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Sex-related effect of early training on stress in young trotters as expressed by heart rate

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The study explored the hypothesis that mares, which are more sensitive than stallions, react to stress with higher increase in heart rate (HR).
A group was studied of 101 clinically healthy Standardbred trotters aged 11-18 months, during their daily training routine. The horses were divided into two groups according to sex (51 colts and 50 fillies). All the horses took part in a standard training session consisting of harnessing to the sulky, moving from the stable and a 45 minutes exercise on a sand track. Before the start of training session a belt with a transmitter for telemetric HR registration was placed on each horse.
The measured HR did not show any differences in HR between colts and fillies either at rest, during handling and harnessing as well as during exercise and after it.

KEY WORDS: heart rate / horses / sex / stress / training

Telemetric measurement of heart rate (HR) is a good non-invasive technique which is used to estimate the functioning of the autonomic nervous system. Over the past decades, HR measuring has been used in horse research to analyse the changes in sympathovagal balance related to diseases, and psychological as well as environmental stressors [Rietmann *et al*. 2004, Terkelsen *et al*. 2005, Kingston *et al*. 2006]. It was also used to evaluate the individual characteristics of horse temperament [McCann *et al*. 1988]. Generally, exposition of horses to novel objects or situations increases

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HR [McCann *et al*. 1988, Jezierski and Górecka 2000]. Habituation to fear-eliciting situations, however, lowers the HR and weakens fear reactions in horses [Shanahan 2003, Christensen *et al*. 2008]. The effect of the estrous cycle on the HR response of mares was also studied, indicating that mares in dioestrous were more receptive to the studied situations than those in estrous [Górecka *et al.* 2004, Hedberg *et al*. 2005].

Standardbred trotters begin training when they are less than a year old, *i.e*. are still growing. A training schedule (including handling, harnessing, transient social isolation and work load) can cause emotional arousal in horses, as demonstrated by HR increase [Reynolds *et al*. 1993]. The HR during the handling test in 12- and 18-month old horses was found to be affected also by sex [Jezierski *et al*. 1999]. The difference in HR between the stallions and mares was also observed during the first phase of race training in Purebred Arabian horses (own data, not published). Moreover, sex has an impact on the career longevity in race horses, with mares having a significantly lower indicator of survival probability than stallions [Sobczyńska 2007].

In humans, the dominant sympathetic nervous activity in males and predominance of parasympathetic nervous activity in females causes the sex-related differences in HR in response to the stress [Sato and Miyake 2004]. Gender-specific sympathetic responses to mental stress were observed in human neonates and adults [Davis and Emory 1995, Ross *et* al. 2001, Schmaus *et* al. 2008]. Estrogens acting directly in the brain decrease the stress response [Takuma *et al*. 2007] while testosterone increases aggressive behaviour and cardiac response to stressors [van Honk *et al*. 2001]. Also, the influence of sex steroid hormones on HR in response to psychosocial stress is well known in humans, but was less extensively studied in horses.

The problem of the influence of sex on physiological reaction to stress in worm-blood horses, is especially worth noting. Mares and stallions often take part in the same competitions or races, regardless of sex. The aim of this study was to compare the effect of stress related to early training on HR in young Standardbred between mares and stallions. The hypothesis was developed that mares, which are more sensitive than stallions, react with higher increase in HR in response to stress.

Material and methods

Horses

A group was considered of 101 clinically healthy, young Standardbreds gathered in one of the trotter training centres in Poland, and aging from 11 to 18 months. The horses had been at the centre for about 4 months before the start of this study. They were observed during the preparation for their training session and during exercise. The training session was part of the usual training routine. The type and course of training were in accordance with the rules developed by the trainer preparing the studied group of horses for races. All horses were exercised five days a week. On the day of training, they were individually moved from their stables to the boxes in the harness stable. Next, they were handled, moved to the corridor and harnessed to the sulky. Lastly, they left the harness stable and started working on the training track. The heart rate (HR) was recorded in two periods: (1) over three consecutive days in December, when the horses were at the age of 11-months, and (2) over four consecutive days in July, when the horses were at the age of 18-months. The horses were divided into two sex groups: 51 colts and 50 fillies.

Training Session Protocol

During the investigation, all horses took part in a standard exercise. The exercise consisted of a 45 min trot on a sand track while the speed was increased from 15 to 30 km/h. Before the horses start the training session, an elastic belt with a transmitter for telemetric HR registration (POLAR OY ELECTRO, Finland) was put around the chest of each horse. The other sensor was installed on sulky for speed measuring (POLAR S725 with speed sensor). After activation of the telemetric set the horses were left alone in the boxes for about 5 min to achieve the resting values of HR. Then the horses were moved from the boxes and prepared to exercise in a manner described above. For all the subjects, HR was continuously measured in 5 s intervals. To identify the beginning and the end of handling, moving, harnessing and starting work, the drivers had to press the lap bottom of the telemeter on starting and terminating the following activity, thus to identify the area of analysis. The data were downloaded from transmitter to computer using interface.

To evaluate the sexual maturity of 11-months old trotters, the blood samples from the jugular vein *via* venipuncture were withdrawn from randomly selected 10 colts and 10 fillies to determine the levels of testosterone (T) and 17β-estradiol (E). The fillies did not show any external oestrus symptoms. The blood was withdrawn after the end of HR measuring. Samples were collected in tubes containing EDTA and immediately centrifuged for the plasma separation. The plasma samples were stored at -20°C until further analysis.

Analytical

The Polar Precision Performance programme was used to analyse the obtained HR and speed data. The levels of plasma T and E were determined using the radioimmunoassay (RIA) kits (Diagnostic Systems Laboratories, WEBSTER, USA) and expressed as ng/ml and pg/ml, respectively.

Statistical

The results were presented as means with standard deviations (SD). Comparisons between the groups of horses were done using ANOVA and the Tukey's t-test (Statistica 6.0). The statistical significance was accepted at the level of $P \leq 0.05$.

Results and discussion

HR in colts and fillies are shown in Figure 1. The HR at rest, during handling and harnessing as well as during exercise within the range of speed from 15 to 20 km/h and after termination of trot is presented. The authors decided to neglect the results obtained during the work at the speed higher than 20 km/h because, under those conditions, the predominance of physical over mental stress can occur. Data were compared considering only the sex as a factor of analysis. No significant differences were observed between HR obtained in fillies and colts at rest (means = 34.2±2.82 *vs*. 35.0±4.37, respectively) and during following steps of training session, e.g. during harnessing, leaving the stable, walking and trotting with a speed of 15 km/h (means = 42.3±13.4 *vs*. 48.3±16.3, 71.5±16.1 *vs*. 73.4±15,1, 81.2±9,71 *vs*. 77.5±10.6 and 99.6±6.82 *vs*. 97.2±8.11, respectively). The low standard deviations indicate that individual HR variation was really non-significant. The original HR graph for two individual colts investigated in this study is shown in Figure 2.

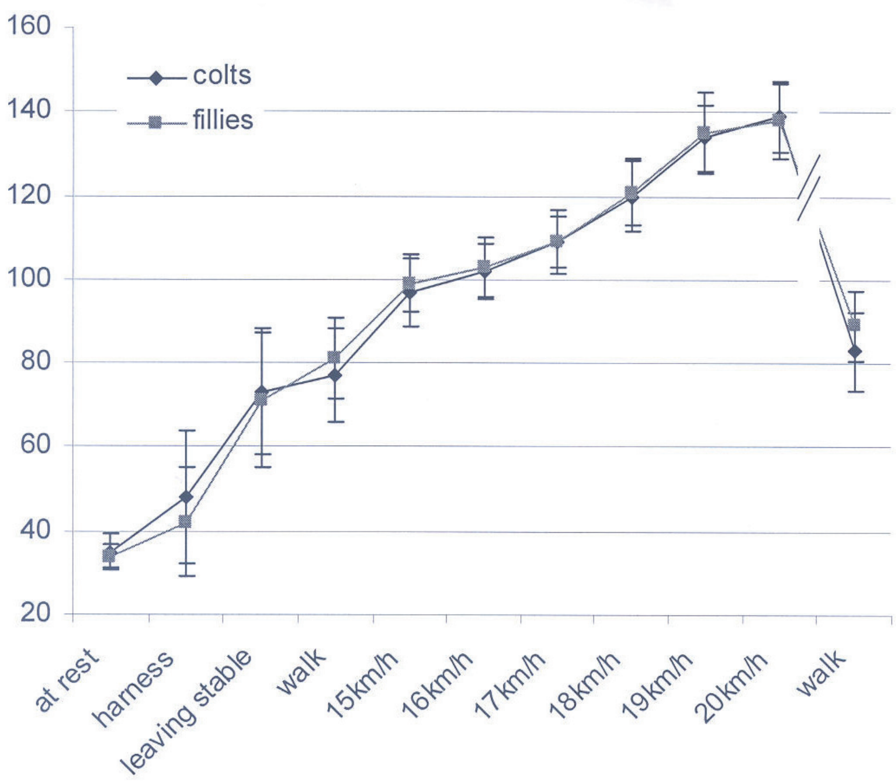

Fig. 1. Heart rate in Standardbred colts and fillies during following phases of daily training routine (means with SD).

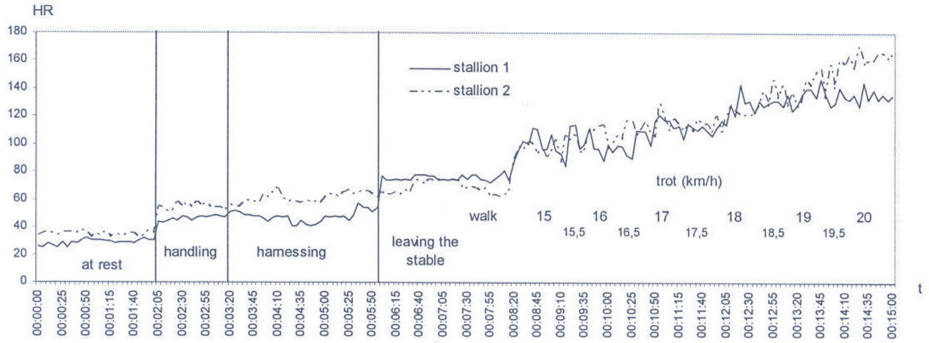

Fig. 2. The copies of HR graphs in two stallions with extremely different results of measurement.

Unexpectedly the HR did not show any differences between colts and fillies either at rest, during handling and harnessing or during exercise and after it. This was contrary to authors' earlier observations (unpublished) showing that Purebred Arabian mares had a higher HR than stallions of the same breed during the first phase of training. Physical activity is an important HR-increasing factor, but HR is related also to emotional factors. Jezierski and Górecka [2000] reported that a social isolation in a stable caused a significant increase in HR in adult horses. HR increased also just before the start of a competition as a result of the psychogenic response to stress [Reynolds *et al*. 1993]. In race horses, only the saddling before training stimulated the sympathoadrenal axis [Podolak *et al.* 2006]. In the study by McCann *et al*. [1988], yearlings classified as "nervous" on the basis of their behaviour showed higher HR during an emotionality scoring procedure than "normal" ones. Moreover, the presence of their handler reduced the HR in horses when exposed to a novel object [Górecka *et al*. 2007]. The stage of oestrus cycle did not affect the mare's HR response during mental tests [Hedberg *et al*. 2005]. However, the HR measured during a routine oestrus control was found to be associated with the physiological status of the mare [Górecka *et al.* 2004].

In humans, sex influenced the HR during applied exercise tests in a number of studies [e.g. Balady *et al*. 1990, Fuster *et al*. 2008]. Also during mental stress HR was higher in females than in males [McAdoo *et al*. 1990, Kilgour and Carvalho 1994]. However, Matsuo *et al*. [2003] showed that HR response to exercise was not affected by the gender or menstrual cycle. Moreover, in the study by Khaksari *et al*. [2005], the increase in HR under stress was higher in males than in females.

HR depends on the activity of both the sympathetic and parasympathetic nervous system. A sympathetic predominance increases HR whereas increased vagal activity leads to HR reduction. Interestingly, Sato and Miyake [2004] reported that during subjective mental workload, the sympathetic nervous activity of females appeared less dominant than that of males.

The exact mechanism by which gonadal steroids might affect the HR response to different stressors is not fully understood. Estrogens initiate the nervous cell action by binding to intracellular receptors. They then act not only on gene expression, but they also influence neuronal activity *via* quick interaction with intracellular metabotropic receptors in the medial preoptic nucleus, what regulates sexual behaviour [Dewing *et al.* 2007]. The acute effect of E on Ca^{2+} fluxes in rat cardiac muscle was also showed [Buitrago *et al*. 2000]. The exposure of rat ventricle to physiological levels of E also increased HR [Buitrago *et al*. 2000]. Estrogens modulate also K^+ channel activity in hypothalamic neurons that are involved in regulating numerous homeostatic functions including motivated behaviour and stress responses [Kelly *et al*. 2002, Malyala *et al*. 2005, Roepke *et al*. 2007]. Chronic stress can induce psychopathology by rearranging dendritic branching pattern and disturb the intracellular system of signal transmission. The cyclic E administration to ovariectomized rats subjected to stress, preserved the integrity of signal transduction cascades [Gerrits *et al*. 2006, Takuma *et al*. 2007]. Estrogens are also considered to be a regulatory factor for neuronal networks that maintain GABAergic neurotransmission [Ikeda *et al*. 2006]. The increase in GABAergic neuron activity, has an inhibitory effect on the other nervous systems. Moreover, a remarkable intersex difference in estrogenic regulation of neurotransmission in male *vs*. female GABAergic networks was shown by Zhou *et al.* [2005]. Treatment with estradiol led to increase in synaptic activity in neurons derived from males and decrease in those from females. As a result, the activity of GABA-dependent neurons in females can increase under the influence of estrogens action. GABA receptors interact also with T which results in a reduction of anxiety-like behaviour [Svensson *et al*. 2003, Fernández-Guasti and Martinez-Mota 2005]. The treatment with T, reduced the fear-related behaviours, but did not change HR in studied heifers [Boissy and Bouissou 1994], ewes [Vandenheede and Bouissou 1993] or monkeys [Rejeski *et al*. 1990]. On the other hand, the T-treatment induced cardiac accelerative responses to mental stressors in women [van Honk *et al*. 2001, Hermans *et al*. 2007]. All the results mentioned above indicate that gonadal steroids are involved in the complex process of HR regulation in response to mental and physical stimuli. However, the end effect of the action of the sex steroids on HR is difficult to predict without carrying out an experiment.

As sex steroid hormones can affect the HR response to stress, and because the horses investigated in this study were very young, it was interesting to note their levels of sexual maturity. In the group of 11-month old fillies, plasma E concentration was only detectable in five animals (mean = 10.6±10.9 pg/ml, figures non-tabulated). This is the level characteristic for periovulatory period, what is the proof that five fillies mentioned were sexually mature [Kosiniak-Kamysz and Wierzbowski 2004]. The levels of T and E were detectable in all 11-month-old colts investigated (mean = 0.62±0.21 ng/ml and 24.7±21.2 pg/ml, respectively, n=10, figures non-tabulated). These were typical values for young, sexually mature stallions [Wichmann *et al*. 1984, Kosiniak-Kamysz and Wierzbowski 2004]. The obtained results showed that the majority of young horses investigated in this study were sexually mature just when they reached the age of 11 months.

In summary, the stress reflected by the HR in Standardbred trotters investigated was not found related to sex while preparing the horses for the training session and during slow exercise. Further studies are necessary to clarify the role of gender in HR specific response to more intense stress which may occur during race career of trotters.

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Płeć młodych kłusaków a ich tętno uwarunkowane stresem wywoływanym wczesnym treningiem

S t r e s z c z e n i e

Celem podjętych badań było określenie liczby tętna (HR) młodych kłusaków w zależności od ich płci podczas rutynowych zabiegów związanych z procesem treningu. Przed rozpoczęciem badań zakładano, że klaczki jako bardziej wrażliwe niż ogierki, będą reagowały na stresujące sytuacje większym wzrostem HR.

Obserwacją objęto 101 zdrowych koni rasy Standardbred w wieku 11-18 miesięcy. W zależności od płci badanych zwierząt podzielono je na dwie grupy: 51 ogierków i 50 klaczek. Badania rozpoczęto od założenia urządzeń telemetrycznych rejestrujących HR do specjalnego popręgu oraz rejestrujących prędkość zaprzęgu montowanych w sulkach. Po wyprowadzeniu koni z boksów i zaprzęgnięciu rozpoczynał się trening złożony z biegu kłusem po piaszczystej bieżni z wzrastającą prędkością w zakresie od 15 do 20 km/h.

Nie odnotowano wpływu płci na wyniki pomiaru HR tak w spoczynku, jak i podczas kolejnych etapów sesji treningowej.