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RESEARCH ARTICLE

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Successive approximation of horses to their first work on a treadmill: The effect of previous loading into a trailer

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Abstract

A horse learning about the entrance to narrow, cage-shaped places may be challenging both for the horses as well as for the owners. For some behaviors, such as loading into a trailer or climbing onto a treadmill, the final behavioral goal can be achieved by working towards it in stages. This study compared the successive approximation of horses to their first work on a treadmill with horses hardly ever loaded (HE L) and regularly loaded (R L) into a trailer. Fourteen horses were divided into two groups (HE L $n = 7$ and R L $n = 7$) based on their experiences of entering into a trailer. All horses were taught using four stages of successive approximation. The average lead time was longer in the HE L than in the R L group, both in the first (HE L: 33.8 ± 12.4 s; R L: 17.6 ± 12.9 s; $p = 0.035$) and last stages (HE L: 12.0 ± 10.3 s; R L: 3.7 ± 1.0 s; $p = 0.032$) of trials. With the subsequent repetitions of each step, the heart rate decreased in both groups. Very few behaviors indicating fear or unwillingness ("rearing," "sideways," and "backwards") were observed. Horses that were regularly loaded exhibited signs of relaxation. The successive approximation of horses to the first work on a treadmill differed and may depend on the previous experiences with loading and travelling in the confined space of a trailer.

KEYWORDS
horse, lead time, learning, shaping, treadmill

1 | INTRODUCTION

For some behaviors, such as jumping a fence or loading into a trailer, the final behavioral goal can be worked towards in stages. In these cases, reinforcement may be used to shape the behavior toward the goal by a process known as successive approximation or shaping (Mills & Nankervise, 1998). In this process, the behaviors are built gradually step-by-step; however, each step should differ only slightly from the previous one. When the horse has achieved the targeted behavioral goal, it receives a reward, so each step is reinforcing (Starling et al., 2016). The evaluation of the progress of successive approximation may be performed using indicators such as the time of leading, the heart rate, the evasive behaviors, and the stress behaviors (Mills & Nankervise, 1998; Padalino, 2015; Stomp et al., 2018; Yngvesson et al., 2016). The learned link between the behavioral response and the reward is what learning theorists call a contingency. It is an important part of operant conditioning, representing associative learning when the horse undertakes a voluntary activity that brings about a reward or allows it to avoid an aversive outcome (McGreevy et al., 2018; McGreevy & McLean, 2007). Operant conditioning involves training using rewarding or aversive consequences carried out to increase or decrease the likelihood (frequency, duration,

or intensity) of a response (McLean & Christensen, 2017). If a response increases in frequency, duration, or intensity, the behavior is reinforced. On the other hand, if the likelihood of response reduces, the behavior is punished. Both adding a reward and removing an aversive stimulus may be used to increase the likelihood of a response, and these are called positive reinforcement and negative reinforcement, respectively. Similarly, adding a reward and removing an aversive stimulus may cause deterioration in behavior, and these are called positive punishment and negative punishment (Starling et al., 2016). McLean and Christensen (2017) suggested that the current nomenclature of "positive" and "negative" in both reinforcement and punishment modalities may be misleading, and therefore, they suggested using more appropriately termed "addition" or "removal" reinforcement and punishment, respectively. Because in a positive/addition reinforcement, the addition of a pleasant stimulus takes place to reward a desired response as well as in a positive/addition punishment, the addition of an aversive stimulus is used to punish an undesired response. Similarly, in a negative/removal reinforcement, an aversive stimulus is removed to reward a desired response, whereas in negative/removal punishment, a desired stimulus is removed to punish an undesired response (McLean & Christensen, 2017).

Both successive approximation and addition reinforcement have been used successfully to teach a horse to enter a trailer. Ferguson and Rosales-Ruiz (2001) suggested shaping, which is the systematic application of additional reinforcement to successive approximations of a goal behavior (Cooper et al., 2007), as an effective technique in a study with five horses that were a problem to load into a trailer. Innes and McBride (2008) compared removal and addition reinforcements during training to perform a range of challenges including trailer loading. The authors confirmed that horses trained by addition reinforcement were more motivated to participate in the training sessions and exhibited more trial and error-type behavior than horses trained by removal reinforcement. Likewise, Padalino (2015) recommended addition reinforcement as a training method during transport. Both successive and single addition reinforcements increase the horses' control over their environment, the restricted space in a trailer, through choice while simultaneously reducing fearfulness by desensitizing and counter-conditioning the horse to stressful stimuli (Coleman et al., 2008). We hypothesized that these experiences with loading and travelling in the narrow space of a trailer may prepare horses for work with other restricted equipment like a treadmill. Horses, as innately neophobic animals, may demonstrate a natural fear of restricted spaces, similar to the tight, narrow space of a trailer (Yngvesson et al., 2016) as well the narrow, cage-shaped space inside a treadmill (Van de Putte et al., 2006) or starting gates (Witkowska-Piłaszewicz et al., 2021). For most horses, the training and management of horses for transportation presents several challenges and can result in the manifestation of transport-related problem behaviors (Yorke et al., 2017). A treadmill is also small, elevated, and enclosed, therefore similar to loading into a trailer; the first work on a treadmill can often be challenging. Because the treadmill is commonly used to investigate poor performance problems and perform upper-airway dynamic endoscopies (Bayly et al., 2019; Franklin et al., 2006), the training of the first and subsequent work on a treadmill becomes increasingly important for horses' owners, handlers, trainers, and veterinarians. A treadmill is also a popular tool in equine gait analysis (Serra Bragança et al., 2018; Stutz et al., 2018), as well as lameness (Bachi et al., 2018; Byström et al., 2018) and hoof balance (Bellenzani et al., 2012; Kau et al., 2020) evaluation, or horses' rehabilitation (Nankervis et al., 2017).

This study aimed to compare the indicators of successive approximation of horses to the first work on a treadmill with horses that were hardly ever and regularly loaded into a trailer. Three categories of indicators were taken into account, time of leading, heart rates, and behaviors both evasive and stress. We hypothesized that horses with extensive experience of trailer loading were expected to show more favorable behaviors and react more calmly to the first time they entered and worked on a treadmill. The behavior and physiology of leisure horses were studied in 10 weeks of successive approximation based on four experimental obstacle structures and the final treadmill test work.

2 | MATERIAL AND METHODS

2.1 | Horses

The study was conducted on 14 mature Polish Warmblood horses (six mares and eight geldings, mean age 14.1 ± 4.7 years, mean weight 546.3 ± 47.8 kg). All the horses were housed in individual stalls with the same management in the Didactic Stable of Horse Breeding Division at Warsaw University of Life Sciences (WULS). The horses were fed three times a day with a dose of oats and hay personalized to each horse to maintain an optimal, healthy condition without obesity, and had daily access to a sandy paddock no less than 6 h per day. All horses were in daily leisure use, namely, recreational riding 1 to 2 h a day, 5 days a week, and were daily led forward and stopped at a halter/rope signal during routine care of the horse. All horses were owned by WULS and purchased at 3–4 years of age and their entire history of use, including trailer loading, was known in detail. Seven horses (three mares and four geldings, mean age 15.1 ± 3.8 years; minimum 10.0; 25% percentile 12.0; median 15.0; 75% percentile 18.0; maximum 21.0) that had been loaded and occasionally transported (two to three times throughout their lives) were considered to be unfamiliar with loading and transportation. Those horses were included in the hardly ever loaded (HE L) group. Seven horses (three mares and four geldings, mean age 13.0 ± 5.4 years; minimum 10.0; 25% percentile 10.0; median 10.0; 75% percentile 20.0; maximum 22.0) had been regularly loaded into a trailer every month in the previous 5 years of leisure use. The horses did not show stress during loading and transportation and entered and exited the trailer easily with no evasive behaviors. Those horses were included in the regularly loaded (R L) group. All the horses had never been worked or loaded on any treadmill before. All procedures took place in a familiar environment they used daily and did not cause them any pain, suffering, or damage. Horses' health status was inspected before the experiment

according to veterinary standards (Davidson, 2018). The horses were clinically healthy, with no dental disorders or any signs of sense disorders, no clinical signs of lameness or musculoskeletal injury; they had no history of laminitis and demonstrated a comparable condition and athletic ability. The study was approved by the II Local Ethical Committee on Animal Testing in Warsaw on behalf of the National Ethical Committees on Animal Testing (No WAW2/035/2018, day 27.04.2018).

2.2 | Study design

The successive approximation of horses to the first work on a treadmill was compared between the horses that were hardly ever and regularly loaded into a trailer, because of the similarity narrow spaces of trailer and treadmill (Figure 1). A treadmill with transparent sidewalls was used (Master-Sport treadmill, Skarzysko-Kamienna, Poland). The inside dimensions were the following: height of 1.95 m (including platform height of 0.45 m), width of 1.20 m, length between the bars at the front and back of 4.20 m, and total length of 6.80 m (including two ramps each with a length of 1.30 m).

The successive approximation was conducted in four steps with gradually increasing enclose of investigated space and wall-filling, using three imitations treadmill and the real treadmill (Figure 2). Each step differed only slightly from the previous one. Each trial was started at the same site of the imitation treadmill or the real treadmill, 5 m in front of it. In the first step, the imitation treadmill made only of poles and stands was used. The horse was led forward to walk between two walls 1.20 m apart. Each wall was built of two 3-m poles suspended on stands at a height of 0.60 and 1.20 m (Figure 2a). The first step was repeated once. In the second step, the imitation treadmill made of poles, stands, and a rubber mat was used. The horse was led forward to walk between two walls, each built with six 3-m poles suspended at a height from 0.40 to 1.50 m. The ground between the walls was made of a rubber mat with the following dimensions: width of 1.20 m and length of 3.00 m (Figure 2b). The second step was repeated three times. In the third step, the imitation treadmill made of poles, stands, and a platform was used. The horse was led forward to walk between two walls, each built of six 3-m poles; however, the ground between the walls was a specially prepared platform. The platform dimensions were the following: the height was 0.45 m, the width was 1.20 m, the length was 3.00 m, and the ramp length was 1.00 m with a rubber mat on it (Figure 2c). The third step was repeated three times. In the fourth step, the real treadmill was used for the first time (Figure 2d). The fourth step was repeated three times. The interval between steps or their repetitions always lasted 7 days. Finally, after the next 7 days, the horses started the first work on the treadmill in a walk. The walk was conducted with speed of up to 1.6 m/s and lasted for 5 min. The whole successive approximation lasted about 10 weeks. The protocol of the successive approximation was previously documented (Masko et al., 2020).

The imitation treadmill or real treadmill was situated in the middle of the riding hall, well known to all the horses. The indoor arena was familiar, and a handler who led the horse was also familiar. The horse was asked to follow the handler walking over one of the imitation treadmills or the real treadmill, without any other horse present. Horses were not forced to follow. Each trial was conducted with a handler walking beside the horse and holding a lead rope attached to the horse's halter. The handler did not apply any pressure to the horse's head at any time. The targeted behavioral goal was to enter the obstacle structures imitating the treadmill or the real treadmill and stop. The trial was finished when all four hoofs were put on the ground inside the imitation treadmill or the real treadmill. When the horse achieved the targeted behavioral goal, it received a reward, so each step was reinforcing. The addition reinforcement included a few pieces of carrot or apple with the voice command "great." The duration of each trial, from starting walking to stopping inside the treadmill, was not longer than 10 min. Thereafter, the horse was led forward outside and was brought back to the stable. An inappropriate

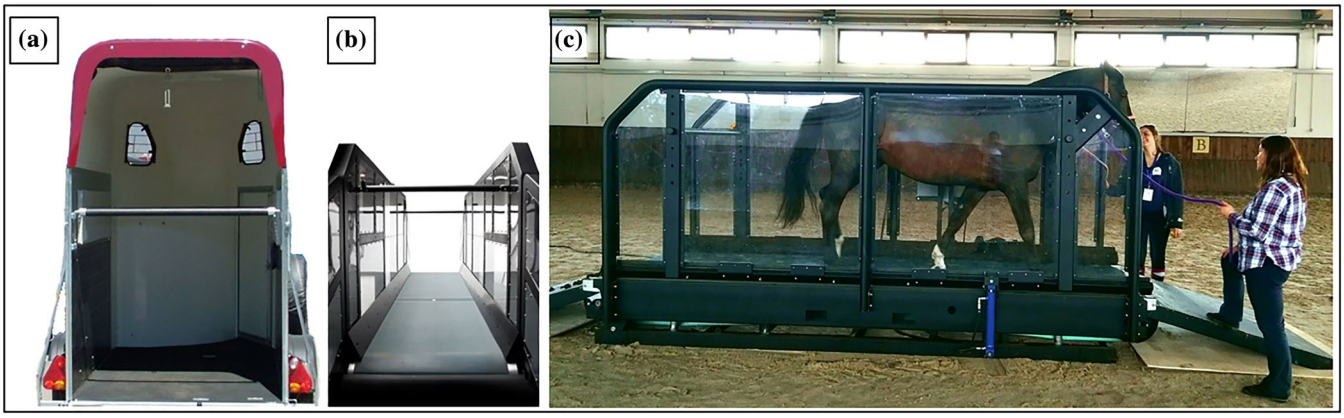

FIGURE 1 The similarity of the narrow spaces of trailer (a) and treadmill (b) and the first work on a treadmill after the successive approximation (c)

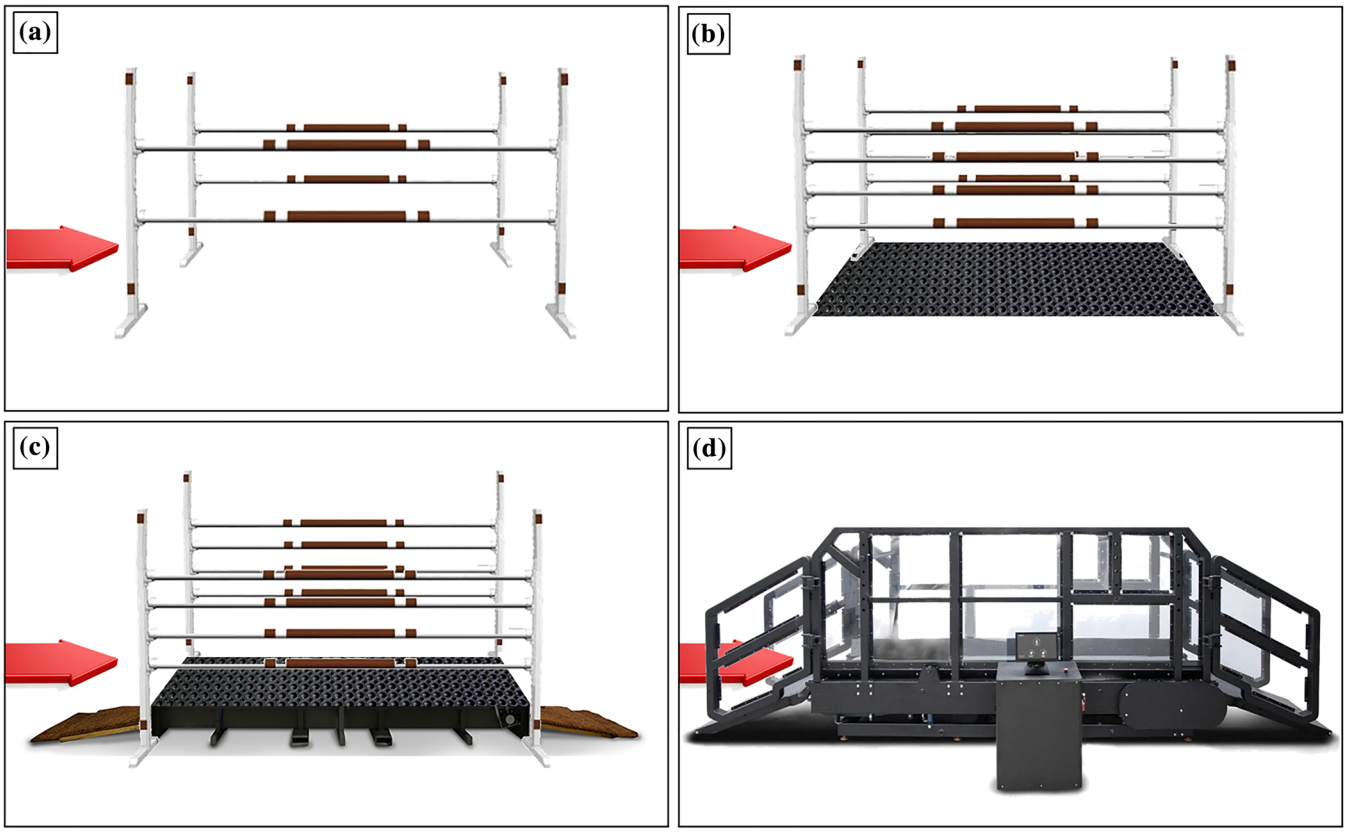

FIGURE 2 Four steps of the successive approximation. In the first step, the imitation treadmill built from poles and stands was used (a). In the second step, the imitation treadmill built from poles, stands and rubber mat was used (b). In the third step, the imitation treadmill built from poles, stands, and platform was used (c). In the fourth step, the real treadmill was used (d). The position from which the test was started has been marked with a red arrow

response for a leading included rearing, walking backwards, walking sideways, or kicking. When the horse displayed these inappropriate behaviors, the addition punishment was used. It included a strengthening of the pressure from the rope with the "stop" voice command.

2.3 | Data collection

Behavioral observations were carried out by a person standing outside the imitation treadmill or treadmill. The horses had unrestricted visibility at all times. Because the second step of successive approximation, each trial was recorded with a wide-angle camera (GoPro, Hero 3; GoPro, Inc., San Mateo, CA, USA). The camera was placed on the edge of the indoor arena, and the horse was visible without any restriction at all times. The observer marked the points and the behaviors on the prepared sheet in real time and, after the trial, compared his observations with the recordings on the video.

The response of horses being led was observed. The times of them being led were measured from the start of the walk to the end of the trial, when all four hoofs were put on the ground inside the imitation treadmill or the real treadmill. If any behavior occurred during the work, a point was marked as an occurrence (*n*). Due to the different duration of the trials, the occurrence of a particular behavior has not been presented as a frequency. The description of the analyzed behaviors is summarized in Table 1. Heart rate (beats per minute [bpm]) was measured using a Polar Equine (Polar Electro Oy, Kempele, Finlandia) placed on the left side of the horse just behind the front leg. The heart rate for each horse was measured at three times during each trial: the first time, immediately before the trial (HR before); the second time, inside the imitation treadmill or real treadmill when all four hoofs were set down (HR inside); and the third time, 30 s after the horse left the imitation treadmill or real treadmill and stopped behind it (HR after). All trials were performed in a controlled environment (air temperature $20.2 \pm 1.6°C$ and humidity $45.4 \pm 0.8\%$) in an indoor riding arena protected from wind and sun radiation and connected to the stable in which horses were housed. The measured heart rate was assessed in relation to the normal heart rate in horses under similar environmental conditions, which in rest is 28–40 bpm (Aiello & Moses, 2016). The results obtained were represented as a data series of the time the horses were being led, the frequency of each behavior and three measurements of the heart rate, compiled for the HE L and R L groups, respectively.

TABLE 1 Description of behaviors analyzed during evaluation of successive approximation to the first work on a treadmill

Behavior	Definition
Snorting	The horse forcefully expulses of air through the nostrils, preceded by a raspy inhalation sound.
Refusing to go forward	The horse's four legs are standing still for a minimum of 5 s.
Stopping	The horse stops and four legs are standing still no more than 5 s.
Turning	The horse stops, turns its head, and tries to change its direction of the movement.
Walking backwards	The horse stops and changes its direction of movement so that it increases the distance from the treadmill or its imitation by going backwards.
Walking sideways	The horse changes its direction of movement with or without stopping so that it does not increase the distance from the treadmill or its imitation by going sideways.
Rearing	The horse rears with its front legs. Note that it is when at least one front leg is lifted more than 10 cm from the ground with an upwards move of the withers.
Kicking	The horse kicks. Note that it is when at least one hind leg is lifted more than 10 cm from the ground with an upwards move of the hindquarters.
Whinnying	The horse vocalizes.
Defecating	The horse drops feces.
Shaking	The trembling of the horse's muscles is visible.
Chewing	The movements of the horse's mouth and teeth are visible.
Ears	The attention or moving of ears is visible.
Nostril	A change of nostril shape from normal to flared is visible.
Tail	A change of tail position from free to raised or cowered is visible.
Legs	The horse is stepping.

Note: Behavior descriptions adapted from the studies of Yngvesson et al. (2016), Stomp et al. (2018), and Padalino (2015).

2.4 | Data analysis

Univariate marginal distributions were tested independently for each data series using a univariate Kolmogorov–Smirnov test. Due to non-Gaussianity of the data, the comparison of the HE L/R L data series was assessed using a Mann–Whitney test. For the strictly numerical data, the time the horses were being led and the heart rates, the data series from the first, the second, and the third repetitions of each steps of successive approximation were compared using the Kruskal–Wallis test with Dunn's multiple comparisons test. The differences were considered statistically significant at the level of $p < 0.05$ and were marked in tables with consecutive letters (only for the HE L/R L) and with bold font (for p values of all significant comparisons). All statistical analysis was performed using Graph Pad Prism 6 software (GraphPad Software Inc., Avenida De La Playa La Jolla, CA, USA).

3 | RESULTS

3.1 | The first step

The average time the horses were being led (mean ± SD) was longer ($p = 0.035$) in HE L (33.8 ± 12.4 s) than in R L (17.6 ± 12.9 s). The heart rate (mean ± SD) was higher in HE L than R L both before (HE L: 33.4 ± 4.0 bpm; R L: 29.1 ± 1.5 bpm; $p = 0.032$) and during (HE L: 59.7 ± 4.6 bpm; R L: 49.7 ± 7.3 bpm; $p = 0.012$) trial, whereas the horses calmed down similarly after leaving the treadmill imitation (Table 2).

In the first step, evasive behavior (only "stopping") was shown in the HE L group but not in R L (Tables 3 and 4). Among stress behaviors, "whinnying," "defecating," "shaking," and "legs" did not occur in both groups (Table 5). The other stress behaviors ("ears," "nostril," "tail," and "chewing") were more intensive in the HE L group than in the R L (Table 6).

3.2 | The second step

The gradually decreasing time the horses were led was demonstrated with the next repetitions of the trial for both groups: HE L ($p = 0.001$) and R L ($p = 0.001$). The time being led was shorter in R L than HE L in the second step (R L: 21.4 ± 6.3 s; HE L: 42.9 ± 16.0 s; $p = 0.029$) and third (R L: 9.3 ± 4.5 s; HE L: 29.3 ± 17.7 s; $p = 0.008$) repetitions, but not in the first one (R L: 77.1 ± 29.3 s; HE L: 102.9 ± 29.3 s; $p = 0.286$). The heart rate before the trial was higher in HE L than R L in all repetitions, first (HE L: 33.4 ± 4.1 bpm; R L: 29.0 ± 1.5 bpm; $p = 0.049$), second (HE L: 32.1 ± 3.1 bpm; R L: 28.3 ± 1.2 bpm; $p = 0.042$), and the third (HE L: 33.5 ± 2.2 bpm; R L: 30.2 ± 0.7 bpm; $p = 0.035$), with no differences between repetitions. The heart rate inside the imitation treadmill was higher in the first repetition than in the second and third in both the HE L ($p = 0.0001$) and R L groups ($p = 0.0001$), with no differences between second and third repetitions. The heart rate inside the imitation treadmill was higher in HE L than R L in all repetitions, first (HE L: 79.7 ± 7.2 bpm; R L: 60.1 ± 6.5 bpm; $p = 0.0006$), second (HE L: 55.7 ± 8.3 bpm; R L: 46.0 ± 3.1 bpm; $p = 0.014$), and third (HE L: 56.3 ± 9.1 bpm; R L: 46.3 ± 2.9 bpm; $p = 0.008$) repetitions. There were no differences in heart rate after the trail in the first (HE L: 55.0 ± 10.5 bpm; R L: 45.1 ± 5.6 bpm; $p = 0.507$) and second (HE L: 43.1 ± 3.7 bpm; R L: 40.1 ± 1.3 bpm; $p = 0.584$) repetitions; however in a third repetition, the heart rate in the R L group was lower than in the HE L group (HE L: 43.6 ± 2.6 bpm; R L: 39.1 ± 1.2 bpm; $p = 0.023$). After the trial, the depression of the heart rate with the repetitions was observed in both groups (Table 2).

In the second step, evasive behavior (only "stopping" and "backwards") was observed in the HE L but also not in the R L

TABLE 2 The values (mean ± SD) of time of leading and heart rates (HR) measured before, inside, and after passing the imitation treadmill or treadmill divided into four steps of successive approximation

		Time of leading (s)			HR before (bpm)			HR inside (bpm)			HR after (bpm)		
Step	**Rep.**	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***
First	One	33.8 ± 12.4†	17.6 ± 12.9‡	**0.035**	33.4 ± 4.0†	29.1 ± 1.5‡	**0.032**	59.7 ± 4.6†	49.7 ± 7.3‡	**0.012**	42.6 ± 3.4†	41.3 ± 2.6†	0.599
Second	First	102.9 ± 29.3†	77.1 ± 29.3†	0.286	33.4 ± 4.1†	29.0 ± 1.5‡	**0.049**	79.7 ± 7.2†	60.1 ± 6.5‡	**0.0006**	55.0 ± 10.5†	45.1 ± 5.6†	0.507
	Second	42.9 ± 16.0†	21.4 ± 6.3‡	**0.029**	32.1 ± 3.1†	28.3 ± 1.2‡	**0.042**	55.7 ± 8.3†	46.0 ± 3.1‡	**0.014**	43.1 ± 3.7†	40.1 ± 1.3†	0.584
	Third	29.3 ± 17.7†	9.3 ± 4.5‡	**0.008**	33.5 ± 2.2†	30.2 ± 0.7‡	**0.035**	56.3 ± 9.1†	46.3 ± 2.9‡	**0.008**	43.6 ± 2.6†	39.1 ± 1.2‡	**0.023**
p		**0.001**	**0.001**		>0.999	0.842		**0.0001**	**0.0001**		0.109	0.108	
Third	First	111.4 ± 23.9†	57.6 ± 9.2‡	**0.031**	32.1 ± 4.0†	28.9 ± 1.5‡	**0.030**	61.4 ± 11.6†	48.0 ± 4.8‡	**0.018**	43.3 ± 4.2†	38.9 ± 0.9‡	**0.035**
	Second	85.7 ± 22.7†	57.6 ± 9.2†	0.225	33.4 ± 4.1†	29.1 ± 1.5‡	**0.049**	54.4 ± 11.0†	44.1 ± 2.0‡	**0.045**	42.0 ± 4.1†	37.4 ± 3.4‡	**0.048**
	Third	77.1 ± 12.6†	57.6 ± 9.2†	0.106	33.4 ± 4.1†	29.1 ± 1.5‡	**0.049**	53.1 ± 6.0†	44.3 ± 4.6‡	**0.011**	41.4 ± 5.4†	36.1 ± 2.4‡	**0.015**
		0.046	>0.999		>0.999	>0.999		0.620	0.860		0.802	0.842	
Fourth	First	173.6 ± 62.5†	31.4 ± 26.6‡	**0.002**	35.1 ± 2.5†	31.4 ± 2.5‡	**0.040**	96.4 ± 10.3†	81.3 ± 16.0‡	**0.047**	47.9 ± 9.4†	42.7 ± 2.6†	0.215
	Second	115.7 ± 30.2†	28.1 ± 30.6‡	**0.006**	35.1 ± 2.5†	31.4 ± 2.5‡	**0.040**	101.0 ± 18.3†	76.3 ± 15.7‡	**0.017**	47.3 ± 6.7†	41.1 ± 1.8†	0.140
	Third	87.8 ± 31.1†	15.3 ± 10.2‡	**0.022**	33.4 ± 4.0†	29.1 ± 1.5‡	**0.049**	84.7 ± 17.4†	61.4 ± 13.3‡	**0.006**	43.9 ± 7.5†	38.0 ± 3.1†	0.080
p		**0.048**	0.807		>0.999	>0.999		0.072	0.120		0.664	0.802	
First work	One	12.0 ± 10.3†	3.7 ± 1.0‡	**0.032**	35.1 ± 2.4†	31.4 ± 2.5‡	**0.029**	119.6 ± 19.1†	97.1 ± 26.7†	0.138	44.3 ± 3.4†	42.8 ± 3.4†	0.446

Note: In the second, third, and fourth steps, subsequent repetitions (rep.) were indicated. The data series are compared within rows between the hardly ever loaded group (HE L) and regularly loaded group (R L) and within the column between repetitions of each step. Differences between measurements are indicated with superscript symbols (†, ‡). The $p < 0.05$ was considered significant and marked with bold font.
Abbreviations: bpm, beats per minute; HE L, the hardly ever loaded group; HR, heart rates; *p*, the level of marginal significance; rep., repetitions; R L, regularly loaded group; s, second; SD, standard deviation.

TABLE 3 The values (mean ± SD) of occurrence of following behaviors: snorting, refusing, stopping, and turning divided into four steps of successive approximation

		Snorting			Refusing			Stopping			Turning		
Step	Rep.	HE L	R L	*p*	HE L	R L	*p*	HE L	R L	*p*	HE L	R L	*p*
First	One	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.85 ± 0.31†	0.0 ± 0.0‡	**0.005**	0.0 ± 0.0†	0.0 ± 0.0†	–
Second	First	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.71 ± 0.49†	0.0 ± 0.0‡	**0.023**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Second	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.48 ± 0.21†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Third	0.0 ± 0.0†	0.48 ± 0.21‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
Third	First	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.58 ± 0.30†	0.0 ± 0.0‡	**0.012**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Second	0.0 ± 0.0†	0.38 ± 0.14‡	**0.021**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.42 ± 0.25†	0.0 ± 0.0‡	**0.030**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Third	0.0 ± 0.0†	0.48 ± 0.21‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
Fourth	First	0.0 ± 0.0†	0.38 ± 0.14‡	**0.021**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.58 ± 0.30†	0.0 ± 0.0‡	**0.012**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Second	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.58 ± 0.30†	0.0 ± 0.0‡	**0.012**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Third	0.0 ± 0.0†	0.48 ± 0.21‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.42 ± 0.25†	0.0 ± 0.0‡	**0.030**	0.0 ± 0.0†	0.0 ± 0.0†	–
First work	One	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.48 ± 0.21†	0.38 ± 0.14‡	**0.048**	0.0 ± 0.0†	0.0 ± 0.0†	–

Note: In the second, third, and fourth steps, subsequent repetitions (rep.) were indicated. The data series are compared within rows between hardly ever loaded group (HE L) and regularly loaded group (R L). Differences between measurements are indicated with superscript symbols (†, ‡). The $p < 0.05$ was considered significant and marked with bold font.
Abbreviations: HE L, the hardly ever loaded group; *p*, the level of marginal significance; rep., repetitions; R L, regularly loaded group; SD, standard deviation.

TABLE 4 The values (mean ± SD) of occurrence of following behaviors: walking backwards, walking sideways, rearing, and kicking divided into four steps of successive approximation

		Walking backwards			Walking sideways			Rearing			Kicking		
Step	Rep.	HE L	R L	*p*	HE L	R L	*p*	HE L	R L	*p*	HE L	R L	*p*
First	One	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
Second	First	0.48 ± 0.21†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
	Second	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
	Third	0.48 ± 0.21†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
Third	First	0.42 ± 0.25†	0.0 ± 0.0‡	**0.030**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
	Second	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
	Third	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
Fourth	First	0.58 ± 0.30†	0.0 ± 0.0‡	**0.012**	0.38 ± 0.14†	0.38 ± 0.14†	>0.999	0.38 ± 0.14†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–
	Second	0.58 ± 0.30†	0.0 ± 0.0‡	**0.012**	0.38 ± 0.14†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.0 ± 0.0†	0.0 ± 0.0†	–
	Third	0.48 ± 0.21†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–	0.38 ± 0.14†	0.0 ± 0.0‡	**0.032**	0.0 ± 0.0†	0.0 ± 0.0†	–
First work	One	0.0 ± 0.0†	0.0 ± 0.0†	–	0.42 ± 0.25†	0.0 ± 0.0‡	**0.030**	0.48 ± 0.21†	0.38 ± 0.14‡	**0.048**	0.0 ± 0.0†	0.0 ± 0.0†	–

Note: In the second, third, and fourth steps, subsequent repetitions (rep.) were indicated. The data series are compared within rows between hardly ever loaded group (HE L) and regularly loaded group (R L). Differences between measurements are indicated with superscript symbols (†, ‡). The $p < 0.05$ was considered significant and marked with bold font.
Abbreviations: HE L, the hardly ever loaded group; *p*, the level of marginal significance; rep., repetitions; R L, regularly loaded group; SD, standard deviation.

TABLE 5 The values (mean ± SD) of occurrence of following behaviors: whinnying, defecating, shaking, and chewing divided into four steps of successive approximation

		Whinnying			Defecating			Shaking			Chewing		
Step	**Rep.**	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***
First	One	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.71 \pm 0.49^{\dagger}$	$0.14 \pm 0.28^{\ddagger}$	**0.042**
Second	First	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.48 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.048**	$0.48 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.048**
	Second	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.032**	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–
	Third	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–
Third	First	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.032**	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–
	Second	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.032**	$0.38 \pm 0.14^{\dagger}$	$0.38 \pm 0.14^{\dagger}$	>0.999
	Third	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.38 \pm 0.14^{\dagger}$	$0.48 \pm 0.21^{\ddagger}$	**0.048**
Fourth	First	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.53 \pm 0.10^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.049**	$0.38 \pm 0.14^{\dagger}$	0.48 ± 0.21^{b}	**0.048**
	Second	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.53 \pm 0.10^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.049**	$0.38 \pm 0.14^{\dagger}$	$0.38 \pm 0.14^{\dagger}$	>0.999
	Third	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.48 \pm 0.21^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.008**	$0.38 \pm 0.14^{\dagger}$	$0.38 \pm 0.14^{\dagger}$	>0.999
First work	One	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.75 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.042**	$0.48 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.048**

Note: In the second, third, and fourth steps, subsequent repetitions (rep.) were indicated. The data series are compared within rows between the hardly ever loaded group (HE L) and regularly loaded group (R L). Differences between measurements are indicated with superscript symbols (†, ‡). The $p < 0.05$ was considered significant and marked with bold font.
Abbreviations: HE L, the hardly ever loaded group; *p*, the level of marginal significance; rep., repetitions; R L, regularly loaded group; SD, standard deviation.

TABLE 6 The values (mean ± SD) of occurrence of following behaviors: ears, nostril, tail, and legs divided into four steps of successive approximation

		Ears			Nostril			Tail			Legs		
Step	**Rep.**	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***	**HE L**	**R L**	***p***
First	One	$1.14 \pm 0.90^{\dagger}$	$0.43 \pm 0.29^{\ddagger}$	**0.046**	$0.86 \pm 0.29^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.031**	$0.86 \pm 0.49^{\dagger}$	$0.29 \pm 0.30^{\ddagger}$	**0.042**	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–
Second	First	$0.86 \pm 0.29^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.021**	$0.89 \pm 0.25^{\dagger}$	$0.43 \pm 0.29^{\ddagger}$	**0.033**	$1.14 \pm 0.90^{\dagger}$	$0.43 \pm 0.29^{\ddagger}$	**0.002**	$0.48 \pm 0.21^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.008**
	Second	$0.78 \pm 0.57^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.038**	$0.78 \pm 0.57^{\dagger}$	$0.48 \pm 0.21^{\ddagger}$	**0.048**	$1.01 \pm 0.62^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.038**	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.021**
	Third	$0.75 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.042**	$0.75 \pm 0.33^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.042**	$0.78 \pm 0.57^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.043**	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–
Third	First	$1.01 \pm 0.62^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.038**	$0.75 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.042**	$0.53 \pm 0.10^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.049**	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.021**
	Second	$0.86 \pm 0.29^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.002**	$0.75 \pm 0.21^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.008**	$0.78 \pm 0.57^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.008**	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.021**
	Third	$0.78 \pm 0.57^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.008**	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–	$0.48 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.048**	$0.0 \pm 0.0^{\dagger}$	$0.0 \pm 0.0^{\dagger}$	–
Fourth	First	$0.78 \pm 0.57^{\dagger}$	$0.78 \pm 0.57^{\dagger}$	>0.999	$0.53 \pm 0.10^{\dagger}$	$0.48 \pm 0.21^{\dagger}$	0.064	$0.89 \pm 0.25^{\dagger}$	$0.53 \pm 0.10^{\ddagger}$	**0.038**	$0.53 \pm 0.10^{\dagger}$	$0.53 \pm 0.10^{\dagger}$	>0.999
	Second	$0.78 \pm 0.57^{\dagger}$	$0.78 \pm 0.57^{\dagger}$	>0.999	$0.53 \pm 0.10^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.049**	$0.86 \pm 0.29^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.021**	$0.53 \pm 0.10^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.049**
	Third	$0.78 \pm 0.57^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.042**	$0.48 \pm 0.21^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.048**	$0.78 \pm 0.57^{\dagger}$	$0.38 \pm 0.14^{\ddagger}$	**0.038**	$0.38 \pm 0.14^{\dagger}$	$0.0 \pm 0.0^{\ddagger}$	**0.021**
First work	One	$0.53 \pm 0.10^{\dagger}$	$0.48 \pm 0.21^{\dagger}$	0.064	$0.53 \pm 0.10^{\dagger}$	$0.53 \pm 0.10^{\dagger}$	>0.999	$0.38 \pm 0.14^{\dagger}$	$0.38 \pm 0.14^{\dagger}$	>0.999	$0.53 \pm 0.10^{\dagger}$	$0.48 \pm 0.21^{\dagger}$	0.064

Note: In the second, third, and fourth steps, subsequent repetitions (rep.) were indicated. The data series are compared within rows between hardly ever loaded group (HE L) and regularly loaded group (R L). Differences between measurements are indicated with superscript symbols (†, ‡). The $p < 0.05$ was considered significant and marked with bold font.
Abbreviations: HE L, the hardly ever loaded group; *p*, the level of marginal significance; rep., repetitions; R L, regularly loaded group; SD, standard deviation.

group. "Stopping" was noted in the first and second repetitions, whereas it was "backwards" in the first and third repetitions (Tables 3 and 4). Among stress behaviors, "whinnying" and "defecating" did not occur in either group. The other stress behaviors ("shaking," "chewing," "ears," "nostril," "tail," and "legs") were more intensive in the HE L in than the R L group. The occurrence of "shaking" and "chewing" decreased with the repetitions. "Snorting" was observed only in the third repetition in R L but not in HE L (Tables 5 and 6).

3.3 | The third step

The time the horses were being led was shorter ($p = 0.031$) in the R L group (average from I, II, and III: 57.6 ± 9.2 s) than in the HE L group in the first repetition, with no differences between repetitions in R L and gradually decreased ($p = 0.046$) in HE L (I: 111.4 ± 23.9 s; II: 85.7 ± 22.7 s; III: 77.1 ± 12.6 s). There were no differences between time being led in the R L and HE L groups during the second and third repetitions. The heart rate was always higher in HE L than in R L, as well as before (average from I, II, and III: HE L: 33.0 ± 3.9 bpm; R L: 29.1 ± 1.4 bpm), during (average from I, II, and III: HE L: 56.3 ± 10.1 bpm; R L: 45.5 ± 4.2 bpm), and after (average from I, II, and III: HE L: 42.2 ± 4.4 bpm; R L: 37.8 ± 2.6 bpm) passing the imitation treadmill. No differences between the repetitions of each measurement were observed (Table 2).

In the third step, evasive behaviors (only "stopping" and "backwards") were observed in the HE L but not in the R L group. "Stopping" occurred in the first and second repetitions, whereas "backwards" only in the first repetition. "Snorting" occurred more frequently in the R L group, not only in the third but also in the second repetition; however, it still did not occur in the HE L group (Tables 3 and 4). "Shaking" was noted in the first and second repetitions of the HE L group, whereas there was "chewing" in the second and third repetitions of both groups. However, "shaking" in the third repetition occurred more often in the R L group than in the HE L group. The other stress behaviors like "ears," "nostril," "tail," and "legs" were more intensive in HE L than R L, with a tendency to decrease along repetitions (Tables 5 and 6).

3.4 | The fourth step

The gradual decrease ($p = 0.048$) in the time being led in the HE L group (I: 173.6 ± 62.5 s; II: 115.7 ± 30.2 s; and III: 87.8 ± 31.1 s) was noted again; however, a still shorter working time in R L (average from I, II, and III: 24.9 ± 13.9 s) than in HE L was demonstrated. The heart rate was lower in R L than in HE L before (average from I, II, and III: HE L: 34.6 ± 3.0 bpm; R L: 31.6 ± 1.6 bpm) and during (average from I, II, and III: HE L: 94.0 ± 10.5 bpm; R L: 73.1 ± 9.8 bpm) but not after (average from I, II, and III: HE L: 46.3 ± 7.7 bpm; R L: 40.6 ± 3.1 bpm) passing the treadmill, with no differences between repetitions (Table 2).

The evasive behaviors ("stopping" and "backwards") were noted more frequently in the HE L group, but not in R L, and manifested the tendency to decrease with the repetitions. In both groups, single "rearing" and "sideways" occurred in the first and second repetitions of the trial. Increasingly "snorting" occurred more frequently in the R L than in HE L group in the first and the third repetition; however, it still did not occur in the HE L group (Tables 3 and 4). "Shaking" was noted in the first and second repetition of the R L group and all three repetitions of the HE L group, whereas there was "chewing" in all repetitions of both groups. The other stress behaviors like "ears," "nostril," "tail," and "legs" occurred in both the HE L and R L groups, again with a tendency to decrease along repetitions and with slight differences between groups (Tables 5 and 6).

3.5 | The first work on a treadmill

The time being led was also shorter ($p = 0.032$) in the R L group (3.7 ± 1.0 s) than in the HE L group (12.0 ± 10.3 s). Higher ($p = 0.029$) heart rate in HE L (35.1 ± 2.4 bpm) than in R L (31.4 ± 2.5 bpm) was noted only before climbing the treadmill, whereas not during work (HE L: 119.6 ± 19.1 bpm; R L: 97.1 ± 26.7 bpm; $p = 0.138$) and after it (HE L: 44.3 ± 3.4 bpm; R L: 42.8 ± 3.4 bpm; $p = 0.446$) (Table 2).

In the case of the first work on the treadmill, the evasive behaviors were observed in R L ("stopping" and "rearing") and HE L ("stopping," "rearing," and "sideways"); however, in R L the occurrences were lower. There was no evidence of "snorting" in either group (Tables 3 and 4). The stress behaviors, including "chewing" and "shaking", occurred more frequently in HE L than in R L. Other stress behaviors were noted in both groups with no differences between HE L and R L (Tables 5 and 6).

4 | DISCUSSION

In both cases, climbing the treadmill and loading into a trailer, the horses are asked to enter a small, narrow space limited at the sides by the walls, which caused a stress reaction. Naïve horses are stressed by being loaded into a trailer (Christensen et al., 2006; Lee et al., 2001), whereas the behavioral signs of stress decrease with increasing vehicle size (Ferguson & Rosales-Ruiz, 2001). Previous research demonstrated that training, following learning theory, reduced fear when being loaded (Yngvesson et al., 2016), and increased the threshold for unwanted natural reactions, such as flight, as well as increased sensitivity and response to human signaling (Visser et al., 2002). Our results suggest that horses with a lot of previous experience of entering a narrow space needed less time and exhibited less stress when entering the treadmill for the first time.

We find it concerning that horses regularly loaded into a trailer did not show any evasive behaviors until they passed the imitation treadmill built with poles. During the first time climbing on the treadmill, those horses exhibited a single "rearing" or "sideways" behavior.

However, the evasive behaviors such as "stopping" and "walking backwards" occurred in this group much less frequently than in horses which were hardly ever loaded. It could be seen that some of the horses from the latter group walked forward, stopped, and/or walked backwards, and then moved forward again repeatedly. Considering that the signals for forward, stop, and back were given by the same staff and hence in similar situations, we find it likely that those different reactions in response to similar forward/stop signals may be a result of previous experiences with loading into a narrow space. Likewise, working time was longer for horses in HE L than R L group, whether imitation treadmill regardless of its type or treadmill was used. The observed effect decreases with the progression of successive approximation. It is visible as a gradual decrease in working time when horses were worked only with poles (both groups) and next with poles and platform or treadmill (only the HE L group). Finally, both groups achieved the behavioral target and went inside the treadmill without evasive behaviors. Learning to climb treadmills was therefore equally effective in both groups of horses but it progressed quicker in regularly loaded horses.

Interestingly, regularly loaded horses started "snorting" during the third repetition of the second step of successive approximation, second and third repetitions of the third step, and first and third repetitions of the fourth step. Because "snorting" was considered as an indicator of a relaxation phase associated with positive emotions (Stomp et al., 2018), we confirmed that "snorting" was expressed even more by horses in a good mood. It may be related to using a food reward as an additional reinforcement by which horses are easily motivated by food or titbits (Williams et al., 2004). Moreover, the advantage of successive approximation is that it enables horses to have many successes on the way to learning the final behavior, which is likely to encourage positive emotional states associated with training (Starling et al., 2016). In this case, the addition reinforcement occurred frequently. Yngvesson et al. (2016) suggested that if the aversive stimuli, in this case entrance to a new imitation treadmill, are presented in connection with a food reward, the counter condition can occur. In the group of regularly loaded horses, the counter condition resulted in an expression of positive emotions, whereas in a group of horses which are hardly ever loaded were only in progression in learning.

In our investigation, we found very few behaviors indicating fear or unwillingness, like single "rearing," "sideways," or "backwards" only when they were climbing onto the treadmill, and no "refusing," "turning," "kicking," "defecating," or "whinnying" during the whole investigation. However, the changes in heart rate showed that horses in both groups were physiologically aroused. The heart rate was always lower in regularly loaded horses, which indicates their better preparation for accepting frightening stimuli. Following Yarnell et al. (2013), we interpreted increased heart rate as a reliable sign of fear of the new situation, in this case probably entering the narrow space. The previous work reports the dramatic increase in heartbeat during loading into and unloading from the vehicle (Waran & Cuddeford, 1995). In the other one, it was shown that a similar increase in heart rate when entering the trailer was significantly reduced after the 3 days of training (Yngvesson et al., 2016). We did not observe a reduction of heart rate depending on the repetitions of the trial when horses were using the imitation treadmill.

It should be taken into account that the horse is a social animal. In social species, the hypothalamic-pituitary-adrenocortical axis contributes to physiological and behavioral mechanisms that support a superorganism (i.e., group) structure that enhances the survival of children and grandchildren. Thus, in social species, social isolation is a potent stressor that often leads to increases in cortisol (Hawkley et al., 2012). In this experience, it cannot be excluded that the part of the stress symptoms observed may be related to the fact that the horses were alone in the indoor arena. On the other hand, all horses examined here have previously worked in the same riding arena alone in their daily activities. Because all the horses have been kept in the same stable for at least 5 years and have been worked daily in the same riding arena with the same leisure usage, the impact of place and isolation between them may be comparable. Moreover, the riding hall was connected with a short corridor with the stable where this and the other horses have been housed, which may have further limited the impact of social isolation. It should also be emphasized that the handler who led the horse was familiar and sociality between animals and persons could partially compensate for the lack of a temporary relation with another horse. In this case, even if we observe stress as the sum of the new situation and social isolation, the result is visible.

The biggest disadvantage of these studies is that it cannot be assumed that the horses examined differed not only in their trailer experience but also that they experienced many more novel and/or different situations. However, all horses were owned by WULS and purchased at 3–4 years of age and their entire history, including the trailer loading, was known in detail. It means that each horse experienced the same environment of the didactic stable of the WULS at least 6 years before the study, which is enough to fully and comparable behavioral, psychoneuroendocrine, and psychoneuroimmune adaptation to environment (Budzyńska, 2014). All horses exhibit training levels, including both entrance into the trailer and transportation by the trailer, appropriate to group HE L or R L, comparable within group. All horses have never taken part in sports competitions. All horses in R L group were loaded into a trailer during the practice labs with the students and, in general, year by year, it was the same horses. Nevertheless, the results obtained should be treated as preliminary studies for further, better-planned experiments. The slightly different horses' experiences might cause the difference between HE L and R L and should be taken into account when individually assessing the suitability of our results. In further research, the inclusion of prebreaking horses and horses grazed on pasture through almost time in the year groups will provide much more valuable data. Likewise in further research, the difference of individual innate behavior and temperament of each horse as well as the impact of genetic problems should be investigated. Such continuation of research is required to deepen and provide new knowledge on the successive approximation of horses to their work in narrow spaces.

Despite the above disadvantages, it seems likely that regularly loaded horses generalize easier to new situations. We speculated that the regularly loaded horses developed many adaptable responses to enter the trailer during learning, which can be used in a similar situation. In contrast, horses that were hardly ever loaded must merely endure a new stressor, the entrance into narrow space, probably corresponding with the higher secretion of cortisol and more clearly correlated behaviors. We did not measure the concentration of cortisol in blood or saliva; therefore, the above speculations require further research.

In conclusion, the successive approximation of horses to the first work on a treadmill differed depending on their previous experience of loading and travelling in the narrow confines of a trailer. All the horses achieved the behavioral target and followed the handler without resistance into the treadmill; however, the group with trailer experience needed less time for the learning process. Regularly loaded horses also exhibited signs of a relaxation phase during the shaping process that could be associated with positive emotions following the additional reinforcement. The results obtained should be treated as preliminary studies for further experiments.

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CONFLICT OF INTEREST

Authors declare no conflict of interests for this article.

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