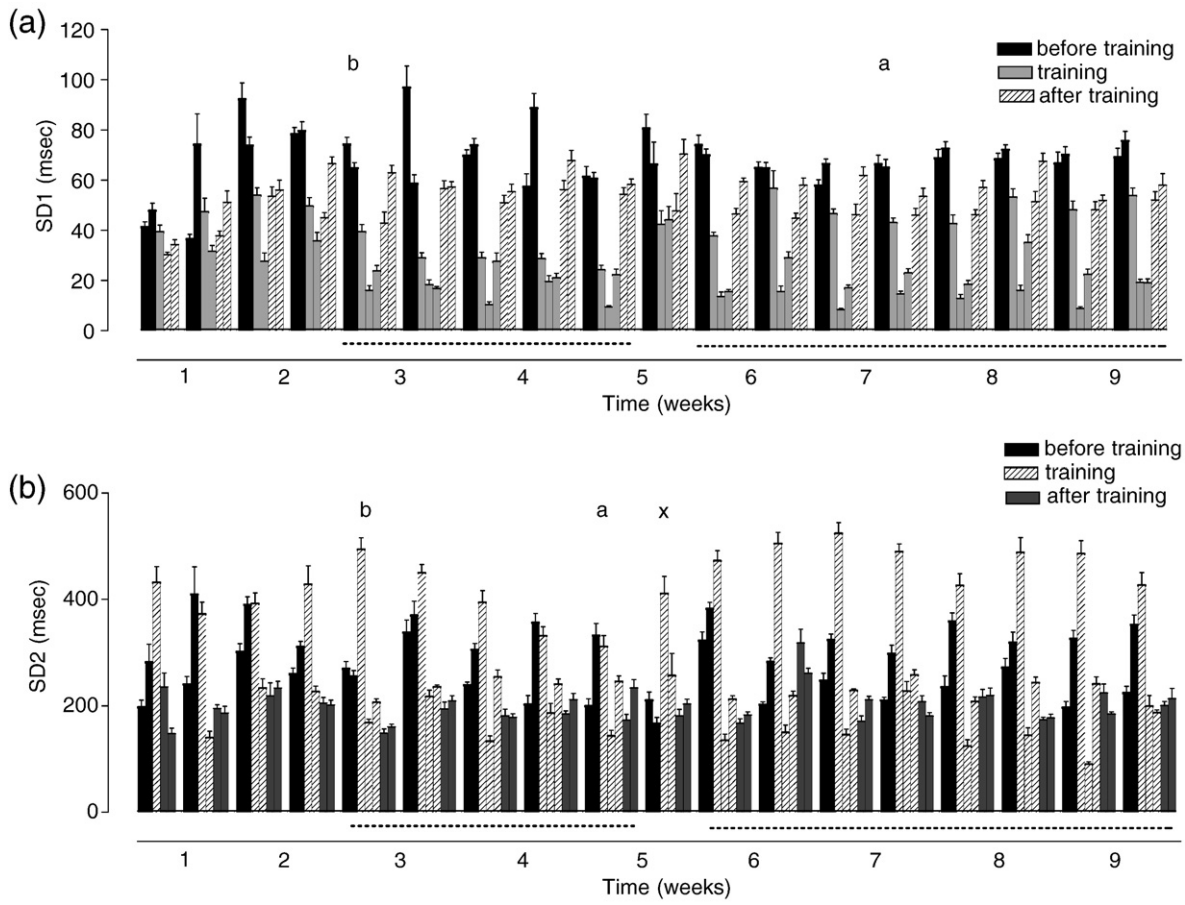

Fig. 5. Geometric mean HRV variables (a) SD1 and (b) SD2 in response to individual training units throughout a 12-week training program in stallions ($n=9$). Blocks of columns represent individual training units and two training units per week were analysed. Dashed line indicates days when horses were ridden, on the other days training occurred without a rider. Significant ($p<0.05$) changes for all individual training units except those marked with x. a, b: AUC values marked with "a" differ significantly from the day of first mounting of a rider (marked "b"; $p<0.05$).

useful to prepare them for being ridden. Achieving some sort of control over the horse in-hand seems to facilitate control under the rider and may help the horse to overcome fear when being mounted presumably through a process of generalisation (McGreevy and McLean, 2007; McGreevy et al., 2009). Sex differences in the response to stressors have been reported and depend on the species investigated. Corticosterone release in response to certain stressors was more pronounced in female than in males rats (Heinsbroek et al., 1991) and free cortisol increased more in female than in male rats exercised on a treadmill (Brown et al., 2007). In contrast, in humans, a more pronounced response to psychological stress was found in men compared to women (Collins and Frankenhauser, 1978; Kirschbaum et al., 1992). Differences in the stress response may also exist between mares and stallions, however, our study protocol did not allow exactly the same training protocol in the two groups. Thus, although both groups could be followed individually, they are not directly comparable at all times.

Stallions and mares in our study also differed with regard to stabling. However, based on behavioral but not physiological data it has been suggested that pasture-kept or group-stabled horses adapt more easily to training (Rivera et al., 2002) and showed less stress-related behavior (Visser et al., 2008) than stalled horses. In contrast, in our study, group-housed mares showed a slightly more pronounced stress response than the individually housed stallions. Therefore, although effects of stabling in our study cannot be excluded, they were less evident than effects of the different handling experience in mares and stallions.

Each training unit was associated with an increase in cortisol release determined by salivary cortisol concentrations. Changes in salivary cortisol concentrations paralleled changes in heart rate and increased with each training unit and decreased rapidly thereafter. Highest cortisol concentrations in saliva were found shortly after training, indicating a slight delay compared to cortisol release into plasma (Schmidt et al., 2009). As for changes in RR interval, also cortisol release might be caused in part by the physical activity of the horses and independent from any stress response. Cortisol release occurred together with changes in HRV and thus can be considered at least in part stress-induced. Cortisol concentrations in samples obtained immediately after riding were loosely, but significantly correlated with RR interval and with all HRV variables except SDRR. A closer correlation might have been found in saliva samples obtained directly during riding, however saliva sampling was not possible in moving horses. Cortisol concentrations differed between individual training units. When horses were lunged only on individual days during the later stages of the training program, i.e. after having been ridden several times, cortisol release in response to lunging was low, indicating habituation to training during the observation period. Because cortisol concentrations decline rapidly after any transient cortisol release and initial increase (Schmidt et al. 2010a), the sampling regime does not allow us to link cortisol release with specific steps of individual training units such as mounting the horse. Overall, salivary cortisol concentrations reached in response to training were markedly lower than concentrations determined with the same analytical techniques in horses during road transport. Peak cortisol concentrations in response to transport were 3- to 4-fold higher (Schmidt et al., 2010a; Schmidt et al., 2010b) than in response to equestrian training in the current study. Thus, although riding and training are clearly associated with an increase in hypothalamo-adrenocortical activity, any stress response

was less pronounced than the response to transport. Interestingly, training-induced salivary cortisol concentrations were higher in mares than in stallions. As for RR interval and HRV, this difference can be due to sex differences or attributed to the different handling experience of the two groups of animals prior to initial equestrian training.

In conclusion, the initial training of young horses following the system of classical European equitation is a stress challenge for the animals but less stressful than other anthropogenic stressors to which domestic horses are exposed on a regular basis. The most pronounced stress reaction occurred in response to the horses being mounted by a rider, a situation resembling an imminent and potentially lethal threat under natural conditions. Our results strengthen the importance of a careful approach taking into account the natural behavior repertoire of the animal when training young horses for equestrian sports.

Acknowledgments

The authors are grateful to the team of Neustadt State Stud for support with the horses and to Stiftung Forschung für das Pferd, the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management and the German Equestrian Federation for financial support. The expert assistance of Edith Klobetz-Rassam and Julia Maderner with the laboratory analysis is kindly acknowledged.

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