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Rozprawa doktorska

Potencjał sygnałów dźwiękowych do tworzenia wirtualnych ogrodzeń pastwiskowych dla koni

The potential of sound signals to create virtual grazing fences for horses

Rozprawa doktorska wykonana w Katedrze Hodowli i Użytkowania Koni

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Liczba punktów w roku publikacji: **MEiN = 100; IF = 3,000**

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Publikacja nr 3 (P3)

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Liczba punktów w roku publikacji: **MEiN = 140; IF = 2,700**

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Spis treści

Streszczenie

Koncepcja wirtualnych ogrodzeń (VF) zakłada utrzymanie zwierząt na wyznaczonym obszarze bez stosowania fizycznej bariery. Celem rozprawy było określenie, czy samodzielnie stosowane sygnały dźwiękowe (bez asocjacji z impulsem elektrycznym) mają potencjał do tworzenia wirtualnych ogrodzeń pastwiskowych dla koni oraz zbadanie wpływu wybranych czynników na efektywność tej bariery. Materiał badawczy stanowiło łącznie 50 dorosłych koni gorącokrwistych. Przeprowadzono szereg testów behawioralnych oceniających: 1) wpływ efektu nowości i typu pochodzeniowego dźwięku (antropogeniczne, zwierzęta drapieżne i niedrapieżne, przyroda nieożywiona) na wyzwalanie odpowiedzi antydrapieżniczej i stopień postrzegania zagrożenia, 2) znaczenie poziomu motywacji do zdobycia określonych zasobów i dystansu ekspozycji na dźwięk (siły efektu zaskoczenia) na skuteczność bariery dźwiękowej i pobudliwość emocjonalną koni oraz 3) zróżnicowanie reakcji względem dźwięku w zależności od indywidualnych cech koni i kontekstu pojawienia się dźwięku. Analizowano czas i częstotliwość różnych zachowań, głównie wskazujących na wzrost czujności i unikanie bodźca audytoryjnego, liczbę punktów za reakcję względem dźwięku przyznanych na podstawie ustalonej skali, czas latencji oraz częstość (HR) i zmienność (HRV) rytmu serca koni (parametry RR, RMSSD, HF, LF, LF/HF). Wykazano, że nagłe i nieoczekiwane dźwięki wpływają na wyzwalanie odpowiedzi antydrapieżniczej u koni, co wskazuje na pewien potencjał bodźców audytoryjnych do tworzenia wirtualnych ogrodzeń. Niewiele dźwięków postrzeganych było jako zagrażające, a efekt taki uzyskiwano jako wypadkową nieprzewidywalności i efektu nowości. Kluczowym czynnikiem modyfikującym skuteczność bariery dźwiękowej jest motywacja do zdobycia określonych zasobów, redukowana istotnie w przypadku motywacji społecznej. Zależność społeczna maskuje efekt stresora audytoryjnego, co wskazuje na znaczenie grupy społecznej jako buforu. Efektywność sygnałów dźwiękowych jest zatem zbyt niska, aby mogły one tworzyć samodzielne, wirtualne bariery. Nie można wykluczyć wykorzystania dźwięków jako dodatkowego wzmocnienia nietrwałych fizycznych rozwiązań, jednak wymagałoby to dalszych badań. Ze względu na pobudliwość emocjonalną koni, należałoby wówczas uwzględnić odpowiednio duży dystans, po przekroczeniu którego wyzwalany byłby dźwięk.

Słowa kluczowe: koń, behawior antydrapieżniczy, wirtualne ogrodzenia, dźwięk

Summary

The concept of virtual fencing (VF) involves keeping animals within a designated area without the use of a physical barrier. The aim of this dissertation was to determine whether self-applied sound signals (without association with electrical impulse) have the potential to create virtual grazing fences for horses and to investigate the influence of selected factors on the effectiveness of this barrier. The study material consisted of a total of 50 adult warm-blooded horses. Several behavioural tests were conducted to assess 1) the influence of novelty effect and sound origin type (anthropogenic, predatory and non-predatory animals, inanimate nature) on the triggering of the anti-predatory response and the degree of threat perception, 2) the importance of the level of motivation to acquire specific resources and the sound exposure distance (the strength of the surprise effect) on the effectiveness of the sound barrier and the emotional arousal of horses, and 3) the variation of responses to sound according to individual horse characteristics and the context of sound appearance. The time and frequency of various behaviours were analysed, mainly indicating increased vigilance and avoidance of the auditory stimulus, the number of points for reaction to sound awarded based on an established scale, latency time, the heart rate (HR) and heart rate variability (HRV) of the horses (RR, RMSSD, HF, LF, LF/HF parameters). Sudden and unexpected sounds were shown to influence the triggering of the anti-predator response in horses, indicating some potential for auditory stimuli to create virtual fences. Only few sounds were perceived as threatening (behavioural-physiological response) and the effect was a combination of unpredictability and novelty effect. A key factor modifying the effectiveness of the sound barrier is the motivation to acquire specific resources, reduced significantly for social motivation. Social dependence masks the auditory stressor effect, indicating the importance of the social group as a buffer. The effectiveness of auditory cues is therefore too low to create independent virtual barriers. However, the use of sounds as additional reinforcement of impermanent physical solutions cannot be excluded, but this requires further research. Due to the emotional arousal of horses, a sufficiently large distance would then have to be included, beyond which the sound would be triggered.

Keywords: horse, anti-predator behaviour, virtual fences, sounds

Wstęp

Badania nad behawiorem antydrapieżniczym dostarczają szerokiej wiedzy, m.in. z zakresu relacji ofiara – drapieżnik [Hebblewhite i Pletscher, 2002; King i in., 2012], wrodzonego i nabytego rozpoznawania sygnałów pochodzących od naturalnego wroga [Adcock i Tucker, 2020; Chamaillé-Jammes i in., 2014; Hawkins i in., 2004] oraz wpływu domestykacji [Kluever i in., 2008] i długotrwałej izolacji od drapieżnika [Blumstein, 2002; Blumstein i in., 2004; Li i in., 2011] na zachowanie lub utratę behawioru antydrapieżniczego. Zmianę wzorca pokarmowego, lokomotorycznego i wzrost czujności w odpowiedzi na sensoryczne bodźce identyfikujące drapieżcę potwierdzono u zwierząt gospodarskich [bydło: Adcock i Tucker, 2020; Kluever i in., 2009; Welp i in., 2004, owce: Arnould i in., 1998; Arnould i Signoret 1993], w tym u koni [Christensen i Rundgren, 2008; Janczarek i in., 2020a; Janczarek i in. 2020b; Watts i in., 2020]. Wyniki tych badań mogą mieć cenny aspekt praktyczny, bowiem domowe kopytne mogą wpływać negatywnie na bioróżnorodność środowiska poprzez spałowanie kory, szybkie zjadanie siewek, zgryzanie pędów, łamanie gałęzi w wyniku ocierania się czy nadmierne zdeptywanie gleby wpływające na zmianę jej właściwości [Janicka i Wilk, 2022]. W przypadku nietrwałych ogrodzeń i utrzymania w systemach otwartych, zwierzęta mogą stanowić fizyczne zagrożenie skutkujące np. zderzeniami z pojazdami [Ramos i in., 2021]. Wykazanie odpowiedzi na stosowanie naturalnych repelentów, tj. odoranty drapieżników może pomóc w ochronie upraw, zarządzaniu wypasem [Apfelbach i in., 2005; Pfister i in., 1990] i redukcji kolizji drogowych z udziałem zwierząt [Andreassen i in., 2005]. Innym rozwiązaniem mogą być tzw. wirtualne ogrodzenia (virtual fences – VF) [Campbell i in., 2018].

Koncepcja VF zakłada utrzymywanie zwierząt na ograniczonym obszarze bez stosowania fizycznej bariery [Lomax i in, 2019]. Wirtualne ogrodzenia powstały w odpowiedzi na potrzebę zapewnienia kompromisu pomiędzy kosztami, nakładami pracy a optymalizacją wypasu. System ten pozwala na kontrolę rozmieszczenia zwierząt w środowisku [Marini i in., 2018a] i zwiększenie skuteczności wykorzystania pastwiska przez zwierzęta, zapobiegając zarówno nadmiernemu, jak i niedostatecznemu zjadaniu zielonki [Lomax i in., 2019]. Niewidzialne bariery mogą stanowić alternatywę dla tradycyjnych ogrodzeń fizycznych, których stawianie nie zawsze jest możliwe [Campbell i in., 2017]. Szczególnie często niewidzialne bariery stosowane są na rozległych obszarach rolniczych, w przypadku których pełne grodzenie generowałoby wysokie koszty [Jouven i in., 2012]. Mogą one również znaleźć zastosowanie na terenach trudnych do zagospodarowania oraz obszarach chronionych. Ponadto brak fizycznej bariery charakteryzuje się dużą elastycznością grodzenia [Lee i Campbell, 2021; Umstatter, 2011].

Najbardziej popularny system VF korzysta z obroży GPS i opiera się na asocjacji dźwięku z impulsem elektrycznym [Lee i Campbell, 2021; Marini i in., 2018b], chociaż znane jest też łączenie impulsu elektrycznego i innych sygnałów (światło, wibracje) [Bishop-Hurley i in., 2007]. Zbliżenie się zwierzęcia do ustalonej wcześniej granicy skutkuje emitowaniem sygnału dźwiękowego, zapowiadającego impuls elektryczny w przypadku kontynuacji marszu. Bodziec dźwiękowy nabiera po pewnym czasie cech bodźca warunkowego i jego percepcja przez zwierzę powinna być wystarczająca do powstrzymania dalszej migracji [Lomax i in., 2019]. W ciągu ostatnich kilkudziesięciu lat badano działanie różnych niefizycznych rozwiązań [Campbell i in., 2017]. Wirtualne ogrodzenia dedykowano i testowano głównie na bydle i w mniejszym stopniu na małych przeżuwaczach [Herlin i in., 2021]. Pomimo, że przy niektórych patentach wspomniano o zastosowaniu ich dla koni [Umstatter, 2011], brakuje badań naukowych na ten temat.

Wiele badań dotyczących innych gatunków zwierząt wskazuje na wysoki potencjał tradycyjnego rozwiązania VF w utrzymywaniu zwierząt na wyznaczonym obszarze. Chociaż większość zwierząt stosunkowo szybko uczy się działania VF [Campbell i in., 2017; Lomax i in., 2019; Markus i in., 2014; Marini i in., 2018a, Marini i in., 2018b], obserwuje się duże zróżnicowanie indywidualne w tempie adaptacji do systemu, a tym samym w liczbie otrzymywanych impulsów [Herlin i in., 2021]. Może to wynikać ze zróżnicowanej wrażliwości percepcyjnej oraz zdolności uczenia się [Campbell i in., 2018; Marini i in., 2018a]. Choć bodziec elektryczny wyzwala podobną reakcję stresową jak różne, powszechne procedury towarzyszące obsłudze zwierząt, zastosowanie prądu może nadal budzić obawy społeczne [Lee i Campbell, 2021; Umstatter i in., 2011]. Pewnym kompromisem mogłoby być poszukiwanie alternatywnych rozwiązań, np. niezależnych od prądu wirtualnych barier dźwiękowych [Butler i in., 2004; Butler i in., 2006; Umstatter i in., 2013; Umstatter i in. 2009]. Metoda ta ma pewne ograniczenia i charakteryzuje się niższą skutecznością w porównaniu z tradycyjnym systemem VF, niemniej może przynajmniej częściowo wspomóc zarządzanie wypasem [Umstatter i in., 2013; Umstatter i in. 2009]. W tym kontekście dźwięk może działać jako bodziec irytujący, drażniący (działanie opóźnione) albo ostry sygnał alarmujący, wyzwalający stosunkowo szybką reakcję unikania u zwierzęcia [Umstatter i in., 2013]. Nie wiadomo jednak, czy i jakie dźwięki byłyby postrzegane przez konie jako zagrażające i czy wyzwalałyby one dostateczną odpowiedź antydrapieżniczą.

Koń w toku swojej ewolucji rozwinął życie w grupie i ucieczkę jako główne strategie przetrwania [Placci, 2020] pełniące odpowiednio funkcję pierwotnej i wtórnej obrony przed drapieżnikami [Apfelbach i in., 2005]. Jako potencjalna ofiara w łańcuchu troficznym jest niezwykle wrażliwy na bodźce środowiskowe, co pozwala na szybką detekcję i identyfikację stresora [Marliani i in., 2021; Rørvang i in., 2020]. Chociaż większość koni w Europie jest obecnie utrzymywana w systemie stajennym [Rose–Meierhöfer i in., 2006], izolacja od drapieżników nie uchroniła tych zwierząt od działania różnych stresorów, głównie pochodzenia antropogenicznego [Cooper i Albentosa, 2005]. W przypadku koni nowe obiekty, wydarzenia lub dźwięki mogą wywoływać wzrost czujności, a nawet ucieczkę [Squibb i in., 2018]. Ponieważ większość bodźców rozpraszających w środowisku zwierzęcia stanowią bodźce audytoryjne, mają one szczególne znaczenie jako czynniki wpływające na zachowanie [Murphy i Dalton, 2014]. Można zakładać, że sygnały dźwiękowe działają jako repelenty, jeśli mają istotne znaczenie biologiczne [Aflitto i Hofstetter, 2014]. Jednocześnie, nawiązując do hipotezy risk – disturbance, bodźce pochodzące ze środowiska antropogenicznego wyzwalają u zwierząt reakcje analogiczne jak obecność drapieżnika [Frid i Dill, 2002]. Prawdopodobnie konie mogą generalizować obecne w środowisku stresory postrzegając je jako potencjalne zagrożenie drapieżnikiem [Christensen i Rundgren, 2008; Janczarek i in., 2020a], a efekt nowości i zaskoczenia może wpływać na zmianę zachowania koni, nawet jeśli zastosowane sygnały nie mają znaczenia biologicznego [Rochais i in., 2017]. Bodźce nagłe i nieoczekiwane są w tym kontekście prawdopodobnie nawet bardziej istotne niż sam efekt nowości ze względu na pewne podobieństwo do poruszających się drapieżników [Christensen i in., 2005]. To z kolei może wskazywać na potencjał dźwięków różnego pochodzenia do tworzenia wirtualnych ogrodzeń pastwiskowych. Należy jednak mieć na uwadze wpływ czynników ograniczających efektywność bariery, które trudno jest kontrolować. Indywidualna zmienność w reaktywności może znacznie różnicować odpowiedź poszczególnych koni i być trudna do przewidzenia.

Reaktywność koni względem różnych bodźców jest modyfikowana motywacją powiązaną z emocjami ciekawości i strachu [Kozak i in., 2018], a podłożem zróżnicowania reaktywości mogą być odmienne cechy temperamentu [Visser i in., 2002]. Wysoki poziom motywacji do zdobycia określonych zasobów może skłaniać zwierzęta do podejmowania interakcji ze stresorem, których osobnik nie podjąłby się przy jej niższym poziomie [Butler i in., 2004; Jouven i in., 2012]. Konie charakteryzuje zróżnicowana wrażliwość sensoryczna [Rørvang i in., 2020], a wymiary osobowości tj. wrażliwość dotykowa, smakowozapachowa, wzrokowa i słuchowa są stabilne w kontekście i czasie [Lansade i in., 2008c]. Biorąc pod uwagę ewolucję konia jako wysoce społecznego roślinożernego zwierzęcia uciekającego [Marliani i in. 2021], kluczowymi wymiarami temperamentu mogącymi różnicować sposób postrzegania zagrożenia i wpływającymi na motywację osobnika są lękliwość i stadność [Lansade i in. 2008a, Lansade i in. 2017; Górecka-Bruzda i in. 2022]. Stadność, która bywa nazywana również motywacją społeczną czy przyciąganiem społecznym, oznacza reaktywność na separację od grupy i potrzebę przebywania w pobliżu stada [Lansade i in. 2008a]. Dla koni izolacja społeczna jest szczególnie silnym stresorem, który wpływa negatywnie na funkcjonowanie organizmu [Kay i Hall 2009; Żelazna i Jezierski 2018]. W sytuacji, kiedy bezpośredni kontakt nie jest możliwy, konie dążą do nawiązania przynajmniej pośrednich interakcji [Yarnell i in., 2015], co wskazuje na wysoką motywację społeczną. Ta jednak może różnić się pomiędzy poszczególnymi osobnikami. Zwierzęta zależne społecznie, w przeciwieństwie do niezależnych i bardziej samodzielnych, źle znoszą izolację i mają trudności z funkcjonowaniem z dala od grupy [Burattini i in., 2020]. Z tych powodów społeczne uwarunkowania koni mogą stanowić czynnik istotnie różnicujący reakcje względem dźwięków i powinny być uwzględnione przy weryfikacji skuteczności wirtualnej bariery.

W odniesieniu do przedstawionego stanu wiedzy oraz charakterystyki konia jako gatunku, w rozprawie podjęto próbę określenia potencjału sygnałów dźwiękowych do tworzenia niezależnych od prądu, wirtualnych ogrodzeń pastwiskowych. Uwzględniono przy tym wpływ motywacji i cech indywidualnych, jako czynników ograniczających skuteczność tego typu rozwiązań.

Hipotezy i cele badań

Hipotezy

W rozprawie doktorskiej przyjęto założenie, że sygnały dźwiękowe mają pewien potencjał do tworzenia niezależnych od prądu, wirtualnych ogrodzeń pastwiskowych, ale efekt ten będzie zależał od różnych czynników. W związku z tym w poszczególnych etapach badań weryfikowano hipotezy cząstkowe:

1. Reakcje koni na sygnały dźwiękowe zależą w większym stopniu od efektu nieprzewidywalności dodatkowo wzmacnianego efektem nowości, niż od pochodzenia tych sygnałów w kontekście biologicznym (P1).
2. Samodzielnie zastosowany dźwięk może działać jako niewidzialna bariera, ale reakcje behawioralne koni i fizjologiczna odpowiedź organizmu będą zależały od poziomu motywacji konia oraz od siły efektu zaskoczenia (P2).
3. Konie o zróżnicowanym stopniu zależności społecznej mogą reagować odmiennie względem niespodziewanego dźwięku pojawiającego się w różnych sytuacjach. Motywacja społeczna maskuje objawy strachu i mobilizuje konie do konfrontacji ze stresorem. Dlatego też, pomimo możliwej odmiennej reakcji lękowej wobec dźwięku pojawiającego się poza kontekstem socjalnym, reakcje osobników zależnych i niezależnych od stada mogą być zbliżone, gdy ten sam bodziec wystąpi w kontekście socjalnym (P3).

Cele

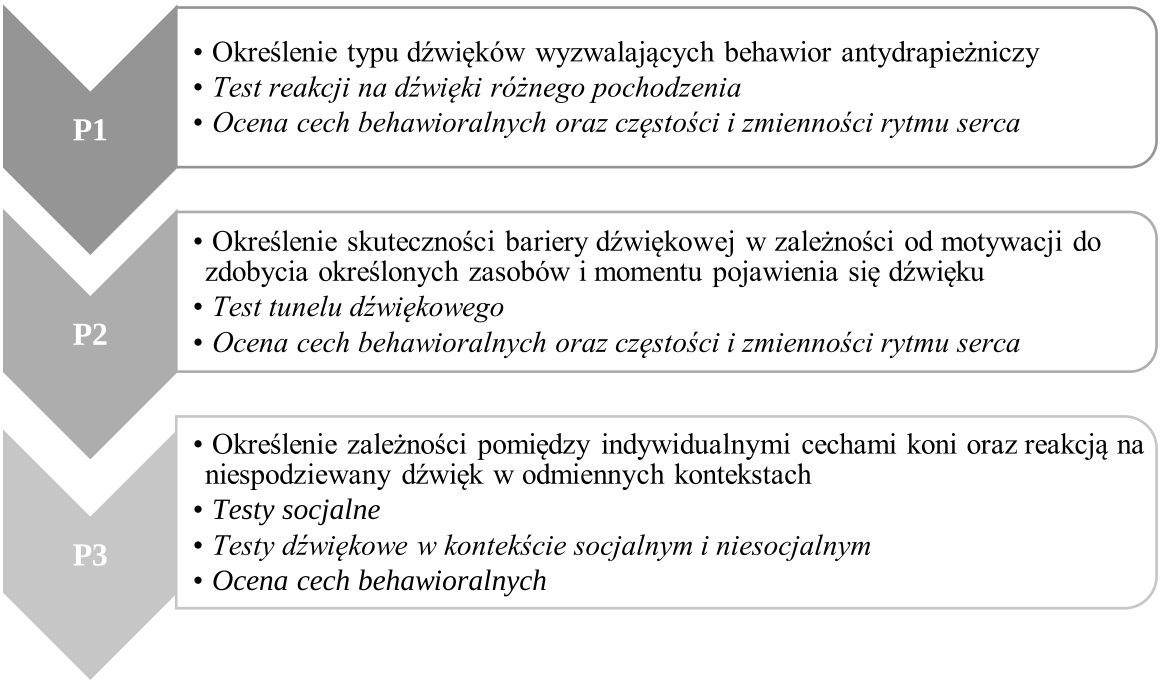
Celem rozprawy było określenie, czy samodzielnie stosowane sygnały dźwiękowe mają potencjał do tworzenia wirtualnych ogrodzeń pastwiskowych dla koni oraz zbadanie wpływu wybranych czynników na skuteczność tej bariery. Wyznaczono następujące cele szczegółowe:

1. Określenie reakcji behawioralnej i pobudliwości emocjonalnej koni na dźwięki różnego pochodzenia oraz pogrupowanie tych dźwięków względem efektu, jaki wywierają na odpowiedź behawioralno-fizjologiczną organizmu (P1);
2. Ocena skuteczności bariery dźwiękowej w zależności od poziomu motywacji pokarmowej i społecznej koni oraz siły efektu zaskoczenia bodźcem (P2);
3. Ocena wpływu wybranych wskaźników behawioralnych i biologicznych koni na zmienność reakcji zwierząt w zależności od kontekstu zastosowanego bodźca dźwiękowego (P3).

Materiał i metody

Etapy badań

Badanie obejmowało trzy główne etapy, które zostały kolejno zaprezentowane w publikacjach wchodzących w skład cyklu rozprawy doktorskiej (ryc. 1). W pierwszym etapie (P1) określono, jaki typ dźwięku ze względu na pochodzenie ma potencjał wirtualnej bariery poprzez wyzwalanie behawioru antydrapieżniczego u koni. Następnie, w etapie drugim (P2), przeprowadzono autorski test tunelu dźwiękowego badający skuteczność tej bariery w zależności od motywacji konia do zdobycia określonych zasobów i siły efektu zaskoczenia dźwiękiem. Na podstawie tych wyników w etapie trzecim (P3) skupiono się na indywidualnych, przede wszystkim społecznych cechach koni, ograniczających efektywność dźwięków jako samodzielnych, wirtualnych ogrodzeń pastwiskowych.



P1

- Określenie typu dźwięków wyzwalających behawior antydrapieżniczy
- *Test reakcji na dźwięki różnego pochodzenia*
- *Ocena cech behawioralnych oraz częstości i zmienności rytmu serca*

P2

- Określenie skuteczności bariery dźwiękowej w zależności od motywacji do zdobycia określonych zasobów i momentu pojawienia się dźwięku
- *Test tunelu dźwiękowego*
- *Ocena cech behawioralnych oraz częstości i zmienności rytmu serca*

P3

- Określenie zależności pomiędzy indywidualnymi cechami koni oraz reakcją na niespodziewany dźwięk w odmiennych kontekstach
- *Testy socjalne*
- *Testy dźwiękowe w kontekście socjalnym i niesocjalnym*
- *Ocena cech behawioralnych*

Ryc. 1. Kolejne etapy badań wchodzących w skład cyklu publikacji rozprawy doktorskiej

Materiał

Badaniami objęto łącznie 50 koni gorącokrwistych, w tym 23 klacze i 27 wałachów w wieku 5-16 lat. Ze względu na ryzyko habituacji koni do bodźców audytoryjnych w wyniku ekspozycji na kilkadziesiąt różnych dźwięków podczas pierwszego etapu badań (P1), 20 koni biorących w nim udział nie wykorzystano ponownie w kolejnych etapach. W etapie drugim zbadano 30, a w trzecim 20 koni wybranych losowo spośród osobników z etapu drugiego.

Konie utrzymywane były w systemie stajennym w indywidualnych boksach ścielonych słomą i wyposażonych w żłób, paśnik, lizawkę i automatyczne poidło. Karmione były trzy razy dziennie paszą treściwą (owies, dodatek otrębów) i sianem łąkowym, a w sezonie pastwiskowym pobierały również zielonkę. Konie były wypuszczane na pastwiska i wybiegi na min. 4 h dziennie w grupie znanych i zaprzyjaźnionych osobników. Użytkowane były jako konie dydaktyczne i rekreacyjne i pracowały pod siodłem maksymalnie 2 h dziennie przez 6 dni w tygodniu. Zwierzęta te były pod stałą kontrolą lekarza weterynarii i codzienną obserwacją swoich opiekunów. Wszystkie konie biorące udział w badaniu były klinicznie zdrowe i nie wykazywały zaburzeń zachowania.

Testy i pomiary behawioralne

Konie zostały poddane szeregowi testów behawioralnych oceniających czas [s] i/lub częstotliwość [częst.] określonych zachowań (P1, P2, P3), pojawienie się danego zachowania w jednostce czasu (system 0-1; [Altman, 1974]) (P1, P3) oraz reakcję wyrażoną za pomocą autorskich skal behawioralnych (P2, P3). Zastosowano obserwację metodą focal-sampling [Altman, 1974].

Pliki audio .mp3 z nagraniami badanych dźwięków wykorzystane w badaniach pobrano z internetu (http://www.orzelorla.pl, http://danielbialogard.pl, http://xn--odgosy-5db.pl, https://www. youtube.com, https:// www.salamisound.com, https://www.pacdv.com (dostęp: 15.03.2021)), zapisano w pamięci komputera, a następnie poddano obróbce technicznej z wykorzystaniem programu Audacity 2.4.2 [Audacity, 2020]. Usunięto fragmenty ciszy (brak istotnego dźwięku w nagraniu >2 s), połączono fragmenty nagrania w celu otrzymania pliku audio o pożądanej długości (60 s; P1, 20 s; P2, P3) i zależnie od potrzeby, wzmocniono dźwięk o maksymalnie 22 dB. Nagrania odtwarzano z intensywnością ok. 80 dB (P1, P2), 100 dB (P3; socjalny test dźwiękowy) i 40 dB (P3; niesocjalny test dźwiękowy) za pośrednictwem urządzenia Samsung Galaxy A02s (Samsung Electronics Co., Ltd., South Korea) połączonego przez Bluetooth z głośnikiem JBL Charge 4.

Obserwacje prowadzono *in situ* ze względu na konieczność przebywania eksperymentatora w pobliżu głośnika (połączenie Bluetooth do 10-15 m) oraz niezbędną obsługę koni w etapie drugim i trzecim (P2, P3). W etapie pierwszym (P1) eksperymentator (n = 1) był niewidoczny dla koni, natomiast w trakcie pomiarów w kolejnych etapach badań eksperymentatorzy (n = 2) stali spokojnie w odległości 10 – 15 m i nie zakłócali przebiegu testu, a wszystkie konie były przyzwyczajone do ich obecności. Ponadto przebieg testów rejestrowano za pomocą kamery Sanyo XACTI w celu uzupełnienia obserwacji.

Test reakcji na dźwięki różnego pochodzenia

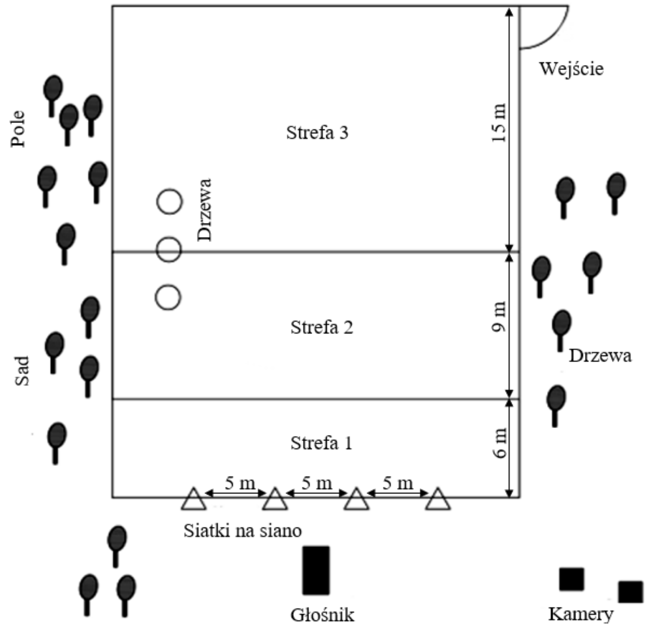
W teście tym oceniano reakcje koni względem 40 potencjalnie znanych (n = 16) i nieznanych (n = 24) dźwięków pochodzenia antropogenicznego (A; n = 11), należących do ptaków i ssaków niedrapieżnych (tzw. zwierząt neutralnych) (N; n = 14), do dziko żyjących ssaków drapieżnych i ich form udomowionych (D; n = 9) oraz pochodzących z nieożywionego środowiska naturalnego (Ś; n = 6). Dźwięki podzielono na 8 grup składających się z 5 dźwięków obejmujących min. trzy różne typy pochodzeniowe. W ciągu 4 kolejnych tygodni koniom prezentowano dźwięki z poszczególnych grup według schematu: dwie grupy w tygodniu w odstępie 2-3 dni (tab. 1). W obrębie każdej grupy dźwięki odtwarzano kolejno co 30 min.

Tab. 1. Schemat odtwarzania dźwięków w trakcie testu reakcji na dźwięki różnego pochodzenia [Janicka i in., 2022b (P1)].

Nr grupy	Tydzień badania	Kolejność odtwarzania dźwięków
I	1	Syrena policyjna (A, ZN) – kwiczenie świni (N, NZ) – szum strumyka (Ś, NZ) – alarm budzika (A, NZ) – pianie bażanta (N, ZN)
II	1	Uderzenie dzwonu (A, NZ) – krzyk myszołowa rdzawosternego (N, NZ) – wycie szympansa (N, NZ) – trzaski gałęzi+szum liści (Ś, ZN) – aplauz (A, ZN)
III	2	Burza (Ś, ZN) – przejeżdżająca lokomotywa (A, NZ) – gra na trąbce (A, ZN) – beczenie owiec (N, NZ) – ryk lwa (D, NZ)
IV	2	Warczenie rysia (D, NZ) – miauczenie kotów (D, ZN) – pracujący odkurzacz (A, NZ) – klangor mew srebrzystych (N, NZ) – szum wiatru (Ś, ZN)
V	3	Ryk jelenia (N, NZ) – kukanie kukułki (Ś, ZN) – przelatujący samolot (A, ZN) – szum fal morskich (Ś, NZ) – trąbienie słonia (N, NZ)
VI	3	Ruch na jezdni (A, ZN) – szum rzeki (Ś, NZ) – furkot kszyka (N, NZ) – okrzyki sroki (N, ZN) – szczekanie psa (D, ZN)
VII	4	Pracująca kosiarka (A, ZN) – ryk niedźwiedzia (D, NZ) – wycie wilka (D, NZ) – warczenie+ryk tygrysa (D, NZ) – krakanie kruka (N, ZN)
VIII	4	Warczenie lamparta (D, NZ) – ryk goryla (N, NZ) – krzyk sępa (N, NZ) – odgłosy tłumu ludzi (A, ZN) – warczenie+ryk pumy (D, NZ)

Dźwięki: ZN – potencjalnie znane, NZ – potencjalnie nieznane, A —pochodzenia antropogenicznego, N – należące do tzw. zwierząt neutralnych, D – dziko żyjących drapieżników i ich form udomowionych, Ś – pochodzące z nieożywionego środowiska naturalnego

Konie testowano w ich zwyczajowych grupach (2-4 osobniki) na znanym, ziemnym padoku zapewniającym odizolowanie od zbędnych bodźców (np. ruch pojazdów, pieszych). Na padoku tym przygotowano plac eksperymentalny pozwalający na ocenę wpływu dźwięków na behawior pokarmowy (siatki na siano), lokomotoryczny i orientację w przestrzeni (podział na strefy) (ryc. 2). Celem wprowadzenia łatwo dostępnego pokarmu w postaci siana w siatkach było również zachęcenie koni do pozostawania w strefie 1 (najbliżej źródła dźwięku) i ocena wystąpienia lub braku zmiany lokalizacji po zastosowaniu dźwięku (strefa 1 – 3).

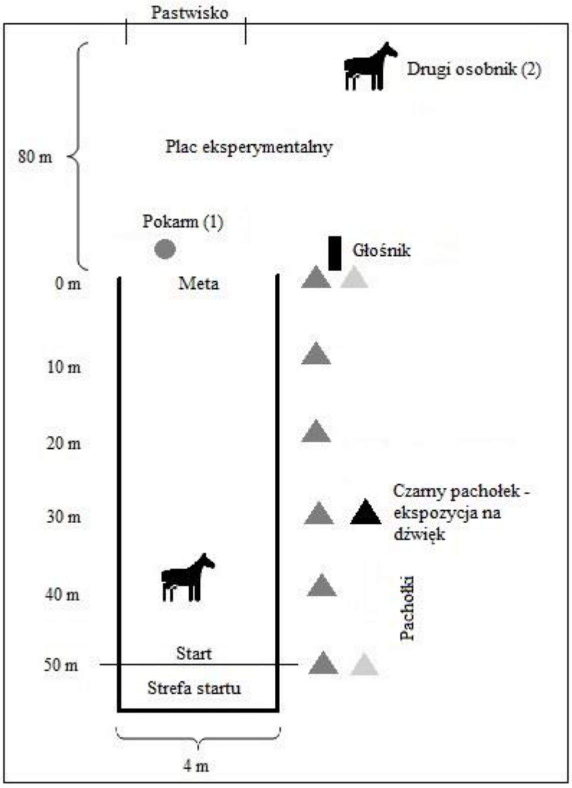


Ryc. 2. Schemat placu eksperymentalnego wykorzystanego podczas testu reakcji na dźwięki różnego pochodzenia [Janicka i in., 2022b (P1)].

Test pozwolił na ocenę, które dźwięki są postrzegane jako zagrażające i wyzwalają mechanizmy behawioru antydrapieżniczego, a więc powodują pojawienie się zachowań obronnych i zredukowanie udziału czynności nieistotnych w danym momencie dla przeżycia danego osobnika [Apfelbach i in., 2005; Janicka i Wilk, 2022]. Oceniano zatem zmiany udziału zachowań – wzrost udziału czujności (przebywanie w pozycji alarmowej) [s], lokomocji (związana z niepokojem) [s] [Janczarek i in., 2020a; Janczarek i in. 2020b], defekacji [częst.], chrapania [częst.], gwałtownego wydmuchiwania powietrza [częst.] [Lansade i in. 2008b; Scopa i in., 2018], reakcji wzdrygnięcia [częst.] [Adcock i Tucker, 2020], czasu przebywania w dalszych strefach placu eksperymentalnego [s] oraz zmniejszenie udziału zachowań pokarmowych [s, częst.], interakcji społecznych [s], odpoczynku [s], zachowań komfortowych [s, częst.] [Apfelbach i in., 2005], skrócenie czasu przebywania w strefie 1 [s], a także wystąpienie zachowań, takich jak przerwanie dotychczasowej czynności i oddalenie się od źródła dźwięku bezpośrednio po jego odtworzeniu [system 0-1]. Za wyjątkiem dwóch ostatnich (ocena wyłącznie po zastosowaniu dźwięku), reakcje koni oceniano w trzech 5-minutowych odcinkach (B0, B1, B2): 5 min przed odtworzeniem dźwięku (B0), 5 min od momentu odtworzenia dźwięku (B1) oraz przez następujące bezpośrednio kolejne 5 min (B2).

Test tunelu dźwiękowego

W celu oceny wpływu czynników motywujących konie do przemieszczania się oraz znaczenia momentu pojawienia się dźwięku (siły efektu zaskoczenia) dla skuteczności wirtualnej bariery, opracowano prosty test tunelu dźwiękowego. W trakcie testu konie przemieszczały się w stronę pastwiska korytarzem (55 x 4 m) wyznaczonym za pomocą plastikowych tyczek i taśmy do pastucha. Końcowa część tunelu była otwarta, co zapewniało możliwość swobodnego wyjścia (ryc. 3).



Ryc. 3. Schemat tunelu dźwiękowego [Janicka i in., 2022a (P2)]; (1), (2) - pierwszy i drugi wariant badania (motywatory); pachołki – ustawione co 10 m w celu ułatwienia obserwacji (po dwa pachołki na starcie i na mecie); czarny pachołek – umieszczony na dystansie 30, 15 lub 5 m od linii mety, wskazujący moment odtwarzania dźwięku; strefa startu – oczekiwanie na rozpoczęcie testu.

Test składał się z dwóch wariantów: w pierwszym z nich motywatorem do przemieszczania się był pokarm znajdujący się 3 m za linią mety (W1; nagroda pokarmowa), natomiast w drugim przypadku, ok. 70 m za linią mety przebywał znany członek stada, uprzednio odczulony na zastosowany dźwięk (W2; nagroda społeczna). Połowa koni została poddana testowi w kolejności W1 → W2 w odstępie 1 tygodnia, a pozostałe zwierzęta w odwrotnej kolejności. Przed przystąpieniem do testu konie podzielono na 3 równe grupy (po 5 klaczy i 5 wałachów w każdej) i losowo przyporządkowano im jeden z trzech dystansów, po osiągnięciu którego odtwarzany był dźwięk: 30, 15 lub 5 m od głośnika znajdującego się na linii mety. Dystans ten oznaczano uprzednio pachołkiem w innym kolorze, aby ułatwić eksperymentatorowi wykrycie właściwego momentu odtworzenia nagrania. Każdy wariant (W1, W2) obejmował 3 etapy (A, B, C). Etap A polegał na zaznajomieniu konia z kierunkiem przemieszczania się i wskazaniu miejsca nagrody. Na tym etapie nie wykonywano pomiarów. Konie były prowadzone przez znanego eksperymentatora w kierunku mety min. jeden raz. Warunkiem ukończenia tego etapu było chętne (brak zatrzymywania, rozglądania się, pewny ruch naprzód) przemieszczanie się w kierunku nagrody (2-4 powtórzenia). W etapie B (próba kontrolna) konie przemieszczały się samodzielnie, dokonywano pomiarów ustalonych zmiennych behawioralnych, jednak nie wprowadzano bodźca eksperymentalnego (dźwięk o cechach futurystycznych). W etapie C (próba eksperymentalna) po osiągnięciu przez konia odpowiedniego dystansu (30/15/5 m od linii mety) odtwarzano dźwięk.

Podczas etapu B i C oceniano czas potrzebny na pokonanie tunelu [s] i na tej podstawie obliczono udział [%] stępa, kłusa, galopu, całkowitej lokomocji i czujności. Notowano również częstotliwość chrapania i defekacji w trakcie trwania testu. Dodatkowo w etapie C określono wystąpienie efektu bariery (1 – kontynuacja marszu, 2 – zatrzymanie, 3 – odejście (stęp), 4 – ucieczka (kłus/galop)) i czas latencji mierzony jako czas od odtworzenia dźwięku do zatrzymania, odejścia lub ucieczki. Za maksymalny czas trwania testu przyjęto 5 min. Po upływie tego czasu test przerywano, a udział ocenianych zachowań obliczano w stosunku do 300 s.

Testy socjalne

Na podstawie wyników testu tunelu dźwiękowego podjęto próbę zbadania, czy reakcja względem bariery dźwiękowej jest związana z indywidualnymi uwarunkowaniami koni, takimi jak cechy społeczne i ogólna lękliwość względem niespodziewanego dźwięku (podrozdział *Testy dźwiękowe w kontekście socjalnym i niesocjalnym*). Z tego powodu przed przystąpieniem do właściwych testów wykonano dwa testy oceniające socjalne tendencje koni.

Test zależności społecznej – test areny

W teście zbadano zróżnicowanie reakcji koni na stres separacyjny i występowanie cechy zależności/niezależności od stada, określanej jako trudność/możliwość funkcjonowania bez wsparcia członków grupy społecznej [Burattini i in., 2020; Stachurska i in., 2021]. Test polegał na umieszczeniu każdego z koni na niewielkim padoku i 5-minutowej separacji od stada (okres nasilonych objawów na nietypową sytuację [Wolff i in., 1997]). Oceniano czas trwania [s] zachowań wskazujących na niepokój i negatywną pobudliwość emocjonalną, takich jak energiczny chód, stanie w pozycji alarmowej, kłus, galop oraz częstotliwość defekacji, rżenia i chrapania [Lansade i in., 2008b; Laurijs i in., 2021; Seaman i in., 2002]. Na podstawie procentowego udziału zachowań ocenianych jako „czas trwania" względem całkowitego czasu obserwacji konie przydzielono do jednej z trzech grup wskazujących na stopień zależności społecznej. Konie niezależne (N) określono jako te, u których poniżej 15% czasu obserwacji stanowiły zachowania wskazujące na niepokój (n = 9), konie umiarkowanie zależne (UZ) poświęciły na takie zachowania 15–50% czasu (n = 5), natomiast zależne od stada (Z) – ponad 50% czasu (n = 6).

Test interakcji społecznych – wprowadzenie na padok

Test pozwolił na zbadanie potrzeby koni do nawiązywania interakcji z pozostałymi członkami stada. Obejmował 10-min obserwację koni [Seaman i in., 2002] po porannym wypuszczeniu na padok i odnotowywaniu co 30 s metodą 0-1 [Altman, 1974] wszystkich zaobserwowanych zachowań opisanych w etogramie. Oceniano występowanie zachowań afiliacyjnych (pielęgnacja społeczna, kładzenie głowy na ciele drugiego konia, zabawa społeczna, podążanie, podejście afiliacyjne) i agonistycznych (zachowania agresywne/grożenie: gryzienie, kopanie, markowanie gryzienia/kopania, grożenie głową, próba uderzenia przednią kończyną, wspinanie się, odgonienie, pościg oraz zachowania uległe: ustępowanie, unikanie zachowań agresywnych/grożenia) [McDonnell, 2003]. Test został powtórzony trzykrotnie w ciągu trzech kolejnych dni.

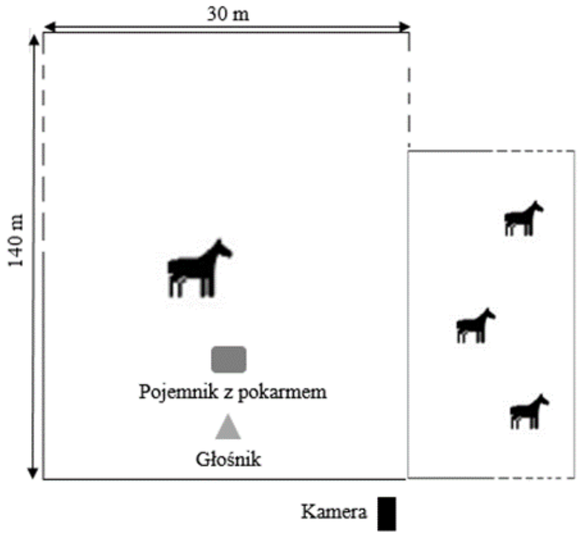
Testy dźwiękowe w kontekście socjalnym i niesocjalnym

Przeprowadzono dwa testy dźwiękowe różniące się tłem pojawienia się dźwięku: bez kontekstu socjalnego (próba kontrolna; stacjonarny test z pokarmem) i z motywacją społeczną (próba eksperymentalna; przemieszczanie się w kierunku stada). Każdy z testów został wykonany dwukrotnie (faza 1, faza 2) w odstępie około roku, aby zmniejszyć ryzyko przyzwyczajenia do dźwięków. Podczas testów wykorzystano dwa różne dźwięki: dźwięk

A – kwik świni, wydawany podczas niepokoju [Çavuşoğlu i in. 2020] i dźwięk B – gwałtowny dźwięk przypominający zakłócenia radiowo-telewizyjne czy trzaski. Wyboru dźwięków dokonano na podstawie obserwacji podczas testu reakcji na dźwięki różnego pochodzenia [Janicka i in. 2022b] (P1) oraz podczas porównywania reakcji na dźwięki odczulone i nowe (badanie własne, nieopublikowane). W pierwszej fazie badania dźwięk A był odtwarzany podczas próby kontrolnej, a dźwięk B podczas próby eksperymentalnej. Odwrotną kolejność zastosowano w drugiej fazie. Ponadto 10 losowo wybranych koni zostało w pierwszej kolejności poddanych próbie eksperymentalnej, a następnie próbie kontrolnej. W przypadku pozostałych 10 koni kolejność procedur została odwrócona. Test kontrolny i eksperymentalny przeprowadzono w odstępie 6 – 8 dni.

Test dźwiękowy w kontekście niesocjalnym (próba kontrolna)

W teście tym sprawdzano reakcję lękową koni względem nowego i niespodziewanego dźwięku, gdy reakcja ta nie jest zakłócona przez motywację społeczną i wymuszanie ruchu naprzód poprzez umieszczoną w odległości nagrodę. Cechę tę kontrolowano poprzez dostosowanie warunków testu do rutyny koni (pomiar w godzinach popołudniowych lub wieczornych – powrót z pastwiska do stajni/na wybiegi w pobliżu stajni, obecność na sąsiadującym padoku trzech znanych koni, uprzednio odczulonych na zastosowane dźwięki oraz dostarczenie atrakcyjnego, łatwo dostępnego pokarmu). Plac eksperymentalny (140 x 30 m) wyznaczono plastikowymi tyczkami i taśmą do pastucha. W pobliżu krótszego boku znajdującego się bliżej stajni umieszczano pojemnik z posiekaną marchwią (ryc. 4).



Ryc. 4. Schemat placu eksperymentalnego wykorzystanego podczas testu dźwiękowego w kontekście niesocjalnym

Test rozpoczynano w momencie, gdy eksperymentator przyprowadził konia na miejsce startu (ok. 3 m od pojemnika z pokarmem) i ustawił go głową skierowaną w stronę pojemnika. W tym czasie drugi eksperymentator umieszczał w nim marchew. Konia uwalniano z uwiązu i pozwalano mu podejść do pojemnika, a eksperymentatorzy opuszczali plac. Po upływie 30 s od momentu rozpoczęcia pobierania pokarmu odtwarzano dźwięk i rozpoczynano obserwację.

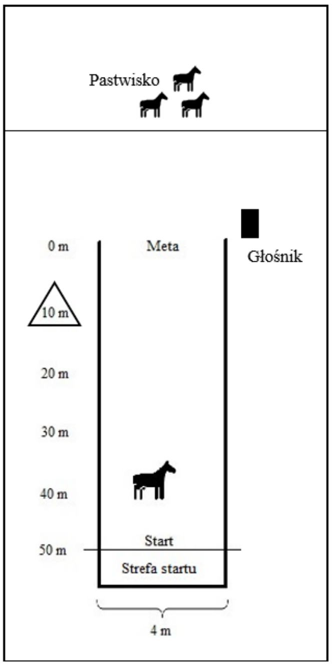
Jako obiektywną miarę reaktywności koni względem stresujących bodźców zastosowano czas latencji do wznowienia pobierania pokarmu [Górecka-Bruzda i in., 2011]. Za maksymalny czas trwania testu przyjęto 5 min. Jeśli po upływie tego czasu koń nie powrócił do pojemnika, uzyskiwał wynik 301 s [Górecka-Bruzda i in., 2011]. Ponadto opracowano 5-punktową skalę behawioralną opisującą zachowanie konia na niespodziewany dźwięk, gdzie 1 oznaczało brak, a 5 najwyższy poziom reakcji lękowej (tab. 2).

Tab. 2. Skala behawioralna do oceny reakcji koni podczas testu dźwiękowego w kontekście niesocjalnym [Janicka i in., 2023] (P3)

Punkty	Reakcja behawioralna
1	Koń nie zwraca uwagi na dźwięk, w nieprzerwany sposób kontynuuje pobieranie pokarmu, jest spokojny.
2	Koń podnosi głowę, nastawia uszy i kontynuuje jedzenie. Nie wykazuje strachu, nie odchodzi od pojemnika z pokarmem.
3	Koń unosi głowę, nastawia uszy, odchodzi na kilka kroków, przygląda się, ostrożnie wraca i ponawia jedzenie, może wystąpić chrapanie.
4	Koń gwałtownie wyjmuje głowę z pojemnika i odskakuje. Kręci się, jest zaniepokojony, może rzucać głową, ostrożnie powraca do pokarmu, wykazuje lęk.
5	Koń gwałtownie odskakuje, odchodzi/ucieka na znaczny dystans, chrapie, długo zwleka z powrotem lub nie powraca w ogóle.

Test dźwiękowy w kontekście socjalnym

W teście tym wykorzystano opracowaną uprzednio procedurę testu tunelu dźwiękowego [Janicka i in., 2022a] (P2) wprowadzając pewne modyfikacje: motywatorem do przemieszczania się było wyłącznie stado znajdujące się na pastwisku, a dźwięk odtwarzano, kiedy każdy z koni osiągnął dystans 10 m od linii mety (głośnika) (ryc. 5).



Ryc. 5. Schemat placu eksperymentalnego wykorzystanego podczas testu dźwiękowego w kontekście socjalnym

Oceniano efekt bariery dźwiękowej [skala 1-5], ukończenie testu w czasie trwania dźwięku (20 s) [skala 1-2] oraz zachowanie po opuszczeniu tunelu [skala 1-6] (tab. 3).

Tab. 3. Zmienne behawioralne oceniane podczas testu dźwiękowego w kontekście socjalnym [Janicka i in., 2023] (P3)

Zachowanie	**Skala ocen**
Efekt bariery	Reakcja na dźwięk; 1 – kontynuacja ruchu naprzód, 2 – zwolnienie, 3 – zatrzymanie, 4 – odejście (stęp), 5 – ucieczka (kłus/galop)
Ukończenie tunelu	Opuszczenie tunelu przed upływem 20 s dźwięku; 1 – nie, 2 – tak
Zachowanie na mecie	Zachowanie po opuszczeniu tunelu; 1 – swobodny stęp/odpoczynek w pobliżu linii mety, 2 – swobodny stęp/odpoczynek z dala od linii mety, 3 – nerwowe rozglądanie się, brak lokomocji, 4 – stęp i nerwowe rozglądanie się, 5 – kłus/galop i nerwowe rozglądanie się, 6 – brak opuszczenia tunelu w ciągu 20 s

Analiza częstości i zmienności rytmu serca

W teście reakcji na dźwięki różnego pochodzenia (P1) oraz w teście tunelu dźwiękowego (P2) dokonano również nieinwazyjnych pomiarów częstości (HR) i zmienności (HRV) rytmu serca w celu oceny pobudliwości emocjonalnej koni podczas ekspozycji na dźwięki. Wśród analizowanych zmiennych znalazły się te, których spadek (RR, RMSSD, HF) lub wzrost (HR, LF, LF/HF) wskazuje na dominację współczulnego układu nerwowego [Borell i in., 2007; Mendonça i in., 2019];

- HR [liczba uderzeń serca/min] – średnia częstość pracy serca (analiza czasowa),
- RR [ms] - interwały pomiędzy kolejnymi załamkami R zespołu QRS (analiza czasowa)

(tylko w P1),

- RMSSD [ms] – odchylenie standardowe różnic pomiędzy kolejnymi odstępami NN (analiza czasowa),
- HF [ms^2] – składowa mocy widma wysokich częstotliwości (0.07–0.5 Hz),
- LF [ms^2] – składowa mocy widma niskich częstotliwości (0.005–0.07 Hz),
- LF/HF [%] – stosunek składowej mocy widma niskich i wysokich częstotliwości; wskazuje na równowagę współczulno-przywspółczulną, z przesunięciem w kierunku dominacji współczulnej.

Pomiarów dokonywano z wykorzystaniem zestawów firmy Polar: urządzeń monitorujących pracę serca typu RS800CX (P1) i Polar Vantage M (P2), nadajników H2 (P1) i H10 (P2) oraz elektrody Polar Pro. Elastyczne paski z elektrodami zakładano na klatkę piersiową koni, a nadajnik umieszczano po lewej stronie na wysokości serca. W celu zoptymalizowania kontaktu elektrody ze skórą konia i poprawy przewodnictwa, wewnętrzną stronę paska na wysokości elektrody pokrywano żelem do EKG [Kovács i in. 2015]. Następnie, po uprzednim zsynchronizowaniu z nadajnikiem i stoperem, monitor mocowano do paska na wysokości mostka konia. Zestawy zakładano i rozpoczynano pomiar w stajni, co najmniej 10 (P2) lub 15 min (P1) przed rozpoczęciem badania. Zapis rejestrowano w sposób ciągły, a po wprowadzeniu danych do komputera i usunięciu artefaktów (zastosowano filtry o niskiej, średniej (P1) lub bardzo niskiej (P2) mocy) wybrane odcinki analizowano w programie Polar Pro Trainer 5 (P1) i Kubios HRV Standard 3.5.0 (P2). Zastosowano analizę trzypunktową, porównując HR i HRV wyjściowe, proceduralne i poproceduralne [Laborde i in., 2017]. Podczas testu reakcji na dźwięki różnego pochodzenia (P1) analizowano, analogicznie jak w przypadku oceny behawioru, trzy 5-minutowe odcinki (B0, B1, B2): 5 min przed odtworzeniem dźwięku (B0), 5 min od momentu odtworzenia dźwięku (B1) oraz przez następujące bezpośrednio kolejne 5 min (B2). Podczas testu tunelu dźwiękowego (P2) analizowano trzy 1-minutowe odcinki (S1, S2, S3): bezpośrednio przed (S1), od momentu odtworzenia (S2) i podczas piątej minuty po odtworzeniu dźwięku (S3) (próba C). Przyjęto, że wzrost dominacji współczulnej/spadek aktywności przywspółczulnej będzie wskazywał na postrzeganie dźwięku jako zagrażającego [Christensen i in., 2005; Janczarek i in., 2020a; Janczarek i in., 2021].

Analiza statystyczna

Analizę statystyczną wykonano w programie lme4” R [Bates i in., 2021] (P1), Statistica 13.3 [Statistica, 2017] (P1) oraz SAS 9.4 [SAS, 2013] (P2, P3). Normalność rozkładu analizowanych cech zbadano za pomocą testów Kolmogorov-Smirnova i Shapiro-Wilka. Z wyjątkiem parametrów HRV uzyskanych w teście tunelu dźwiękowego (P2), uzyskane dane nie miały rozkładu normalnego. Dla wszystkich wykonanych testów statystycznych przyjęto poziom istotności $p < 0.05$. Dla przejrzystości, w artykule nr 2 i nr 3 wyniki przedstawiono w postaci średnich i odchyleń (P2) lub błędów standardowych (P3).

W artykule nr 1 główna analiza dotyczyła wpływu typu pochodzeniowego dźwięku ($n = 4$; A, N, D, Ś) i rodzaju dźwięku ($n = 40$, tab. 1) na zachowanie i pracę serca koni w trzech odcinkach 5-minutowej obserwacji (okresy B0, B1, B2). Ze względu na dużą liczbę uzyskanych wyników przeprowadzono kilkuetapową selekcję i analizę statystyczną. W pierwszym etapie za pomocą ANOVY weryfikowano wpływ czynnika stałego „okres obserwacji” ($n = 3$) na wartość każdej behawioralnej i fizjologicznej zmiennej zależnej podczas odtwarzania danego dźwięku ($n = 40$) lub dźwięku danego pochodzenia ($n = 4$). Następnie dla istotnych i bliskich istotności modeli „pochodzenie dźwięku/dźwięk*zmienna zależna” przeprowadzono procedurę liniowych modeli mieszanych i obliczono zmiany (wzrost lub spadek) wartości danej cechy w okresie B1 i B2 w stosunku do B0. Jako efekt stały przyjęto okres obserwacji, a w efektach losowych uwzględniono zróżnicowanie reakcji koni. Z dalszej analizy wyeliminowano modele, w przypadku których nie stwierdzono istotnych zmian w okresie B1. W kolejnym etapie określono, które modele spełniają założenia o wyzwalaniu behawioru antydrapieżniczego i wzroście aktywności współczulnego układu nerwowego. Dla modeli tych przeprowadzono test kontrastów Tukeya z „okresem obserwacji” jako czynnikiem stałym i na tej podstawie porównano reakcję behawioralną i fizjologiczną koni podczas odtwarzania każdego dźwięku pomiędzy okresem B1 i B2. W ostatnim etapie modele zostały pogrupowane według kategorii „efekt dźwięku" i „typ zmiennej zależnej”. Ze względu na efekt dźwięku, dźwięki zakwalifikowano jako zagrażające (zagrożenie I stopnia; wpływ na zachowanie i pracę serca), zakłócające (zagrożenie II stopnia; wpływ na czujność i inne cechy behawioralne), łagodnie alarmujące (zagrożenie III stopnia; wpływ na czujność) i neutralne (niezagrażające; brak wpływu na behawior antydrapieżniczy i aktywację współczulną). Ze względu na typ zmiennej zależnej, na którą wpływały dźwięki, modele przypisano do kategorii: zajmowana strefa, behawior pokarmowy, lokomocja, czujność, pobudliwość emocjonalna. Na podstawie udziału dźwięków znanych i nieznanych (pierwsza analiza) oraz różnego pochodzenia (druga analiza) w puli modeli przypisanych do poszczególnych kategorii, przeprowadzono test chi-kwadrat Pearsona. Określono procentowy udział dźwięków danego typu w każdej kategorii biorąc pod uwagę ich udział [%] w ogólnej puli dźwięków. Ten sam test wykonano analizując, czy dźwięki z poszczególnych kategorii „efektu dźwięku" (n = 4) powodowały pojawienie się zachowań ocenianych metodą 0-1.

W artykule nr 2 w oceniano zmianę zachowania koni pod wpływem czynników stałych: „etap badania" (B, C) i „wariant badania" (pokarm/nagroda społeczna) (pierwsza analiza) oraz „dystans odtwarzania dźwięku" (30/15/5 m) i „wariant badania" (druga analiza). Przy analizie zmian HRV w trzech 1-minutowych odcinkach pomiaru w etapie C czynnikami stałymi były „odcinek pomiaru" i „wariant badania" (trzecia analiza) oraz „odcinek pomiaru" i „dystans odtwarzania dźwięku" (czwarta analiza). Istotność wpływu analizowanych czynników na HRV obliczono za pomocą analizy wariancji, a istotne średnie porównano za pomocą testu Tukey'a HSD. W przypadku cech behawioralnych wykonano test Kruskala-Wallisa, a istotne wyniki porównano metodą Dwassa, Steela i Critchlow-Flignera (DSCF).

W artykule nr 3 pierwsza analiza polegała na ocenie wpływu czynnika stałego „zależność społeczna" (n = 3) na reakcje koni w testach dźwiękowych, podczas izolacji od stada oraz na częstotliwość nawiązywania różnych interakcji społecznych (test Kruskala-Wallisa). Istotność różnic w reakcji koni z poszczególnych poziomów zależności społecznej obliczono metodą DSCF. W kolejnym etapie, za pomocą korelacji rang Spearmana, zbadano występowanie zależności pomiędzy wynikami uzyskanymi przez konie w testach behawioralnych (druga analiza). Dodatkowo oceniono wpływ płci (trzecia analiza) oraz wieku (czwarta analiza) na zachowanie koni we wszystkich testach behawioralnych. Różnice w reakcji klaczy (n = 10) i wałachów (n = 10) oraz młodszych (n = 10; 6-10 lat) i starszych koni (n = 10; 11-16 lat) zbadano z wykorzystaniem testu U Manna-Whitneya.

Omówienie wyników

Zaobserwowano, że tylko osiem z 40 zastosowanych dźwięków nie spełniło żadnych założeń dotyczących wpływu na behawior antydrapieżniczy i aktywację układu współczulnego. Większość dźwięków miała słaby lub umiarkowany wpływ na reakcje koni (behawioralne zmienne zależne), a tylko osiem z nich można uznać za stresogenne, powodujące zmiany behawioralno-fizjologiczne (tab. 4).

Tab. 4. Dźwięki pogrupowane według kategorii efektu dźwięku (różne stopnie percepcji zagrożenia) na podstawie ich wpływu na zachowanie i pracę serca koni [Janicka i in., 2022b] (P1)

Kategoria efektu dźwięku	**Reakcja**	**Dźwięk**	**Całkowita liczba istotnych modeli**	**X Rozkład procentowy (ZN/NZ)**	**Y Rozkład procentowy (A/N/D/Ś)**
Zagrażające (I stopień zagrożenia)	behawioralno-fizjologiczna odpowiedź stresowa	sroka, świnia, szympans, myszołów rdzawosterny, kszyk, słoń, strumyk, jeleń	n = 49 (w tym fizjologiczne: n = 16)	a* ZN: 25,27 NZ: 74,73	a** A: 0,00 N: 86,73 D: 0,00 Ś: 13,27
Zakłócające (II stopień zagrożenia)	zakłócenie behawioru	kukułka, koty, wilk, sęp, ryś, lampart, syrena policyjna, pies, wiatr, owca	n = 29	ZN: 64,79 NZ: 35,21	b** A: 6,25 N: 24,75 D: 55,50 Ś: 13,50
Łagodnie alarmujące (III stopień zagrożenia)	wzrost czujności	odkurzacz, burza, aplauz, lew, mewy, budzik, tygrys, ruch na jezdni, puma, trzaski gałęzi, bażant, niedźwiedź, tłum, goryl	n = 15	ZN: 50,00 NZ: 50,00	A: 35,37 N: 13,90 D: 29,02 Ś: 21,71
Dźwięki neutralne (brak zagrożenia)	brak	dzwon, lokomotywa, trąbka, samolot, fale morskie, rzeka, kruk, kosiarka	n = 0	Z % dźwięków ZN: 60,10 NZ: 39,90	Z % dźwięków c** A: 52,79 N: 8,37 D: 0,00 Ś: 38,84

X, Y – rozkład procentowy istotnych modeli (dźwięk*zmienna zależna) uzyskanych dla różnych typów dźwięków w każdej kategorii efektu dźwięku; dźwięki ZN/NZ: a*χ2 = 10,47, p = 0,001; dźwięki A/N/D/Ś: a**χ2 = 82,86, p = 0,00; b**χ2 = 17,67, p = 0,001. Z – dla kategorii dźwięków neutralnych obliczono odsetek określonych rodzajów dźwięków: c**χ2 = 8,82, p = 0,03. Przy obliczeniach uwzględniono procentowy udział danego typu dźwięku w całkowitej puli dźwięków.

Efekt dźwięku zdecydowanie częściej wpływał na zmiany zachowania niż na częstośći (HR) i zmienność (HRV) rytmu serca koni. W odpowiedzi na zakłócający bodziec dźwiękowy konie istotnie dłużej przebywały w pozycji alarmowej wykazując czujność, więcej czasu poświęcały na lokomocję, krócej pobierały pokarm, natomiast częściej podchodziły do siatek z sianem. Wśród zmian parametrów częstości i zmienności rytmu serca koni wskazujących na dominację układu współczulnego obserwowano przede wszystkim wzrost HR i spadek RR. Wpływ dźwięku był przeważnie krótkotrwały i utrzymywał się przez 5 min.

Uwzględniając efekt dźwięku na zmienne behawioralne i/lub HR oraz HRV stwierdzono, że nowość bodźca audytoryjnego miała istotne znaczenie jedynie w przypadku dźwięków zakwalifikowanych jako zagrażające, czyli mających potencjał wyzwalania behawioralno-fizjologicznej reakcji stresowej (74,73% istotnych modeli) (tab. 4). Za tego typu działanie odpowiadały niemal wyłącznie dźwięki należące do ptaków i ssaków niedrapieżnych (86,73% istotnych modeli). W przypadku sygnałów dźwiękowych wpływających na zakłócenie behawioru (wzrost czujności + zmiana min. jednej dodatkowej cechy behawioralnej) stwierdzono największy udział odgłosów należących do zwierząt drapieżnych (55,50% istotnych modeli), a w następnej kolejności tzw. neutralnych (24,75%). Rozkład udziału różnych typów pochodzeniowych w kategorii dźwięków wpływających na wzrost czujności był zbliżony, jednak przeważały dźwięki antropogeniczne (35,37%). Wśród dźwięków neutralnych znalazły się głównie antropogeniczne (52,79%) i pochodzące z nieożywionego środowiska naturalnego (38,84%). Generalnie dźwięki powodowały zmianę 14 zmiennych zależnych pogrupowanych w 5 kategorii (tab. 5).

Tab. 5. Kategorie zmiennych zależnych (i zmienne zależne), na które wpływały dźwięki różnych typów (ZN/NZ, A/N/D/Ś) [Janicka i in., 2022b] (P1)

Kategoria zmiennej zależnej	Ogólny wpływ (%)	X Rozkład procentowy (ZN/NZ)	Y Rozkład procentowy (A/N/D/Ś)
Zajmowana strefa (n=3; strefa 1, 2, 3)	6,67	ZN = 60,10 NZ = 39,90	A = 0,00 N = 36,39 D = 42,49 Ś = 21,12
Behawior pokarmowy (n=4; czas i częstotliwość pobierania siana, innych zachowań pokarmowych i ogółem)	15,63	ZN = 45,69 NZ = 54,31	A = 8,71 N = 61,86 a** D = 21,31 Ś = 8,12
Behawior lokomotoryczny (n=2; stęp, lokomocja ogółem)	17,50	ZN = 45,41 NZ = 54,59	A = 8,00 N = 62,77 b** D = 29,23 Ś = 0,00
Czujność (n=1)	75,00	ZN = 46,46 NZ= 53,54	A = 15,84 N = 32,21 c** D = 34,55 Ś = 17,40
Pobudliwość emocjonalna (n=4; HR, RR, RMSSD, LF/HF)	6,67 (uwzględniając wyłącznie HR, RR: 17,50)	ZN = 17,51 a* NZ = 82,49	A = 0,00 N = 75,08 d* D = 0,00 Ś = 24,92
Zmienne zależne ogółem (n=14)	16,61	ZN = 42,78 NZ = 57,22	A = 8,78 N = 51,27 e** D = 25,78 Ś = 14,17

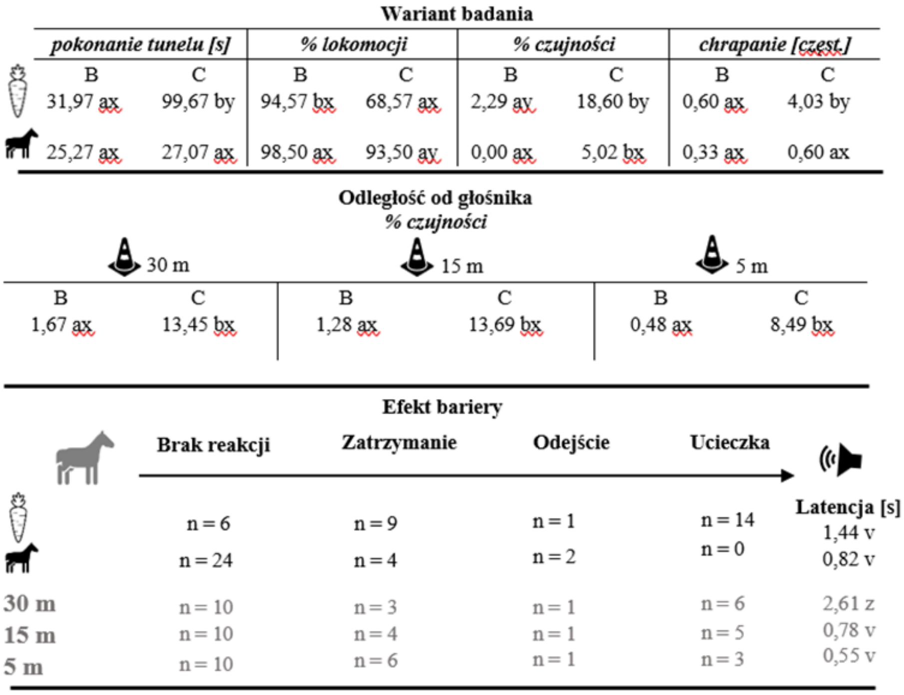
X, Y – rozkład procentowy istotnych modeli (dźwięk*zmienna zależna) uzyskanych dla różnych typów dźwięków w każdej kategorii zmiennej zależnej (uwzględniając procentowy udział każdego typu dźwięku w całkowitej puli dźwięków); dla dźwięków ZN/NZ: a*$\chi2$ = 5,60, p = 0,02; dla dźwięków A/N/D/Ś: a**$\chi2$ = 18,63, p = 0,00; b**$\chi2$ = 11,55, p = 0,01; c**$\chi2$ = 12,50, p = 0,01;

d**χ2 = 22,96, p = 0,00; e**χ2 = 44,62, p = 0,00. Ogólny wpływ (%) dźwięków – odsetek istotnych modeli w danej kategorii zmiennej zależnej względem wszystkich możliwych do uzyskania modeli w danej kategorii.

Podstawową reakcją koni na pojawienie się dźwięku było przerwanie dotychczasowej czynności i przybranie pozycji alarmowej (czujność; 75% możliwych w tej kategorii modeli). Ogółem, za większość istotnych zmian odpowiadały odgłosy należące do ptaków i niedrapieżnych ssaków (51,27% istotnych modeli), a w następnej kolejności do ssaków drapieżnych (25,78%). Efekt nowości dźwięku był widoczny przede wszystkim we wpływie na pobudliwość emocjonalną koni (82,49% istotnych modeli). W pozostałych kategoriach rozkład modeli z udziałem dźwięków znanych i nieznanych był zbliżony. Uwzględniając typ pochodzeniowy, za zmiany HR i HRV odpowiadały przede wszystkim odgłosy nieznanych zwierząt neutralnych (75,08%).

Wyniki pierwszego etapu badań (P1) pozwoliły w efekcie na wstępną ocenę czy dźwięki różnego pochodzenia mogą wpływać na wyzwalanie reakcji antydrapieżniczej, a więc czy mają pewne cechy repelentów i potencjał do wykorzystania w tworzeniu niewidzialnych ogrodzeń. W kolejnym etapie (P2) kontynuowano ocenę tego potencjału za pomocą testu tunelu dźwiękowego i rozpoczęto prace nad analizą czynników wpływających na efektywność bariery dźwiękowej. Wykazano, że jest ona w znacznym stopniu zależna od motywacji zwierzęcia, a więc od atrakcyjności zasobów znajdujących się poza barierą (tab. 6).

Tab. 6. Wyniki odnoszące się do reakcji behawioralnej koni podczas testu tunelu dźwiękowego (P2)



Wariant badania

	pokonanie tunelu [s]		*% lokomocji*		*% czujności*		*chrapanie [częst.]*	
	B	C	B	C	B	C	B	C
🥕	31,97 ax	99,67 by	94,57 bx	68,57 ax	2,29 ay	18,60 by	0,60 ax	4,03 by
🐴	25,27 ax	27,07 ax	98,50 ax	93,50 ay	0,00 ax	5,02 bx	0,33 ax	0,60 ax

Odległość od głośnika
% czujności

30 m		15 m		5 m	
B	C	B	C	B	C
1,67 ax	13,45 bx	1,28 ax	13,69 bx	0,48 ax	8,49 bx

Efekt bariery

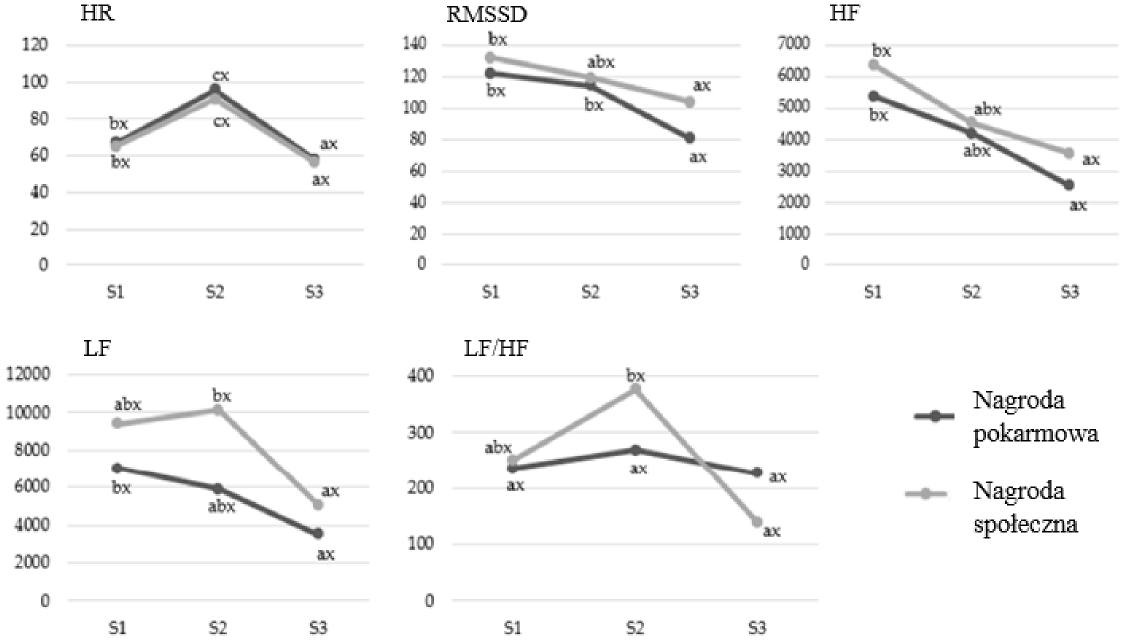
🐴	**Brak reakcji**	**Zatrzymanie**	**Odejście**	**Ucieczka**	**Latencja [s]**
🥕	n = 6	n = 9	n = 1	n = 14	1,44 v
🐴	n = 24	n = 4	n = 2	n = 0	0,82 v
30 m	n = 10	n = 3	n = 1	n = 6	2,61 z
15 m	n = 10	n = 4	n = 1	n = 5	0,78 v
5 m	n = 10	n = 6	n = 1	n = 3	0,55 v

Wyniki oznaczone różnymi literami różnią się istotnie przy p < 0,05 pomiędzy etapem B i C testu (litery a, b), pomiędzy wariantami badania (pokarm, nagroda społeczna) i dystansami ekspozycji na dźwięk (30, 15, 5 m) dla tej samej zmiennej behawioralnej w obrębie tego samego etapu (litery x, y) oraz dla czasu latencji pomiędzy wariantami lub dystansami ekspozycji na dźwięk (litery v, z).

Skuteczność bariery wynosiła 80% (24 konie odpowiedziały zatrzymaniem lub zmianą kierunku ruchu w stronę przeciwną), gdy motywatorem był pokarm i spadała do 20% w przypadku chęci dołączenia do znanego konia. W takim przypadku, inaczej niż w teście z pokarmem, czas pokonania tunelu nie ulegał wydłużeniu i odnotowano zdecydowanie mniejszy wzrost czujności po odtworzeniu dźwięku niż w przypadku wariantu z pokarmem (0,00 → 5,02% vs. 2,29 → 18,60%). Uwzględniając jedynie sytuacje, gdy bariera była skuteczna, czas latencji w obu wariantach był zbliżony. Dystans ekspozycji na dźwięk miał mniejszy wpływ na reakcje behawioralne koni. Skutkował jedynie wzrostem czujności (zbliżony dla trzech dystansów) i różnicował czas latencji, gdzie siła efektu zaskoczenia była najmniejsza na dystansie 30 m od źródła dźwięku (2,61 vs. 0,78 - 15 m i 0,55 s - 5 m). Niemniej efekt bariery był porównywalny.

Analizując fizjologiczną odpowiedź na barierę dźwiękową zauważono, że przesunięcie w kierunku aktywacji współczulnej lub przywspółczulnej różniło się w zależności od parametrów – zmiany HR, RMSSD i HF wskazywały na dominację współczulną, a LF i LH/HF przywspółczulną (ryc. 5, 6). Zarówno w odniesieniu do wpływu motywatora (ryc. 5), jak i efektu dystansu ekspozycji na dźwięk (ryc. 6), zmiany HR pojawiały się bezpośrednio po zastosowaniu dźwięku (S2), ale efekt ten utrzymywał się krótko. Obserwowano wówczas znaczny wzrost wartości w okresie S2, a następnie istotny spadek w okresie S3, czyli w piątej minucie po zastosowaniu dźwięku. W przypadku parametrów HRV zmiany były opóźnione i nie obserwowano natychmiastowego wpływu dźwięku. Istotne zmiany rejestrowano w okresie S3 w porównaniu do okresu S1 lub S2. W teście tunelu dźwiękowego nie można wykluczyć wpływu ruchu na analizowane parametry, co omówiono w publikacji nr 2.

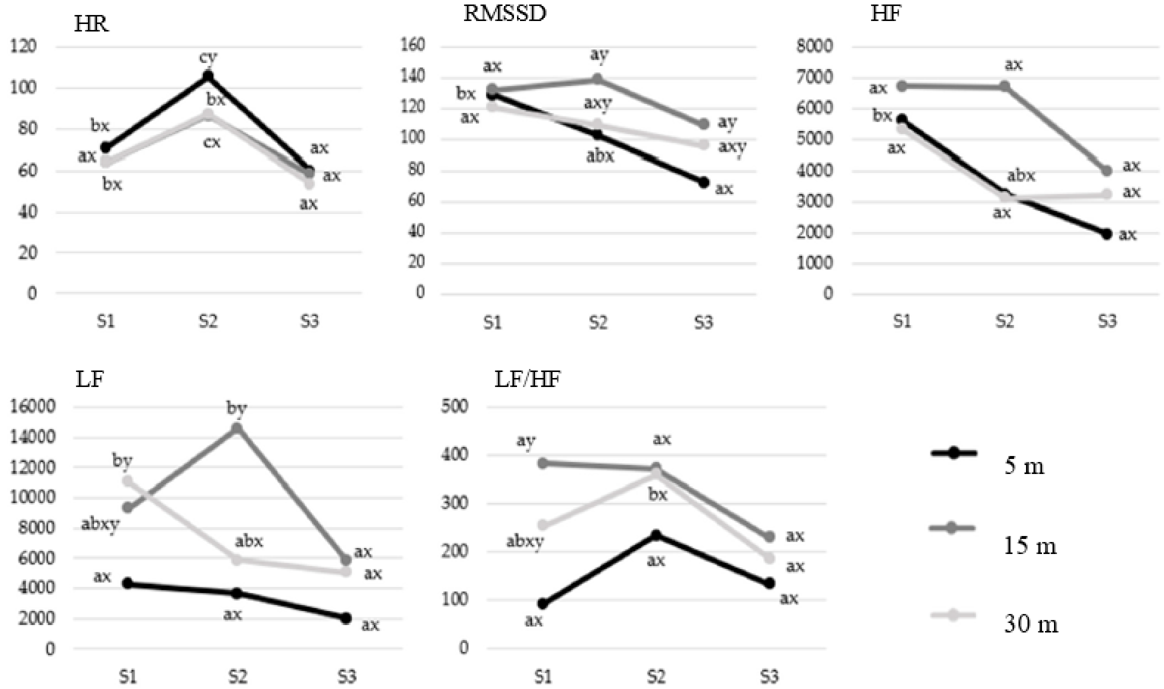
Nie stwierdzono różnic w reakcji fizjologicznej w okresie S1, S2 i S3 między dwoma wariantami testu (ryc. 5). Zaobserwowano jedynie zmiany w reakcji na barierę w obrębie każdego z wariantów. W wariancie z pokarmem odnotowano spadek HF w okresie S3 w stosunku do S1 i spadek RMSSD w okresie S3 w stosunku do S2. W wariancie z nagrodą społeczną zarówno zmiana HF, jak i RMSSD była widoczna w okresie S3 w stosunku do S1. Stopniowa relaksacja organizmu (spadek LF, LF/HF) w piątej minucie po zastosowaniu dźwięku była widoczna głównie w przypadku uzyskania nagrody społecznej (szybka regeneracja w okresie S3 względem S2). Podczas wariantu z pokarmem stwierdzono jedynie spadek LF w okresie S3 w stosunku do S1.



Ryc. 5. Zmiany parametrów częstości i zmienności rytmu serca koni podczas testu tunelu dźwiękowego (P2) w zależności od wariantu badania (pokarm, nagroda społeczna); wyniki oznaczone różnymi literami różnią się istotnie przy $p < 0{,}05$ w ramach tego samego wariantu badania w kolejnych okresach testowych S1 (przed), S2 (od momentu) i S3 (po odtworzeniu dźwięku) (litery a, b) lub pomiędzy różnymi wariantami w tym samym okresie (litery x, y) [Janicka i in., 2022a] (P2).

Odmiennie niż w przypadku wpływu motywatora, dystans ekspozycji na dźwięk w większym stopniu różnicował odpowiedzi HR i HRV. Aktywacja układu współczulnego była najbardziej wyraźna w przypadku najsilniejszego efektu zaskoczenia dźwiękiem (5 m). Największy wzrost HR bezpośrednio po zastosowaniu dźwięku zaobserwowano dla dystansu 5 m. Ponadto opóźniony i stopniowy (zmiana w okresie S3 w stosunku S1) spadek RMSSD i HF stwierdzono jedynie na tym dystansie. Z kolei zmiany parametrów LF i LF/HF w piątej minucie po zastosowaniu dźwięku wskazywały na zmianę aktywacji w kierunku dominacji przywspółczulnej dla dystansów 15 m (LF) i 30 m (LF, LF/HF), czego nie stwierdzono dla dystansu 5m.

Podsumowując można stwierdzić, że rodzaj dostępnych zasobów wpływał w większym stopniu na odpowiedź behawioralną i skuteczność wirtualnej bariery. Z kolei dystans ekspozycji na dźwięk miał mniejsze znaczenie w kontekście efektu bariery, ale w większym stopniu różnicował odpowiedź fizjologiczną (tab. 6, ryc. 5, ryc. 6).



Ryc. 6. Zmiany parametrów częstości i zmienności rytmu serca koni podczas testu tunelu dźwiękowego (P2) w zależności od dystansu ekspozycji na dźwięk (30, 15, 5 m); wyniki oznaczone różnymi literami różnią się istotnie przy p < 0,05 w ramach tego samego dystansu w kolejnych okresach testowych S1 (przed), S2 (od momentu) i S3 (po odtworzeniu dźwięku) (litery a, b) lub pomiędzy różnymi dystansami w tym samym okresie (litery x, y) [Janicka i in., 2022a] (P2).

W kolejnym etapie analizowano zróżnicowanie reakcji względem dźwięku w zależności od indywidualnych cech koni i kontekstu pojawienia się bodźca audytoryjnego. Zaobserwowano, że reakcja koni na krótkotrwałą separację od stada była zróżnicowana, stąd dokonano podziału na trzy stopnie zależności społecznej (rozdział Materiał i metody – *Test zależności społecznej – test areny*). Cecha ta była jednak związana wyłącznie z niepokojem podczas oddzielenia od grupy (dłuższy czas lokomocji, czujności) i nie wiązała się z zaangażowaniem w interakcje międzyosobnicze podczas normalnego, niezakłóconego przebywania na padoku w stadzie (test interakcji społecznych) (tab. 7). W stacjonarnym teście dźwiękowym bez kontekstu socjalnego stwierdzono istotne różnice w reakcji lękowej koni zależnych i niezależnych społecznie. Konie o wyższym stopniu zależności miały dłuższy czas latencji do ponowienia pobierania pokarmu, a ich reakcja na niespodziewany dźwięk była bardziej gwałtowna (więcej punktów w skali behawioralnej). Charakteryzowały się zatem wyższą lękliwością wobec nowego, niespodziewanego dźwięku. Efekt ten jednak nie był już widoczny podczas testu dźwiękowego w trakcie próby dołączenia do stada. Konie ze wszystkich trzech grup reagowały wówczas podobnie. Korelacje pomiędzy wynikami testów behawioralnych wykazały, że zależność społeczna (niepokój podczas separacji) koreluje w pewnym stopniu z ogólną lękliwością na nieznany dźwięk (*A, *B; tab. 7) oraz że osobniki, które charakteryzowały się wyższą lękliwością w teście dźwiękowym bez kontekstu socjalnego, reagowały też silniej (więcej punktów w skali behawioralnej) na barierę dźwiękową (*C, *D; tab. 7). Niemniej jednak skuteczność tej bariery była niska i ograniczała się głównie do zwolnienia chodu.

Tab. 7. Różnice w zachowaniu koni o różnym stopniu zależności społecznej podczas testów socjalnych i dźwiękowych (P3)

Zmienna	**Konie zależne** (N = 6)	**Konie umiarkowanie zależne** (N = 5)	**Konie niezależne** (N = 9)
Test zależności społecznej [s] – ogółem „niepokój” *A, *B			
Energiczny stęp	188,67 ± 22,04 c	34,00 ± 7,31 b	0,00 ± 0,00 a
Kłus	23,33 ± 8,44 b	3,60 ± 2,91 ab	1,22 ± 1,22 a
Galop	2,67 ± 1,26 a	2,40 ± 1,47 a	0,56 ± 0,55 a
Czujność	45,83 ± 10,36 b	46,00 ± 13,85 b	9,44 ± 3,02 a
Test interakcji społecznych [częst.]			
Zachowania afiliacyjne	23,67 ± 3,29 a	24,80 ± 4,69 a	19,11 ± 4,34 a
Zachowanie dominacyjne	4,17 ± 1,92 a	2,00 ± 0,71 a	4,22 ± 1,14 a
Zachowania uległe	3,83 ± 0,54 a	2,00 ± 0,71 a	1,88 ± 0,35 a
Testy dźwiękowe w kontekście socjalnym (KS) i niesocjalnym (NS)			
Efekt bariery (KS1) *C, *D	2,19 ± 0,20 a	1,80 ± 0,19 a	1,98 ± 0,16 a
Ukończenie tunelu (KS2)	1,81 ± 0,07 a	1,83 ± 0,07 a	1,78 ± 0,06 a
Zachowanie na mecie (KS3)	2,64 ± 0,27 a	2,00 ± 0,30 a	2,41 ± 0,27 a
Latencja – pokarm (NS1) *A, *C	196,75 ± 20,69 b	129,30 ± 26,40 ab	61,67 ± 12,43 a
Reakcja na dźwięk (NS2) * B, *D	3,92 ± 0,18 b	3,30 ± 0,33 ab	2,78 ± 0,13 a

Średnie (± błąd standardowy) oznaczone różnymi literami różnią się istotnie w wierszach przy p < 0,05 (DSCF). Najważniejsze istotne korelacje oznaczono tymi samymi symbolami (we wszystkich przypadkach p < 0,001): *A = 0,354; *B = 0,397; *C = 0,325; *D = 0,350. Jednostki: KS1 [skala 1-4], KS2 [skala 1-2], KS3 [skala 1-6], NS1 [s], NS2 [skala 1-5]

Analiza wpływu czynnika badanych grup wiekowych nie wykazała jego istotnego znaczenia w różnicowaniu zachowań koni w żadnym z testów przeprowadzonych w etapie 3 (P3). Z kolei różnice między klaczami a wałachami widoczne były w reaktywności podczas separacji (klacze – silniejszy niepokój) i ogólnej lękliwości wobec dźwięku poza kontekstem socjalnym (klacze – silniejsza reakcja lękowa). Klacze były też bardziej niespokojne po przejściu tunelu i oczekiwaniu na połączenie ze stadem (więcej punktów w skali behawioralnej).

Tab. 8. Różnice w zachowaniu klaczy i wałachów podczas testu zależności społecznej (niepokój) oraz testów dźwiękowych w kontekście socjalnym (KS) i niesocjalnym (NS) (P3)

Zmienna	**Klacze**	**Wałachy**
Niepokój [%]	45,37 ± 6,78 b	24,07 ± 5,71 a
Latencja – pokarm (NS)	167,50 ± 17,48 b	70,70 ± 13,27 a
Reakcja na dźwięk (NS)	3,60 ± 0,18 b	2,90 ± 0,15 a
Efekt bariery (KS)	2,20 ± 0,16 a	1,80 ± 0,14 a
Ukończenie tunelu (KS)	1,78 ± 0,05 a	1,82 ± 0,05 a
Zachowanie na mecie (KS)	2,83 ± 0,23 b	1,83 ± 0,21 a

Średnie (± błąd standardowy) oznaczone różnymi literami różnią się istotnie w wierszach przy p < 0,05 (DSCF).

Dyskusja

Konie charakteryzują się dużą wrażliwością na bodźce środowiskowe, a cecha ta umożliwiła im przetrwanie przez miliony lat [Marliani i in., 2021; Minero i Canali, 2009; Rørvang i in., 2020]. Różnorodne, nieoczekiwane wydarzenia mogą zwiastować potencjalne zagrożenie, dlatego ich szybka detekcja za pomocą zmysłów może decydować o sukcesie danego osobnika [Janczarek i in., 2020a]. Reakcje koni w pierwszym etapie badań własnych (P1) potwierdziły, że konie pozostają czujne na bodźce audytoryjne pojawiające się w znanym im otoczeniu. Z perspektywy ewolucyjnej bodźce zakłócające powinny być analogiczne do ryzyka drapieżnictwa (hipoteza risk-disturbance) [Frid i Dill, 2002]. Chociaż większość dźwięków w badaniu własnym wywierała jedynie słaby lub umiarkowany wpływ na zmianę zachowania zwierząt, to sygnały te przynajmniej w pewnym stopniu wpłynęły na behawior antydrapieżniczy koni. Wzrost czujności, która stanowi istotę obrony przed drapieżnikami [Apfelbach i in., 2005], obserwowano jako odpowiedź na zdecydowaną większość spośród 40 zastosowanych dźwięków. Konie również mniej czasu poświęcały na pobieranie pokarmu (ale częściej ponawiały tę próbę) i dłużej chodziły. Podobne wyniki w zakresie reakcji domowych kopytnych na sygnały olfaktoryczne, wizualne lub audytoryjne pochodzące od drapieżników uzyskali wcześniej inni autorzy [Adcock i Tucker, 2020; Janczarek i in., 2020b; Kluever i in., 2008; Kluever i in., 2009]. Ponadto dźwięki, które były postrzegane jako najbardziej zagrażające, skutkowały wzrostem aktywacji współczulnej, co jest zgodne z obserwacjami Janczarek i in. [2021] podczas ekspozycji koni na odgłosy drapieżników. Zmiany w badaniu własnym dotyczyły jednak niemal wyłącznie wzrostu HR i spadku RR, tak więc zastosowane dźwięki w niewielkim stopniu wpływały na parametry HRV. Generalnie zarówno zmiany behawioralne, jak i fizjologiczne miały charakter krótkotrwały i wywoływały niewielką pobudliwość emocjonalną koni [Hall i in., 2018]. Behawior antydrapieżniczy jest kosztowny w utrzymaniu, dlatego z punktu widzenia wydatku czasowo-energetycznego, jak również bezpieczeństwa ludzi, obniżenie czujności u udomowionych zwierząt jest korzystne [Chuard i in., 2020; Rørvang i in., 2020]. Dokonanie podziału dźwięków w zależności od efektu, jaki wywierały na zachowanie i pracę serca koni pozwoliło na dostrzeżenie, że reakcja ta była w pewien sposób stopniowana w zależności od typu pochodzeniowego dźwięku lub łącznego efektu pochodzenia i nowości. Tylko kilka dźwięków skutkowało behawioralno-fizjologiczną reakcją stresową (zagrożenie I stopnia). Pozostałe dźwięki powodowały różne zmiany behawioralne (zagrożenie II stopnia), wyłącznie wzrost czujności (zagrożenie III stopnia) lub brak jakichkolwiek zmian (dźwięki neutralne). Podobnych wniosków dostarcza badanie Janczarek i in. [2021], w którym reakcja koni na wokalizację grupy drapieżników była bardziej intensywna niż na odgłosy pojedynczych zwierząt. Zgodnie z hipotezą threat-sensitive predator avoidance, zwierzęta powinny dostosowywać sposób i siłę reakcji względem presji drapieżniczej proporcjonalnie do postrzeganego zagrożenia [Brown i in., 2009]. W naturze reagowanie adekwatne do stopnia zagrożenia pozwala gatunkom ofiar zminimalizować wydatek energetyczny i inne koszty unikania drapieżnika [Papworth i in., 2013]. Obserwacje te można również przenieść na konie utrzymywane przez człowieka i narażone na różne stresory antropogeniczne. W pierwszym etapie badań własnych wykazano, że konie nie postrzegały badanych dźwięków jako tak samo zagrażających i reagowały na nie odmiennie. Potencjał sygnałów dźwiękowych do tworzenia wirtualnej bariery jest zatem zależny od zastosowanych dźwięków. Rodzajem dźwięków, które miały najsilniejszy wpływ na konie (reakcja behawioralno-fizjologiczna) były odgłosy zwierząt neutralnych, ale w większości nietypowych i nieznanych dla badanych koni (szympans, słoń, świnia, jeleń, myszołów rdzawosterny, kszyk). Pod względem podziału na efekt dźwięku, odgłosy należące do zwierząt drapieżnych działały przede wszystkim jako bodźce zakłócające i łagodnie alarmujące. Teoretycznie to dźwięki o istotnym znaczeniu biologicznym powinny wywierać silniejszy efekt repelentu [Aflitto i Hofstetter, 2014]. Trudno jednoznacznie stwierdzić, co było przyczyną takiego zjawiska. Osłabioną reakcję na odgłosy drapieżników można wytłumaczyć faktem, że w dzisiejszych czasach konie są w dużej mierze wolne od presji drapieżników, zwłaszcza te trzymane w stajniach na obszarach miejskich [Janczarek i in., 2020b]. Może to również wynikać z odmiennych parametrów technicznych dźwięku, co zasugerowali Janczarek i in. [2020a] porównując reakcje koni na odgłosy drapieżników znanych i nieznanych ich przodkom. Jak wskazują Rochais i in. [2017], nowość, a także nieoczekiwany charakter bodźca wpływają na jego rozpraszający efekt, bowiem trudno jest zignorować zdarzenia, które nie są spodziewane w danym kontekście [Murphy i Dalton, 2014]. W pierwszym etapie badań własnych takimi dźwiękami były głównie odgłosy wydawane przez nietypowe ptaki i niedrapieżne ssaki, a w następnej kolejności ssaki drapieżne. Dźwięki antropogeniczne i pochodzące z przyrody nieożywionej były w większości postrzegane jako łagodnie niepokojące lub neutralne, nawet jeśli część z nich była koniom nieznana. Prawdopodobnie tego typu dźwięki, stanowiące tło dla wielu innych wydarzeń, są generalizowane i nie były niczym zaskakującym dla koni utrzymywanych w systemie stajennym, a więc nie skupiały ich uwagi w znaczący sposób [Pálsdóttir i in., 2014; Stachurska i in., 2017]. Nowak i in. [2016] zaobserwowali, że obecność człowieka była postrzegana przez małpy jako zagrożenie w zależności od tego, czy w danym habitacie mogły się go spodziewać. Można zatem wnioskować, że potencjał dźwięków do tworzenia niezależnej od prądu wirtualnej bariery będzie wynikał przede wszystkim z nieprzewidywalności bodźca audytoryjnego w danym kontekście i w mniejszym stopniu jego nowości. Uwzględniając jednak wpływ dźwięku na reakcje koni można stwierdzić, że o ile zakłócający efekt (zmiana behawioru) można wyjaśnić efektem zaskoczenia, o tyle postrzeganie dźwięku jako zagrażającego (zmiana behawioru i parametrów pracy serca) wynika z nietypowego kontekstu jego wystąpienia, jak również jego nowości. Wyniki testu reakcji na 40 dźwięków (P1) potwierdziły więc pierwszą hipotezę pracy. Dodatkowo wykazano, że wysoko alarmujące dźwięki wpływając na zmianę zachowania w kierunku uogólnionej reakcji antydrapieżniczej mają pewien potencjał do tworzenia wirtualnej bariery pastwiskowej.

Wyniki testu w tunelu dźwiękowym (P2) pozwoliły na dalszą weryfikację głównej hipotezy. Ponownie potwierdzono, że niespodziewany i nieistotny biologicznie dźwięk może być postrzegany jako zagrożenie i może wpływać na zmianę kierunku przemieszczania się zwierząt. Niemniej całkowita skuteczność wirtualnego ogrodzenia wyniosła 50%, co wcześniej stwierdzili też Umstatter i in. [2009] w badaniu przeprowadzonym na krowach. Taki wynik uniemożliwia więc komercyjne stosowanie dźwięków jako niezależnych wirtualnych ogrodzeń [Umstatter i in., 2013]. Nie eliminuje to jednak możliwości wykorzystywania dźwięków jako wsparcia dla konwencjonalnych, nietrwałych ogrodzeń, np. w miejscach krytycznych (w pobliżu drogi, upraw). Wymagałoby to dalszych, szczegółowych badań nad skutecznością tego rodzaju wzmocnienia. Wykazano również, że czynnikiem istotnie modyfikującym efektywność wirtualnej bariery jest motywacja do zdobycia określonych zasobów. Przy jej niższym poziomie, kiedy atraktantem był pokarm, skuteczność wynosiła 80%. Przy wysokiej motywacji do przebywania w pobliżu znanego członka stada, współczynnik sukcesu spadł jednak do 20%. W badaniu Jouven i in. [2012], obecność innych osobników nie powstrzymywała niektórych owiec od przekroczenia ustalonej granicy, pomimo wcześniejszego skojarzenia dźwięku z impulsem elektrycznym. Konie są zwierzętami wysoce zmotywowanymi do podejmowania interakcji społecznych [Yarnell i in., 2015; Søndergaard i in., 2011], co potwierdzono w niniejszym badaniu. Czas pokonania tunelu uległ znacznemu wydłużeniu, zmniejszył się udział lokomocji i wzrosła częstotliwość chrapania jedynie podczas wariantu z pokarmem. W przypadku obu wariantów testu wydłużeniu uległ czas czujności, jednak był on istotnie krótszy w obecności drugiego osobnika. Wyniki te wskazują, że motywacja społeczna może działać jak bufor socjalny, który pomaga koniom stawić czoła stresorom [Ricci–Bonot i in., 2021].

W podobny sposób można również częściowo wyjaśnić zmiany parametrów pracy serca. Generalnie zarówno w odniesieniu do wariantu testu, jak i dystansu ekspozycji na dźwięk, bezpośrednio po odtworzeniu nagrania wzrastało tylko HR. Parametry HRV zmieniały się stopniowo albo w kierunku dominacji współczulnej albo przywspółczulnej, co mogło wynikać z konfliktowego charakteru testu – konfrontacji ze stresorem i następującej po niej nagrodzie. Chociaż nie zaobserwowano różnic w częstości i zmienności rytmu serca koni pomiędzy dwoma wariantami, to aktywacja przywspółczulna w piątej minucie po zastosowaniu dźwięku, a więc stopniowa relaksacja organizmu (parametry LF, LF/HF), była widoczna głównie w przypadku uzyskania nagrody społecznej. Może to wskazywać na znaczenie obecności drugiego osobnika w łagodzeniu objawów stresu [Christensen i in., 2008].

Drugi analizowany czynnik – dystans ekspozycji na dźwięk (siła efektu zaskoczenia) w niewielkim stopniu różnicował odpowiedź behawioralną na zastosowaną barierę, a jej skuteczność na każdym z trzech dystansów utrzymywała się na zbliżonym poziomie. Siła efektu zaskoczenia była najmniejsza w przypadku dystansu 30 m, co zaobserwowano w postaci najdłuższego czasu latencji do reakcji zwierzęcia. Dystans ekspozycji na dźwięk miał z kolei istotne znaczenie dla fizjologicznej odpowiedzi organizmu. Aktywacja współczulna była najsilniej zaznaczona dla dystansu 5 m, a stopniową zmianę w kierunku dominacji przywspółczulnej (relaksację organizmu) odnotowano wyłącznie dla dystansów 15 i 30 m. Możliwość odpowiednio wczesnego wykrycia stresora audytoryjnego miała zatem pozytywny wpływ na pobudliwość emocjonalną koni. W naturze szybkie wykrycie drapieżnika zapewniało przetrwanie [Christensen i Rundgren, 2008], jednak to wykazywanie czujności, a więc monitorowanie otoczenia, odgrywa kluczową rolę w lokalizowaniu zagrożenia [Apfelbach i in., 2005]. Rozważając wykorzystanie dźwięków we wspieraniu tradycyjnych metod grodzenia należy uwzględnić odpowiednią odległość wyzwalającą odtwarzanie dźwięku. Na podstawie wyników testu, biorąc pod uwagę skuteczność dźwięku jako bariery i jego wpływ na dobrostan zwierząt, można stwierdzić, że korzystne jest wyznaczanie większych odległości. Test tunelu dźwiękowego pozwolił na potwierdzenie drugiej hipotezy (P2). Jednak pomimo wykazania pewnego potencjału wykorzystywania dźwięków do tworzenia samodzielnych wirtualnych ogrodzeń, jego wyniki były powodem do odrzucenia głównej hipotezy pracy. Innym czynnikiem, który mógłby znacząco redukować ten potencjał, jest ryzyko habituacji, na które wskazali Butler i in. [2006]. Ze względu na odrzucenie głównej hipotezy, w niniejszej pracy nie kontynuowano badań nad tym zjawiskiem.

Dalsza analiza reakcji koni względem niepokojącego sygnału dźwiękowego wskazała na znaczenie indywidualnych cech zwierząt w różnicowaniu odpowiedzi. W teście zależności społecznej nie wszystkie konie reagowały takim samym niepokojem na oddzielenie od stada, a więc ich wrażliwość na stres separacyjny była zróżnicowana. Potwierdzono tym samym występowanie cechy temperamentu określanej jako stadność czy zależność [Burattini i in., 2020], badanej już przez Wolff i in. [1997], Lansade i in. [2008b] czy Górecką-Bruzdę i in. [2022]. Dodatkowo, podobnie jak Górecka-Bruzda i in. [2022], zauważono, że cecha ta jest silniej zaznaczona u klaczy niż wałachów. Nie zależała ona jednak od wieku konia i ogólnej tendencji do nawiązywania interakcji międzyosobniczych, a raczej od potrzeby pozostawania w grupie. Stąd najlepiej można ją zaobserwować podczas izolacji socjalnej [Wolff i in., 1997; Seaman i in., 2002; Lansade i in. 2008b]. Korelowała ona natomiast z lękliwością koni wobec nieoczekiwanego bodźca audytoryjnego pojawiającego się bez kontekstu socjalnego. Na zależność pomiędzy lękliwością a reaktywnością na izolację wskazują również wcześniejsze badania Briard i in. [2015] przeprowadzone na koniach oraz Villalba i in. [2009] na owcach. Tak jak przewidywano formułując trzecią hipotezę pracy (P3), konie o różnym poziomie zależności społecznej reagowały na nagły dźwięk odmiennie podczas testu w niespołecznym, ale nie w społecznym kontekście, kiedy to przemieszczały się w kierunku stada przebywającego na pastwisku. Efekt bariery dźwiękowej był nieznaczny w przypadku koni z każdej grupy. Motywacja do dołączenia do grupy była zbyt silna, aby niespodziewany, nowy dźwięk mógł powstrzymać zarówno niezależne, jak i zależne konie przed dalszą wędrówką. Porównując wyniki obu testów dźwiękowych można wywnioskować, że silna motywacja społeczna niejako maskuje strach wywołany przerażającym bodźcem. Ponownie wykazano więc efekt buforu społecznego wcześniej opisany przez Christensen i in. [2008] oraz Ricci-Bonot i in. [2021]. Wyniki badań własnych wskazują na wysoce społeczną naturę konia jako czynnik uniemożliwiający wykorzystanie sygnałów dźwiękowych do tworzenia samodzielnych ogrodzeń pastwiskowych. Cennym aspektem badania było też zwrócenie uwagi na fakt, że próbując przewidzieć zachowanie zwierzęcia, należy brać pod uwagę kontekst pojawienia się danego bodźca. Nawet jeśli odpowiedzi względem różnych stresorów mogą korelować przy rozpatrywaniu danego zmysłu [Lansade i in., 2008c], odmienne okoliczności i motywacje osobnika sprzyjają różnicowaniu jego reakcji.

Podsumowanie i wnioski

1. Konie pozostają czujne na bodźce audytoryjne w swoim otoczeniu i różnicują reakcje na różne dźwięki w zależności od stopnia postrzeganego zagrożenia.
2. Zakłócający efekt dźwięku objawiający się zmianą zachowania wynika z nietypowego kontekstu jego wystąpienia. Z kolei postrzeganie dźwięku jako zagrażającego jest wypadkową nieprzewidywalności i efektu nowości i skutkuje behawioralno-fizjologicznymi zmianami organizmu konia.
3. Nagłe i nieoczekiwane dźwięki wpływają na wyzwalanie odpowiedzi antydrapieżniczej u koni, co wskazuje na pewien potencjał bodźców audytoryjnych do tworzenia wirtualnych ogrodzeń.
4. Motywacja do zdobycia określonych zasobów jest kluczowym czynnikiem modyfikującym skuteczność bariery dźwiękowej. Przy niższym poziomie motywacji, kiedy atraktantem jest pokarm, jej efektywność może być stosunkowo wysoka. Chęć dołączenia do grupy społecznej istotnie ją jednak obniża.
5. Dystans, po przekroczeniu którego wyzwalany jest dźwięk, decyduje o sile efektu zaskoczenia. Jego znaczenie jest mniejsze w kontekście skuteczności bariery, ale istotnie wpływa na pobudliwość emocjonalną koni. Korzystne jest więc wyznaczanie odpowiednio dużych dystansów od ustalonej granicy wirtualnej bariery.
6. Reakcje koni względem nieoczekiwanego dźwięku mogą się różnić w zależności od kontekstu jego wystąpienia i społecznych cech zwierząt. Wysoki i niski poziom zależności społecznej nie różnicuje odpowiedzi względem stresora dźwiękowego w kontekście socjalnym, ale jego pojawienie się poza kontekstem socjalnym skutkuje najsilniejszą reakcją lękową koni zależnych. Motywacja społeczna może zatem maskować strach wywołany przerażającym bodźcem.

Podsumowując, efektywność wysoko alarmujących dźwięków bez uprzedniej asocjacji z prądem jest zbyt niska, aby bodźce audytoryjne mogły być używane jako samodzielne wirtualne bariery. Wpływają na to czynniki, które trudno jest kontrolować, takie jak motywacja do przekroczenia ustalonej granicy, a szczególnie silna potrzeba dołączenia do członków stada. Rozważając jednak wykorzystanie sygnałów dźwiękowych dla dodatkowego wzmocnienia nietrwałych fizycznych rozwiązań, należy uwzględnić efekt nieprzewidywalności i zaskoczenia konia przez barierę dźwiękową przy jednoczesnym zachowaniu odpowiednio dużego dystansu wyzwalającego dźwięk.

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Praca oryginalna *Original paper*

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

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

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

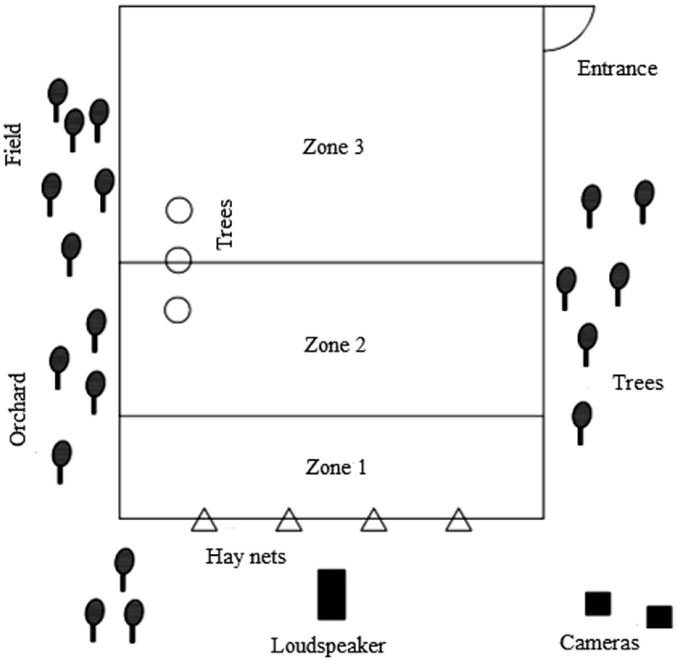

Fig. 1. The layout of the experimental paddock

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*χ^2 = 10.47, p = 0.001. For AS/NAS/PS/IES: a**χ^2 = 82.86, p = 0.00; b**χ^2 = 17.67, p = 0.001. For the neutral sound category (no impact) percentage of certain types of sounds was calculated: c**χ^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%20 Report%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.

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Can Sound Alone Act as a Virtual Barrier for Horses? A Preliminary Study

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Simple Summary: The concept of virtual fencing involves containing animals in a restricted area without a physical barrier by using audio and electric stimuli. Electrical pulses may initiate public concerns, and therefore, alternative aversive stimuli should be tested. As prey animals, horses are particularly perceptive to environmental stimuli. We assumed that a self-applied acute alarming sound may act as an invisible barrier for horses. Thirty horses moved individually through a designated corridor towards food or social reward when the sound was played at one of three distances (30, 15 or 5 m) from a defined line. In the case of food reward, the virtual barrier had an 80% success rate, making the horses respond with flight, going away or stopping. However, the possibility of social interactions reduced the efficacy to 20%. The sound exposure distance did not influence the barrier effectiveness but varied cardiac response with the highest stress level for a distance of 5 m. In general, sound alone has a limited potential as a deterrent and therefore cannot be applied independently. The motivation to move and the sound exposure distance are important factors for sound barrier effectiveness and its impact on the welfare of the horses.

Abstract: Virtual fencing is an innovative alternative to conventional fences. Different systems have been studied, including electric-impulse-free systems. We tested the potential of self-applied acoustic stimulus in deterring the horses from further movement. Thirty warmblood horses were individually introduced to a designated corridor leading toward a food reward (variant F) or a familiar horse (variant S). As the subject reached a distance of 30, 15 or 5 m from a finish line, an acute alarming sound was played. Generally, a sudden and unknown sound was perceived by horses as a threat causing an increase in vigilance and sympathetic activation. Horses' behaviour and barrier effectiveness (80% for F vs. 20% for S) depended on motivator (F/S), while the cardiac response indicating some level of stress was similar. The motivation for social interactions was too strong to stop the horses from crossing a designated boundary. Conversely, the sound exposure distance did not vary the barrier effectiveness, but it differentiated HRV responses, with the strongest sympathetic activation noted at a distance of 5 m. Thus, the moment of a sound playback has important welfare implications. Due to the limited potential of sound as a virtual barrier, auditory cues cannot be used as an alternative for conventional fencing.

Keywords: horse; sound; virtual fencing; grazing management; motivation; perception

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1. Introduction

Virtual fences have been developed as an alternative to conventional fencing in order to comprise the trade-off between optimal grazing, costs, time and labour investments [1]. Since the system provides an enclosure without a physical barrier, it is characterized by fencing flexibility [2]. It has the potential to protect environmentally sensitive areas and brings benefits to wildlife conservation [3]. Over the last four decades, multiple types of fenceless systems have been studied, and several of them have been patented [4]. The targeted deterrent to prevent the animals from crossing a defined line can vary (e.g., audio, vibration, light) [5], but virtual fencing usually involves electric shock [6]. The most common system uses collar-mounted GPS and works by pairing an audio tone with an electric stimulus. As the animal approaches the previously established boundary, the collar emits an audio cue. Further forward movement results in receiving an electrical pulse, however, stopping or turning around prevents an aversive stimulus [7]. As shown by Marini et al. [8], the sheep's responses were directed precisely at the auditory cue since they quickly reused the previously restricted area once it was included again. Campbell et al. [4,9] made similar observations for cattle. This is an important aspect in terms of an efficient grazing management tool [8]. Nevertheless, it cannot be excluded that animals memorise the locations where they received an aversive stimulus [2].

Although virtual fencing is not 100% effective in manipulating animal behaviour [10], many studies indicate its high potential in keeping animals in a designated area [1,2,4,7,8]. In the initial period after the introduction of an invisible barrier, the animals cannot avoid receiving the electrical pulse, and acute stress symptoms may occur. In order to meet welfare requirements, it is necessary that the animals quickly learn how to respond to the virtual fence [3]. However, individual differences were found in the rate of adaptation to the system as manifested by the different number of shocks the animal receives [11]. Although many studies show the level of stress during the learning period is comparable to that associated with many everyday procedures, the application of virtual fencing may still raise public concern for potential welfare issues [3]. Additionally, using electric shock collars for dogs is banned in some countries, and this law may also be extended to other species [12]. For these reasons, alternative repellent methods are also studied. In this case, sounds of different origins may serve as aversive stimuli [6,13–15].

Sound may act as an acute alarming or irritating stimulus, resulting in various behavioural reactions—rapid or delayed, respectively [6]. The volume of the sound may be stable [14] or changeable depending on the distance from the defined line. The closer the animal is to the boundary, the louder the sound is [15]. The audio cue may be emitted from a device attached to the animal [14,15] or from loudspeakers placed in a designated line [6]. The use of sounds as independent aversive stimuli has certain limitations related to the risk of habituation [13] and lower efficiency compared to traditional virtual barriers [6,14]. Nevertheless, Umstatter et al. [6] showed that auditory cues may reduce the time spent in excluded zones.

Virtual fences are mainly designed and tested for cattle [3] and, to a lesser extent, small ruminants [1,8]. Even if some of the patents targeted horses [16], there is a lack of scientific studies in this field. Ungulates can threaten biodiversity through sprig gnawing, rapid seedlings eating and bark stripping. Moreover, comfort behaviour such as rubbing against trees may result in the destruction of vegetation [17]. But it is not clear if sound alone may act as a repellent for grazing horses. Horses evolved as prey animals and have therefore developed a range of adaptations to facilitate predator avoidance [18]. Even though under domestic conditions the risk of predation is relaxed [19], horses experience many stressful situations on a daily basis. Different procedures, novel sounds or objects may all trigger the motivation to flee [20]. High sensory sensitivity is vital for controlling the surroundings and reacting quickly when necessary [21]. In the wild, horses use visual, auditory and olfactory cues to detect a predator [22]. With regard to the localisation of potential danger, the ability to hear high frequencies is particularly important [23]. Although predators are generally silent while hunting, many species respond to their vocalisation, as it directly indicates the presence of a predator [22,24]. Studies on predator recognition in domestic ungulates show they still exhibit antipredator behaviour when exposed to predators' odours, sounds or visual signs [25–27] and thus, these cues may have some potential to modify the grazing activity of prey [28]. Aflitto and DeGomez [29] suggest that acoustic stimuli may act as repellents if they are biologically relevant. However, according to the risk-disturbance hypothesis [30], animal reactions towards different frightening stimuli may derive from a generalised antipredator defence mechanism. In the previous study [31], we observed that horses responded mostly to the sounds of unusual animals, like pigs or chimpanzees, and not to the vocalisation of predators. Rochais and Hausberger [32] also noted that novelty and unexpectedness provide the distraction potential of the sounds. In prey species, responses to suddenness are probably even more pronounced than those triggered by novelty, given the similarity to moving predators [33]. This may be indicative of the repellent potential of acute alarming sounds in grazing management.

Due to the lack of direct knowledge in the field of developing virtual fences for horses, we conducted a preliminary study to determine the potential of a self-applied acoustic stimulus to deter a horse from further movement. Referring to the antipredatory responses of the horses [20], an acute alarming sound effect was chosen [12]. Because horses remain vigilant to environmental stimuli and employ fast threat detection [33], we hypothesised that sound alone may act as an invisible barrier. However, we predicted that the behavioural and cardiac responses of the horses would depend on the resources available beyond the designated boundary (food vs. social reward) and on the moment of the sound.

2. Materials and Methods

2.1. Animals and Housing

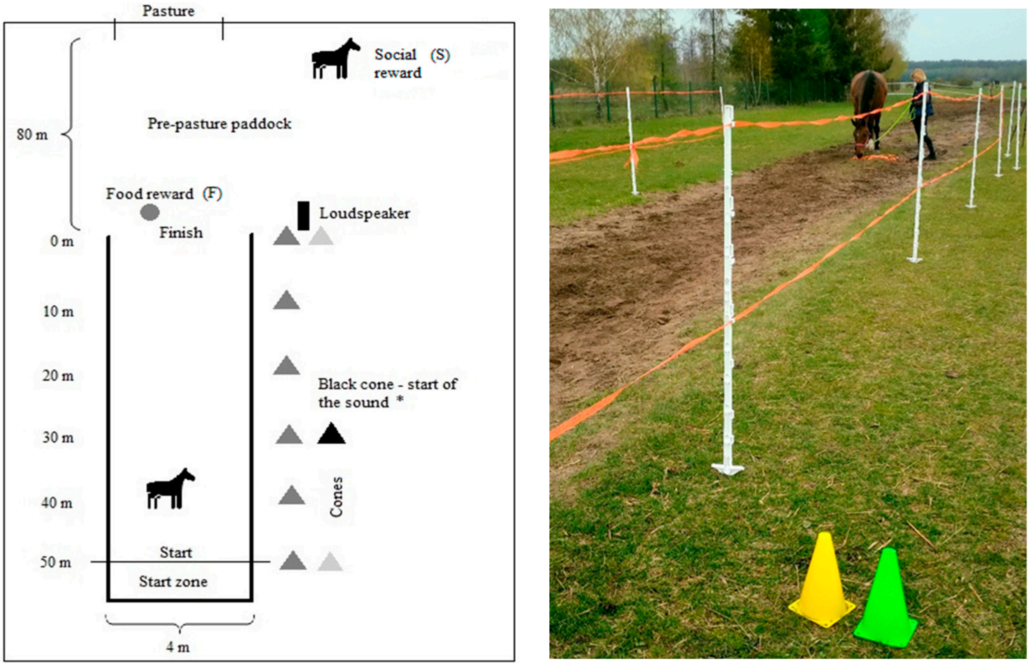
The study involved 30 adult warmblood horses aged 6–16 years (15 mares and 15 geldings). All of the horses were kept in individual box stalls in the same horse riding centre for at least two years. Each box was bedded with wheat straw and equipped with a crib, hay feeder, salt lick and an automatic waterer. The subjects were fed a mixture of oats and bran three times a day and had unlimited access to hay. They were used as leisure horses and worked under the saddle for a maximum of 2 h a day in the afternoon or evening hours. The horses were released to an expansive pasture or several paddocks for at least six hours a day. They were all familiar with the pasture and the pre–pasture paddock (experimental paddock) leading towards it. The subjects were under permanent veterinary control and were constantly observed by an experienced caretaker during daily handling. No physical or behavioural disorders were found before or after the study.

2.2. Procedures of the Sound Tunnel Test

2.2.1. General Conditions and Test Preparation

The virtual barrier potential of the auditory stimulus was assessed during the sound tunnel test (STT) that was conducted in spring 2022. It consisted of playing back a recording of a futuristic sound while a horse moved towards a known pasture (providing locomotory, grazing and social opportunities) through a designated corridor (sound tunnel; ST). The ST was set up five days before the start of the study to let the horses get familiar with the novelty in their environment for two hours a day. Prior to testing, the horses were familiarised with the movement direction through the ST, with five runs being completed. The pasture was separated from the experimental paddock (pre-pasture paddock), where the ST was placed, by a permanent fence of metal railings. However, the tested horse was able to remain in visual and auditory contact with the herd staying at an average distance of approximately 200–400 m from the tunnel. While one of the horses was subjected to STT, none of the other horses involved in the study was in the pasture at the time to avoid the risk of habituation to the experimental sound. They all stayed in the stable building located about 300 m from the experimental paddock while waiting for the test. After completing the STT, the horse was brought to the paddock situated next to the stable. After the last horse had completed the test, all individuals were led to the pasture. The experiment was not carried out on windy (<0.3 m/s) or rainy days to reduce the risk of interfering with the perception of the sound stimulus.

The horses were brought to the experimental paddock in the morning hours by a known caretaker. After 5 min of the rest, they were individually introduced to the ST—a 55 m long and 4 m wide corridor designated with 1.55 m plastic poles and tape for an electric fence, commonly used to demarcate a grazing area (Figure 1a,b). It was open-closed from one side (start) and opened on the end side (finish), letting a horse roam freely after it finished a test. The first 5 m was the 'start zone', where the subject waited for the test to begin while being held by a known caretaker. Yellow cones were placed every 10 m outside the tunnel to ease the observer's control of a horse's movement through the corridor from a distance of 10 m. Additionally, three green cones were used to mark a start and finish line and the place where the sound was played as the horse reached it (sound exposure distance).



(**a**) (**b**)

Figure 1. (**a**). The outline of a sound tunnel and an experimental paddock. (F), (S)—the first and second variants of the study (two separate tests); cones—subsequent 10 m distances from the loudspeaker (two cones at the start and finish); black cone—positioned 30, 15 or 5 m from the finish line (*), indicating the moment of sound playback; start zone—waiting for the test to begin. (**b**). The end part of the sound tunnel and a horse obtaining a food reward after familiarity with the tunnel.

2.2.2. Carrying Out the Test

The horses were randomly divided into three experimental groups of 10 subjects depending on the sound exposure distance and comprising one of the following distances from the loudspeaker placed at the finish line:

- First group—30 m
- Second group—15 m
- Third group—5 m

Each group included five mares and five geldings (to minimise the risk of the gender impact on the results) and was exposed to the sound at the same distance throughout the study. The sound–indicating cone (the green cone) (Figure 1b) was relocated over one of three distances to facilitate the experimenter playing the sound on time. Each horse was subjected to two tests on two separate days:

- Variant F—the motivator (additionally to a near pasture) for movement was a food reward
- Variant S—the motivator (additionally to a near pasture) for movement was a familiar and friendly horse that was not included in the study (social reward)

Prior to the study, we interviewed the horse keeper. The caretaker indicated a horse that was the most friendly towards the other individuals and the least inclined to agonistic behaviour. The interval between the two variants was one week. The food reward was placed 3 m behind the end of the ST, whereas a known conspecific stood around 70 m further to minimise the sound impact on it. Additionally, it had been habituated to the experimental sound before the study. The familiar horse was not attached or confined to allow the horses to have social contact after each test. However, to prevent locomotory behaviour during tests, the familiar horse was provided with attractive food while the first horse was being tested.

Fifteen randomly selected horses were subjected to the STT in the following order: variant F and then variant S. The other individuals were tested in reverse order to reduce the risk of the order of variants affecting the horses' reactions (Table 1). Five horses were tested on a given day.

Table 1. The order of the test variants.

Day	Variant	Group of the Horses	Day	Variant	Group of the Horses
Week 1			Week 2		
1	Variant F	a	7	Variant S	a
2	Variant S	b	8	Variant F	b
3	Variant F	c	9	Variant S	c
4	Variant S	d	10	Variant F	d
5	Variant F	e	11	Variant S	e
6	Variant S	f	12	Variant F	f

Each variant (F, S) of the STT consisted of three stages:

- A—recalling the direction of movement and showing the reward; no measurements
- B—a control trial; behavioural measurements
- C—an experimental trial; behavioural and cardiac activity measurements

During stage A, a horse was led by a known caretaker from the start to the finish line of the ST, where it received a reward (variant F: food, variant S: social contact). If the individual went actively and willingly with a caretaker in the second trial, stage A was considered completed. The horses needed 2–4 repetitions of the first stage. In stage B, the caretaker released the subject from the rope in the start zone. The horse was al-lowed to move through the tunnel on its own. It was then brought back to the start zone again. In stage C, which immediately followed stage B, a sound was introduced while the horse moved through the tunnel on its own. Horses were freely released for the next 5 min after the sound was played.

2.2.3. Sound Stimulus

The futuristic sound used in the study was played with a wireless speaker (JBL Charge 4, rated power of 30 W) connected to a Samsung Galaxy A02s device (Samsung Electronics Co., Ltd., Suwon-si, Republic of Korea) via Bluetooth. It let the experimenter play the sound from a distance of 10 m from the ST, where he stayed during the test. The 20-s recording was prepared in Audacity 2.4.2. software by combining short fragments of several seconds into one and amplifying the entire recording by 12 dB. The sound was violent and novel for subjects and was played at a sound intensity level (LA) of approximately 80 dB measured at a distance of 1 m from the loudspeaker to provide its acute alarming features [12,13]. This intensity has been proven to have only a short-term effect on the cardiac response [31]. If the horse reached the finish line before the end of the recording time, the recording was stopped.

2.3. Behavioural Data Collection and Analyses

The time of STT completion and the time or frequency of certain behaviours were recorded separately for stages B and C for both variants F and S of the study (Table 2). Two dependent variables, i.e., 'barrier effect' (the first reaction to the sound scored on a 4-point scale indicating a sound barrier effect) and 'latency time' (latency time to respond to the sound), were determined only for stage C. Furthermore, 'latency time' was measured only for the horses that stopped, went away or ran away in response to the sound stimulus. Because the time needed to complete an STT varied between the horses, behaviours measured per unit time [s] (locomotory behaviour, vigilance) were calculated as a ratio of a certain behaviour to the tunnel completion time [%] and were used in this form in the statistical analysis. If the horse did not leave the tunnel within 5 min, the test was considered finished, and the percentages of individual behaviours were calculated in relation to 300 s.

Table 2. Behaviours assessed during stages B and C of each test variant (F, S).

Behaviour	Description
Tunnel completion [s]	Time taken by the horse to cross the tunnel from the start line to the finish line
Walk/trot/canter [%]	Time spent walking/trotting/cantering
Locomotion [%]	Total time of locomotion (walk, trot and canter)
Vigilance [%]	Time spent standing alert; standing still with elevated neck, intently orientated head and pinnae [34]
Snoring [freq.]	Total number of a very short raspy inhalation sound [35]
* Defecation [freq.]	Total number of repetitions of the elimination of faeces
** Barrier effect [scale 1–4]	The first reaction to the sound; 1—continuing forward movement, 2—stopping, 3—going away, 4—running away
** Latency time [s]	Latency time to respond to the sound; assessed only for horses that stopped, went away or ran away in response to the sound stimulus

%—percentage of time spent on a certain behaviour regarding the total time of tunnel completion; freq.—the number of repetitions, * behaviour eliminated from the analysis due to the variability of results close to or equal to zero, ** variables assessed only in stage C.

2.4. Cardiac Activity Data Collection and Analysis

The emotional arousal of the horses in response to the sound barrier was measured based on the parameters of heart rate variability (HRV) using Polar Vantage M telemetric devices. An elastic strap with electrodes (Polar Pro) and a transmitter (H10) attached to it was fastened around the horse's chest so that the electrodes were adjacent to the left side of the horse at the heart level. To optimize electrode-skin contact, the inner side of the electrode part of the strap was covered with ECG gel. Horses were familiarised with the equipment for three subsequent days before the start of the study. On each experimental day, devices were put on the horses in the stable at least 10 min before the test. The devices were synchronized with electronic timers to enable controlling the moment of sound playback and then to monitor heart activity changes during the successive periods of the test. Recordings were carried out continuously and stopped 5 min after the sound was played. Due to the character of the study, cardiac responses were collected during the moderate movement. To facilitate the assessment of the sound impact on the emotional arousal of the horses, heart rate recordings were analysed in three 1—minute subsequent periods [36]: directly before the sound (starting parameters—pre-sound period; S1), from the moment of the sound playback (sound period; S2) and during the fifth minute after the sound was played (post-sound period; S3). The 1-min HRV measurements were chosen due to the short-lasting character of the test and the sound stimulus used (20 s) and were proved to provide a reliable assessment of the parameters of HRV [36]. A 3-point measurement allowed to monitor the immediate (S2) and delayed (S3) effects of the sound on the cardiac responses of the horses. Heart function was measured with HR (bpm) and heart rate variability (HRV) parameters that reflect the modulating effect of the autonomic nervous system on cardiac activity [37,38]:

- RMSSD (ms)—standard deviation of differences between successive IBIs (time domain),
- HF (ms^2)—high-frequency component (0.07–0.5 Hz) of HRV determined by spectral analysis (frequency domain),
- LF (ms^2)—low-frequency component (0.005–0.07 Hz) of HRV determined by spectral analysis (frequency domain),

- LF/HF (%)—low frequencies/high-frequencies ratio (frequency domain).

An increase in HR reflects sympathetic activity, in LF and LF/HF reflect a mix of sympathetic and vagal influences towards a sympathetic shift, whereas RMSSD and HF increases directly reflect parasympathetic (vagal) activity. Laborde et al. [36] strongly recommend drawing conclusions based on HRV indices that reflect clearly identified physiological systems with a theoretical underpinning, such as parameters of the vagal tone being measured through RMSSD (in the time domain) and HF (in the frequency-domain). Thus, RMSSD and HF (to check whether the results are consistent) were taken as the main variables. LF and LF/HF were used to additionally discern differences between the impact of the studied factors on the reactions of the horses. Heart rate recordings were analysed with Kubios HRV Standard 3.5.0 software. Very low threshold filters of beat correction were used to eliminate artefacts when necessary.

2.5. Statistical Analyses

The statistical analysis was performed with SAS 9.4 software [version 9.4 by SAS Institute Inc., Cary, NC, USA]. The behavioural data, but not HRV data, showed deviations from normality assumptions (checked by Kolmogorov-Smirnov and Shapiro-Wilk tests). For this reason, non-parametric tests were used for behavioural variables, whereas parametric tests were conducted for HRV measurements. Non-parametric analyses were performed using a Wilcoxon assessment, a Kruskal-Wallis test and Dwass, Steel and Critchlow-Fligner multiple comparisons. Statistical analyses for HRV were conducted using the procedures of the general linear model and the least mean squares. In the case of behavioural analysis, the qualitative variables included the combinations of traits: test variant (F or S reward)_stage of the study (B or C) and distance from the speaker (30, 15 or 5 m)_stage of the study (B or C). For better clarity, the variables were presented and discussed as mean values ± standard deviation (SD). Only comparisons within the same variant (FB–FC, SB–SC) or distance (5B–5C, 15B–15C, 30B–30C) or between different variants (FB–SB, FC–SC) or distance (5B–15B–30B, 5C–15C–30C) within the same stage were pictured and discussed as other comparisons (e.g., FB–SC, 5B–15C) were not the subject of the interest. In the case of cardiac measurements, the independent variables were: the variant of the study (F or S), the distance from the loudspeaker (30, 15 or 5 m) and the stage of the sound tunnel test (B or C). The interactions of 'the variant' or 'the distance' and the stage of the study were analysed. In addition to the main factors, the differences between mares and geldings were checked during the STT. For all analyses, the level of significance was set at $p < 0.05$. Only significant results are presented in the results section.

3. Results

Prior to the experiment, the heart rate variability resting parameters that were to be assessed during the study were measured. Mares were characterized by higher values of the HR (39.0 bmp) and LF (2170.5 ms^2) compared to geldings (37.7 bmp and 1797.1 ms^2, respectively). Except for HR (higher values for mares), there were no differences in behavioural and cardiac variables between mares and geldings during the STT ($p > 0.05$). For this reason, the factor 'gender' was excluded from further analysis.

3.1. Behavioural Responses

The variant of the study indicating the motivation to move through the ST (food or social reward) had a greater impact on behavioural responses of the horses to the unknown sound than the distance at which the horse was exposed to it (30, 15 or 5 m from the loudspeaker) (Table 3). Regarding the studied variant (F or S), significant differences were observed in four compared to one behavioural variables for the effect of the sound exposure distance.

Table 3. Differences in horses' behaviour (means ± SD) in two stages of the sound tunnel test (B—control trial, without the sound and C—experimental trial, with the sound), depending on the test variant and sound exposure distance.

Behavioural Variable	**Study Factors**	**Stage of the Study** **B**	 **C**
Test variant (food—F/social—S reward)			
Tunnel completion time [s]	F	31.97 ± 20.27 ax	99.67 ± 97.95 by
	S	25.27 ± 15.90 ax	27.07 ± 25.45 ax
Total locomotion [%]	F	94.57 ± 6.28 bx	68.57 ± 25.07 ax
	S	98.50 ± 5.01 ax	93.50 ± 13.21 ay
Vigilance [%]	F	2.29 ± 3.98 ay	18.60 ± 14.29 by
	S	0.00 ± 0.00 ax	5.02 ± 12.32 bx
Snoring [freq.]	F	0.60 ± 1.99 ax	4.03 ± 4.42 by
	S	0.33 ± 1.30 ax	0.60 ± 2.30 ax
Sound exposure distance (5/15/30 m from the speaker)			
Vigilance [%]	5	0.48 ± 2.13 ax	8.29 ± 10.38 bx
	15	1.28 ± 3.58 ax	13.69 ± 18.75 bx
	30	1.67 ± 3.20 ax	13.45 ± 18.75 bx

Means marked with different letters differ significantly at $p < 0.05$ within the rows (letters a, b) or within the columns for the same behavioural variable (letters x, y).

3.1.1. The Variant of the Study

Except for vigilance (higher during variant F; $p < 0.05$), horses reacted similarly ($p > 0.05$) during the control trial (B) both when the reward was food and social contact (Table 3). Depending on the studied variant, significant changes in behaviour between stages B and C were noted ($p < 0.05$). Horses needed more time to complete an STT (C: 99.67 ± 97.95 s, B: 31.97 ± 20.27 s), spent less time on total locomotion (C: 68.57 ± 25.07%, B: 94.57 ± 6.28%), were more vigilant (C: 18.60 ± 14.29%, B: 2.29 ± 3.98%) and snored more frequently (C: 4.03 ± 4.42, B: 0.60 ± 1.99) when the motivation to move forward was food. When individuals moved towards a familiar horse, only the percentage of time spent alert (C: 5.02 ± 12.32%, B: 0.00 ± 0.00%) changed significantly. The time required to get through the tunnel and the time of remaining vigilant were relevantly longer during variant F compared to variant S of the study ($p < 0.05$). Additionally, horses spent less time on locomotion and snored more when provided with food than social reward ($p < 0.05$).

3.1.2. Sound Exposure Distance

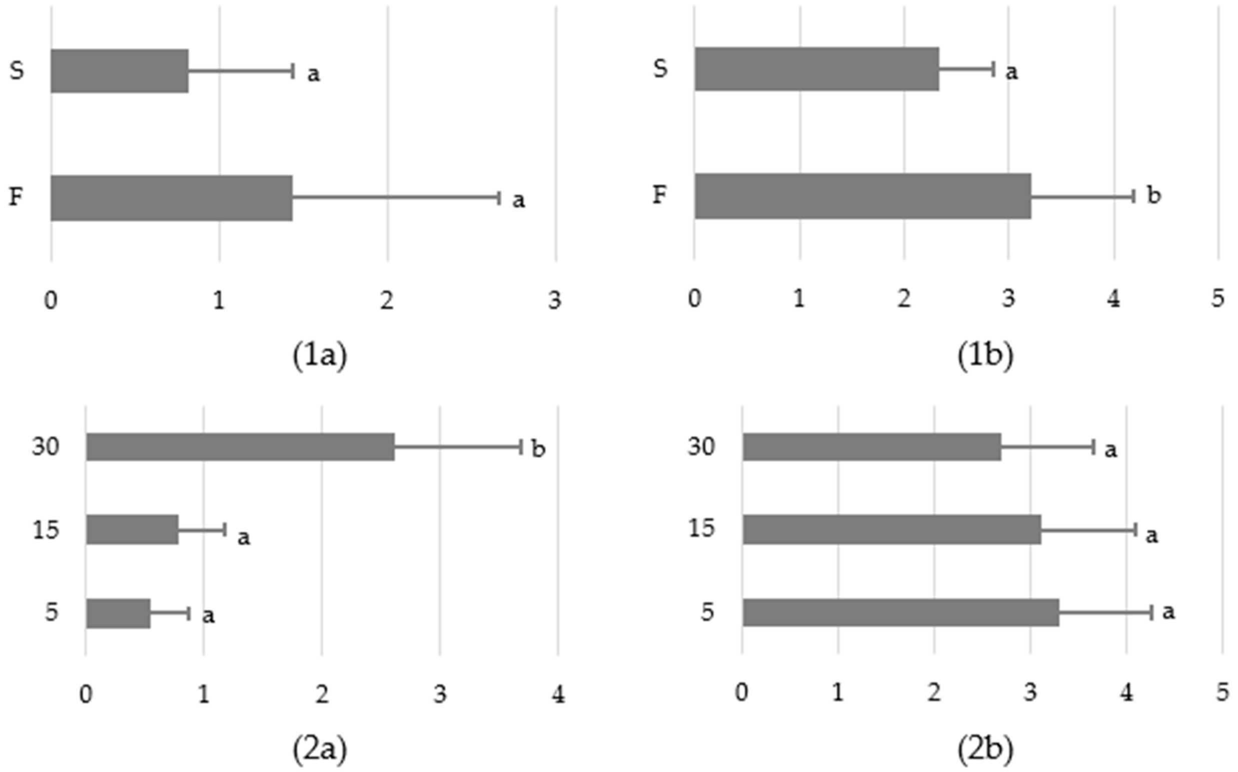
Regarding the distance to the finish line at which a horse was exposed to the experimental sound (Table 3), only vigilance increased significantly in stage C within each distance (5 m: 17 times, 15 m: 10 times; 30 m: 8 times; $p < 0.05$). However, there were no differences in the duration of standing alert between three distances during stages C and B of the study ($p > 0.05$).

3.1.3. The Sound Barrier Effect

Overall, in the two variants of the study, the sound barrier was 50% effective (30 out of 60 desired reactions). The virtual barrier effect (stopping, going away or running away after the sound appearance) was noted in the case of 24 horses in variant F (success: 80%) of the STT, whereas only six animals responded with stopping or going away towards the start zone during variant S (success: 20%) (Table 4). Regarding success rate, horses gained more points in behavioural scale (the sound barrier effect was more pronounced) when they had no possibility of direct social contact (F: 3,21 ± 0,98 vs. S: 2,33 ± 0,52; $p < 0.05$) after the STT completion (Figure 2). The time of this response did not differ between the test variants ($p > 0.05$). An inverse relationship was observed for the effect of the sound exposure distance. The latency time was relevantly longer ($p < 0.05$) at a distance of 30 m from the loudspeaker in comparison to 15 and 5 m (about 3 and 5 times, respectively), but no differences in the barrier effect of the sound were noted ($p > 0.05$). However, there was a trend that horses tested at 30 m reacted more calmly (Table 4).

Table 4. Number of the horses that ignored, stopped, went or ran away after the sound application regarding the variant of the study and the sound exposure distance.

Study Factors	Ignoring	Stopping	Going Away	Running Away
		Variant of the study		
Food reward	6	9	1	14
Social reward	24	4	2	0
		Sound exposure distance		
5 m	10	3	1	6
15 m	10	4	1	5
30 m	10	6	1	3



(1a) (1b)

(2a) (2b)

Figure 2. (**1a**). The impact of the test variant (food reward—F/social reward—S) on latency time to respond to the sound [s]. (**1b**). The impact of the test variant on the effect of the sound barrier assessed on a 4-point scale. (**2a**). The impact of the sound exposure distance (30, 15 or 5 m) on latency time to respond to the sound [s]. (**2b**). The impact of the sound exposure distance on the effect of the sound barrier assessed on a 4-point scale. Means marked with different letters differ significantly at $p < 0.05$.

3.2. Cardiac Activity Responses

Although all included cardiac indices changed during the sound tunnel test, shifts towards vagal or sympathetic activation differed depending on the HRV parameters (Figures 3 and 4). When changes were observed, LF and LF/HF, as well as RMSSD and HF, dropped gradually, both when regarding the motivator (Figure 3) and the sound exposure distance effect (Figure 4). It was also noted that heart rate changes (HR) were immediate but short-term (significant growth in S2 period and then drop in S3 period), whereas changes in HRV parameters were delayed (drop in S3 compared to S1; gradual drop or S2 period; rapid drop)—they did not differ between sound and pre-sound period in any case.

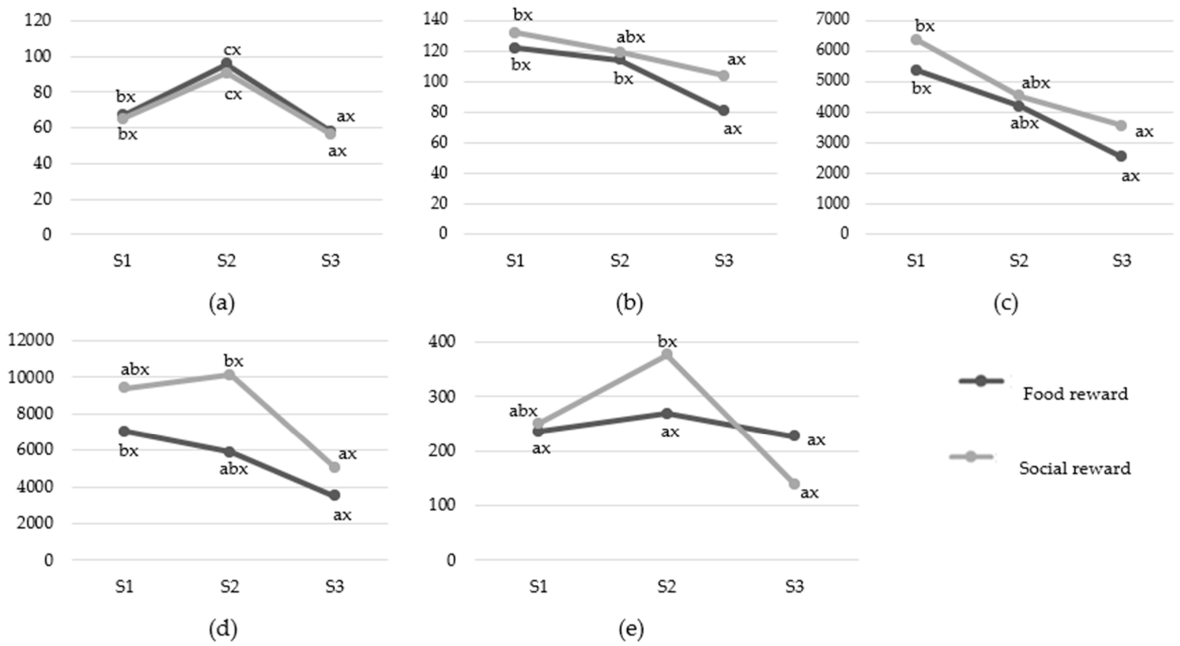


Figure 3. Mean values of: (**a**) HR [bpm]; (**b**) RMSSD [ms]; (**c**) HF [ms^2]; (**d**) LF [ms^2]; (**e**) LF/HF [%] in subsequent test periods: pre-sound (S1), sound (S2) and post-sound (S3) period depending on the test variant (motivator: food/social reward). Means marked with different letters differ significantly at $p < 0.05$ within the same variant of the study in subsequent test periods S1, S2 and S3 (letters a, b) or between different variants within the same period (letters x, y).

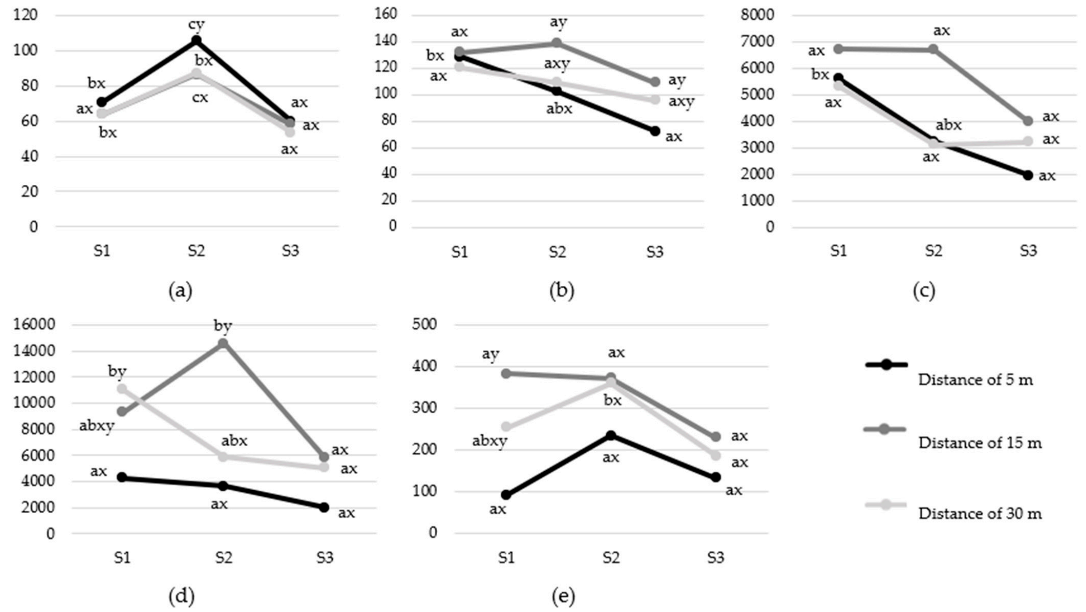


Figure 4. Mean values of: (**a**) HR [bpm]; (**b**) RMSSD [ms]; (**c**) HF [ms^2]; (**d**) LF [ms^2]; (**e**) LF/HF [%] in subsequent test periods: pre-sound (S1), sound (S2) and post-sound (S3) period depending on the sound exposure distance (30/15/5 m). Means marked with different letters differ significantly at $p < 0.05$ within the same distance in subsequent test periods S1, S2 and S3 (letters a, b) or between different distances within the same period (letters x, y).

3.2.1. The Variant of the Study

There were no differences ($p > 0.05$) in pre-sound (S1), sound (S2) or post-sound (S3) measurements between the two test variants (Figure 3). Only changes within the variants were observed ($p < 0.05$).

HR increased rapidly just after the sound appearance and dropped relevantly in the fifth minute after sound exposure in comparison to S2 and S1 in both variants of the study. RMSSD decreased between S3 and S2 (a delayed, but rapid drop), while HF decreased between S3 and S1 (delayed, gradual drop) during variant F of the study. In the case of variant S, both RMSSD and HF values were characterized by a delayed, gradual drop. There was a deferred decrease in LF value in S3 compared to the S1 period when the horses were provided with a food reward, but no changes in LF/HF were noted. On the contrary, LF and LF/HF dropped rapidly between S3 and S2 measurements when the horses were allowed direct social contact. Among HRV indices, LF (variant S) and LF/HF (both variants) increased after the sound was played, but these changes were not statistically significant ($p > 0.05$).

3.2.2. Sound Exposure Distance

Considering the effect of the sound exposure distance (Figure 4), the values of the HRV parameters during the pre-sound period did not differ significantly in most of the cases ($p > 0.05$). The only differences were found between 5 and 30 m for LF and between 5 and 15 m for LF/HF, with higher values for 30 m and 15 m, respectively. Similarly, only single differences were noted in corresponding measurements between different distances during the sound period ($p < 0.05$). The highest increase in HR was noted for the distance of 5 m. The lowest values of RMSSD after sound playback were found for the horses tested at a distance of 5 m, but they were significantly lower than the values for the 15 m distance exclusively. In turn, LF during the S2 period measured at the distance of 15 m was significantly higher than those for 5 m and 30 m. In the post-sound period, the only relevant difference concerned RMSSD, which was meaningfully lower ($p < 0.05$) for the horses exposed to the sound at 5 m (72.34 ± 43.35 ms) compared to 15 m (109.08 ± 64.81 ms).

Within the variant of the study, the direction of HR changes was similar to the food and social reward variants of the study (Figure 3). A rapid increase in HR during the sound period and then a significant drop ($p < 0.05$) in the post-sound period were observed for each distance (Figure 4). LF and LF/HF did not change during the test in the case of horses from 5 m group ($p > 0.05$). A drop ($p < 0.05$) in both parameters was noted for 30 m (LF: S3 vs. S1, LF/HF: S3 vs. S2), and a drop in LF was also visible for 15 m (S3 vs. S2). RMSSD and HF changed significantly only in the horses tested at a 5 m distance. In both cases, a delayed drop of these parameters in S3 compared to the S1 period ($p < 0.05$) was noted. In the case of RMSSD (15 m), LF (15 m) and LF/HF (5 and 30 m), the increase was observed during the S2 period, but these changes were not statistically significant ($p > 0.05$).

4. Discussion

The current study revealed that a non-biologically relevant acute alarming sound is generally perceived by horses as threatening and, therefore, triggers avoidance or flight responses in the opposite direction. Similar observations were made by Umstatter et al. [14], who found that cows exposed to audio stimuli turned away from a designated line. These results show that self-applied acoustic stimulus may have some potential to manipulate the locomotion and dispersion of horses on pastures. However, a general success rate of 50%, which is in agreement with the results obtained by Umstatter [14], eliminates the commercial use of the sounds as independent virtual fences. Umstatter et al. [6] came to the same conclusions in the previous study by testing irritating sounds to control the location of cows. For these reasons, acoustic stimuli may be rather used as a support for conventional fencing. Similar recommendations were made for a traditional (paired with electric shock) virtual fencing system to reduce the risk of animals accessing roads or public areas [3]. We observed that the efficiency of the sound barrier decreased from 80% when the motivator to cross the boundary was a food reward to 20% in the case of social reward. This clearly indicates that a sound alone, even frightening, is too weak to stop a horse from further movement when it is highly motivated to obtain certain resources. Another concern is the risk of rapid habituation to sounds, which was observed by Butler et al. [13] and which would further reduce the potential of audio stimuli as independent barriers. In the current study, we did not investigate this factor due to its limited implications (weak potential of sound barrier). However, habituation should be an important factor when testing the effectiveness of virtual fencing and attempts to reduce this effect should be investigated. Due to the high vigilance and perceptual sensitivity of horses [21,33], even subtle changes in sound playback may have a significant impact on reducing the rate of habituation.

The time needed to complete a sound tunnel increased significantly only when the food reward was provided, whereas delaying further walking is crucial for the barrier effect. Moreover, Jouven et al. [10] noted that the effectiveness of the virtual fence for sheep declined in the presence of conspecifics, despite the previous association of the audio cue with an electrical pulse. It is commonly known that horses are highly motivated to any kind of social interaction [39,40], which was also observed in our study. Considering only successful trials, there were no differences in latency time to stop and go or run away in response to the sound in both variants of the study. If horses reacted to the sound, their response was just as quick. This fact has an evolutionary origin, as fast threat (predator) detection ensured survival [41]. Regarding the prolonged time of sound tunnel completion, the percentage of time spent on locomotory behaviour decreased after the sound application in the food reward variant of the study. This provides some potential for auditory stimuli as a grazing management tool. Using the sound barrier made the horses more vigilant, which is thought to be a primary antipredator defence mechanism [28], but the time of standing alert was much shorter in the presence of the second horse. Moreover, the frequency of snoring, acting as stress-releasing behaviour to restore the homeostasis [42], increased only during the food reward variant of the study. Altogether, these results may suggest that social motivation acts simultaneously as a social buffer that helps horses face a frightening stimulus [43]. Furthermore, the tested subjects observed a calm and habituated companion, which may reduce fear reactions in stressful situations significantly more than a non-experienced companion [44]. This finding is important in a social context but limits the possibility of using sounds as independent invisible barriers. However, the current study does not provide an answer to how horses exposed to a sound barrier in a group would react. Social impact on horses' readiness to cross the sound boundary was visible in behaviour, but no differences in HRV parameters were found between the two variants of the study. The trend in changes in cardiac activity in both cases was similar.

In general, regarding both main study factors (motivator and sound exposure distance), the moment of the sound appearance had a significant influence only on HR responses. HRV indices were mostly characterized by a gradual drop in their values. HRV reflects the modulating effect of ANS on cardiac responses [37]. In the previous study [31], we showed that a 1-min recording was not sufficient to influence HRV responses (RMSSD, LF, HF, LF/HF), and mainly only HR and RR changes were noted. In that particular case, horses were in the known paddock in a group and were not supposed to border a line. In the current study, horses were encouraged to move forward when a sudden, frightening sound appeared. These circumstances had an impact on HRV changes, but the response was delayed. Another reason for these differences may be the measurement time—5 min in the previous [31] and 1 min in the current study.

Considering the food and social reward variants of the study, the RMSSD and HF decrease in the post-sound period indicates a decrease in vagal tone, hence, higher sympathetic activity a few minutes after the sound appearance [37]. Simultaneously, lower values of LF (both variants) and LF/HF (social reward variant) in the post-sound period reflect some relaxation in SNS. This variation in HRV parameters may be due to the 'conflicting' character of the test. Although an aversive stimulus induced a stress response, obtaining the reward mitigated its negative effects. As shown by Safryghin et al. [45], the physiological response depends on the context that evoked the changes. An HR increase was seen both in positive and negative contexts. Thus, some conflicting contexts may have been reflected in HRV changes in our study. In terms of the companion of a second horse, a slight increase in LF/HF (activation towards SNS) was observed directly after sound application, followed by a rapid decrease in the fifth minute after auditory stimulus appearance. This result may suggest the importance of the presence of a companion in alleviating stress symptoms [44].

Besides emotional arousal, an HR increase in the sound period might also have been influenced by a flight reaction, which was noted in 14 out of 60 cases. However, even if it occurred, escape in trot or canter was usually limited to a few seconds. Since gaits faster than walking occur in nature as a defence against predators [46], a horse's response to the acute alarming sound might have been analogous. Lenoir et al. [47] observed that HR increased, while RMSSD, LF and HF decreased, during intensive, compared to moderate, physical effort. Although a gradual drop in HRV parameters was noted, the increasing intensity of movement was not a rule in the our study, which was also confirmed by the significant drop in HR values in the post-sound measurement. Thus, it suggests that changes in HRV may have been a result of a virtual barrier impact. All things considered, the impact of the changes in movement in response to a sound based on HRV parameters cannot be excluded, and this is a weakness of the current paper.

Regarding the influence of the sound exposure distance, we observed the opposite behavioural-physiological response than for the impact of a motivator. Behavioural variables did not differ between horses tested at three distances (30 m, 15 m or 5 m), whereas there was a clear tendency in HRV changes between the groups. This finding is in accordance with an experiment conducted by Safryghin et al. [45], who showed that a behavioural response may not reflect the physiological state of the organism. We observed that the only behavioural variable that depended on the sound playback was vigilance. No matter the distance, horses were more alert during the sound application test stage. Only the time to respond to the sound varied between three distances. Horses tested at 30 m reacted the slowest to the sound barrier, but still, a latency time of 2.61 ± 1.07 s was sufficient to stop the horses from further walking. Moreover, the immediate reactions of the horses to the auditory stimulus showed some tendency to be the calmest, which may have safety implications [20,33].

In terms of cardiac responses, the most disturbing was the sound played at a distance of 5 m. In the wild, sudden stimuli are particularly important in inducing an avoidance response [41], so audio cues played at a distance of 5 m from the horse may have been perceived as more threatening. Moreover, sounds at this distance were heard as louder, which, according to Butler et al. [13], might have resulted in stronger responses. Only HR changes towards SNS activation were visible immediately after audio cue exposure in each experimental group. The delayed decrease of RMSSD and HF values, suggesting an increasing level of stress after sound appearance, was noted only for the lowest horse–loudspeaker distance. Additionally, PNS activation was visible for greater sound exposure distances by the drop of LF (for 15 m) and LF/HF (for 15 m and 30 m) and not for 5 m, which indicated some relaxation after obtaining a reward. These results show that the possibility of detecting a frightening stimulus had a positive effect on the emotional arousal of the horses. Vigilance plays a crucial role in detecting and localising a stressor [28], and horses tested at a distance of 30 m had the most time to react. When considering the use of sounds in the developing of virtual fences or supporting traditional fencing methods, an appropriate distance triggering the sound playback should be taken into account. Regarding the efficiency of sound as a barrier and its welfare implications, we recommend longer distances in establishing an animal–loudspeaker 'triggering point' when using stationary speakers.

5. Conclusions

An acute alarming sound affected the antipredator defence mechanism of the horses, suggesting some potential as a virtual barrier. However, the motivation for social interactions was too strong for a sound alone to stop the horses from crossing a designated boundary. Due to its insufficient efficacy, the self-applied acoustic stimulus cannot be used independently. Nevertheless, its possible useas a support for conventional fencing cannot be completely excluded, e.g., in areas with a higher risk of animal escapes by increasing vigilance and redirecting of movement, In this case, further research would be needed, as the solution would require the design of a suitable and cost-effective automated system.

The sound stimulus affected the cardiac responses of the horses, indicating at least partial activation of the sympathetic nervous system. To achieve a compromise between efficiency, safety and welfare concerns, the moment of sound playback should allow the horses to detect it early enough.

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ORIGINAL PAPER

Does social motivation mitigate fear caused by a sudden sound in horses?

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Abstract

Living in a herd has multiple advantages for social species and is a primary survival strategy for prey. The presence of conspecifics, identified as a social buffer, may mitigate the individual stress response. Social isolation is, therefore, particularly stressful for horses, which are gregarious animals. However, they are not equally vulnerable to separation from the group. We tested whether more and less socially dependent horses and independent individuals would differ in their responses to novel and sudden sounds occurring in two contexts: non-social and social motivation. Twenty warmblood horses were first exposed to two social tests: to evaluate the level of social dependence (rate of restless behaviour; social isolation) and the quantity and the quality of interactions in which they were involved (stay on a paddock). Two fear audio tests were then performed to compare the responses to sudden sounds while feeding (non-social motivation; control trial) and while moving towards the herd (social motivation; experimental trial). Socially dependent horses showed more pronounced avoidance behaviour and needed much more time to resume feeding during the control trial. Hence, dependent individuals appeared to be more fearful. However, during an experimental trial, horses of both groups tended to ignore the sound or paid only limited attention to the stimulus, continuing to move forward towards their conspecifics. Thus, social motivation may mitigate fear caused by a frightening stimulus and make fearful and dependent horses more prone to face a potentially stressful event. This finding should be taken into account in horse training and management.

Keywords Horse · Gregariousness · Social dependence · Fearfulness · Social buffer

Introduction

Horses' reactivity to different stressors and environmental stimuli is modified by the animal's motivation, fearfulness or curiosity (Kozak et al. 2018). The variety of these responses may stem from differences in coping styles or temperamental traits (Visser et al. 2002) and has major implications in animal husbandry (Rothmann et al. 2014). Responding to suddenness and novelty may compromise both the safety of the handler and the welfare of a horse (Acton et al. 2020; Vidament et al. 2021). It is, therefore, crucial to be aware of the possible reactions of the horses (Corgan et al. 2021). Temperament is defined as any characteristic of an individual that appears to be stable over both time and situation (Roberts et al. 2016). Fearfulness and gregariousness, i.e. reactivity to social isolation from conspecifics (Lansade et al. 2008b), are among the most well-known and particularly interesting dimensions of equine temperament (Lansade et al. 2008a, 2017; Górecka-Bruzda et al. 2022). Indeed, these traits relate directly to the evolution of the horse, which is a highly social prey animal that lives in a herd and remains in constant readiness to detect and avoid the threat (Marliani et al. 2021).

A stable and organised social structure, manifested by dominance and submissive interactions, enables horses to avoid the deleterious energy and health costs that would be incurred in aggressive encounters (Narciso et al. 2021). The group members help to improve foraging strategies, provides learning opportunities (Krueger and Heinze 2008; Krueger et al. 2014; Mendonça et al. 2021) and facilitates localising and avoiding a potential threat (Hothersall and Casey 2012). For social species, living in a herd is the

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primary antipredator defence mechanism (Apfelbach et al. 2005). Although predation pressure is relaxed in domestic horses (Janczarek et al. 2021), they experience multiple anthropogenic stressors (Squibb et al. 2018). According to the risk-disturbance hypothesis, various sounds, objects and events may trigger a response analogous to the presence of a predator (Frid and Dill 2002). In this context, social support from other members of the herd consists of social buffering that mitigates individual stress levels (Hartmann et al. 2012).

When the alternative is no possibility of social interaction, horses are highly motivated to make any kind of physical contact (full, head or muzzle) with other individuals (Søndergaard et al. 2011). They strengthen relationships through affiliative approaches, mutual grooming or keeping proximity (Wolter et al. 2018). However, if direct contact is not possible, they at least strive for indirect interactions (Yarnell et al. 2015). For horses, social isolation is a particularly strong stressor that negatively affects their behavioural and physiological reactivity (Kay and Hall 2009; Żelazna and Jezierski 2018). When returning to the group after a previous restriction of social opportunities, horses may manifest a rebound effect, which refers to increased intensity of interactions with conspecifics (Christensen et al. 2002a). Stress elicited by social isolation may even moderate mild somatic pain (Reid et al. 2017). However, horses do not respond to the separation from the group with the same strength (Wolff et al. 1997). Socially dependent individuals have difficulty functioning when out of the herd. In contrast, independent animals are not excessively bonded to other horses or humans and are self-reliant when left away from the herd (Burattini et al. 2020). In the previous study (Janicka et al. 2022a), we showed that the motivation for social interaction rapidly decreased the potential of a self-applied acoustic stimulus in creating virtual barriers. Only 20% of horses turned back or stopped after the playback of acute alarming sound (sudden, unexpected) when the second horse was present in the excluded zone. In turn, when food reward was offered in that zone, the virtual barrier was 80% effective and made the horses respond with flight, going away or stopping.

Here, we assessed if horses characterised by different levels of social dependence would differ in their fear reaction to the sudden sounds occurring in two different contexts: social (while moving towards the herd) and non-social (while consuming attractive food). Given the significance of the social nature of horses, we hypothesised that socially dependent and independent individuals may show different fear responses in the non-social, but similar and weak responses in the social context, which would highlight the importance of social motivation in reducing the effect of frightening stimulus.

Materials and methods

Ethical statement

All experimental procedures were carried out in accordance with the European directive 2010/63/EU and Polish laws—the Act of January 23, 2021—Law on the organization of breeding and reproduction of farm animals (Journal of Laws 2021, Item 36) and were approved by the Local Ethics Review Committee for Animal Experiments (no 58/2022), Lublin, Poland.

Animals and living conditions

The study involved 20 adult warmblood horses (aged 6–16 years; 10.6 ± 3.6 years), comprising ten mares and ten geldings. The horses knew each other as they had been housed in the same equestrian centre for at least two years. They were released to an extensive pasture or occasionally to several paddocks for about six hours a day, where they could engage in social interaction. All of the subjects were familiar with the paddocks and pastures used in the study. Thus, habituation to the environment was not necessary.

The subjects were kept in individual box stalls bedded with wheat straw and equipped with a manger, hay feeder, salt lick and an automatic waterer. They were fed three times a day with a mixture of oats and bran and had unlimited access to hay. The horses worked under the saddle for a maximum of two hours a day, six days a week. The subjects were under permanent veterinary control (periodic physical examination, assessment during the movement, anamnesis, monitoring heart rate) and were regularly observed by an experienced caretaker during daily handling. Additionally, they were examined one week before and after the study (the above procedures). No physical or behavioural disorders were found before or after the study.

The tests were carried out on calm, windless days (< 0.3 m/s) during the spring period of 2022 and 2023. The location of the testing area reduced unnecessary stimuli which could interfere with the perception of the sound stimulus and affect horse's reactions, including auditory stimuli, movement of vehicles, pedestrians, or other horses. No sudden and loud noises (e.g. dog barking, tractor noise, or other equipment) interrupted the testing procedures.

Two adult female experimenters handled the horses during the study. They were known to the horses, as they were also their caretakers and looked after them five days a week.

Social tests

Prior to the main part of the experiment (Sound tests), the horses were subjected to two different social tests to assess the level of social dependence and engagement in social interactions. The tests were conducted on separate, consecutive days.

Social dependence test–arena test

Dependence on the other members of the social group was assessed during the herd (social) dependence test. The test consisted of separation from a herd that stayed on the pasture about 300 m from a tested subject. Horses were individually released to a small paddock (400 m^2) and were observed for a subsequent five minutes using the focal animal sample method (Altmann 1974). This time appeared to be sufficient because individual differences in reactions to an unusual situation were shown to be the strongest within the first few minutes (Wolff et al. 1997).

Behaviours that were assessed during the test were chosen based on the ethogram used in the arena test by Seaman et al. (2002), but some modifications were implemented. The most frequent behavioural variables indicating negative emotional arousal during isolation were vocalisation, defecation, locomotion and vigilance (Lansade et al. 2008b). Thus, certain behaviours assessed by Seaman et al. (2002) (standing, investigation, tail posture, paw, urination) were waived and the traits: ‘canter’ and ‘snoring’ (instead of snorts produced in positive context (Laurijs et al. 2021)) were added to the ethogram used in the current study. Behaviours assessed as frequency (whinny, snoring and defecation) were primarily recorded. However, since in the current study they were displayed only rarely by a few horses, they were not included in the Table 1 and were not considered when dividing horses into groups with different levels of social dependence.

Table 1 Behaviours used to assess social dependence during herd dependence test (modified ethogram worked out based on Seaman et al. (2002))

Behaviour	Description
Sustained walk (s)	Walking energetically, looking in front or around
Trot (s)	A two-beat gait
Canter (s)	[a]A three-beat gait
Vigilance (s)	Standing still with an elevated neck, intently orientated head and ears

[a]Added behaviour (modification of the ethogram)

After conducting and analysing the data from the social dependence test, horses were divided into three groups of social dependence. The level of social dependence was determined on the self-established percentage time of displaying restless behaviours based on variation of horses’ responses:

- independent (I): < 15% (n = 9) of the observation time was accounted for by restless behaviour–horses able to function without the social support of conspecifics (Stachurska et al. 2021),
- moderately dependent (MD): 15–50% (n = 5),
- dependent (D): > 50% (n = 6)–horses not self-reliant, reactive to social isolation from conspecifics (Burattini et al. 2020).

Social interaction test–introduction to a paddock

To determine the engagement in social interactions, the horses were observed just after being released into a large paddock (5000 m^2) in the morning (8.00 a. m.). The procedure was repeated on three consecutive days, with a single observation session lasting ten minutes. This observation time was taken from the study of Seaman et al. (2002), who assessed the dominant and submissive behavioural responses of horses after reintroduction to the group.

Affiliative and agonistic interactions (McDonnell 2003) (Table 2) in which each focal animal was involved were recorded every 30 s using the one-zero sampling method (Altmann 1974). The total number of affiliative, aggressive/

Table 2 Behaviours recorded during social interaction test (McDonnell 2003)

Affiliative behaviour	Aggressive and threatening behaviour
Mutual grooming (nipping/nuzzling/rubbing each other’s neck, mane, chest, back, rump or tail)	Head, bite or kick threat
Laying the head on the neck, back or rump	Bite, kick
Approach (forward movement toward another horse and staying close)	Head bump
Following (moving along behind another horse, no attempt to attack)	Strike (one or both forelegs rapidly extended forward to contact another horse, often accompanied by a squeal or snort)
Playing with conspecifics (any reaction or sequence of behaviour during play in motion, occurring socially)	Rear (raising of the forelegs into the air, supporting the body on the hind legs only)
	Herding (combination of head threat and ears laid back with forward locomotion)
	Chase (pursuit of another horse, usually at a gallop)

Submissive behaviour–avoidance of aggressive/threatening interaction threatening and submissive behaviours exhibited by each horse was then calculated. The measurement of spatial proximity (horses standing with body contact or within two horse-lengths), commonly used to assess social bonds (Wolter et al. 2018), was not taken into account because of the short-term character of the social test, whereas horses show a mean latency of changing the spatial distribution of group members every 8 min (Christensen et al. 2002b).

Sound tests

Sound tests were the startling fearfulness tests, assessing horses' reactions to a sudden auditory stimulus (Seaman et al. 2002). However, these tests were conducted in two different contexts: without (a control trial) and with (an experimental trial) a social motivation. Each sound test was carried out twice (two stages) with an interval of about a year (to reduce the likelihood of habituation to the sounds). Two different sounds were used during these tests: sound A (squeal of a pig, produced during distress (Çavuşoğlu et al. 2020)) and sound B (futuristic characteristics; similarity to radio and TV interference, crackling). The sounds were selected based on the results of our previous studies: comparing reaction to 40 sounds of different origin (Janicka et al. 2022a), assessing the possibility to use sound stimulus in creating virtual barrier (Janicka et al. 2022b) and comparing reactions to 40 known and four novel sounds (unpublished data). Sounds that were chosen elicited similar and strongest avoidance behaviour. Both sounds were unknown to the study horses. In the first stage of the study (stage 1; 2022 year), sound A was played during the control trial and sound B during the experimental trial. The reverse order was then used in the second stage (stage 2; 2023 year). In addition, 10 randomly selected horses were first subjected to the experimental and then to the control trial and for the remaining 10 horses, the order of procedures was reversed. The experimental trial was conducted in the morning (7.30–10.30) when horses were normally released to the extensive pasture to induce the motivation to move forward and to join the herd. In turn, the control trial was carried out in the afternoon/evening hours (15.30–19.30) when horses were brought back to the stable and were used to stay in boxes or paddocks near the stable (reduction of social motivation). The interval between two trials was at least six days (6–8 days; differences caused by poor weather conditions: rain or wind > 0.3 m/s).

Non-social motivation sound test (NMST)–a control trial

The horses were individually subjected to a controlled assessment of their fear response to a new and unexpected sound while eating (non-social motivation sound test; NMST). The testing arena (140 × 30 m) was designated with tape for an electric fence on one of the known paddocks. It was located next to another paddock, providing proximity (approximately 50 m) to conspecifics during the test to avoid the impact of social isolation stress and lack of concentration on the food provided. The three horses in the neighbouring paddock (the same animals throughout the experiment, visible to study subjects) were habituated to the test sound a few days before the study.

A plastic container for the later provision of attractive food was placed at the front of the testing arena. A loudspeaker (JBL Charge 4, rated power of 30W) was positioned 1 m in front of the container, allowing the horse a long escape distance after the sound playback, if necessary. Prior to the test, horses were taught to feed from the container.

Each horse was released into a testing arena 15 min before the start of the test to habituate to the experimental setting. The first experimenter (E1) then brought the horse to the start point (3 m from the container) and waited for the test to begin. At this time, the second experimenter (E2) put chopped carrots inside the container. The horse was unleashed (by E1) and could start feeding, and both experimenters left the arena. After 30 s, E2 played a sudden sound (20 s, 40 dB; first stage–sound A, second stage–sound B) via Bluetooth by a Samsung Galaxy A02s device (Samsung Electronics Co., Ltd., South Korea). As the maximum range of Bluetooth was approximately 15 m, experimenters had to stay at this distance during the test in order to play the recording at the right moment. However, they stood calmly, did not disrupt the test and the horses were used to their presence (they were also their caretakers).

Latency to respond to the stimulus was proved to be the objective and easy measure of horses' responsiveness in fear tests (Górecka-Bruzda et al. 2011). Therefore, latency to resume feeding (s) was measured in the current study. If a horse did not approach the food within 300 s, it was given 301 s (Górecka-Bruzda et al. 2011). Additionally, a 5–point behavioural scale describing horses' reactions after sound playback was developed. Receiving 5 points indicated the highest level of fear (Table 3).

Social motivation sound test (SMST)–an experimental test

The social motivation sound test (SMST) was a modified version of the sound tunnel test developed in the previous study (Janicka et al. 2022a) to assess the potential of the sounds in creating electric-free virtual fences. During the test, the horses moved through the same corridor (55 m length, 5 m width, designated with tape for an electric fence) leading towards a known pasture, where the other members of the herd (except for individuals included in the study) were staying. The start–end of the corridor was closed after the introduction of a horse to a start zone (first 5 m), whereas the finish-end was permanently open,

Table 3 Behavioural scale used to assess horses' responsiveness during the sudden sound test

Points	Behavioural response
1	The horse pays no attention to the sound, continues to take food in an uninterrupted manner and is calm
2	The horse raises its head, puts up its ears and resumes feeding. It is not spooked, and it does not walk away from the food
3	The horse lifts its head, ears up, walks away a few steps, looks at it, approaches cautiously and starts to eat, may snoring
4	The horse abruptly removes its head and jumps back. Spins, impatient, may toss head, hesitantly approaches food, still spooked
5	The horse abruptly jumps back, walks away/runs a considerable distance, snorts, is afraid to approach food for a very long time (hesitant, tense) or does not approach at all

allowing the animal to leave it after each run. The experimental paddock, at which the corridor was designated, was separated from the pasture by metal railings. There was a loudspeaker placed outside the corridor at the finish line. The day before the SMST, horses were brought into the experimental paddock for two hours to familiarise themselves with the corridor.

SMST was conducted in the morning hours on three consecutive days (three trials). During each trial, horses went through the corridor at least three times: (1) being led by the experimenter (E1), (2) freely, without the experimenter (E1), and (3) freely, but with playing a sudden sound by E2 (20 s, 100 dB; first stage–sound B, second stage–sound A) when the individual reached a distance of 10 m from the finish line. The initial two trials evaluated the horse's willingness to walk towards the pasture and other horses located approximately 150 m away. If the horse exhibited this behaviour, a third trial was conducted to observe its reactions. Before each trial, the study subject was held on the line in a start zone by E1. The second experimenter (E2) waited in a distance of 15 m from the loudspeaker to play the sound at the right moment after the horse was released by E1.

We showed that the motivation for social interaction rapidly decreased the audio virtual fencing effect (Janicka et al. 2022a). Thus, we focused on subtle differences in reaction to the sound between horses with different levels of social characteristics. The sound barrier effect, completion of the SMST and behaviour after completion of the test were recorded (Table 4).

Statistical analyses

The statistical analysis was performed with the SAS 9.4 software (version 9.4, SAS Institute Inc., Cary, NC, USA). Since the data did not conform to a normal distribution, as determined by the Kolmogorov–Smirnov and Shapiro–Wilk tests ($p < 0.05$), non-parametric tests were applied. Correlations between the results of behavioural tests were checked with + Spearman's rank correlation. Kruskal–Wallis test was used to evaluate if social dependence affected the horses' responses to the sounds and engagement in affiliative and dominant-submissive interactions. Dwass-Steel-Critchlow-Fligner (DSCF) multiple comparisons were then performed to check for the significance of differences between the groups. Additionally, the differences between mares ($n = 10$) and geldings ($n = 10$) and between younger ($n = 10$; 6 – 10 years) and older horses ($n = 10$; 11–16 years) during the tests (Mann–Whitney U test), as well as between two stages of the study (Wilcoxon test) were checked. Even though the significance of results was calculated via non-parametric tests, for better clarity and readability for readers, the results were presented in tables as mean value ± standard error of the mean (SE). The minimum level of significance for all tests used was accepted at $p < 0.05$.

Results

Stage of the study differences

No significant differences were found between the results of sound tests in two stages of the study (the revised order

Table 4 Behavioural variables assessed during social motivation sound test

Behaviour	Description
Barrier effect (scale 1–5)	Reaction to the sound; 1–continuing forward movement, 2–slowing down, 3–stopping, 4–going away, 5–running away
Completion of the test (scale 1–2)	Leaving a corridor before the end of the 20 s sound; 1–no, 2–yes
Finish behaviour (scale 1–6)	Behaviour after completion of the test; 1–free walking/resting near the finish line; 2–free walking/resting away from the finish line, 3–looking around nervously + no locomotion, 4–walking + looking around nervously, 5–trot/canter + looking around nervously, 6–not reaching a finish line within 20 s of the sound

Table 5 Comparison between reactions in social and non-social sound tests in dependence of the stage of the study

Variable	Stage 1	Stage 2
Latency to resume feeding in NMST (s)	104.45 ± 16.97 a	133.75 ± 16.31 a
Behavioural scale in NMST (1–5)	3.60 ± 0.18 a	2.90 ± 0.15 a
Barrier effect in SMST (1–5)	3.05 ± 0.17 a	3.45 ± 0.17 a
Completion of the SMST (1–2)	1.82 ± 0.05 a	1.78 ± 0.05 a
Finish behaviour in SMST (1–6)	2.50 ± 0.25 a	2.17 ± 0.21 a

Means (± SE) marked with different letters differ significantly between stage 1 and stage 2 of the study at $p < 0.05$ (Wilcoxon test)

NMST non-social motivation sound test, *SMST* social motivation sound test, *stage 1* squeal of a pig (sound A) as control sound and futuristic sound (sound B) as experimental sound, *stage 2* changed order of the sounds

of sounds used in the control and the experimental trial) ($p > 0.05$) (Table 5). Horses responded similarly, no matter which sound (A–squeal of a pig, or B–futuristic sound) was used as control or experimental. For this reason, further comparisons between the reaction in two stages were waived.

Dependence on the social group and need for social interactions

Most of the horses (n = 11) showed more (n = 6; dependent) or lesser (n = 5; moderately dependent) signs of anxiety when they were physically isolated from the group (Table 6). Of all the other horses, those categorised as dependent spent the most time demonstrating a sustained walk (DSCF = 3.8730, p = 0.0170; DSCF = 5.0767, p = 0.0010; in comparison to moderately dependent and independent, respectively). The time of vigilance was similar in the dependent and moderately dependent groups (DSCF = 0.2582, p = 0.9818) and definitely longer than in independent horses (DSCF = 4.1779, p = 0.0088; DSCF = 4.2567, p = 0.0074, respectively). Canter rarely occurred and did not differ significantly between the groups ($p > 0.05$). Dependent horses trotted (DSCF = 3.9485, p = 0.0145) more than independent horses but not moderately dependent individuals (DSCF = 2.7749, p = 0.1217; DSCF = 2.9863, p = 0.0875, respectively).

Although horses responded differently to social isolation, the quantity and the quality of interactions during their stay on a paddock (social interaction test) were similar between the groups ($p > 0.05$). The horses usually displayed affiliative interactions, whereas dominance and submissive behaviours were rarely observed. For this reason, agonistic behaviours were not included in further analysis.

Reactions to sounds

All the horses (n = 20) moved willingly towards the pasture in SMST in the first two trials and were subjected to the third trial (the proper test), in which their reactions were assessed.

Horses' responses to a sudden sound in NMST varied, but they generally interrupted feeding for some time (stage 1; n = 17, stage 2; n = 18) and showed movement in the opposite direction (stage 1; n = 13, stage 2; n = 16). In contrast, they rarely stopped forward movement (stage 1; n = 4, stage 2; n = 5) and turned back towards the start zone (stage 1; n = 2, stage 2; n = 1) in SMST. Their reactions were usually limited to slowing down after hearing a sound while moving towards the other herd members.

Table 6 Comparison of the time budget of horses with different levels of social dependence based on the social dependence test

Behaviour	Dependent (N = 6)	Moderately dependent (N = 5)	Independent (N = 9)
Time budget			
Sustained walk (s)	188.67 ± 22.04 c	34.00 ± 7.31 b	0.00 ± 0.00 a
Trot (s)	23.33 ± 8.44 b	3.60 ± 2.91 ab	1.22 ± 1.22 a
Canter (s)	2.67 ± 1.26 a	2.40 ± 1.47 a	0.56 ± 0.55 a
Vigilance (s)	45.83 ± 10.36 b	46.00 ± 13.85 b	9.44 ± 3.02 a
Frequency of social interactions			
Affiliative interactions	23.67 ± 3.29 a	24.80 ± 4.69 a	19.11 ± 4.34 a
Dominance behaviour	4.17 ± 1.92 a	2.00 ± 0.71 a	4.22 ± 1.14 a
Submissive behaviour	3.83 ± 0.54 a	2.00 ± 0.71 a	1.88 ± 0.35 a

Means (± SE) marked with different letters differ significantly in rows at $p < 0.05$ (DSCF)

Table 7 Impact of the social dependence of the horses on reactions to sounds

Behaviour	Social characteristics of the horses		
	Dependent (N = 6)	Moderately dependent (N = 5)	Independent (N = 9)
Sound barier (SMST)	2.19 ± 0.20 a	1.80 ± 0.19 a	1.98 ± 0.16 a
Completion of SMST	1.81 ± 0.07 a	1.83 ± 0.07 a	1.78 ± 0.06 a
Finish behaviour (SMST)	2.64 ± 0.27 a	2.00 ± 0.30 a	2.41 ± 0.27 a
Refeeding latency (SST)	196.75 ± 20.69 b	129.30 ± 26.40 ab	61.67 ± 12.43 a
Behavioural scale (SST)	3.92 ± 0.18 b	3.30 ± 0.33 ab	2.78 ± 0.13 a

Means (± SE) marked with different letters differ significantly in rows at $p < 0.05$ (DSCF)

Dependent horses were more fearful during NMST than independent horses (Table 7). They needed much more time to resume feeding after the sound was played (DSCF = 6.0376, $p < 0.0001$) and had higher scores on the behavioural scale, indicating the strength of the fear response (DSCF = 6.3439, $p < 0.0001$). In spite of this, no differences were found between the groups during the SMST ($p > 0.05$). The sound barrier effect was poor and usually limited to slowing down or stopping for a while, and eventually, most of the horses finished the test.

There were no correlations between the horses' reactions in two different sound tests, except for the first reaction after the sound playback (behavioural scale) and latency to refeed in NMST and barrier effect in SMST (Table 8). In the case of the horses that gained more points on the behavioural scale (stronger fear response) and needed more time to resume feeding, the sound barrier effect was greater (Spearman's rank correlation; $r_s = 0.350$, $p < 0.0001$ and $r_s = 0.325$, $p = 0.0003$, respectively). However, these correlations were weak.

Both refeeding latency ($r_s = 0.354$, $p < 0.0001$) and behavioural scale scoring ($r_s = 0.397$, $p < 0.0001$) were weakly correlated with the time spent on restless behaviour in the herd dependence test. Simultaneously, restlessness (indicating the level of herd dependence) and the responses during SMST were not correlated ($p > 0.05$).

There was no correlation between the frequency of affiliative interactions and social dependence (restlessness) ($r_s = 0.172$, $p = 0.1898$). However, in the case of the horses that interacted more, the sound barrier effect was stronger (more points in the behavioural scale) ($r_s = 0.416$, $p < 0.0001$) and the horses finished the test with a lower probability ($r_s = -0.349$, $p < 0.0001$). They also received more points on the behavioural scale during NMST ($r_s = 0.491$, $p < 0.0001$) and had longer refeeding latencies ($r_s = 0.315$, $p = 0.0005$).

Sex and age differences

Mares were more restless when separated from the group during the herd dependence test (Mann–Whitney U test; U = 256.50, $p = 0.0037$), needed more time to resume feeding after sound playback in NMST (U = 1156.50, $p = 0.0007$) and responded to this sound more violently (they had a higher score on the behavioural scale) (U = 1260.00, $p = 0.0046$) than geldings (Table 9). Showing affiliative interactions was common and comparable (U = 427.50, $p = 0.7412$). Although mares showed submissive behaviour more often (U = 297.00, $p = 0.0234$), agonistic interactions were rarely presented. No differences between mares and geldings were found in response to the sound during SMST ($p > 0.05$). The sound barrier effect (stopping/limiting further movement) was weak, and both mares and geldings

Table 8 Correlations between the results of behavioural tests

Sudden sound test and social tests	Sudden sound test, herd dependence test and social motivation sound test					
	Latency (NMST)	Behav. (NMST)	Restles.	Barrier (SMST)	Compl. (SMST)	Finish (SMST)
Latency (NMST)	–	–	0.354*	0.325*	−0.128	0.149
Behav. (NMST)	–	–	0.397*	0.350*	−0.170	0.063
Restles	–	–	–	0.060	0.040	0.146
Affiliative behaviour	0.315*	0.491*	0.172	0.416*	−0.350*	0.058

*Statistically significant correlations (in all cases $p < 0.001$)

Latency (NMST) refeeding latency in NMST, *behav. (NMST)* behavioural scale in NMST, *restless.* restless behaviour (%) in herd dependence test, *barrier (SMST)* effect of sound barrier in SMST, *compl. (SMST)* completion of SMST, *finish (SMST)* finish behaviour in SMST, *NMST* non-social motivation sound test, *SMST* social motivation sound test

Table 9 Sex and age differences during social and sound tests

Variable	Horse groups (sex/age)	
	Mare	Gelding
Restless behaviour (%)	45.37 ± 6.78 b	24.07 ± 5.71 a
Affiliative interactions (freq.)	21.50 ± 1.72 a	22.30 ± 2.19 a
Dominance behaviour (freq.)	3.50 ± 0.67 a	3.80 ± 0.60 a
Submissive behaviour (freq.)	3.00 ± 0.29 b	2.00 ± 0.22 a
Latency to resume feeding in NMST (s)	167.50 ± 17.48 b	70.70 ± 13.27 a
Behavioural scale in NMST (1–5)	3.60 ± 0.18 b	2.90 ± 0.15 a
Barrier effect in SMST (1–5)	2.20 ± 0.16 a	1.80 ± 0.14 a
Completion of the SMST (1–2)	1.78 ± 0.05 a	1.82 ± 0.05 a
Finish behaviour in SMST (1–6)	2.83 ± 0.23 a	1.83 ± 0.21 b
	Younger (6–10 years)	Older (11–16 years)
Restless behaviour (%)	35.50 ± 4.36 a	33.93 ± 4.83 a
Affiliative interactions (freq.)	25.20 ± 1.19 b	18.60 ± 1.42 a
Dominance behaviour (freq.)	3.20 ± 0.31 a	4.10 ± 0.54 a
Submissive behaviour (freq.)	2.60 ± 0.19 a	2.40 ± 0.19 a
Latency to resume feeding in NMST (s)	99.0 ± 15.73 a	139.20 ± 17.32 a
Behavioural scale in NMST (1–5)	3.30 ± 0.18 a	3.20 ± 0.17 a
Barrier effect in SMST (1–5)	2.05 ± 0.15 a	1.95 ± 0.15 a
Completion of the SMST (1–2)	1.75 ± 0.06 a	1.85 ± 0.05 a
Finish behaviour in SMST (1–6)	2.45 ± 0.23 a	2.22 ± 0.23 a

Means (± SE) marked with different letters differ significantly between mares and geldings or younger and older horses at $p < 0.01$ (Mann–Whitney U test)

NMST non-social motivation sound test, *SMST* social motivation sound test

finished the test with a similar frequency. The only difference concerned behaviour after completion of the SMST. Mares were more agitated while waiting to be reunited with the herd ($U = 1030.00$, $p < 0.0001$).

There were no differences between the responses of younger and older horses during the herd dependence test, NMST and SMST ($p > 0.05$). Only during the social interaction test younger individuals showed significantly more affiliative interactions ($U = 1134.00$, $p = 0.0005$).

Discussion

Horses evolved as prey species for which living in a herd is a survival strategy (Marliani et al. 2021). Thus, isolation from conspecific is a particularly strong stressor for horses (Kay and Hall 2009). However, similarly to Wolff et al. (1997), Lansade et al. (2008b) and Górecka-Bruzda et al. (2022), we showed that they were not equally vulnerable to separation from the herd. The differences in isolation anxiety were mainly displayed by various engagement in sustained walking, vigilance and trotting, whereas neighing, snorting and defecation were infrequent. In turn, Lansade et al. (2008b) point to the frequency of neighing as the most interesting behavioural parameter, which was well correlated with other parameters measured in the same isolation tests, such as defecation, locomotion and vigilance. The differences in these observations may be due to the individual characteristics of the horses. Also some possibility of auditory contact between the study subjects and the herd (staying in a distance of 300 m) can not be entirely excluded, which is a limitation of our study. Besides the existence of a trait 'social dependence', the isolation test also revealed the importance of the factor 'sex' on gregariousness. We noticed that, compared to geldings, mares were more restless when separated from the herd. This observation is in agreement with the study of Górecka-Bruzda et al. (2022), who showed that mares were more socially dependent than geldings. The current study further showed that social dependence is not manifested by engagement in social interactions. After the introduction to a large paddock during the social interaction test, the horses presented a wide range of affiliative interactions, including mutual grooming, following, approach and motor playing. The frequency of affiliative relationships did not vary between horses of different levels of gregariousness, or between mares and geldings. The similar engagement in interactions of horses with different social characteristics stresses the importance of social contact with conspecifics.

Additionally, the fact that horses with different levels of gregariousness made a similar number of social interactions indicates that correlation between engagement in affiliative interactions and higher agitation after sound playback both in a non-social and social motivation context were due to greater fearfulness rather than social dependence. We further confirmed that social dependence does not result from the need for direct interactions but rather from the very need to stay close to the herd and is best observed during social isolation (Le Scolan et al. 1997; Wolff et al. 1997; Seaman et al. 2002; Lansade et al. 2008b; Lansade and Simon 2010). However, we did not consider spatial proximity between the horses in the social interaction test, which, as one of the social bond measurements, could show correlations with social dependence. It would be worthwhile to include this trait in future research. In the current study agonistic interactions were rare and limited exclusively to threatening, so it was not possible to compare such behaviour in dependent and independent individuals. Similarly, Kimura (1998) noticed that during spring season, thus, when breeding season starts, dominance–submission interactions were not frequent between free-ranging mares. We also observed horses in spring, but in the case of our study the results could stem from a short period of observation. Furthermore, unlike Kimura's (1998) study, our experiment was not conducted at different seasons of the year. The limitation of social interaction test after introduction to a paddock (even if simple and easy to repeat) in assessing the full range of equine social behaviour should be taken into account in further studies.

As we predicted, horses of different levels of social dependence varied in their responses to the sudden sound during the test in a non-social (but not a social) motivation context when they moved towards a herd on a pasture. Horses classified as dependent were more fearful compared to independent individuals in a non-social motivation context. They responded to the sound more violently (jumped back and walked/run away more often) and needed significantly more time to resume feeding than self-reliant animals. Briard et al. (2015) also noted that fearless horses were simultaneously less gregarious. In turn, Villalba et al. (2009) showed that sheep cautious in accepting new food were more reactive during social isolation. Indeed, in many species, these traits have been used to characterise 'bold' and 'shy' behavioural types (Briard et al. 2015). We found no correlations between the rate of restlessness during separation from the herd and the results of the social motivation sound test. Horses' reactions were usually limited to slowing down after hearing the sound or eventually to stopping for a while. Even when hesitating, they decided to move forward to stay closer to the herd. Thus, the motivation to join the group was too strong for an unexpected, novel sound to stop both independent and dependent horses from further movement. Jouven et al. (2012) showed that some of the sheep, previously trained not to exceed the virtual fence, followed their conspecifics regardless of the electric shocks they received. Since, in our study, dependent and moderately dependent individuals showed a greater level of fear during a control sound test and then reacted similarly to independent horses in the social motivation sound test, we may conclude that the strong need to stay in proximity to members of the group mitigates fear caused by a frightening stimulus. As reported by Burattini et al. (2020), boldness and independence are two important behavioural traits that influence horses' fearfulness, assertiveness and sociability. Socially dependent horses are not at ease when left alone, away from the herd. Instead, they rely more on their conspecifics (Burattini et al. 2020). However, it should be noted that sound tests used in our study differed in the nature of the motivational conflict. In the non-social motivation test horses had to choose between staying and eating attractive food or fleeing (stay-avoidance conflict), whereas in social motivation test they had to decide whether to continue moving forward towards the herd or to stop/turn back to avoid the frightening stimulus (moving forward-avoidance conflict). Also, unlike in the test with food, the horses had no reason to stay near the loudspeaker once they had passed it. The mentioned differences between two tests may be the reason for limited correlations between horses' responses to sound playback in different motivational conflicts. Lansade et al. (2008c) showed that within each sense, the greater the horses' response to one stimulus, the greater their response to the other. However, as it was shown in our study, it is important to regard the context of presenting a testing stimulus during behavioural observations.

In the current study, also mares, that were shown to be more socially dependent than geldings, were definitely more anxious during a control sound test but reacted the same as the geldings during the sound test in the social motivation context. Thus, willingness to be reunited with conspecifics reduced the effect of a frightening stimulus. The only difference concerned behaviour after completion of a test, when the horses were waiting to join the herd. Again, the mares were more agitated, similar to the social isolation test (social dependence test). Lansade et al. (2022) noted that weaned horses still preferred their dam as well as other mares from their natal group after five months of separation, but this tendency was stronger in fillies than in colts. A stronger need to stay in a group in the case of mares may be due to the social structure of the species. In the wild, horses lived in groups comprising mainly females, and this is probably why social support is particularly important for them (Górecka-Bruzda et al. 2022). However, it should be noted that the geldings may have reacted differently than it would be seen under natural conditions in stallions.

The results of the current study may have practical implications. Horse owners should be aware of the existence of social dependence in horses and its correlations with fearfulness. However, similarly to Christensen et al. (2008) and Ricci-Bonot et al. (2021), we confirmed the role of conspecifics as a social buffer and the importance of social motivation to confront a stressor. For example, in the study of Dai et al. (2019), horses were taught loading into a trailer in groups, which allowed their gregarious nature to be used in the learning process. We conclude that this factor should be considered in horse management when handling or schooling animals.

Conclusions

Horses are highly motivated to stay close to their conspecifics, but they differ in their responses to social isolation. Socially dependent horses are not at ease being left alone outside the herd, in contrast to those more independent. However, while on the paddock, they engage in social interactions in a similar way. Dependent individuals are generally more fearful than independent animals. However, when facing sudden, novel audio stimulus in a social motivation context (motivation to join a herd), they react similarly to more self-reliant horses. Thus, social motivation may mitigate fear caused by a frightening event in horses. This finding should be taken into account in horse training and management.

Author contribution The conception for the paper was conceived by WJ and IW. The experiments were designed by WJ and IW and performed by WJ. The data were analyzed by WJ and TP. The paper was written by WJ and was revised critically by IW and TP. All authors read and agreed to the published version of the manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval All experimental procedures were carried out in accordance with the European directive 2010/63/EU and Polish laws—the Act of January 23, 2021—Law on the organization of breeding and reproduction of farm animals (Journal of Laws 2021, Item 36) and were approved by the Local Ethics Review Committee for Animal Experiments (no 58/2022), Lublin, Poland.

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(1) Wiktoria Janicka, Izabela Wilk, Magdalena Ryżak (2022). Horses' perception of a threat posed by sounds of different origin. Med. Weter. 78(8), 401-413.

Mój udział wynosił 85% i polegał na opracowaniu koncepcji, założeń metodologicznych, zaplanowaniu i przeprowadzeniu badań, przygotowaniu bazy danych, udziale w analizie statystycznej, opisie i interpretacji wyników badań, przygotowaniu rycin i wizualizacji danych, napisaniu pierwszej wersji manuskryptu, redakcji publikacji, korespondencji z redakcją i korekcie prac po recenzjach.

(2) Wiktoria Janicka, Izabela Wilk, Tomasz Próchniak, Iwona Janczarek (2022). Can Sound Alone Act as a Virtual Barrier for Horses? A Preliminary Study. Animals 12(22), 3151.

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Mój udział wynosił 85% i polegał na opracowaniu koncepcji, założeń metodologicznych, zaplanowaniu i przeprowadzeniu badań, przygotowaniu bazy danych, udziale w analizie statystycznej, opisie i interpretacji wyników badań, przygotowaniu rycin i wizualizacji danych, napisaniu pierwszej wersji manuskryptu, redakcji publikacji, korespondencji z redakcją i korekcie prac po recenzjach.

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Mój udział wynosił 10% i polegał na pomocy w opracowaniu koncepcji i założeń metodologicznych oraz wsparciu merytorycznym.

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Uniwersytetu Przyrodniczego
w Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Wiktoria Janicka, Izabela Wilk, Magdalena Ryżak (2022). Horses' perception of a threat posed by sounds of different origin. Med. Weter. 78(8), 401-413.

Mój udział wynosił 5% i polegał na pomocy w przeprowadzeniu badań i interpretacji wyników.

Magdalena Ryżak
Podpis