



Heart rate variability in Konik and purebred Arabian horses in response to different predator vocalisations



I. Janczarek^a, A. Stachurska^{a,*}, W. Kędzierski^b, E. Wnuk Pawlak^a, I. Wilk^a, K. Zygłewska^a, A. Paszkowska^a, M. Ryzak^c, A. Wiśniewska^a

^a Department of Horse Breeding and Use, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, 20-950 Lublin, Poland

^b Department of Biochemistry, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, 20-950 Lublin, Poland

^c Institute of Agrophysics, Polish Academy of Sciences, 20-290 Lublin, Poland

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ABSTRACT

The current predation threat of domestic horses is generally low, and horses do not know predators' frightening cues. We studied whether horses still recognise predation threats. The aim of the study was to analyse the emotional response of purebred Arabian horses (**Arabian**) and Polish Konik horses (**Konik**) to an Arabian panther (*Panthera pardus nimr*) (**panther**) growl and a grey wolf (*Canis lupus*) (**wolf**) howl. Panther vocalisation was known to Arabian ancestors, whereas ancestors of Konik knew wolf vocalisation. The response to the howls of golden jackals (*Canis aureus*) (**jackal**), which did not prey on equids, was also studied comparatively. Two groups of 10 adult horses of each breed were subject to predator sounds of one predator daily for 5 min during a turn out on pasture. The test was performed for 18 days in total. The sound of each predator was interchangeably featured from one loudspeaker for 3 days followed by four loudspeakers simultaneously to imitate a group of predators for 3 days. The horses' emotional agitation in response to the sounds was measured based on the parameters of heart rate variability (**HRV**) using telemetric devices. The results showed that the predators' sounds were identified by horses as stressful or neutral. Horses generally retained their anti-predator responses even in the current habitat, which typically lacks predation cues. The results are not always coherent and may demonstrate that the response is somewhat attenuated. The wolf howl elicited a stronger response in Koniks. The panther growl more strongly influenced Arabians, whereas the jackal howl minimally elicited an agitation in the horses. The differentiated response of the two horse breeds to the three predator species suggests that the response is an innate adaptation to the predation risk in the habitat of the breed ancestors. This response occurs regardless of the emotional arousal specific to a breed, and the frightening cue is not the sound *per se* but the possible attack of predators. Horses display a type of understanding of the sound meaning. Their HRV response seems to be adequate for the threat signalled by the sound.

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Implications

Attacks by predators on horses occur on a global scale; however, predator vocalisations are typically unknown by horses maintained under domestic conditions. Vocalisations were used in this study to determine heart rate variability responses, which indicate emotional agitation in horses. Two studied horse breeds developed in different habitats and once threatened by different predators exhibited stronger responses to predators known by their ancestors. This finding may reflect an innate adaptation of horses to the conditions of their ancestors' habitat and may be important when introducing outlander horses into locations that are not free of predators.

Introduction

After millions of years of living in the wild, horses and their ancestors developed appropriate responses to various dangerous stimuli, which enabled them to survive in different habitats and to co-exist with predators (Ahmadinejad et al., 2010). Horses mainly shared open grasslands with their predators (Christensen and Rundgren, 2008). Wild horses recognise predators by visual, auditory and olfactory stimuli. The horse's strongest response is to flee from a fearful cue (Leiner and Fendt, 2011). Large carnivores may induce direct lethal effects but can also influence ungulates by non-lethal effects, including changing distributions towards a less risky habitat or increasing vigilance levels while grazing (Kuijper et al., 2013). Domesticated horses are not only provided with food, water and harbour but are also protected from predators; thus, these horses are increasingly dependent on humans. Due to domestication and habitat changes, present horse behaviours could have been

* Corresponding author.

E-mail address: anna.stachurska@up.lublin.pl (A. Stachurska).

altered (Leiner and Fendt, 2011). Studies on other farm animals, e.g. cattle (*Bos taurus*) and sheep (*Ovis aries*), demonstrate that during domestication and selection for calmness, the animals did not completely lose the ability to respond to the predation threat, but the threshold of the response presumably increased (Dwyer, 2004; Laporte et al., 2010). Horses that were always directly used in various human activities in close contact are particularly selected to be calm; hence, they might lose more of their instinctive reactions. Simultaneously, when considering the recent expansion in the population of grey wolf (*Canis lupus*) (wolf) and wolf conservation (Caniglia et al., 2014), an important question arises regarding whether the anti-predator response in domestic horses is mostly retained or attenuated and whether horses of different origin vary with this respect.

Various horse breeds were formed in different geographic zones and thus were potentially threatened by different predators. For example, the primordial Polish Konik (Konik) breed was formed based on indigenous horses maintained for ages in Eastern Europe (Mackowski et al., 2015). Purebred Arabian (Arabian) horses represent oriental horses, which are considered to be one of the oldest breeds formed centuries ago in the desert-like territories of the Arabian Peninsula (Głażewska, 2010). Formerly, the ancestors of Koniks could have been attacked by packs of wolves that co-existed with ungulates in woody East-Central Europe (Jędrzejewski et al., 2002), whereas the ancestors of Arabians, or at least their foals, could have been threatened by Arabian panthers (*Panthera pardus nimr*) (panther) once widely spread in the Arabian Peninsula (Uphyrkina et al., 2001; Mazzolli, 2009). Currently, wolves and mountain lions (*Felis concolor*) represent the main predators in the world that prey upon free-ranging horses. Specifically, wolves are located in Southern Europe (Meriggi and Lovari, 1996) and Eastern Asia (Duyne van et al., 2009), whereas both species are present in North America (Knopff et al., 2010). Golden jackals (*Canis aureus*) (jackal) do not threaten ungulates; however, they can disquiet them (Lanszki and Heltai, 2002). Jackals are found in the Middle East among other places and started their expansion into Central and Northern Europe in the mid-twentieth century (Trouwborst et al., 2015).

Currently, natural enemies of the horses (even if they occur) are scarce. However, it seems that present-day horse behaviours exhibited in response to disturbance stimuli, such as loud noises, rapidly approaching objects, an aggressive dog or even simple routine procedures, such as vaccination or transportation, evolved from generalised, originally anti-predator responses (Frid and Dill, 2002; Leiner and Fendt, 2011).

Predators' stimuli *per se* may still frighten horses. We used the predators' vocalisations in the study given that earlier studies showed that auditory cues affected the horses more strongly than olfactory cues (Christensen and Rundgren, 2008; Ahmadinejad et al., 2010). These studies report that horses' responses to evade detection and capture by predators are not only behavioural but are also potentially emotional as demonstrated by cardiac activity changes. To the best of our knowledge, all previous studies tested individual horses, and no study has evaluated the response of a social herd of horses to predation. It was hypothesised that due to the evolution and co-existence of the horse ancestry with predators in common geographic territories where the breeds were formed, the present horse response to the predator threat might be differentiated.

When a horse is frightened, the sympathetic component of the autonomic nervous system is activated and catecholamines are secreted. In contrast, parasympathetic branch activity decreases in this situation. The balance between the sympathetic (stimulating) and parasympathetic (inhibiting) systems can be evaluated based on heart rate variability (HRV) analyses (Rietmann et al., 2004; Borell von et al., 2007).

The aim of the study was to analyse the emotional response of Arabian horses and Konik horses to panther vocalisations, which were known to Arabian horse ancestry and unknown to Konik horse ancestry and wolf vocalisations, which were known to Konik ancestry and unknown to Arabian ancestry. The response to the howls of jackals, which did not prey on equids, was also studied as a control cue.

Material and methods

Horses and conditions of maintenance

The study involved 10 Konik mares and 10 Arabian mares. The horses were maintained in two facilities located at a distance of 60 km (Koniks: 51°32'N, 22°97'E and Arabians: 50°96'N, 23°07'E) where no cases of predator stalking were observed. The horses did not have any previous contact with the studied predators. The horses in both facilities constituted similar groups. Specifically, the horses were 5–15 years old (Koniks: 102.4 ± 39.4 months, Arabians: 108.2 ± 42.5 months), clinically sound, in the second trimester of pregnancy (apart from one barren Arabian mare), and lacked behavioural disturbances and excessive excitability. The horses were broodmares, which were never used for riding or had not been used for riding for at least 3 years. The two facilities exhibited similar conditions: located in the countryside, far from roads, and in silent places not exposed to any external influences. Each horse group was maintained in a stable in box-stalls that were 3 m × 3 m in size, bedded with straw, and equipped with a crib in the corner and an automatic waterer. The horses were fed twice daily with meadow hay and oats. They had contact with humans during the everyday handling and mating management. The horses were released into pasture for a minimum of 5 h per day. The devices to measure the cardiac activity (sport-testers) were attached to horses before turning out to familiarise them with the procedure for 1 week before the beginning of the experiment.

Procedure of experiment

The experiment was performed in August and September. The cardiac activity parameters were measured using telemetric Polar ELECTRO OY-RS800CX sport-testers (Polar Electro Oy, Kempele, Finland). The telemetric sets were installed on the animals before releasing to the paddock. An elastic belt with a sport-tester was placed around the chest of the horse, and the tester was activated manually. Next, the horse was left alone in the box for approximately 5 min to give it time to accept the device. The entire horse breed group with activated sport-testers was released into the pasture approximately half an hour before the beginning of the experiment at approximately 9.00 a.m. The pastures in both facilities were nearly squares of 0.5 ha and familiar to the horses. On the pasture, the horses could graze and freely move. The test always began when the horses were located at the central part of the pasture. The experimenter was not visible to the horses. After the end of the test, the horses were led to the stable to take off the sport-testers and then turned out again for almost 5 h.

The vocalisations of three predators were tested: panther growls, wolf howls and jackal howls. Within one experimental stage, one of the predator sounds was featured on 1 day, and the two other predator sounds were successively featured on every two or three of the following days according to the scheme presented in Table 1. The day test always contained three phases: 5 min of silence (pre-sound), 5 min of the predator's sound (sound) and 5 min of silence (post-sound). The test with a different predator sound order was repeated over the two next stages. The horses were subject to an individual sound (IS) emitted from one loudspeaker located 1 m outside of the pasture corner during the first three experimental stages. Within the analogous next three stages, four loudspeakers placed 1 m from each corner were used to feature the group sound (GS), i.e., the same predator vocalisations were simultaneously projected from four directions to imitate a group of predators. The sounds were played using two-way loudspeakers characterised by rated power of 25 W and a speaker frequency response of 20–20 000 Hz (Sony CMT-SBT100). The intervals between the six stages lasted a minimum of 1 week and depended on the weather. Experiments were performed on days without rain and with wind up to 0.5 m/s. The disrupted daily schedule of the tests that were not

Table 1

The schedule of featuring predator sounds during tests of horses on pasture every 2 or 3 days within successive experimental stages separated by a minimum of 1 week.

Individual sound	Group sound
Stage 1	Stage 4
Golden jackal	Golden jackal
Arabian panther	Arabian panther
Grey wolf	Grey wolf
Stage 2	Stage 5
Arabian panther	Arabian panther
Grey wolf	Grey wolf
Golden jackal	Golden jackal
Stage 3	Stage 6
Grey wolf	Grey wolf
Golden jackal	Golden jackal
Arabian panther	Arabian panther

conducted regularly limited instrumental conditioning and anticipation of the test by the horses (McGreevy and McLean, 2007).

The sound level (A-weighted equivalent continuous sound level, LAeq) of the three predator sounds measured at the loudspeaker ranged from 54.0 dB for the jackal to 58.6 dB for the wolf. Analysis of the fast time-constant sound level (LAF) showed that LAF_{min} ranged from 37.7 dB for the panther to 43.6 dB for the wolf, whereas LAF_{max} ranged from 65.7 dB for the wolf to 82.5 dB for the panther. The maximum of the acoustic pressure fell to 1000 Hz in all cases. The wolf sounds were mainly characterised by a low frequency (250–2000 Hz). Jackal sounds were dominated by higher frequencies (630–5000 Hz) and panther sounds exhibited a broader range (250–5000 Hz).

Heart rate variability analysis

The HRV was used to determine the emotional agitation of the horses. Data from the sport-testers were downloaded to a computer with a peripheral IrDA USB 2.0 adapter and then analysed with PolarProTrainer 5.0 (Polar Electro Oy, Kempele, Finland) and Kubios HRV 2.0 software (University of Kuopio, Finland) (Borell von et al., 2007; Tarvainen et al., 2014). The beat-to-beat (RR) recordings were adjusted to eliminate artefacts, and the primary registered cardiac rhythm was controlled to remove stimulation originating from conduction centres other than the sinoatrial node. The low correction factor of the custom filter was used. RR intervals identified by the programme as artefacts were automatically substituted by interpolated intervals calculated from differences between the previous and next RR interval accepted by the programme. This filter was sufficient to eliminate artificial, single artefacts in RR recordings. The verified RR intervals (normal inter-beat intervals, NN; milliseconds, ms) represent a time HRV parameter. Using the recorded data, the programme also calculated the magnitude power of frequency HRV parameters:

- total power of HRV (TP) (Hz, ms²): up to 0.4 Hz, provides the overall picture of HRV and is equivalent to standard deviation of the NN intervals (SDNN) from the time HRV analysis;
- low-frequency component of the power spectrum (LF) (Hz, ms²): ranges from 0.04 to 0.15 Hz, reflects the activity of the sympathetic nervous system;
- high-frequency component of the power spectrum (HF) (Hz, ms²): ranges from 0.15 to 0.40 Hz, represents the activity of the parasympathetic (vagal) system;
- LF/HF: the ratio of spectrum density power from low to high frequencies, provides an indicator of the functional sympathetic-parasympathetic balance.

The data recorded during three 5-min phases of the test were analysed. All-time measurements were made using a hand stopwatch.

The choice of time point of each phase was based on the analysis of the time axis in the figures automatically shown by the software.

Statistical analysis

The measurements from all the horses studied were used in the analysis. The data were tested with regard to the normality of distribution using Kolmogorov–Smirnov and Shapiro–Wilk tests. The tests did not reject the normality of data distribution in any case. Statistical analyses were performed using multifactorial models of Repeated Measure Analysis of Variance for measures of dependent variables repeated in the same horse group as well as multiple T-Tukey's tests with a minimum level of significance of $\alpha = 0.05$. The analysis included the following factors: 5-min test phase (pre-sound, sound and post-sound), horse breed (Konik and Arabian), predator sound (jackal, panther and wolf), type of sound (IS and GS) and the interactions between these factors.

Results

Normal inter-beat intervals

The NN level in the pre-sound phase did not differ significantly between the horse breeds (Fig. 1). The panther IS and GS induced significant decreases in NN in Arabian horses during the sound phase ($P < 0.001$), whereas wolf IS and GS resulted in NN decreases in Konik horses ($P < 0.001$). In the post-sound phase, NN remained reduced with the exception of that noted for Arabians after exposure to the panther IS when it increased again ($P < 0.05$). Significant differences were found between the horse breeds in both sound and post-sound phases of IS and GS. When subject to the panther growl, Arabians consistently exhibited higher NN values than did Koniks. Conversely, the wolf howl elicited lower NN values in Koniks than in Arabians.

Total power of heart rate variability

Fig. 2 shows that the jackal IS and GS did not elicit significant TP changes in Koniks or Arabians. Regarding the panther, only GS induced a response in Koniks, whereas both IS and GS were effective in Arabians ($P < 0.05$). The wolf IS and GS elicited an important TP increase in Koniks ($P < 0.05$ and $P < 0.01$, respectively) but not in Arabians. The TP was typically higher in Arabians than in Koniks. However, for the wolf GS, the reverse effect was observed. The Koniks' TP was higher than the Arabians' TP ($P < 0.05$). Comparing the effects of different predators, it was noted that in the pre-sound phase of IS and GS, the TP did not differ in the horse groups. When IS or GS could be heard, Koniks reacted more strongly to the wolf compared to the panther and jackal, whereas Arabians were more reactive to the panther ($P < 0.05$). In the post-sound phase, IS and GS values tended to be lower. In Koniks, the response to panther GS and wolf GS was significantly stronger compared with the respective IS ($P < 0.05$).

Low-frequency component of the power spectrum

The LF did not differ in the studied horse breeds in the IS and GS pre-sound phases (Fig. 3). The jackal vocalisation did not significantly increase LF as it did not augment TP. The panther IS elicited LF increases exclusively in Arabians, whereas panther GS was effective in both horse breeds ($P < 0.001$). Both IS and GS of the wolf exclusively influenced Koniks ($P < 0.05$ and $P < 0.001$, respectively). While featuring the sound, jackal IS induced higher LF in Arabians, whereas jackal GS resulted in higher LF in Koniks ($P < 0.05$). Arabians were more sensitive to IS and GS of the panther, whereas Koniks exhibited higher LF for the wolf IS ($P < 0.05$) and GS ($P < 0.001$). LF responses to the vocalisations tended to be sustained in the post-sound phase. In general, GS of panther and wolf affected the horse LF more strongly than IS, and the difference was mainly significant in Koniks ($P < 0.05$).

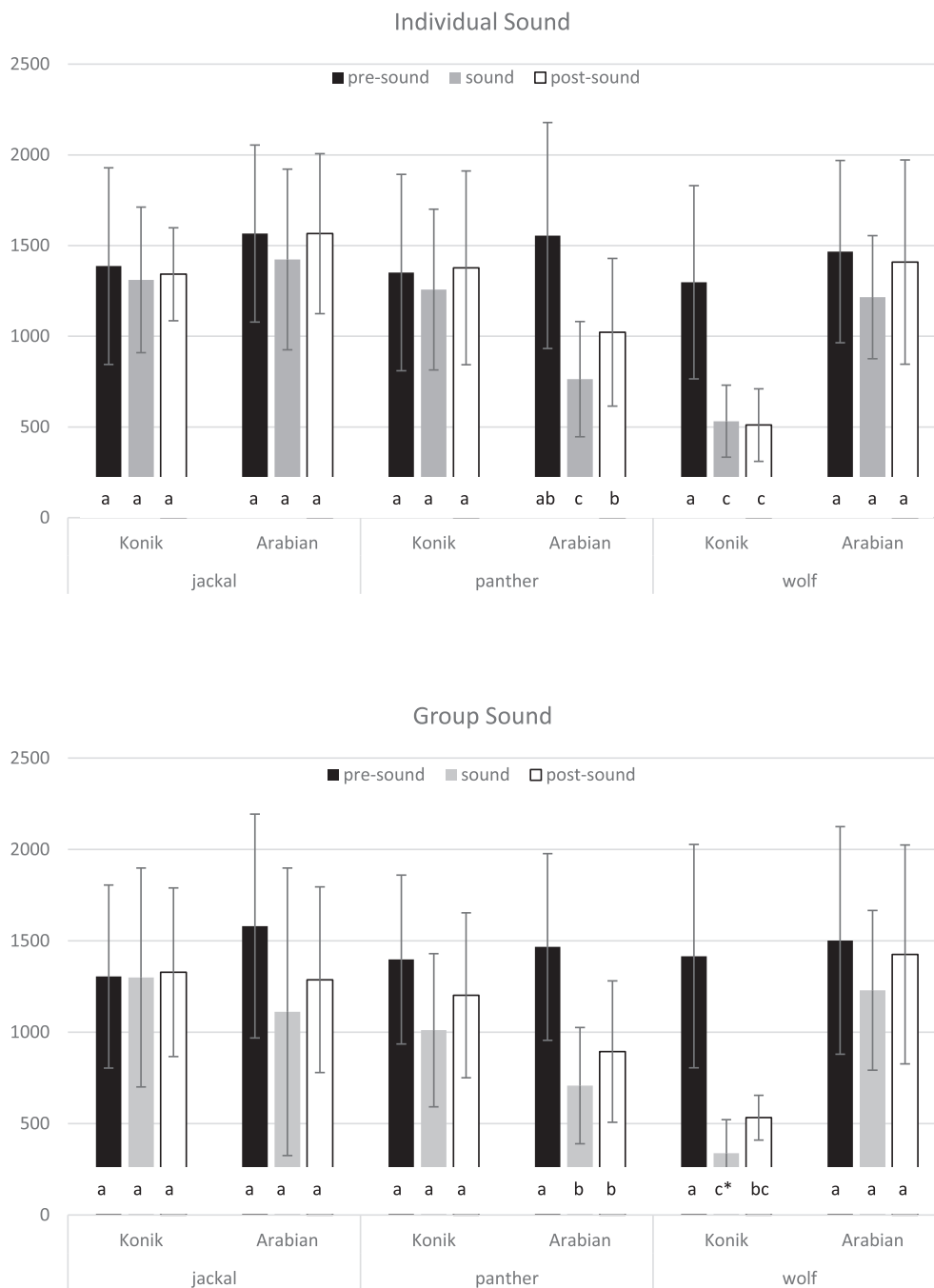


Fig. 1. Normal inter-beat intervals (NN) in horses on pasture subject to predator sound. Significant differences in means ($P < 0.05$) are noted as follows: different letters (a, b, c) indicate significant differences between bars (considering particular breeds, predators and test phases); * indicate significant differences between a bar of individual sound and an analogous bar of group sound.

High-frequency component of the power spectrum

In the pre-sound phase of jackal GS and panther GS, HF was higher in Arabians than in Koniks ($P < 0.05$; Fig. 4). During the sound phases, an HF decrease appeared in response to the jackal IS in Arabians and jackal GS in Koniks ($P < 0.05$). Both IS and GS of the panther did not affect Koniks but reduced HF in Arabians ($P < 0.001$). The wolf subsequently elicited a reduced HF in Koniks for both IS and GS and in Arabians for GS compared with pre-sound phases and the sounds of other predators ($P < 0.05$). In the post-sound phase, the differences tended to remain. High frequency levels were lower when GS was heard compared to IS in three cases, and the opposite result was noted in one case (the post-sound panther phase in Koniks; $P < 0.05$).

Ratio of spectrum power from low to high frequencies

Fig. 5 illustrates the response in terms of LF/HF. The jackal induced an increased GS exclusively in Koniks ($P < 0.05$). The panther elicited an increase in the parameter with the exception of IS in Koniks. The wolf IS and GS exclusively affected Koniks ($P < 0.001$). Different predators influenced the two breeds to different degrees. Specifically, in Koniks compared to Arabians, LF/HF was higher during jackal GS, lower during panther IS and GS and higher during wolf IS and GS ($P < 0.001$). Higher parameters were identified in response to wolf IS and GS in Koniks compared to other vocalisations, whereas panther elicited a higher LF/HF level in Arabians ($P < 0.05$). Fluctuations in the LF/HF in the post-sound phases were generally

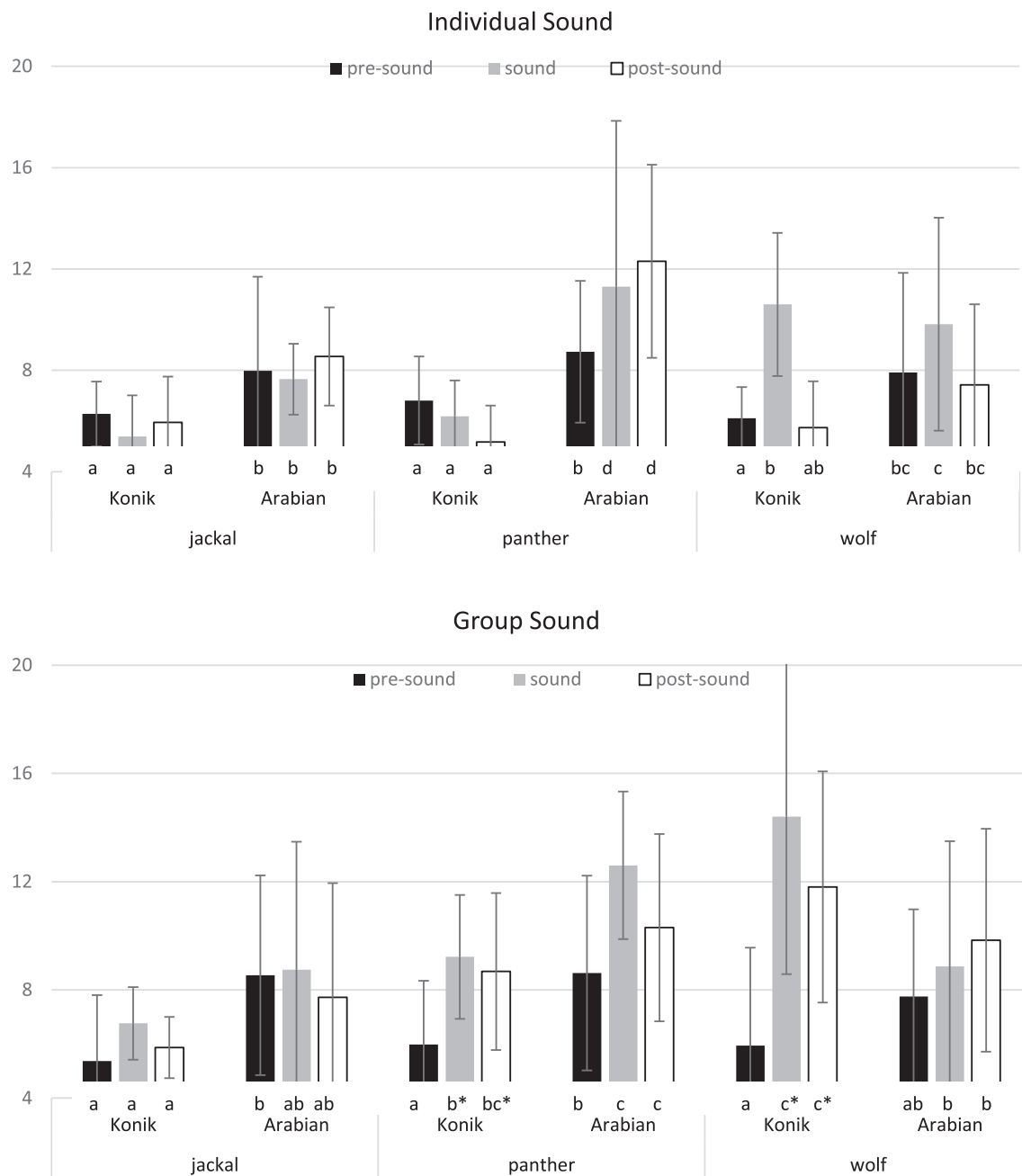


Fig. 2. Total power of heart rate variability (TP) in horses on pasture subject to predator sound. Significant differences in means ($P < 0.05$) are noted as follows: different letters (a, b, c, d) indicate significant differences between bars (considering particular breeds, predators and test phases); * indicate significant differences between a bar of individual sound and an analogous bar of group sound.

similar to those of sound phases. Among all the cases of significant differences between IS and GS effects, LF/HF was higher when GS was featured ($P < 0.05$).

Discussion

The general response to predator's vocalisation

During the study, two different responses of the horses to predators' vocalisations were observed: a decrease in NN and HF along with an increase in TP, LF and LF/HF, or a lack of reaction. Similar changes in HRV parameters were observed during stressful situations that occur during training and reflect the horses' agitation (Schmidt et al., 2010). For example, a decrease in beat-to-beat intervals in naïve horses was observed

when a rider was mounting a standing horse (Schmidt et al., 2010). Even saddling the trained horse induced a decrease in NN (Stachurska et al., 2015). Lowered HF was noted in leisure horses during work in an arena in response to the presence of an audience that was walking and talking (Janczarek et al., 2019). On the other hand, opposite changes in HRV parameters (a decrease in LF and LF/HF as well as an increase in HF) were identified in horses subjected to relaxation techniques, including massage and music (Stachurska et al., 2015; Kędzierski et al., 2017). The horses were relaxed in response to these techniques.

The changes of HRV parameters stated in the studied horses are the result of a shift from parasympathetic to a transient predominance of sympathetic nervous system activity in the sympatho-vagal balance (Rietmann et al., 2004; Schmidt et al., 2010). In general, sympathetic and parasympathetic system activities are antagonistic to each other.

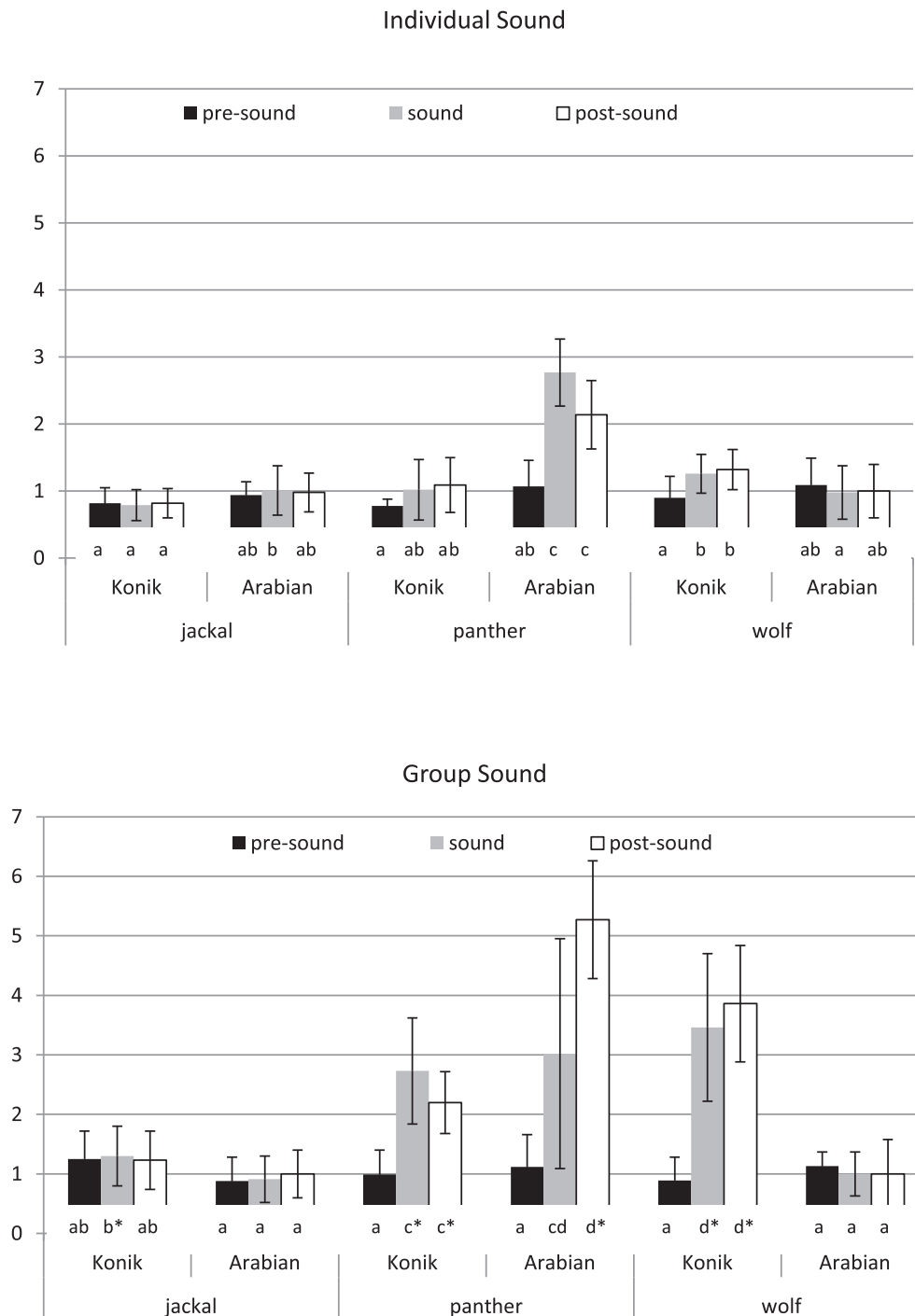


Fig. 3. Low frequency component of the power spectrum (LF) in horses on pasture subject to predator sound. Significant differences in means ($P < 0.05$) are noted as follows: different letters (a, b, c, d) indicate significant differences between bars (considering particular breeds, predators and test phases); * indicate significant differences between a bar of individual sound and an analogous bar of group sound.

An increase in the sympathetic component accelerates the heart rate, whereas an increased tone of the vagal component decelerates the heart rate. The constant influence of the two antagonistic systems on heart activity leads to the variability in heart rate rhythm. This variability is a cyclic occurrence of differences in the heart beat-to-beat intervals. The differences can be registered and analysed as time HRV parameters, such as NN, and frequency HRV parameters (LF, HF and LF/HF). In particular, frequency HRV parameters precisely reflect the predominance of the sympathetic or parasympathetic nervous system

activity that dominates in the current situation (Janczarek et al., 2016b). Thus, the typical decrease in NN and HF with simultaneous increases in TP, LF and LF/HF reported in Arabian horses in response to panther growling and in Konik horses in response to wolf howling indicate that listening to these vocalisations was identified by horses as stressful.

The response of Koniks and Arabians to various predators was different. The wolf howl elicited a stronger response in Koniks, and the panther growl influenced Arabians more strongly. The jackal's howl did

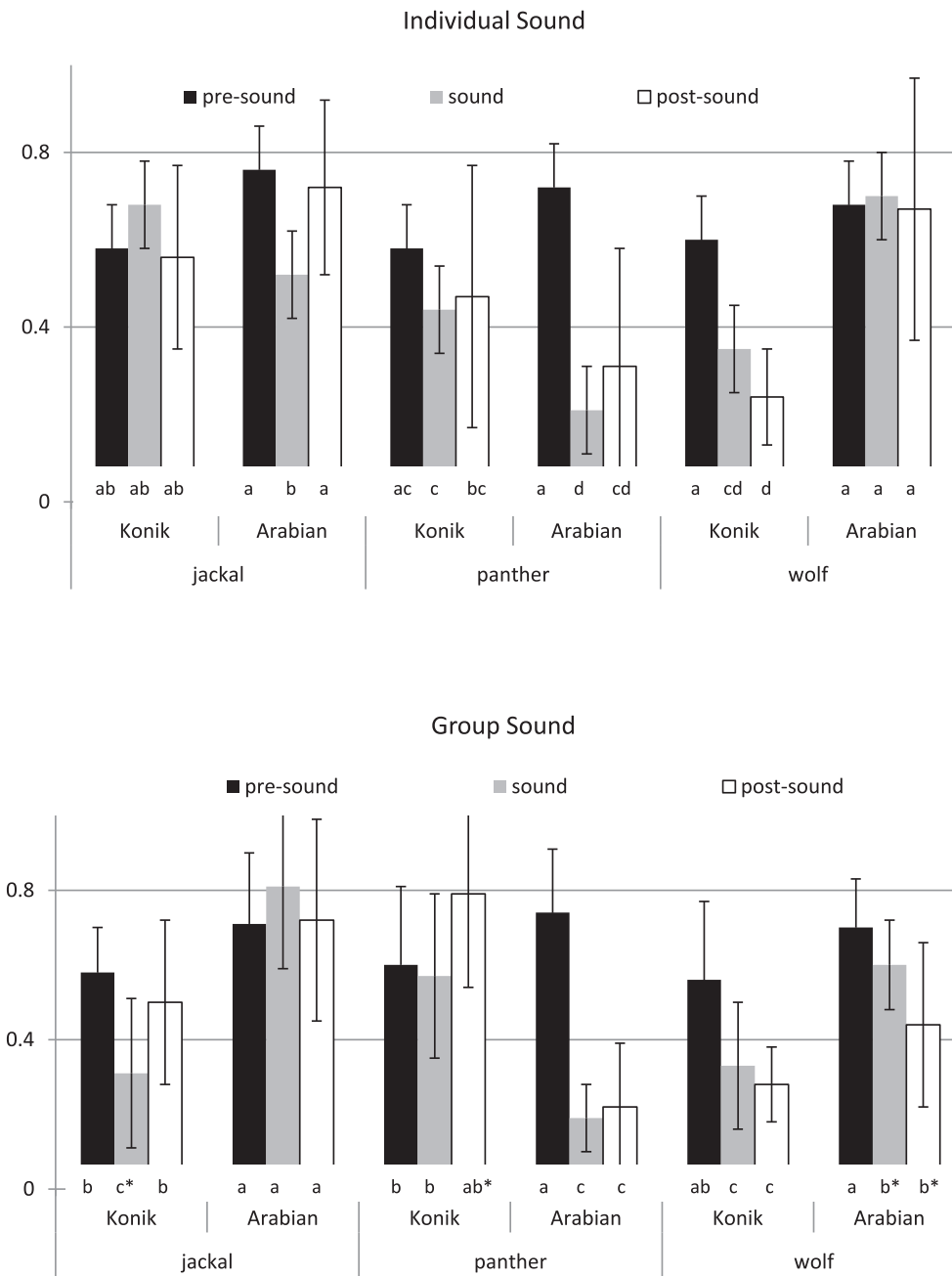


Fig. 4. High frequency component of the power spectrum (HF) in horses on pasture subject to predator sound. Significant differences in means ($P < 0.05$) are noted as follows: different letters (a, b, c, d) indicate significant differences between bars (considering particular breeds, predators and test phases); * indicate significant differences between a bar of individual sound and an analogous bar of group sound.

not elicit agitation in the horses except for GS in Koniks (HF decrease and LF/HF increase). The initial comparative analysis of the predators' sounds revealed that the jackal's howl exhibited the highest frequency. High tones may annoy and scare the horses (Saslow, 2002). It seems that if the response to different predator vocalisations resulted from a fear of an unknown sound *per se* and not a predator attack, a jackal's howl at a high frequency would frighten the horses the most. Thus, the response to other predators known by the horses' ancestry suggests that the frightening cue is not the sound by itself but the possible attack of a panther or wolves. The jackal typically hunts alone or in pairs and does not threaten equids given its small size (Lanszki and Heltai, 2002). The jackal howl in the study was considered as a type of a control cue, and the lack of (or a slight) response to its vocalisation may indicate

that the horses are not disturbed by a predator that has never threatened equids.

The results showed the significant effect of panther and wolf vocalisations on the HRV in horses, which is consistent with the study by Ahmadijeh et al. (2010) who observed behavioural and heart rate responses of Caspian miniature horses to lion faeces and roars. These horses responded to auditory stimuli with a remarkably increased heart rate. The olfactory stimulus evoked behavioural reactions but no significant elevation in the heart rate. When analysing a horse's response to predator stimuli, it should be mentioned that predator odour alone does not frighten domestic horses according to Christensen and Rundgren (2008). An increased heart rate was observed only if the horses were simultaneously presented to an additional sudden stimulus of a moving

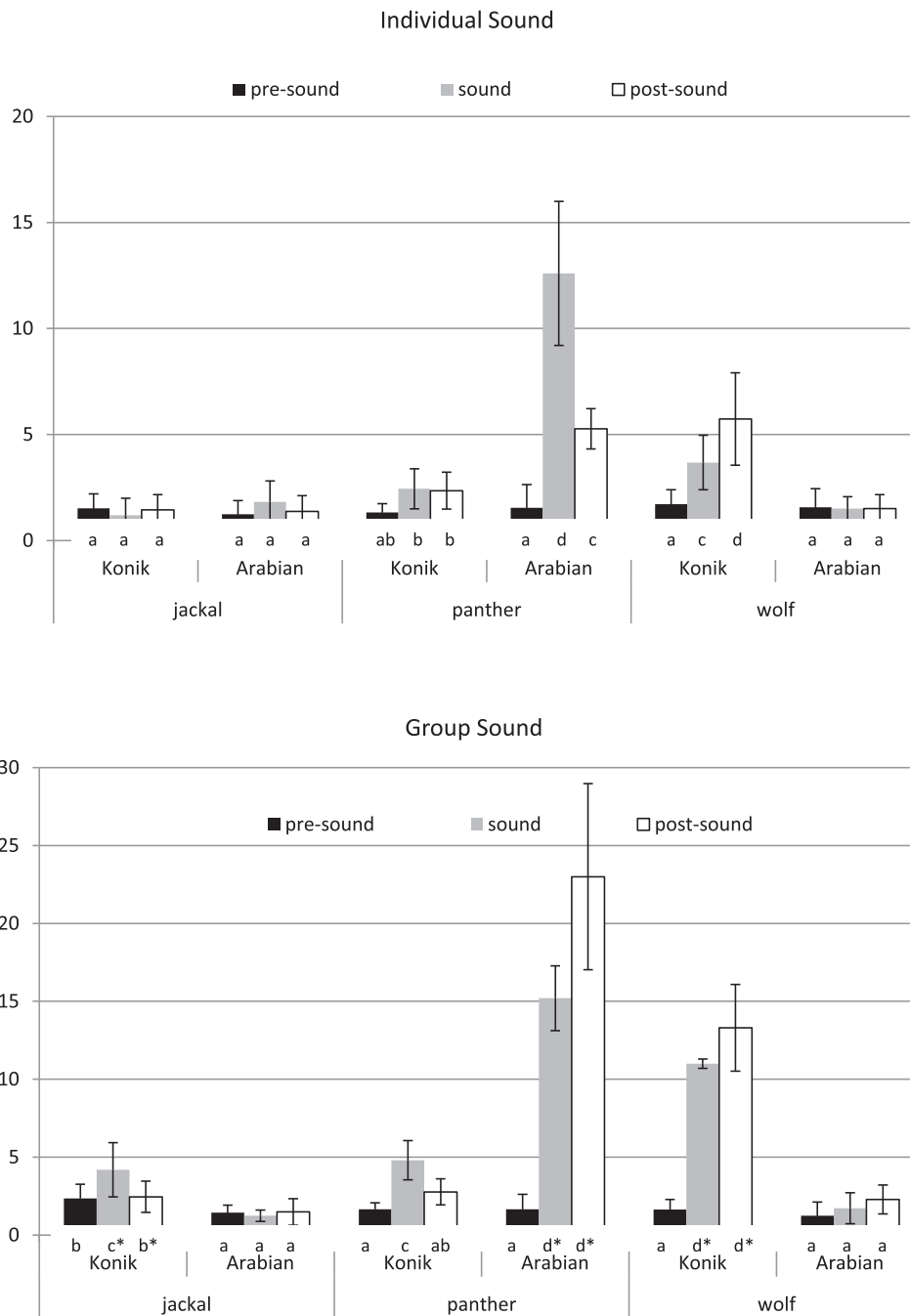


Fig. 5. Ratio of spectrum power from low to high frequencies (LF/HF) in horses on pasture subject to predator sound. Significant differences in means ($P < 0.05$) are noted as follows: different letters (a, b, c, d) indicate significant differences between bars (considering particular breeds, predators and test phases); * indicate significant differences between a bar of individual sound and an analogous bar of group sound.

plastic bag. In this light, the current results of the auditory tests demonstrate the effects of a strong predator threat on the horses and that the effects distinctly vary in different breeds.

Effect of panther growl versus wolf howl in Koniks and Arabians

The panther growl elicited a differentiated response. In Arabians, strong emotional agitation appeared both with IS and GS, whereas in Koniks weak agitation was identified exclusively with GS. The increased agitation was reflected by an increase in sympathetic nervous system activity and a decrease in the parasympathetic activity of the nervous system. The wolf howl induced an opposite response in the two breeds:

a high level of agitation in Koniks and a lack of reaction in Arabians. The direct comparison of the effects of the predator sounds confirms that in almost all of the parameters, the panther more strongly affected the Arabians, whereas the wolf induced a higher level of agitation in Koniks. These findings imply that the hypothesis was confirmed: Arabian horses, the ancestors of which co-existed with the Arabian panther in the Arabian Peninsula, are more excited by the growl of this predator, whereas Koniks, which were historically threatened by wolves in East-Central Europe, are more agitated by a wolf howling. The responses and differences between them do not appear to be accidental but seem to be innate. The results suggest that a type of “genetic memory” exists in horses. To the best of our knowledge, this issue has not been

studied in horses or other farm animals. Innate responses are typically considered in terms of millions years of evolution and natural selection. However, studies on mice discovered that olfactory experiences might be inherited even in subsequent generations (Dias and Ressler, 2013).

Our results are consistent with the study by Dwyer (2014) who found that in the absence of predators in the habitats, the anti-predator strategies of sheep persist. Furthermore, differences in the reactions of less selected hill sheep breeds and highly selected lowland breeds to the threat of predation are noted. The author suggests that a continuum of responsiveness exists between the wild sheep and present sheep breeds. Studies on cattle also showed that artificial selection did not result in the absence of anti-predator behaviours under circumstances free of predator threat (Laporte et al., 2010).

Individual sound versus group sound

When comparing IS and GS effects, IS may be considered typical for the panther, which is currently the largest and most widely distributed cat in the Arabian Peninsula that hunts alone (Mazzolli, 2009). GS is typical for wolves, which hunt in packs (Jędrzejewski et al., 2002). However, the GS of panther, wolf and jackal typically elicited a stronger response than IS, and this observation was noted in both breeds. Only TP was influenced by wolf GS more distinctly in Koniks compared with Arabians. Since the sound coming to the horses from four directions was always more frightening than the sound from one loudspeaker, it seems that the method by which different predator species prey is less important for the horses than the reinforcement of the stimulus.

Excitability of Konik horses versus Arabian horses

In the pre-sound phase, TP and HF indicated higher excitability of Arabians compared to Koniks. However, taking into account other parameters, the emotional arousal was similar in these breeds. Many authors have observed the high emotional excitability of Arabian horses, whereas Koniks, which represent a primitive breed, are expected to exhibit reduced emotional arousal (Janczarek et al., 2016b; Sackman and Houpt, 2019). The lack of a distinct difference in the pre-sound excitability of the breeds in the current results may be attributed to the pasture circumstances during this phase. According to Borell von et al. (2007), the activity of the autonomic nervous system falls into a reference scale only at rest. The current results show that the pasture conditions cannot be considered as comparable to rest. Although staying on pasture relaxes the horses (Janczarek et al., 2016a; Placci et al., 2020), it does not fully relax Koniks. Koniks' increased vigilance on pasture may be connected with the remaining adaptation to natural conditions of this primordial breed. Hence, although the excitability of the two breeds studied differs, these differences were not evident in our study. The results reveal a strong influence of the type of predator on the horse's response, and this response is irrespective of the emotional arousal specific to a breed.

Conclusion

In conclusion, horses exhibit a stronger response to predators known compared to unknown by ancestors of the breeds studied. These findings reflect an innate adaptation of horses to predation in the habitat of their ancestors. The response is irrespective of the excitability of a breed and is not a reaction to the sound *per se*. The horses display a type of understanding of the vocalisation meaning. Their response seems to be adequate to the threat signalled as a sound. Although the predator's vocalisation is not known, the horses instinctively react properly to the risk. These results may be important when introducing outlander horses into a location that is not free of predators. Furthermore, the findings may help to understand some horse behaviours displayed by foals or "difficult" horses. Occasionally, their behaviour

seems to be strange or not appropriate for the circumstances, but it likely results from behaviours assimilated by the ancestors of present horses. The animals have generally retained their anti-predator responses even in the current habitat, which is typically lacking predation cues. The results are not always coherent, which may indicate that the response is somewhat attenuated. Additional studies are warranted to further examine the horse's response to other predator species stimuli and the response of other horse breeds to predation risks.

Ethics approval

Animal care and experimental procedures were in accordance with the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes and were approved by the Local Ethics Review Committee for Animal Experiments (no 27/2016).

Data and model availability statement

None of the data were deposited in an official repository. The data are available upon request.

Author ORCIDS

0000-0001-9032-8840 I. Janczarek; 0000-0003-4616-5583 -A. Stachurska; 0000-0001-7532-805X W. Kędzierski; 0000-0002-9203-523X E. Wnuk-Pawlak; 0000-0001-7958-2303 -I. Wilk; K. Zygłewska1, A. Paszkowska; 0000-0002-6400-5375 -M. Ryżak; 0000-0003-1127-2960 -A. Wiśniewska.

Authors contribution

IJ designed and supervised the study, AW, EWP, MR, KZ and AP performed the tests and analysed the data. IW made the statistical analysis and made the tables and figures. IJ and AS drafted the manuscript. AS and WK prepared the final version of the manuscript which was read and approved by all the authors.

Declaration of interest

The authors declare no conflicts of interest.

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