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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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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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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)