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Horses' perception of a threat posed by sounds of different origin*

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Summary

The aim of the study was to assess the behavioural reaction and emotional arousal of warmblood horses in response to sounds of different origin, and to classify those sounds into neutral ones, those causing a behavioural change and those causing a behavioural and physiological stress response. We tested the hypothesis that the perception of a sound as neutral or potentially threatening does not simply depend on the sound origin *per se*, but rather on the context in which the sound occurrs (predictability), additionally enhanced by the novelty effect. Recordings of 40 sounds, known (KS) and unknown (US), from four groups: anthropogenic (AS), neutral animal (NAS), predator (PS), and inanimate environment sounds (IES), were played to 20 warmblood horses remaining in their familiar paddock. The duration, frequency or occurrence of certain behaviours (e.g. walking, feeding, standing alert, stopping current activity), the heart rate (HR) and heart rate variability (HRV) were measured. The horses' reactions were rather weak and short-term. Most of the sounds resulted in distraction (increase in alertness). The horses ate less, walked and stood alert longer, and had a higher HR after certain sounds were played. The strongest stress response (physiological and behavioural) to NAS, mostly US, was observed. PS caused behavioural disturbance, but no cardiac activity changes. The weakest responses were observed for AS and IES. Modern, stable-kept horses remain vigilant to auditory stimuli in their environment and differentiate their responses to different sounds. The perception of a threat posed by sounds depends on their unpredictability and novelty.

Keywords: horse management, sounds, behaviour, heart rate variability

The adaptive ability of herbivores to assess a threat is based on the trade-off between satisfying basic needs and detecting/avoiding potential dangers (22, 23). A reaction inadequate to the degree of threat may result in unnecessary energy expenditure related to the constant readiness to confront the predator or even in death (19). The domestic horse (*Equus caballus*), as the so-called flight animal, is particularly sensitive to environmental stimuli (50). Although most horses in Europe are kept in stables (51), isolation from predators has not protected these animals from various stressors, which are mainly of anthropogenic origin (20). In the case of horses, new objects, events, or sounds might trigger a stress reaction and, consequently, increased vigilance or flight (54). According to the threat-sensitive predator avoidance hypothesis (14), an animal adjusts the form and intensity of reaction to the degree of the threat perceived. The intensity of such a reaction may be influenced by the current and earlier predation pressure and life experience of the animal (15). The gradation of reaction has been found in representatives of numerous taxa (22, 57), including mammals, as evidenced by the behavioural plasticity of the brown woolly monkey (*Lagothrix poeppigii*) (48) or the European rabbit (*Oryctolagus cuniculus*) (44) facing a threatening factor of various intensity. It was also noted in horses of breeds such as Polish koniks and Arabian horses (33). Their reaction to the sounds of a group of predators was more intense than it was to the sounds of individual animals.

Anti-predator behaviours can be present in animals that have not been exposed to predatory attacks for centuries (9, 41). Examples can be found in numerous studies on various kinds of animals: calves differentiating odorants into neutral and predator ones (1), adult cattle increasing vigilance at the expense reduced grazing time in response to chemical and visual stimuli of the wolf (38), or horses that were confronted

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with sounds of predators known or unknown to their ancestors (33).

However, anti-predator behaviours might also partially or completely disappear (9, 41). The causes of the weakening of the defensive instinct in farm animals include the process of domestication (37), selection for a calm temperament and a gentle character (26), and keeping animals in a safe environment (1, 11). According to the risk allocation hypothesis, high predatory pressure in the past combined with its almost complete absence in modern domestic horses has resulted in their poor expression of anti-predator behaviours (15). Human activity has decreased the ability of horses to coexist with predators in open housing systems (6, 21).

Prey may generalize their antipredator responses to specific predators, and animals closely related to them, depending on the risk of predatory pressure and predator diversity in a given area (23). According to the risk-disturbance hypothesis, human-related stimuli trigger reactions analogous to those elicited by predation risk (24). Therefore, horses probably generalize stressors present in the anthropogenic environment and perceive them as a potential predator threat (18, 32). On the other hand, according to the anthropomorphising tendency, we should assume that in an environment that is free from natural enemies, horses should feel comfortable and ignore biologically irrelevant stimuli (54, 56). We should also assume that their commonly known excitability results only from sensory sensitivity (50).

Horses' responses to auditory stimuli are of interest to many scientists (31, 35, 45, 49, 53). It is well-known that sound signals, depending on their frequency, have different impact on the behaviour of equines (35). Smith et al. (53) found that horses showed different reactions depending on the nature of human non-verbal vocalization. Rochais et al. (49) proved that the effect of novelty and surprise may disturb the behaviour of horses exposed to new auditory stimuli, even if these stimuli are not biologically significant. On the other hand, Huo et al. (31) observed a positive effect of relaxation music on horses' behaviour. Since most distractors are auditory, various sounds are of particular importance as disturbing factors (45). However, it is not fully understood how horses differentiate noises and sounds (both known and unknown) into threatening, neutral, and pleasant ones (50).

There are numerous theories explaining why the reactions of prey to clues of predators are so diverse (10, 15, 23, 24). In order to assess reactions and their gradation on the basis of threat perception, signals of known and unknown predators, as well as alarm voices of conspecifics and heterospecifics, are commonly used (15, 16, 22, 44, 48). However, to the best of our knowledge, horses' behavioural and physiological responses to sounds of different origin, including both natural and synthetic sounds, have not been compared so far. In the case of working horses, it is crucial to know the factors on the basis of which these animals classify various sounds into neutral and potentially threatening (3, 18). Differentiating events in terms of the degree of threat allows animals to avoid unnecessary stress and energy expenditure (42). On the other hand, understanding horses' sensory sensitivity and mechanisms of their reactions, can improve safety during everyday procedures and activities (50, 54).

In our study, we tested horses' reactions to sounds from the anthropogenic environment, inanimate nature, or neutral and predatory animals. In view of predation-related hypotheses and the lack of solid knowledge about how horses classify sounds of different origin and how the process of domestication has influenced their anti-predatory reactions, we hypothesised that horses' reactions to different sounds do not simply depend on the origin of the sound *per se*, but rather on the context in which it is heard (predictability), additionally enhanced by the novelty effect. The aim of the study was to assess the behavioural reaction and emotional arousal of warmblood horses in response to sounds, and to group those sounds into neutral ones, those causing behavioural change and those causing behavioural and physiological stress response.

Material and methods

Animals and their living conditions. The study included 20 leisure warmblood horses (Malopolski (n = 8), Wielkopolski (n = 7), Polish Half-Breed (n = 5)) aged 5-15 years: 8 mares and 12 geldings. All animals were housed at the same equestrian centre located on the outskirts of the city, away from the main thoroughfares in Lublin Voivodeship, Poland. The horses were accustomed to large groups of people, motor vehicles and air traffic due to a nearby airport (5.6 km). The horses were also familiar with other farm and companion animals kept at the centre (alpacas, goats, cows, cats and dogs). Apart from domesticated forms of predators (cats and dogs), they had never come into contact with predatory animals. They were taken care of by five caretakers. The horses worked under the saddle two hours a day, six days a week. Riders were of different ages and had different riding abilities. The animals were also periodically desensitized to different types of visual stimuli by natural training methods. However, they had not been desensitized to any sound stimuli.

All horses were kept in individual box stalls (3.5 m × 3.5 m). The floor in the stalls was covered with straw twice a day. There was a hay feeder, a manger, an automatic waterer and a salt lick in each box stall. The horses were fed three times a day with meadow hay and a grain mix (concentrate) with vitamin supplements. Seasonally, they grazed on pastures for 4-6 hours a day. During the pasture season, they were turned out in sand paddocks with 2 to 7 familiar individuals for a minimum of 4 hours.

Prior to the experiment, horses were constantly observed during daily handling by an experienced caretaker. Additionally, a motionless person test was conducted while the horses were in the box (25, 29). No behavioural disorders were found, and all horses showed a similar response to the unknown person (i.e. voluntary approach and sniffing the human standing still). During the time of the study, all horses were clinically healthy and not injured.

Ethics approval. The procedures were conducted with permission (27/2016 issued on 13 May 2016) from the Local Committee for Ethics in Animal Experimentation, Lublin, Poland.

Experimental procedure. The research was carried out during the spring period of 2021. The horses were divided into 7 groups of 2 to 4 animals that were familiar and friendly with each other. Each group was exposed to 40 sounds of various origins, either known (KS) or unknown (US) to the horses. Four types of sounds were distinguished according to their origin: anthropogenic sounds (AS; n = 11; including, among others, sounds of city traffic, motor vehicles and household appliances), sounds of the so-called neutral animals (NAS; n = 14; birds and non-predatory mammals), sounds of wild predatory mammals (potentially dangerous to horses in a natural environment) (27, http://www.lrgaf.org/articles/Wild%20Horse%20DNA%20Report%202015) and their domesticated forms (PS; n = 9) and sounds from the inanimate environment (IES; n = 6; related to weather and elements of the inanimate environment) (Tab. 1.) Before the experiment, eight sets of sounds were created by selecting five recordings at random, but in such a way that each set included at least three types of sounds in terms of origin. The order of sounds in a given set was the same for all groups of horses (Tab. 1). In each week of the experiment, the animals were presented with two sets of sounds with a break of 2-3 days between one set and the other (both in one week and between the two sets in consecutive weeks of the study). This reduced the likelihood of horses becoming accustomed to the routine of the procedure and presenting automated responses. The research was carried out depending on the weather. The tests were not conducted when atmospheric conditions could interfere with the perception of the sound stimulus (rainfall, wind > 0.3 m/s). That is why the study took several days longer for some horses. However, the main guidelines of the study were followed (a maximum of two sets of sounds a week with a break of 2-3 days). The research was carried out between 7:30 am and 1:30 pm The horses were brought to the place of the experiment (the experimental paddock) from the stables and then taken to the pasture after the end of the study. Two groups of horses were tested each day: one between 7:30 am and 10:30 am (minimum 1.5 h after morning feeding) and the other between 10:30 am and 1:30 pm. Therefore, every week each group was tested once in the morning for one set of sounds and once around noon for the other set. This helped to lower the risk of habituation to the research environment. After entering the experimental paddock, the horses were left for 20-30 minutes, which allowed them to express their behaviour freely. Although the place in which the experiments were conducted was known to the horses, it was necessary to let the animals calm down and satisfy their curiosity and need for exploration after transfer from the stables to the paddock. When horses had not exhibited increased locomotor activity for at least 5 minutes, the starting point of the study was noted, regardless of the paddock space occupied by the animals. The natural behaviour of the horses in the paddock was a subject of interest and a point of reference for statistical analysis.

Tab. 1. Playing order of the sets and sounds within the sets. Additional information in brackets includes the sound origin and the animal's potential familiarity with the sound

Set No	Experimental week*	Playing order
I	1	Police siren (AS, KS) – Squealing and grunting of a pig (NAS, US) – Stream (IES, US) – Alarm clock (AS, US) – Call of a pheasant (NAS, KS)
II	1	Church bell (AS, US) – Call of a red-tailed hawk (NAS, US) – Screaming of a chimpanzee (NAS, US) – Branch cracking + rustling of leaves (IES, KS) – Applause (AS, KS)
III	2	Storm (IES, KS) – Passing locomotive (AS, US) – Playing the trumpet (AS, KS) – Sheep bleating (NAS, US) – Roar of a lion (PS, US)
IV	2	Growling of a lynx (PS, US) – Meowing of cats (PS, KS) – Vacuum cleaner (AS, US) – Mewing of herring gulls (NAS, US) – Wind (IES, KS)
V	3	Bellow of a deer (NAS, US) – Cuckooing of a cuckoo bird (NAS, KS) – Plane flying (AS, KS) – Sound of waves (IES, US) – Trumpeting of an elephant (NAS, US)
VI	3	Road traffic (AS, KS) – River (IES, US) – Flap of a common snipe (NAS, US) – Calls of a magpie (NAS, KS) – Barking of dogs (PS, KS)
VII	4	Lawnmower (AS, KS) – Roar of a bear (PS, US) – Howling of a wolf (PS, US) – Growls and roars of a tiger (PS, US) – Cawing of a raven (NAS, KS)
VIII	4	Growls of a leopard (PS, US) – Grunting of a gorilla (NAS, US) – Screeches of a vulture (NAS, US) – Crowd of people (AS, KS) – Growls and roars of a cougar (PS, US)

Explanations: AS – anthropogenic sounds, NAS – neutral animal sounds, PS – predator sounds, IES – inanimate environment sounds, KS – potentially known sounds, US – potentially unknown sounds. *Due to adverse atmospheric conditions (rainfall, wind > 0.3 m/s), the layout of the procedure changed, resulting in the experiment taking a few more days

Experimental paddock. The sand paddock used for the study was familiar to the horses. It was surrounded by trees and located in the centre area next to an orchard and a field (Fig. 1). The paddock fence was made of metal railings. There were a few trees inside the paddock (about 1.5% of the area). The location of the paddock reduced unnecessary stimuli related to the movement of vehicles, pedestrians, riders and other horses. It was located 300 meters from the stables and other paddocks with horses not participating in the experiment at that time. This prevented animals in the stable from hearing the recordings, so that they were exposed to a given sound only once during the study. The paddock was conventionally divided into three zones along its longer side, depending on the distance from the speaker used for playing the sounds. The speaker was placed 3 meters behind the fence and it was invisible to the horses. Zone 1 was closest to the sound source and extended 6 meters into the test area. There were trees and grass outside the fence (attractive food) that horses could try to reach while being in the experimental paddock. Zone 1 was additionally enriched with four easily accessible nets

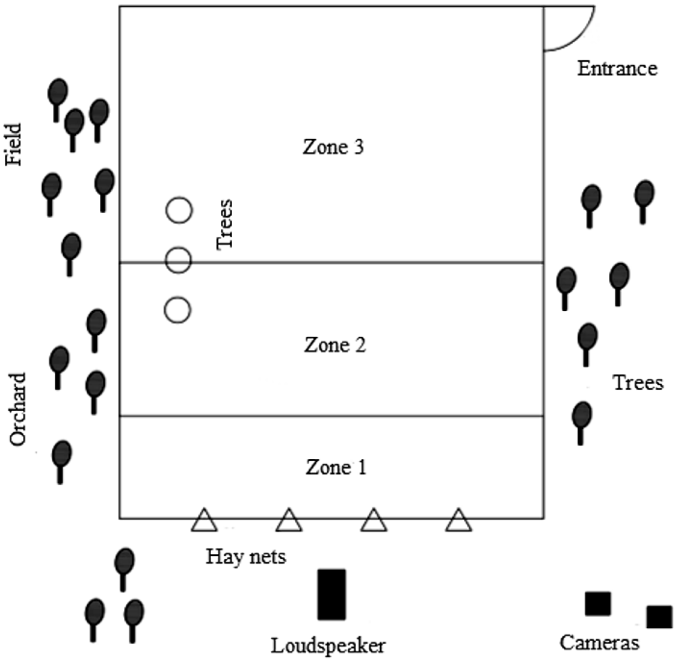

Fig. 1. The layout of the experimental paddock

with hay that were hung on the shorter side of the fence at intervals of 5 meters. This was inteded to encourage the horses to stay in zone 1, where they were most exposed to the experimental stimulus (sound). Zone 2 was set at a distance of 6-15 meters, and zone 3 at a distance of 15-30 meters from the hay nets. Throughout the study, the horses continued to be used for riding for an average of two hours a day. On the experiment day, they were used only in the afternoon and evening hours. Since horses were usually turned out in the pastures or paddocks (including the one where the experiment was held) during test hours and the sound stimulus exposure was short, the experiment did not disturb the normal routine of the animals.

Sounds. All sounds used in the study were downloaded from the following websites: https://www.pacdv.com, http://www.animal-sounds.org, http://www.orzelorla.pl, http://danielbialogard.pl, http://xn--odgosy-5db.pl, https://www.youtube.com, https://www.salamisound.com, https://www.pacdv.com (accessed on 15 March 2021) and saved to computer memory. Then they were prepared for spectral analysis in Audacity 2.4.2. The recordings were amplified, if necessary, by a maximum of 22 dB. Fragments of silence (no significant sound for > 2 seconds) were removed, and the recordings were combined so that each of the 40 sounds lasted about 60 seconds. Then they were subjected to spectral analysis at the Institute of Agrophysics, Polish Academy of Sciences, Lublin. Most of the sounds showed the maximum sound intensity at a frequency of 1250 Hz. The highest frequency at the maximum level of sound intensity was found in the recordings of a pheasant (5000 Hz), an alarm clock (4000 Hz), gulls and a buzzards (3150 Hz), as well as magpies and sheep herds (2500 Hz). The lowest frequency at the maximum sound intensity was recorded for a cat (1000 Hz). In terms of the differentiation of frequency components, the sound intensity level was most differentiated for a crowd of people, thunderstorm, a cat and a deer, whereas the recordings of a river and road traffic were the least diverse. All sounds were played at a sound intensity level (L_A) of 79 ± 4 dB, measured at the loudspeaker before the experiments. This intensity has been proven to have only a short-term effect on the heart rate (55). The recordings were played using a wireless dual-membrane speaker (JBL Charge 4) with a rated power of 30W and noise-and-echo reduction. The speaker was connected to a Samsung Galaxy A02s device (Samsung Electronics Co., Ltd., South Korea) via Bluetooth.

Behavioural data collection and analyses. The behavioural reaction of the horses to sounds of different origin was assessed in three intervals of 5 minutes each: 1) starting parameters (b0; before the recording was played), 2) procedural parameters (b1; from the moment the recording was played) and 3) recovery parameters (b2; another 5 min; the time of returning to the initial parameters) (34). The time interval between playing successive sounds in a given set was 30 minutes (41). Using a detailed ethogram developed by McDonnell (43), as well as the results of previous research on horses' responses to sound signals (18, 32), a behavioural protocol was developed containing a set of behaviours assessed during the study (Tab. 2). During each of the three 5-minute measurements (periods b0, b1, b2), the duration (s) and frequency (number of repetitions) of specific behaviours, as well as the duration (s) of staying in one of the three zones designated in the experimental paddock were measured. Time was measured with an accuracy of one second using a manual stopwatch and it was recorded in the protocol. In addition, immediately after playing the recording (period b1), the occurrence of behaviours such as approaching or moving away from the sound source and stopping certain activities was recorded using the one-zero sampling method (0-1 method) (4), where ‚0' meant "no", and ‚1' meant "the occurrence of a specific reaction". The duration of standing and being alert, eating, moving and staying in zone 1 was of particular importance. The observations were made by the focal-animal sampling method (4) during the tests and on the basis of a later analysis of recordings from video cameras (Sanyo XACTI). In each group of horses, a person conducting the experiment (trained in animal behaviour and invisible to the horses) focused on two individuals, noting the duration and frequency of their behaviours. If there were more than two horses in the experimental paddock, the behavioural activity of the other animals was assessed on the basis of video recordings. Defecation, snorting and snoring were observed only *in situ* because of potential difficulties in observing or hearing them during video analysis. The cameras were located outside the experimental paddock, at a distance of 6 and 8 meters from the right corner of its front part (zone 1). Before the experiment, numerous attempts were made to find the optimal camera setting that would eliminate the risk of „dead zones". Only the combination we used allowed the cameras to cover the entire experimental area.

Cardiac activity data collection and analyses. The influence of the sound signals on the emotional arousal of the horses was determined on the basis of the heart rate (HR) and heart rate variability (HRV). The heart rate was recorded with Polar ELECTRO OY telemetry devices (type RS800CX). The devices included HR monitors and elastic straps with electrodes adjacent to the surface of the body

Tab. 2. Behaviours assessed in the behavioural protocol for each sound during three 5-minute periods (b0, b1, b2)

Behaviour	Unit	Comments
Staying in the zone 1/2/3	time (s)	–
Feeding from hay nets	time (s), freq.	–
Other feeding behaviours	time (s)	Attempts to feed on leaves (inside/outside) and grass outside of the experimental paddock, eating tree bark
Total feeding behaviour	time (s)	Combined time of feeding from hay nets and other feeding behaviours
Walk	time (s)	For at least 2 s
Trot**	time (s)	For at least 2 s
Canter **	time (s)	For at least 2 s
Total locomotion	time (s)	Combined time of walking, trotting, and cantering
Standing	time (s)	Only for resting; standing alert not included
Comfort behaviour (s)	time (s), freq.	Individual grooming and playing
Affiliative behaviours**	time (s)	Mutual grooming, playing with other individuals, positive interactions with other horses
Agonistic behaviours	time (s)	Behaviours indicating domination and/or submission
Standing alert	time (s)	Standing with the neck up; head and ears turned to the direction of the sound's source
Exploration	time (s)	Sniffing and scattering faeces, sniffing elements of the paddock
Defecation**	freq.	–
Snorting	freq.	–
Blowing**	freq.	Nervous expulsion of air through the nostrils
Spooking**	freq.	Startling of a horse related to the potential stressful situation
Interruption resulting in stopping the behaviour	0-1*	Definite, for at least 2 s.
Going away from the sound source	0-1*	–
Approaching the sound source	0-1*	–

Explanations: Freq. – unit of frequency (how many times the behaviour was repeated); *0-1 sampling; 0 – no occurrence of the behaviour, 1 – occurrence of the behaviour; assessed only in b1 period as the first reaction to the sound; ** Behaviours eliminated from regression analysis due to the variability of results close to or equal to zero; b0 – starting parameters (before sound exposure), b1 – procedural parameters (during sound exposure), b2 – recovery parameters (after sound exposure)

and with a transmitter attached to them on the outside (H2). Two days before the experiment, all horses were fitted with the devices in order to habituate them to the research equipment. The straps were fastened around the horses' chest so that the electrodes were on the left side of the horse at the heart level. To optimize conductivity and minimize electrical resistance, the rubber part of the strap where the electrodes were attached was covered with a large amount of ECG gel (39). Then, HR monitors, previously synchronized with a specific transmitter, were attached to the elastic straps at the sternum level. Polar kits were mounted on the horses in the stable and switched on at least 15 minutes before they went to the experimental paddock. Simultaneously with the HR monitor, a manual stopwatch was activated to record the duration of playing individual sounds and use it for later analyses of specific segments in HR recordings. HR recordings were carried out continuously and stopped only after the end of the b2 period for the last sound. The devices were removed in the experimental paddock, and the horses were taken to another paddock. Data from HR monitors were transmitted to a computer by a peripheral IrDA USB 2.0 adapter. Then the recordings were processed and analysed with the Polar Pro Trainer 5 software. Where necessary, low-power and or medium-power filters were used to eliminate individual artifacts. Parameters analysed included those whose increase indicated increased activity of the parasympathetic ANS (RR interval, RMSSD, HF) and sympathetic ANS (HR avg., LF, LF/HF) (12, 33):

- RR interval [ms] – intervals between consecutive R waves of the QRS complex; time domain,
- RMSSD [ms] – root mean square of successive differences; time domain,
- HF [ms^2] – high-frequency component of the power spectrum (0.07-0.5 Hz); frequency domain,
- HR avg. [bpm] – mean heart rate; time domain,
- LF [ms^2] – low-frequency component of the power spectrum (0.005-0.07 Hz); frequency domain,
- LF/HF [%] – the ratio of the low-frequency spectrum to the high-frequency spectrum indicating the sympathetic-parasympathetic balance; frequency domain.

According to recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (40), the duration of each measurement for short-term analyses was identical and amounted to 5 minutes: starting HR/HRV (b0), procedural HR/HRV (b1), and HR/HRV recovery (b2).

Statistical analysis and analytical strategy. The measurements showed deviations from normality assumptions (checked by Kolmogorov-Smirnov test (if n > 50), Shapiro-Wilk test (if n < 50) and visually on histograms). Therefore, a series of transformations were performed before any calculations and modelling. We decided to used the natural logarithm transformation to improve the shape of distributions and standardize them to obtain an identical unit of measurement (expressed as deviations from the average score).

The main analysis concerned the influence of independent variables: sound origin (n = 4; AS, NAS, PS, IES) and sound (n = 40) on the behavioural and cardiac activity of the horses in the three periods of observation (b0, b1, b2). Our analytical strategy was divided into two phases. In the first one, related to the exploration process, effect sizes and significance were sought, whereas in the second one, the modelling process was introduced. In the exploration process, the classic analysis of variance was conducted. When the variance equality was violated, the Welch-corrected ANOVA was performed (36). The analysis aimed to investigate significant differences between the three periods of observation (b0 vs. b1 vs. 2) for each dependent variable while playing a given sound (n = 40). Due to the variability of results equal or close to zero, six dependent variables were eliminated from further analysis (Tab. 2). Then, significant and close to significant cases were modelled by a linear mixed-effects model (7) using the „lme4" R package (8). This analysis was used to calculate the change (increase or decrease) in the value of a given feature during b1 and b2 in relation to b0. After verification, the 'sound origin/sound*dependent variable' models were excluded if significant changes were found only in b2 with respect to the b0 period. Models in which significant changes occurred during b1 or b1 and b2 with respect to the b0 period were left unchanged (since our interest was the impact of the sound played in the b1 period). Then, trends towards change in a specific dependent variable (increase or decrease) under the influence of different sounds during the b1 period were observed. Dependent variables for which the 'sound*dependent variable' models did not show a clear trend of vigilance/stress-eliciting impact of the sound (random increase or decrease in the value of a given feature) and models deviating from a distinct trend for a given dependent variable were excluded from further analysis. Using the „multcomp" R package, multiple comparisons were calculated for selected 'sound origin/sound*dependent variable' models to verify differences between b1 and b2 periods for a given sound/sound origin (30) by the Tukey contrast method. To plot differences between the influence of numerous sounds on horses' reactions, 95% confidence intervals were calculated for regression coefficients.

The selected models were grouped according to the 'sound effect' and 'dependent variable type' categories (see Sound selection). Then, the percentage of significant changes caused by KS/US and AS/NAS/PS/IES was determined (taking into account their percentage in the overall pool of sounds) and Pearson's chi-squared test was conducted (Statistica 13.3 PL). The impact of the 'sound effect' on behaviours assessed by the 0-1 sampling method was examined by the same method. For all analyses, the level of significance was set at $p < 0.05$.

Tab. 3. General impact of the sounds on the horses' reactions

Sound	Increase in the value	Decrease in the value
Behavioural and cardiac responses		
Magpie	zone 3 (s), hay nets (freq.), walk (s), total locomotion (s), standing alert (s), HR (bpm)	hay nets (s), total feeding (s), RR (ms)
Pig	zone 2 (s), hay nets (freq.), walk (s), total locomotion (s), standing alert (s), HR (bpm)	hay nets (s), total feeding (s), RR (ms)
Chimpanzee	hay nets (freq.), standing alert (s), HR (bpm)	hay nets (s), other feeding (s), total feeding (s), RR (ms)
Red-tailed hawk	hay nets (freq.), walk (s), total locomotion (s), standing alert (s), HR (bpm)	total feeding (s), RR (ms)
Common snipe	walk (s), total locomotion (s), standing alert (s), HR (bpm)	hay nets (s), RR (ms)
Elephant	walk (s), total locomotion (s), standing alert (s), HR (bpm), LF/HF (%)	RR (ms)
Stream	standing alert (s), HR (bpm)	RR (ms)
Deer	standing alert (s)	RMSSD (ms)
Behavioural responses		
Cuckoo bird	zone 3 (s), hay nets (freq.), standing alert (s)	zone 1 (s), total feeding (s)
Cats	hay nets (freq.), walk (s), total locomotion (s), standing alert (s)	total feeding (s)
Wolf	zone 3 (s), standing alert (s)	zone 1 (s), total feeding (s)
Vulture	standing alert (s)	hay nets (s), total feeding (s)
Lynx	standing alert (s)	zone 1 (s)
Leopard	hay nets (freq.), standing alert (s)	–
Police siren	standing alert (s)	other feeding (s)
Dog	total locomotion (s), standing alert (s)	–
Wind	standing alert (s)	zone 1 (s)
Ewe	hay nets (freq.), standing alert (s)	–
Vacuum cleaner	hay nets (freq.), walk	–
Sorm	hay nets (freq.)	–
Applause, lion, seagulls, alarm clock, tiger, road traffic, cougar, branch cracking, pheasant, bear, crowd, gorilla	standing alert (s)	–
Church bell, railway engine, trumpet, plane, waves, river, raven, lawnmower	none	none

Results and discussion

General responses to the sounds. Only eight out of 40 experimental sounds did not influence horses at all. Most of the sounds had a weak or moderate impact on the horses (with limited changes in behavioural dependent variables), and only eight of them may be considered as stress eliciting (with both physiological and behavioural changes) (Tab. 3). If an effect was observed, it was greater for behavioural than for cardiac activity (77 vs. 16 out of the 93 selected 'sound*dependent variable' models and 12 vs. 3 out of the 15 selected 'sound origin*dependent variable' models).

In most cases, considering differences between periods b0, b1 and b2 caused by one sound/sound origin (see: x and y for Tab. 4, 5a-d as comparisons between b1-b0 vs. b0, b2-b0 vs. b0 and b2-b0 vs. b1-b0; the difference between b2-b0 vs. b1-b0 indicates the difference in change between b2-b1 period), the effect of the sound was either short-term (it was visible only in the b1 period: b1-b0 and b2-b1; $p < 0.05$; b2-b0; $p > 0.05$) or moderately prolonged when the effect of the sound continued to some degree in the b2 period (b1-b0; $p < 0.05$, b2-b1 and b2-b0; $p > 0,05$). Only a few sounds (chimpanzee, magpie, wolf and red-tailed hawk) caused a prolonged effect (b1-b0 and b2-b0; $p < 0.05$, b2-b1; $p > 0.05$).

With a few exceptions (for the b1 period: frequency of feeding from hay nets, standing alert and RR interval; for the b2 period: locomotor behaviour), there were no differences between the impact of different sounds on the same type of behaviour (a and b for Tab. 4, 5a-d). If the auditory stimuli affected the behavioural or cardiac activity of the horses, their impact was usually similar. Estimates of each of the dependent variables during b0 period did not differ between the sounds.

Behaviours that were most often interrupted due to the sounds were standing alert, feeding and locomotor behaviour. Therefore, only these were included in the tables.

Behavioural responses. The variable that was most influenced by 'the origin of the sound' was standing alert. Each type of sound (AS, NAS, PS, IES) caused significant increase in time devoted to being alert (Tab. 4). This time was longest in the case of PS and

Tab. 4. The impact of the sound origin (IES, AS, PS, NAS) on the duration of standing alert

Type of sound	b0	b1-b0	b2-b0
	Standing alert		
IES	–0.22 xa	0.26 ya	–0.06 xa
AS	–0.23 xa	0.38 ya	–0.02 xa
PS	–0.28 xa	0.80 yb	0.11 xa
NAS	–0.24 xa	1.00 yb	0.14 xa

Explanations: Changes (increase or decrease (–)) in periods b1 and b2 compared to period b0 were calculated. Estimates (b1-b0 vs. b0, b2-b0 vs. b0 and b2-b0 vs. b1-b0) marked with different letters differ significantly at $p < 0.05$ within one type of a sound (horizontally; x, y); between the sounds of different origin based on 95% confidence intervals calculated for regression coefficients (vertically; a, b)

Tab. 5a. The impact of the sounds on the time the horses spent on standing alert

Sound	b0	b1-b0	b2-b0	*Impact of the sound
	Standing alert			
Pig	–0.01 ax	2.58 ay	0.00 ax	21
Chimpanzee	–0.25 ax	1.83 ay	0.40 ax	11
Pheasant	–0.13 ax	1.41 ay	0.43 ax	10
Bear	–0.29 ax	1.55 ay	0.54 ax	5
Vulture	–0.31 ax	1.10 ay	0.01 ax	4
Elephant	–0.26 ax	1.34 ay	0.12 ax	3

Explanations: Only the most impactful sounds (concerning *) were included in the tables.* The number of the sounds that had weaker effect (caused lesser increase in the time of standing alert) than a given sound; based on 95% confidence intervals calculated for regression coefficients

Tab. 5b. The impact of the sounds on the feeding behaviour of the horses

Sound	b0	b1-b0	b2-b0	b0	b1-b0	b2-b0	b0	b1-b0	b2-b0
	Time of feeding from hay nets			Frequency of feeding from hay nets			Total time of feeding behaviour		
Chimpanzee	–0.16 ax	–0.22 ay	–0.06 axy	–0.13 ax	0.20 aby	0.15 ay	0.02 ax	–0.58 ay	–0.16 ax
Magpie	0.19 ax	–0.48 ay	–0.02 axy	–0.06 ax	0.09 ay	0.11 ay	0.29 ax	–0.56 ay	–0.20 axy
Pig	–0.03 ax	–0.51 ay	–0.11 ax	–0.10 ax	0.09 ay	–0.02 ax	–0.03 ax	–0.46 ay	0.07 ax
Vulture	0.13 ax	–0.38 ay	–0.06 axy	–	–	–	–0.07 ax	–0.45 ay	0.10 ax
Common snipe	0.30 ax	–0.38 ay	–0.17 axy	–	–	–	–	–	–
Cuckoo bird	–	–	–	–0.04 ax	0.10 aby	0.02 axy	0.36 ax	–0.27 ay	0.05 ax
Red-tailed hawk	–	–	–	–0.08 ax	0.12 aby	0.00 axy	0.12 ax	–0.37 ay	–0.16 axy
Cats	–	–	–	–0.10 ax	0.29 by	0.03 axy	0.41 ax	–0.34 ay	–0.10 axy
Wolf	–	–	–	–	–	–	0.01 ax	–0.52 ay	–0.43 ay
Leopard	–	–	–	–0.05 ax	0.12 aby	0.02 axy	–	–	–
Ewes	–	–	–	–0.07 ax	0.14 aby	0.10 axy	–	–	–
Vacuum cleaner	–	–	–	–0.03 ax	0.17 aby	0.05 axy	–	–	–
Storm	–	–	–	–0.11 ax	0.11 ay	0.05 axy	–	–	–

Tab. 5c. The impact of the sounds on the duration of the locomotor behaviour of the horses

Sound	b0	b1-b0	b2-b0	b0	b1-b0	b2-b0
	Walking			Total locomotion		
Common snipe	–0.09 ax	0.57 ay	0.16 abxy	–0.36 ax	0.56 ay	–0.02abx
Cats	–0.40 ax	0.58 ay	0.00 abx	–0.13 ax	0.57 ay	0.12abxy
Elephant	–0.06 ax	0.69 ay	–0.43 bx	–0.06 ax	0.64 ay	–0.42ax
Red-tailed hawk	–0.41 ax	0.71 ay	0.87 ay	–0.41 ax	0.66 ay	0.60by
Magpie	–0.34 ax	0.79 ay	0.32 abxy	–0.35 ax	1.07 ay	0.39abxy
Pig	–0.34 ax	0.88 ay	0.09 abx	–0.32 ax	0.94 ay	0.25abx
Vacuum cleaner	–0.31 ax	0.60 ay	0.37 abxy	–	–	–
Dog	–	–	–	–0.14 ax	0.67 ay	0.66aby

Tab. 5d. The impact of the sounds on the cardiac activity of the horses

Sound	b0	b1-b0	b2-b0	b0	b1-b0	b2-b0
	Mean HR (bpm)			RR interval (ms)		
Magpie	0.21 ax	0.26 ay	0.08 axy	–0.29 ax	–0.22 ay	–0.10 axy
Common snipe	0.16 ax	0.38 ay	–0.06 ax	–0.25 ax	–0.37 aby	0.08 ax
Red-tailed hawk	–0.15 ax	0.42 ay	0.19 axy	0.13 ax	–0.47 aby	–0.23 axy
Stream	0.13 ax	0.51 ay	0.03 ax	–0.16 ax	–0.41 aby	0.00 ax
Elephant	–0.11 ax	0.56 ay	0.01 axy	0.07 ax	–0.44 aby	0.03 ax
Pig	–0.06 ax	0.71 ay	0.19 ax	–0.03 ax	–0.69 by	–0.17 ax
Chimpanzee	0.09 ax	0.75 ay	0.03 ax	–0.10 ax	–0.70 by	–0.07 ax
Other						
Elephant; LH/HF (%)	–0.21 x	0.64 y	0.14 xy			
Deer; RMSSD (MS)	–0.03 x	–0.29 y	0.05 x			

Explanations for tables 5a-5d: changes (increase or decrease (–)) in periods b1 and b2 compared to period b0 were calculated. Estimates (b1-b0 vs. b0, b2-b0 vs. b0 and b2-b0 vs. b1-b0) marked with different letters differ significantly from the b0 period at $p < 0.05$ within one type of a sound (horizontally; x, y); between the sounds of different origin based on 95% confidence intervals calculated for regression coefficients (vertically; a, b); b0 – starting parameters (before sound exposure), b1 – procedural parameters (during sound exposure), b2 – recovery parameters (after sound exposure). The tables include only the most impactful sounds, i.e. those that influenced both behavioural and cardiac activity or at least three behavioural variables, including standing alert

NAS sounds. However, the effect of the sound origin was short-term (b1-b0 and b2-b1; $p < 0.05$, b2-b0; $p > 0.05$).

With regard to the 'individual sound' effect, 30 sounds resulted in a significant increase in time spent standing alert. The most distractive sounds concerning the number of the sounds that had weaker effect than a given sound (n), were the following: pig (n = 21), chimpanzee (n = 11), pheasant (n = 10), bear (n = 5), vulture (n = 4) and elephant (n = 3) (Tab. 5a). Their effect was short-lasting: for almost all of the 30 sounds, a significant decrease in vigilance was observed in b2 compared to the b1 period.

In terms of feeding behaviour (Tab. 5b), most of significant changes caused by the sounds occurred in the frequency of feeding from hay nets. The horses approached the nets or resumed eating (after interruption) more often after 10 sounds were played: pig, magpie, cuckoo bird, storm, leopard, red-tailed hawk, ewes, chimpanzee, cats and vacuum cleaner. Cats' meowing resulted in a significantly higher frequency of food intake during the b1 period than the squealing and grunting of a pig, the chatter of a magpie and the sound of a storm. However, apart from these exceptions, there were no differences between the impact of the sounds on each feeding behaviour variable in any period (b0, b1, b2). We observed a significant decrease in the time spent feeding from hay nets (under the influence of the sounds of a chimpanzee, magpie, pig, common snipe and vulture) and total feeding behaviour (chimpanzee, magpie, wolf, pig, vulture, red-tailed hawk, cats, cuckoo bird) during the b1 period compared to the b0 period. Only the chatter of a magpie and the screaming of a chimpanzee (for the frequency of feeding from hay nets) and the howling of a wolf (for total feeding behaviour) induced a prolonged effect (b1-b0 and b2-b0; $p < 0.05$, b2-b1; $p > 0.05$). The other sounds had a moderate effect (b1-b0; $p < 0.05$, b2-b0 and b2-b1; $p > 0.05$) or no effect (b1-b0 and b2-b1; $p < 0.05$, b2-b0; $p > 0.05$) on feeding behaviour in the b2 period.

A distraction effect of the sounds was also seen in the extended time spent on locomotor behaviour (Tab. 5c). Both walking and total locomotion changed significantly when six recordings were played: the sounds of a common snipe, cats, elephant, red-tailed hawk, magpie and pig. We also noted that the sound of a vacuum cleaner had an effect on the 'walking' variable, while dog barking influenced the 'total locomotion' variable. Most of the sounds had a short-term effect (b1-b0 and b2-b1; $p < 0.05$, b2-b0; $p > 0.05$) or a moderate effect (b1-b0; $p < 0.05$, b2-b0 and b2-b1; $p > 0.05$) on locomotor behaviour. The barking of a dog resulted in a prolonged time spent on total locomotion, and the scream of a red-tailed hawk extended the time of walking and total locomotion (b1-b0 and b2-b0; $p < 0.05$, b2-b1; $p > 0.05$). There were no differences between the impact of individual sounds during the b1 period. In the b2 period, the only significant differences were noted in the effects of the trumpet of an elephant and a red-tailed hawk's call on locomotor behaviour (a decrease for the elephant and an increase for the red-tailed hawk).

Cardiac activity responses. We measured the resting parameters of the heart rate (HR) and heart rate variability (HRV) while the horses stayed in box stalls before beginning any experimental procedures. We noted the following values: HR = 36.4 ± 6.0; RR = 1681.0 ± 247.9; RMSSD = 88.5 ± 19.0; HF = 1857.1 ± 1110.7; LF = 1633.8 ± 759.0; LF/HF = 148.8 ± 72.2.

The impact of the 'sound origin' on the cardiac activity of the horses was restricted to NAS. NAS activated the sympathetic nervous system response (an increase in the mean HR and LF/HF and a decrease in the RR interval). For the mean HR and RR interval, this effect was limited to the b1 period. When it comes to LF/HF, the sound origin effect was observable in the b2 period, but it was weakened (b2-b0 and b2-b1; $p > 0.05$).

Eight out of the 40 sounds caused changes in cardiac activity directed towards sympathetic activation. These changes, however, were almost exclusively a rise in the mean HR and a decrease in the RR interval (magpie, common snipe, red-tailed hawk, stream, elephant, pig, chimpanzee) (Tab. 5d). Only two sounds influenced other cardiac parameters. The trumpet of an elephant caused LF/HF to increase, and the bellow of a deer reduced RMSSD in the b1 period. The effect of each sound was either short-term (b1-b0 and b2-b1; $p < 0.05$, b2-b0; $p > 0.05$) or medium-term (b1-b0; $p < 0.05$, b2-b0 and b2-b1; $p > 0.05$). There were no differences between particular sounds for any period in relation to the mean HR. A decrease in the RR interval during the b1 period was greater for pig and chimpanzee sounds compared to magpie.

Sound selection. Based on the selected models that could be perceived as distractive or vigilance eliciting, we assigned the sounds into appropriate categories according to the dependent variable they had influenced and the effect they had caused. The effect of the sound (threat perception) was based on the reaction that it had caused (behavioural and/or cardiac activity changes) and the number of dependent variables it had affected. For each of the categories, we designated a percentage of KS/US and AS/NAS/PS/IES using a method described in Statistical analysis and analytical strategy.

We distinguished five categories of dependent variables (occupied zone, feeding behaviour, locomotor behaviour, standing alert and emotional arousal), and 14 of these dependent variables changed significantly following an auditory stimulus (Tab. 6). Sound stimuli had the greatest impact on the time spent standing alert (75% of the possible models for this variable were significant) and the duration of locomotor (17.50%) and feeding behaviour (15.63%). There was no relationship between KS/US or AS/NAS/PS/IES sounds and the time spent in particular zones ($p > 0.05$). The effect of the sound novelty was observed only for the emotional arousal category (82.49% of the possible models were US), whereas the sound origin effect was also observed for other categories ($p < 0.05$; minimum level of significance for each case). Apart from the standing alert and occupied zone categories, the highest percentage of significant changes for each category was calculated for NAS. In general (total for all variables), the least disturbing sounds for the horses were AS and IES, but there were no differences between KS and US percentages.

Tab. 6. Dependent variable categories (and dependent variables) that were affected by sounds of different types (KS/US, AS/NAS/PS/IES)

Dependent variable category	General impact (%)	X percentage distribution (KS/US)	Y percentage distribution (AS/NAS/PS/IES)
Occupied zone (n = 3; zone 1, 2 and 3)	6.67	KS = 60.10 US = 39.90	AS = 0.00 NAS = 36.39 PS = 42.49 IES = 21.12
Feeding behaviour (n = 4; time and frequency of feeding from hay nets, other and total feeding behaviour)	15.63	KS = 45.69 US = 54.31	AS = 8.71 NAS = 61.86 a** PS = 21.31 IES = 8.12
Locomotor behaviour (n = 2; walk and total locomotion)	17.50	KS = 45.41 US = 54.59	AS = 8.00 NAS = 62.77 b** PS = 29.23 IES = 0.00
Standing alert (n = 1)	75.00	KS = 46.46 US = 53.54	AS = 15.84 NAS = 32.21 c** PS = 34.55 IES = 17.40
Emotional arousal (n = 4; mean HR, RR interval, RMSSD, LF/HF)	6.67 (concerning only HR or RR: 17.50)	KS = 17.51 a* US = 82.49	AS = 0.00 NAS = 75.08 d* PS = 0.00 IES = 24.92
Total for all variables (n = 14)	16.61	KS = 42.78 US = 57.22	AS = 8.78 NAS = 51.27 e** PS = 25.78 IES = 14.17

Explanations: Percentage distribution of significant models obtained for different types of sounds in each dependent variable category; for KS/US: a*$\chi^2 = 5.602$, $p = 0.018$. For AS/NAS/PS/IES: a**$\chi^2 = 18.633$, $p = 0.000$; b**$\chi^2 = 11.546$, $p = 0.009$; c**$\chi^2 = 12.502$, $p = 0.006$; d**$\chi^2 = 22.963$, $p = 0.000$; e**$\chi^2 = 44.620$, $p = 0.000$; X, Y – calculated based on the ratio of the percentage of KS, US and AS, NAS, PS, IES in significant models of each dependent variable category and their percentage in the total number of sounds (n = 40). General impact (%) of the sounds – percentage of significant models among all possible models in each dependent variable category; model – 'sound*dependent variable'

We also determined four categories of sound effect that might be considered as potential degrees of horses' threat perception (Tab. 7).

Sounds were classified as 'threatening' (perceived as a first degree threat) if they affected both behavioural and cardiac activity (at least one impact on behaviour and HR/HRV), since this in-

Tab. 7. Sounds grouped into effect categories (different degrees of a threat perception) according to their impact on behaviour and emotional arousal, including the percentage distribution of KS/US, AS/NAS/PS/IES in each category

Sound effect category	Reaction	Sound	Total number of significant models	X percentage distribution (KS/US)	Y percentage distribution (AS/NAS/PS/IES)
Threatening signal (I degree threat)	physiological and behavioural stress response	magpie, pig, chimpanzee, red-tailed hawk, common snipe, elephant, stream, deer	n = 49	a* KS: 25.27 US: 74.73	a** AS: 0.00 NAS: 86.73 PS: 0.00 IES: 13.27
Disturbing/alarm cue (II degree threat)	behavioural disturbance	cuckoo bird, cats, wolf, vulture, lynx, leopard, police siren, dog, wind, ewe	n = 29	KS: 64.79 US: 35.21	b** AS: 6.25 NAS: 24.75 PS: 55.50 IES: 13.50
Mildly alarming signal (III degree threat)	mildly increased vigilance	vacuum cleaner, storm, applause, lion, seagulls, alarm clock, tiger, road, traffic, cougar, branch cracking, pheasant, bear, crowd, gorilla	n = 15	KS: 50.00 US: 50.00	AS: 35.37 NAS: 13.90 PS: 29.02 IES: 21.71
Neutral sound (no threat)	none	church bell, railway engine, trumpet, plane, waves, river, raven, lawnmower	n = 0	Z Percentage of sounds KS: 60.10 US: 39.90	Z Percentage of sounds c** AS: 52.79 NAS: 8.37 PS: 0.00 IES: 38.84

Explanations: Percentage distribution of significant models obtained for different types of sounds in each sound effect category; for KS/US: a*$\chi^2 = 10.47$, $p = 0.001$. For AS/NAS/PS/IES: a**$\chi^2 = 82.86$, $p = 0.00$; b**$\chi^2 = 17.67$, $p = 0.001$. For the neutral sound category (no impact) percentage of certain types of sounds was calculated: c**$\chi^2 = 8.82$, $p = 0.03$; X, Y – calculated based on the ratio of the percentage of KS, US and AS, NAS, PS, IES in significant models of each sound effect category and their percentage in the total number of sounds (n = 40), Z – calculated based on the ratio of the percentage of KS, US and AS, NAS, PS, IES in each sound effect category and their percentage in the total number of sounds (n = 40); model – 'sound*dependent variable'

dicates a behavioural and physiological stress response and readiness to react quickly when necessary (50). Moreover, 'threatening' sounds also had to increase the duration of standing alert, which is a sign of intensified vigilance, a primary antipredator defence mechanism (51). Second degree threat sounds, also defined as 'disturbing/alarm cues', influenced only behaviour (standing alert and at least one other variable). Three of the sounds (cuckoo bird, cat, wolf) were distinctive in the amount of significant impacts. However, since each of them affected three different categories of behavioural variables (based on Tab. 6) but not cardiac activity, we classified them as disturbing sounds (second degree threat). Mildly alarming sounds (a third degree threat) induced a slight increase in the horses' vigilance, which manifested itself as an extension of time spent standing alert or as changes in other categories of behaviour, not related to standing alert. Neutral sounds did not influence any of the dependent variables and were therefore defined as non-threatening.

The novelty effect of the sounds (KS/US) was visible only for the first degree threat category (Tab. 7). In all possible 'sound*dependent variable' models of the threatening signal category, 74.73% of significant changes were caused by US. There were no significant differences in percentages of KS/US for mildly alarming, disturbing and neutral sounds, even if KS accounted for most of the relevant effects within the two last categories.

The impact of the sound origin was visible for the first and second degree threat categories and for neutral sounds, but not for the third degree threat category (Tab. 7). Models that were perceived as threatening were almost exclusively NAS (86.73%). PS accounted for more than a half of all significant behavioural changes within the disturbing/alarming cue effect. AS (52.79%) and IES (38.84%) had an advantage over other neutral sounds. Generally, AS and IES acted mostly as neutral or mildly alarming signals, NAS as threatening signals, and PS as disturbing or mildly alarming signals.

We measured first reactions of the horses to the sound by observing the presence or absence of the following behaviours: approaching or going away from the sound source and interrupting current activity, taking into account the effect of the sound (threat degrees). The relation between the sound effect and behaviour was noted for 'going away' and 'interruption' variables ($p < 0.0001$) (Tab. 8). Going away from the loudspeaker was most often caused by threatening sounds and least often by neutral sounds. Sounds that were perceived as more threatening made the horses stop their current activity more often.

Threat perception and the context of sound use. From an evolutionary perspective, disturbance stimuli should be analogous to predation risk (24). Prey species respond to such stimuli in a similar way as they would to the presence of a predator (46). We used auditory stimuli of different origin to assess whether, and to

Tab. 8. The impact of the sound effect category on behaviours assessed by the 0-1 sampling method

Sound effect category	*% of 'yes' changes
Going away from the sound source *a	
Threatening signal	23.75
Disturbing/alarm cue	11.50
Mildly alarming signal	14.29
Neutral sound	1.88
Interrupting current activity *b	
Threatening signal	55.63
Disturbing/alarm cue	42.50
Mildly alarming signal	41.07
Neutral sound	15.00

Explanations: *Percentage distribution of 'yes' changes induced by sounds from each sound effect category: a*χ^2 = 34.664, p = 0.000; b*χ^2 = 58.790, p = 0.000. Relationships were significant at $p < 0.05$; 'yes' changes – going away/interruption was observed. Approaching the sound source – no relationship

what degree, stable-maintained horses perceived them as a threat. Horses stood alert for a longer period of time, ate less (but approached hay nets more often), and spent more time walking when exposed to sounds that they perceived as potentially threatening or disturbing. The most frightening sounds induced an increase in the mean HR and a decrease in the RR interval. These were signs of behavioural and emotional agitation (28), and they were in accordance with signs observed by Christensen et al. (17) during a novel sound test. The reactions obtained in our study bear some resemblance to antipredator responses. In an experiment conducted by Janczarek at al. (34), recognition of a predator's sound resulted in increased vigilance; horses also grazed less and trotted or cantered more. In a later study, Janczarek et al. (33) found changes in the HR and HRV of horses. Those changes consisted in sympathetic agitation and occurred after exposure to the sound of a predator. In contrast, we noted an impact of sounds on the mean HR and the RR interval, but not on other HRV parameters. This may stem from differences in perceptual sensitivity of different breeds, as well as differences in maintenance conditions and the duration of sound exposure. Unlike the five-minute stimulus in the study by Janczarek et al. (33), the one-minute stimulus used in our study may not have been disturbing enough to affect heart rate variability. Nevertheless, horses' reactions to different sounds corresponded to responses triggered by predator stimuli, so it cannot be excluded that those reactions were generalized and of primary anti-predatory origin. Survival depends on decision making (24), and fast reaction to unknown or unexpected stimuli is essential to avoid a potential threat (18).

Excessive response to an unthreatening, biologically irrelevant stimulus may expose an animal to an unnecessary energy and time loss (48). Thus, the ability to assess the current risk is crucial (44). In our study, the horses reacted intensively (by behavioural and cardiac changes) only to a few sounds. The other auditory stimuli acted as rather moderate or weak distractors. The stable-kept horses from the experiment by Janczarek et al. (32) showed weak signs of anxiety when exposed to a predator's natural vocalization. Similarly, Brown et al. (13) observed that wild ungulates did not perceive human activity as a predation risk. According to our study, it seems beneficial that the horses' reactions were somewhat limited. Unnecessary responses generate costs for animals (19) and reduce human safety (50). Animals have to choose between acquiring resources (like food) and eliciting antipredator responses (22). Stable-maintained horses are mostly free of predation disturbance, so they can afford to reduce vigilance. As shown in our study, the domestic horses reacted with anxiety to certain sounds, but the majority of the stimuli resulted only in increased alertness. They adjusted their responses depending on whether and how threatening the sound appeared to them at the moment.

In the context of ecology and a free-range system, only sounds of predators (and not those of neutral animals, inanimate environment or anthropogenic) were related to a real threat to horses' survival, since an encounter with a predator could be lethal (27, http://www.lrgaf.org/articles/Wild%20Horse%20DNA%20Report%20 2015). Aflitto and DeGomez (2) point out that acoustic stimuli may have some potential as repellents if they have a biological meaning. However, the type of sounds that had the strongest impact in our study (based on linear mixed-effects model analysis) were neutral animals' sounds. The sounds from this set affected cardiac activity, vigilance, locomotion and feeding behaviour, whereas the other sound types, including predator sounds, had a significant influence only on the duration of standing alert. Thus, the sound origin *per se* did not explain the horses' reactions. Janczarek et al. (32) showed that horses' reactions to predator vocalization were quite weak and that the sound of an unknown predator was perceived as more threatening than the one known to their ancestors. Apart from the novelty effect of the sound, different reactions may also have been caused by differences in the characteristics of predator vocalizations. On the other hand, Watts et al. (59) showed that certain reactions may be explained by the roles mares and stallions play in a herd. In our study, the horses stood alert longest when sounds of a predator or a neutral animal were played. Also, taking into account percentages of sounds of different origin among the relevant 'sound*dependent variable' models, the effects of neutral animals and then of predators were greatest in each dependent variable category. Therefore, the perception of a threat depends on more factors than just the origin of the stimulus.

The novelty, as well as unexpectedness, of a stimulus increases its distraction potential (49). It is difficult to ignore events that are completely out of context (45). With regard to the categories of sound effects (threat degrees), the effect of familiarity or unfamiliarity with a sound was visible only for the threatening sound category, while the effect of sound origin was present in all categories, except for mildly alarming signals. We made analogous observations for the dependent variable categories (effect of familiarity/unfamiliarity with a sound – only emotional arousal; effect of sound origin – five out of six categories). Neutral animal sounds and predator sounds had a greater influence on the horses (higher threat degrees). Anthropogenic sounds and inanimate environment sounds were perceived as mildly alarming or neutral. Nature sounds form a background for other noises and have a calming effect (47). Horses kept in stables are exposed to a variety of different sounds, routines and objects (55). Therefore, anthropogenic sounds were nothing unusual for the horses living in human surroundings and did not attract their attention to any significant degree. Similarly, most of the samango monkeys in an experiment by Nowak et al. (46) did not perceive human presence as a threat, unless it was unexpected. In that case, they showed avoidance behaviour. Thus, unusual stimuli play an important role as disturbance factors (in our study: sounds of predators and other animals). Conversely, sounds and other stimuli are perceived as neutral when they are appropriate (usual) for the context (in our study: anthropogenic and inanimate environment sounds) or simply habitual (49, 60). Most of the neutral animal and predator sounds from the threatening and disturbing effect categories were unusual or at least unexpected in anthropogenic surroundings. Unlike the other most distractive animal sounds, the ones of predators did not result in cardiac activation, but usually affected only behavioural activity to a moderate or small degree. This weakened response may be explained by the fact that nowadays domestic horses are largely free from predator pressure, especially when kept in stables in urban areas (34). This may also be a result of a greater variability and unusuality of neutral animal sounds compared to those of predators that may have been somewhat similar to each other (e.g. roaring, growling). Nevertheless, our results do not refute horses' ability to recognise predator cues, which has been proved by other authors (18, 33, 34, 59). Numerous factors influence the way animals react (1, 52, 54, 58), so results may differ across studies (9, 26, 38). Significant changes in behaviour and cardiac activity in the threatening effect category were caused almost exclusively by potentially unknown sounds. The only dependent variable category where the effect of novelty was visible was the emotional arousal category. This indicates that the unpredictability combined with novelty of auditory stimuli may play an important role and result in horses perceiving sounds as a potential threat. Rochais and Hausberger (49) made similar observations about the distraction effect of auditory stimuli. According to our study, while the disturbing effect of a sound may be explained by the context in which the sound occurs (expectedly or unexpectedly, based on the sound origin), the threatening effect of a sound results from an unusual context of its occurrence as well as its novelty.

The fact that horses adjusted their reactions appropriately to the context is beneficial for both horses and owners (50, 54). Numerous studies, however, have looked for natural repellents to facilitate wildlife and livestock management (5). Sounds of first, second and third degree threats made the horses stop their current activity and had some potential to deter them from approaching the hay nets. Furthermore, the horses ate less and stood alert longer after a sound was played – this could reduce the consumption of fodder. Only one sound, the howling of a wolf, significantly affected the spatial distribution of the horses. The repellent effect of auditory stimuli cannot be excluded, but it needs further research.

Stable-kept warmbloods remain vigilant to auditory stimuli in their environment and recognize sounds of different origin. Most of these sounds have a greater or lesser effect on their behaviour. The weakest response is related to sounds from the inanimate environment or anthropogenic sounds that are part of everyday life for stable-maintained horses and are probably generalized by the animals. A moderate response is observed mainly for domesticated predator sounds as well as unfamiliar predator sounds. The strongest behavioural or behavioural and physiological response is caused by sounds of other, mostly unfamiliar animals, such as a pig, chimpanzee, red-tailed hawk, magpie, common snipe, elephant or deer. The physiological stress response depends on the novelty effect. Thus, the perception of a threat posed by a sound is a result of an unusual context of sound occurrence and the novelty of the sound. However, at this stage of the study, we cannot determine the ranges of specific technical characteristics shared by sounds eliciting similar behavioural and physiological responses. Differences in horses' reactions to sounds of predators and other animals are therefore worth investigating in further studies, focusing, for instance, on the timbre and transformations of length and volume of a sound or on individual characteristics of horses in terms of threat perception. These findings may have future implications for the management and care of horses.

References

1. *Adcock S. J. J., Tuckerm C. B.*: Naïve domestic Bos taurus calves recognize the scent of a canine predator. Anim. Behav. 2020, 164, 173-180.
2. *Aflitto N. C., Hofstetter R. W.*: Use of acoustics to deter bark beetles from entering tree material. Pest Manag. Sci. 2014, 70 (12), 1808-1814.
3. *Ahmadinejad M., Hassani A., Kharazian F.*: The responses of horses to predator stimuli. Int. J. Vet. Res. 2010, 4, 5-9.
4. *Altmann J.*: Observational study of behavior – Sampling methods. Behaviour 1974, 49 (3-4), 227-267.
5. *Apfelbach R., Blanchard C. D., Blanchard R. J., Hayes R. A., McGregor I. S.*: The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neurosci. Biobehav. Rev. 2005, 29 (8), 1123-1144.
6. *Bagchi S., Mishra C.*: Living with large carnivores: predation on livestock by the snow leopard (Uncia uncia). J. Zool. 2006, 268, 217-224.
7. *Bates D., Mächler M., Bolker B., Walker S.*: Fitting linear mixed-effects models using lme4. J. Stat. Softw. 2015, 67 (1), 1-48.
8. *Bates D., Meachler M., Bolker B., Walker S., Christense R. H. B., Singmann H., Dai B., Scheipl F., Grothendieck G., Green P., Fox J., Bauer A., Krivitsky P. N.*:

Linear Mixed-Effects Models using 'Eigen' and S4 (version 1.1-27.1). Package 'lme4. CRAN, 2021.

9. *Blumstein D. T.*: Moving to suburbia: ontogenetic and evolutionary consequences of life on predator-free islands. J. Biogeogr. 2002, 29 (5-6), 685-692.
10. *Blumstein D. T.*: The multipredator hypothesis and the evolutionary persistence of antipredator behavior. ethology 2006, 112, 209-217.
11. *Blumstein D. T., Daniel J. C., Springett B. P.*: A Test of the Multi-Predator Hypothesis: Rapid Loss of Antipredator Behavior after 130 years of Isolation. Ethology 2004, 110 (11), 919-934.
12. *Borell E. von, Langbein J., Després G., Hansen S., Leterrier C., Marchant-Forde J., Marchant-Forde R., Minero M., Mohr E., Prunier A., Valance D., Veissier I.*: Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – A review. Physiol. Behav. 2007, 92, 293-316.
13. *Brown C. L., Hardy A. R., Barber J. R., Fristrup K. M., Crooks K. R., Angeloni L. M.*: The effect of human activities and their associated noise on ungulate behavior. PLoS One 2012, 7 (7), 1-11.
14. *Brown G. E., Macnaughton C. J., Elvidge C. K., Ramnarine I., Godin J.-G. J.*: Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. Behav. Ecol. Sociobiol. 2009, 63, 699-706.
15. *Brown G. E., Rive A. C., Ferrari M. C. O., Chivers D. P.*: The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. Behav. Ecol. Sociobiol. 2006, 61, 9-16.
16. *Chamaillé-Jammes S., Malcuit H., Le Saout S., Martin J.-L.*: Innate threat-sensitive foraging: black-tailed deer remain more fearful of wolf than of the less dangerous black bear even after 100 years of wolf absence. Oecologia 2014, 174, 1151-1158.
17. *Christensen J. W., Keeling L. J., Nielsen B. L.*: Responses of horses to novel visual, olfactory and auditory stimuli. Appl. Anim. Behav. Sci. 2005, 93 (1-2), 53-65.
18. *Christensen J. W., Rundgren M.*: Predator odour per se does not frighten domestic horses. Appl. Anim. Behav. Sci. 2008, 112, 136-145.
19. *Chuard P. J. C., Grant J. W. A., Ramnarine I. W., Brown G. E.*: Exploring the threat-sensitive predator avoidance hypothesis on mate competition in two wild populations of Trinidadian guppies. Behav. Processes 2020, 180, 104225.
20. *Cooper J. J., Albentosa M. J.*: Behavioural adaptation in the domestic horse: potential role of apparently abnormal responses including stereotypic behaviour. Livest. Prod. Sci. 2005, 92, 177-182.
21. *Danvir R. E.*: Multiple-use management of western U.S. rangelands: wild horses, wildlife, and livestock. Hum.-Wildl. Interact. 2018, 12, 5-17.
22. *Ferrari M. C. O., Chivers D. P.*: Learning threat-sensitive predator avoidance: how do fathead minnows incorporate conflicting information? Anim. Behav. 2006, 71, 19-26.
23. *Ferrari M. C. O., Messier F., Chivers D. P.*: Can prey exhibit threat-sensitive generalization of predator recognition? Extending the Predator Recognition Continuum Hypothesis. Proc. Royal Soc. B. 2015, 275, 1811-1816.
24. *Frid A., Dill L.*: Human-caused disturbance stimuli as a form of predation risk. Conserv. Ecol. 2002, 6, 11.
25. *Fureix C., Menguy H., Hausberger M.*: Partners with bad temper: reject or cure? A study of chronic pain and aggression in horses. PLoS One 2010, 5, e12434.
26. *Geffroy B., Samia D. S. M., Bessa E., Blumstein D. T.*: How nature-based tourism might increase prey vulnerability to predators. Trends Ecol. Evol. 2015, 30 (12), 755-765.
27. *Goodwin D.*: Horse behaviour: evolution, domestication and feralization, [in:] Waran N. (ed.): The welfare of horses. Vol. 1: Kluver Academic Publishers, Dordrecht 2002, p. 1-18.
28. *Hall C., Randle H., Pearson G., Preshaw L., Waran N.*: Assessing equine emotional state. Appl. Anim. Behav. Sci. 2018, 205, 183-193.
29. *Henry S., Hemery D., Richard M.-A., Hausberger M.*: Human-mare relationships and behaviour of foals toward humans. Appl. Anim. Behav. Sci. 2005, 93, 341-3628.
30. *Hothorn T., Bretz F., Westfall P., Heiberger R. M., Schuetzenmeister A., Scheibe S.*: Simultaneous Inference in General Parametric Models (version 1.4-17). Package 'multcomp'. CRAN, 2021.
31. *Huo X., Wongkwanklom M., Phonraksa T., Na-Lampang P.*: Effects of playing classical music on behavior of stabled horses. Vet. Integr. Sci. 2021, 19 (2), 259-267.
32. *Janczarek I., Stachurska A., Kędzierski W., Wiśniewska A., Ryżak M., Kozioł A.*: The intensity of physiological and behavioral responses of horses to predator vocalizations. BMC Vet. Res. 2020, 16, 431.
33. *Janczarek I., Stachurska A., Kędzierski W., Wnuk-Pawlak E., Wilk I., Zyglewska K., Paszkowska A., Ryżak M., Wiśniewska A.*: Heart rate variability in Konik and purebred Arabian horses in response to different predator vocalisations. Animal 2021, 15, 100045.
34. *Janczarek I., Wiśniewska A., Chruszczewski M. H., Tkaczyk E., Górecka-Bruzda A.*: Social behaviour of horses in response to vocalisations of predators. Animals 2020, 10, 2331.
35. *Kamieniak J., Marciniak B., Sapuła M.*: Reakcje behawioralne koni poddanych działaniu wybranych bodźców dźwiękowych. Med. Weter. 2010, 66 (6), 413-417.
36. *Kaufmann J., Schering A.*: Analysis of Variance ANOVA, [in:] Balakrishnan C., Colton T., Everitt B., Piegorsch W., Ruggeri F., Teugels J. (eds.): Wiley StatsRef: Statistics Reference Online. John Wiley & Sons, Ltd 2014. Online ISBN: 9781118445112. Available online: https://onlinelibrary.wiley.com/doi/epdf/10.1002/9781118445112.stat06938 (accessed on 28 October 2021).
37. *Kluever B. M., Breck S. W., Howery L. D., Krausman P. R., Bergman D. L.*: Vigilance in cattle: the influence of predation, social interactions, and environmental factors. Rangeland Ecol. Manage. 2008, 61, 321-328.
38. *Kluever B. M., Howery L. D., Breck S. W., Bergman D. L.*: Predator and heterospecific stimuli alter behaviour in cattle. Behav. Processes 2009, 81, 85-91.
39. *Kovács L., Tőzsér J., Kézér F. L., Ruff F., Aubin-Wodala M., Albert E., Choukeir A., Szelényi Z., Szenci O.*: Heart rate and heart rate variability in multiparous dairy cows with unassisted calvings in the periparturient period. Physiol. Behav. 2015, 139, 281-289.
40. *Laborde S., Mosley E., Thayer J. F.*: Heart rate variability and cardiac vagal tone in psychophysiological research – recommendations for experiment planning, data analysis, and data reporting. Front. Psychol. 2017, 8, 213.
41. *Li C., Yang X., Ding Y., Zhang L., Fang H., Tang S., Jiang Z.*: Do Père David's Deer lose memories of their ancestral predators? PLoS One 2011, 6 (8), e23623.
42. *MacLean S. A., Bonter D. N.*: The sound of danger: threat sensitivity to predator vocalizations, alarm calls, and novelty in gulls. PLoS One 2013, 8 (12), e82384.
43. *McDonnell S. M.* (ed.): The equid ethogram: A practical field guide to horse behavior. Eclipse Press, Lexington 2003, pp. 1-375.
44. *Monclús R., Palomares F., Tablado Z., Martínez-Fontúrbel A., Palme R.*: Testing the threat-sensitive predator avoidance hypothesis: physiological responses and predator pressure in wild rabbits. Oecologia 2019, 158, 615-623.
45. *Murphy S., Dalton P.*: Ear-catching? Real-world distractibility scores predict susceptibility to auditory attentional capture. Psychon. Bull. Rev. 2014, 21, 1209-1213.
46. *Nowak K., Hill R. A., Wimberger K., le Roux A.*: Risk-taking in samango monkeys in relation to humans at two sites in South Africa, [in:] Waller M. T. (ed.): Ethnoprimatology: Primate Conservation in the 21st Century. Springer International Publishing, Cham 2016, pp. 301-314.
47. *Pálsdóttir A. M., Persson D., Persson B., Grahn P.*: The journey of recovery and empowerment embraced by nature-clients' perspectives on nature-based rehabilitation in relation to the role of the natural environment. Int. J. Environ. Res. Public Health 2014, 11, 7094-7115.
48. *Papworth S., Milner-Gulland E. J., Slocombe K.*: Hunted Woolly Monkeys (Lagothrix poeppigii) show threat-sensitive responses to human presence. PLoS One 2013, 8 (4), e62000.
49. *Rochais C., Henry S., Hausberger M.*: Spontaneous attention-capture by auditory distractors as predictor of distractibility: a study of domestic horses (Equus caballus). Sci. Rep. 2017, 7, 15283.
50. *Rørvang M. V., Nielsen B. L., McLean A. N.*: Sensory abilities of horses and their importance for equitation science. Front. Vet. Sci. 2020, 7, 663.
51. *Rose-Meierhöfer S., Klaer S., Ammon C., Brunsch R., Hoffmann G.*: Activity behavior of horses housed in different open barn systems. J. Equine Vet. Sci. 2006, 30 (11), 624-634.
52. *Schell C. J., Young J. K., Lonsdorf E. V., Santymire R. M., Mateo J. M.*: Parental habituation to human disturbance over time reduces fear of humans in coyote offspring. Ecol. Evol. 2018, 8, 12965-12980.
53. *Smith A. V., Proops L., Grounds K., Wathan J., Scott S. K., McComb K.*: Domestic horses (Equus caballus) discriminate between negative and positive human nonverbal vocalisations. Sci. Rep. 2018, 8, 13052.
54. *Squibb K., Griffin K., Favier R., Ijichi C.*: Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures. Appl. Anim. Behav. Sci. 2018, 202, 34-38.
55. *Stachurska A., Śniadek J., Janczarek I.*: Pierwsza reakcja częstości skurczów serca koni na różne rodzaje muzyki w stajni. Med. Weter. 2017, 78 (3), 500-504.
56. *Thompson K., Clarkson L.*: How owners determine if the social and behavioral needs of their horses are being met: Findings from an Australian online survey. J. Vet. Behav. 2019, 29, 128-133.
57. *Turney S., Godin J.-G. J.*: To forage or hide? Threat-sensitive foraging behaviour in wild, non-reproductive passerine birds. Curr. Zool. 2014, 60 (6), 719-728.
58. *Visser E. K., van Reenen C. G., Rundgren M., Zetterqvist M., Morgan K., Blokhuis H. J.*: Responses of horses in behavioural tests correlate with temperament assessed by riders. Equine Vet. J. 2003, 35 (2), 176-183.
59. *Watts E. T., Johnson C., Carver S., Butler C., Harvey A. M., Cameron E. Z.*: Maternal protectiveness in feral horses: responses to intraspecific and interspecific sources of risk. Anim. Behav. 2020, 159, 1-11.
60. *Webb N. V., Blumstein D. T.*: Variation in human disturbance differentially affects predation risk assessment in western gulls. Condor 2005, 107, 178-18.

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