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Can Repeated Exposure to Music Mitigate Horses' Reactions to Sudden and Unexpected Stimuli?

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Abstract

The living conditions imposed on horses mean that they are inevitably confronted with situations that can induce stress. Music is a promising tool for managing such situations, but its benefits could be attenuated by repeated exposure. In this study, we aim to determine whether music can mitigate horses' reactions to unexpected stimuli and if playing the same music daily leads to a loss of its efficiency. We compared three groups of 12 horses that were led on a route punctuated by potentially stressful stimuli for 10 consecutive days. Each group of individuals wore headphones and was consistently subjected to one of the three experimental conditions: a "music" test condition during which the same music track was played, a "noise" condition during which pink noise was played, and a "no-music" control condition. We found that music has a relaxing effect on horse behavior and heart rate. Interestingly, parameters with the pink noise were intermediate between the music and no-music conditions. Regarding the music's loss of efficiency through repeated playing, our results show that this tool continues to effectively mitigate the behavioral expression of stress after seven consecutive exposures, but this effect was not found every day. Music can therefore help make human-horse interactions safer by limiting the stress of horses faced with unexpected events, but further investigation is needed to understand the underlying mechanisms and ensure a safe and consistently efficient use in the field.

Keywords

Stress; behavior; heart rate; habituation; equitation science; welfare

1. Introduction

It has been shown that music can be beneficial to animal welfare [1]. Music could specifically alleviate stress and fear responses, as shown for chickens [2], rats [3], and cows during milking [4]. Additionally, studies have shown that music has a calming effect on dogs and chimpanzees [5] and promotes play behavior in piglets [6]. Similarly, in rodents, it can also improve physiological parameters such as weight, heart rate, and sympathetic and parasympathetic system activity [7]. Beneficial effects of music on the emotional state have been observed in horses after exposure in the stables without any particular stressful situation [8–11]. In their study, Carter and Greening [8] explored the impact of four different styles of music that they played in the stables: country music, classical music, jazz music, and rock music. They found different stress and relaxation behaviors according to the style of music. Comparing the impact of the different tracks with each other, they observed more resting behaviors and less alertness and vigilance behaviors when country and classical music were played than when jazz and rock music were played.

The living conditions imposed on horses mean that on a regular basis, they are inevitably confronted with multiple situations that can generate a stressed state. Transport, for example, is clearly recognized as stressful, and several studies have shown it to be a significant stressor, even in experienced horses [12–14]. In addition to these situations, we must consider the occasions where horses are led by humans through environments with unpredictable and random stimuli. Finally, veterinary care is one of the events that generate stress, particularly when carried out in the stall on isolated individuals. All these events, taken separately or cumulatively, can affect the emotional state of the animals. In humans, several teams have demonstrated that listening to music can induce greater relaxation during exposure to stressful events or stop the stress response immediately after exposure [15]. Similarly, in horses, the stress generated by potentially stressful situations such as social isolation, transport, or farriery can be limited by playing Western music [10,16–18], which appears to be an effective solution for improving individual well-being. Regarding cardiac physiology, horses exposed to the playing of the main theme of Forrest Gump displayed a better recovery with a faster return to a basal heart rate after stress compared to horses that were not exposed to music [16,18].

Prolonged and/or repeated states of stress can have harmful consequences in the medium and long term and will jeopardize the well-being of individuals [19]. Improving the management of these situations is a major scientific challenge to improve animal welfare and, in the long term, the safety of human-horse interactions. A more relaxed horse will be less likely to express dangerous behaviors (e.g., defense, escape), thus reducing the risk of accidents. Furthermore, the relationship between a rider and horse is established over time, through repeated interactions and routines. The quality of this relationship is a factor involved in the evaluation of the stressful nature of a situation such as handling or horse van loading [12], and when this relationship is positive, it can be a source of intrinsic rewards for the animals [20]. Thus, limiting negative interactions and reducing the animal's state of stress during routine, recurrent, and necessary interventions (such as transport, veterinary care, etc.) can only be of long-term benefit to the human-horse relationship.

The regular use of music to manage the daily stressful situations encountered by horses inevitably involves the repeated or prolonged exposure of individuals to music. It is therefore essential to address the question of the evolution through time of the calming effect of music on horses to determine if a form of habituation occurs (i.e., a decrease in the efficiency of a stimulus – here the music – induced by multiple exposures to this stimulus).

In the current literature, no study to date has specifically tested habituation to music playing in horses. However, some authors have found that the beneficial effects of music change with prolonged exposure. This is the case, for example, of Stachurska *et al.* [9] in their study on the impact of music when it is played for 5 hours daily in stables. Beyond the calming effect of music through the decrease and stabilization of heart rate, the authors observed a return to the initial cardiac physiology parameters during the fifth month of exposure. Similarly, Wiśniewska *et al.* [11] played music to older horses (over 20 years old) for 3 hours per day in the stable and observed a disappearance of the beneficial effects on cardiac physiology after 3 weeks of treatment. These observations suggest a decrease in the beneficial effects of music that may reflect a habituation phenomenon with extended exposure.

1.1. Study Objectives

With the aim of optimizing the use of music as a stress management tool, this study aims to determine whether music can alleviate horses' reactions to acute stressors and if the daily playing of the same music leads to a decrease in its calming effect. To this end, we compared three groups of 12 horses that were led along a route and were subjected to potentially stressful stimuli (auditory, visual, sudden) for 10 consecutive days. Each group of individuals was subjected to a different experimental condition using headphones: a "music" test condition during which a music track was played continuously through the headphones, a "noise" condition during which pink noise was played continuously through the headphones, and a "no-music" control condition during which the headphones were inactive. The group led in the "music" condition will allow 1) to evaluate the impact of music on the horses' reaction to stressors and 2) to test whether a phenomenon of habituation to music - inducing, therefore, a progressive loss of its calming effect - appears. The group led in the "noise" condition will allow us to test the impact of an auditory track that is not musical. Finally, the group of horses tested in the "no-music" condition will allow us to verify the stressful nature of the route *per se* while being led by a human and to observe whether a decrease in their reactions to the stressors occurs over time.

2. Materials and Methods

2.1. Subjects and Individual Measures and Distribution of Individuals

The 36 horses that participated in the present study were housed in 10m^2 individual stables. They were ridden or taken out to the paddock every day. They were fed daily in the morning, at noon, and at the end of the afternoon with pellets, and some were also provided hay. Water was available *ad libitum* through an automatic waterer located in the stall. It should be noted that the equestrian center did not play any music via the radio.

In order to obtain three comparable experimental groups, their composition was balanced according to the temperament, sex, and age of the individuals. For this purpose, the experimental individuals were subjected to simplified temperament tests [21]: reactivity to an unknown surface, reactivity to an unknown object, and reactivity to suddenness.

We used a semi-random allocation procedure. Random draws were conducted to assign each individual to one of the three experimental groups. Once this was done, we checked the balance of the groups by comparing the mean values of the ages and coordinates of the individual reactions to the unknown surface, unknown object, and suddenness tests.

2.2. Experimental Conditions

The music was played directly into the horse's outer ear using an "audio cap" (see **Figure 1**). This is a traditional earcap equipped with a pair of earphones in front of the pinnae and a pocket to hold a small MP3 player between the two ears. The use of earmuffs is a common practice in horse riding, so the subjects were already familiar with this type of equipment before the study began. However, we familiarized them with the audio cap by fitting it on the horse and playing music for 15 minutes on four occasions prior to the study and checked for the absence of negative or stressful reactions. Additionally, to prevent any potential association between the cap and stress episodes, we also exposed the horses to the cap and music outside the context of stress. To do this, the horses were fitted with the audio cap between two successive trials when they were alone in their home stable.

Finally, as suggested by Wilson *et al.* **[10]**, simple auditory stimulation implies some attenuation of the horse's sound environment, and this noise reduction could partly explain the relaxing effects of music. We therefore included the "noise" condition to ensure that the differences that might be observed in the "music" condition are indeed due to the acoustic and musical parameters of the audio tracks. In this condition, individuals were exposed to pink noise. Pink noise is a random signal with constant energy in the octave bands and is therefore weighted according to the properties of the human audiogram (and, by extension, also those of horses). Comparisons between the music and pink noise conditions would thus allow us to test the contribution of musical characteristics to the effect of music, with potentially a lower stress response in the music condition compared to the noise condition.

2.3. Experimental Situations

The stressful situation we chose was that of leading the horses along a track and subjecting them to unexpected and potentially stressful stimuli. The routes had a mean duration of 6 min 38 s (± 2 min 10 s) and were each composed of five visual and/or sudden stimuli and five auditory stimuli (10 in total). The details of each visual and sudden stimulus are provided in **Table 1**. For the auditory stimuli, we selected animal sounds (a dog or wolf barking, a rooster crowing, a cow mooing or a donkey braying) and human environmental sounds (a whistle, an ambulance siren, a car or truck horn, a motorbike engine starting or a truck engine running). These are short sound recordings (maximum five seconds) that were recorded and kindly made available by Joseph Sardin **[22]**.

It is possible that repeated exposures to the same stressful situation may induce habituation to this type of stress *per se*, thus gradually diminishing its stressful nature. To limit this risk, the stimuli's position and order of appearance were modified from one exposure to another, and different audio stimuli were selected each time. The aim was to prevent the horses from becoming accustomed to the stressful situation, which would prevent the detection of a potential habituation to the music. In addition, for a given stressor type (e.g., throwing a ball), the intensity was gradually increased after each exposure (first exposure: ball thrown 5m in front of the horse, last exposure: ball thrown 3m behind the horse, **Table 1**. For an example, see **Supplementary Material 1**.

Figure 1: Photograph of the audio cap placed on a halter.

2.4. Data Collection and Analysis

All trials were recorded with cameras (GoPro, JVC, or Canon camcorders). Several cameras were used to increase the number of viewing angles. Thus, behavioral observations were conducted retrospectively from the recorded videos using the focal sampling method **[23]**. All observations were carried out blind; i.e., only the experimenter in charge of handling the horses was aware of the assigned conditions for each horse during the experiment.

For our analyses, using the focal sampling method, we recorded the occurrence of the following behaviors during the presentations of the different stimulus categories (visual, sudden, auditory) as well as the duration of the trial (see **Supplementary Material 2** for the description of the different behaviors):

For visual stimuli: alertness; backing away, blowing sound; defecation; gaze towards door; glances; immobility; outward alertness; running away; scratching; sniffing the stimulus objects; sniffing other objects; startled; stopping; trotting.

For sudden and auditory stimuli (ordered by reaction's intensity, see below): orientation of the ears towards the stimulus (1); raising head (2); glances (3); twitches (4); stopping (5); blowing sounds (6); startled (7); backing away (8); turning around (9); running away (10).

To analyze the stress responses to visual stimuli, as the exposure duration depended on the time spent by the individual horse on the route, we calculated the sum of the occurrences of behaviors weighted by the duration of each individual's route. For this purpose, we calculated a coefficient equal to the duration of each individual's trial, divided by the maximum duration achieved by an individual for the same route. We then calculated the mean occurrence of the behaviors displayed during the different routes, weighted by this coefficient.

Table 1: Details of the visual and sudden stimuli along the different routes.

Stimulus	Details
	Visual Category
Unknown surface 1 (SI 1)	Ground tarpaulin (3.5 x 2m) framed by 2 bars
Unknown surface 2 (SI 2)	Ground tarpaulin (3.5 x 2m) framed by 2 bars, with one bar raised by a cube
Framed area 1 (UCPA 1)	Round green carpet (2m diameter) laid on the ground and framed by a 2m high flag (right) and a garden hose (left)
Framed area 2 (UCPA 2)	Round green carpet (2m diameter) laid on the ground and framed by a 2m high flag (right) and a tarpaulin with aluminum foil taped to it, then attached to the wall aluminum attached to the wall
Framed area 3 (UCPA 3)	Round green carpet (2m diameter) placed on the ground and framed by a 2m high flag (right) and a chandelier covered by a tarpaulin with aluminum foil taped to it (left)
Incongruous object 1 (IO 1)	Chandelier covered with a tarpaulin with aluminum foil taped to it
Incongruous object 2 (IO 2)	Passage between IO 1 and two construction site signs
Incongruous object 3 (IO 3)	Passage between two chandeliers surrounded by aluminum
Incongruous object 4 (IO 4)	Two-person tent on the ground
Incongruous object 5 (IO 5)	Two-person tent placed upside down with aluminum foil taped to it
Banner 1 (GP 1)	Orange banner, 7m long creating a 3m wide corridor with the wall
Banner 2 (GP 2)	Orange banner, 8m long in the center of the arena, with two zones marked with studs. Zone 1 = 0 to 2m; zone 2 = 2 to 4m.
Banner 3 (GP 3)	Orange banner, 7m long creating a 2m wide corridor with the wall
Low branch 1 (BB 1)	A 2m bamboo branch suspended 2.5m from the ground by means of two stanchions. Ten 50 cm-long ribbons are attached to each side of the branch
Low branch 2 (BB 2)	A 2m bamboo branch suspended 2.5m from the ground by means of two stanchions. Ten 1m-long ribbons are attached to each side of the branch
	Sudden Category
Flags 1	3 flags of 1m in the center of a construction cone. An experimenter pushes the cone with her foot so that the 3 flags fall in front of the horse, which has been stopped beforehand
Flags 2	Same conditions as "Flags 1," but six ribbons have been attached to the 3 flags
Umbrella 1	Quick opening of an umbrella 3m in front of the horse
Umbrella 2	Quick opening of an umbrella with 20cm-long ribbons at the ends, 3m in front of the horse
Ball 1	Throwing a ball against the arena's boot barrier, 5m in front of the horse
Ball 2	Throwing a ball against the arena's boot barrier, 3m behind the horse

As auditory and sudden stimuli were applied punctually, a simple intensity score was calculated for the analysis of stress responses. To this end, a value was assigned to each behavior in the sudden and auditory categories. Ranging from 1 for ear orientation to 10 for running away, these values increased in incremental steps of 1 according to the order of the behaviors presented above. For example, raising head scores to 2 and turning away scores to 9. In **Supplementary Material 2**, the definitions of the behaviors recorded during the reaction to auditory and sudden stimuli reflected the intensity of the horse response from a simple focus towards the stimulus, to a higher elevation of the head, to the occurrence of body jerks, to a more tense or backward attitude, and ultimately to the flight of the subject.

We also recorded heart rate (HR) which allows the investigation of the activity of the autonomic nervous system (ANS). To achieve this, we used a Polar EQUINE RS800CX heart rate monitor. This tool allows to measure the R-R interval, also referred to as the Interbeat Interval (IBI), which is defined as the time in milliseconds (ms) between two peaks of the R wave of the QRS complex on the trace of an electrocardiogram (ECG). The heart rate measurements started in the stall four minutes before the subject left for the trial and ended in the stall four minutes after it had returned. We considered separately the test phase (i.e., when the horse was being led on the stressful route), the pre-test phase (4 min before leaving the stable), and the post-test phase (4 min after returning to the stable).

2.5. Statistical Analysis

The statistical analyses were conducted using R software version 4.2.1 [24]. None of the variables follow a normal distribution, so they were all statistically analyzed using permutation tests for non-parametric data.

For the analysis of the behavioral variables, the data from 18 of the 36 horses initially subjected to the experiment were analyzed (the videos of the 18 remaining individuals were not exploitable), and the physiological variables (pre-test, test, and post-test heart rate) were analyzed for all individuals (N = 36).

To address the questions concerning the impact of music on the stress state of the horses, we first studied the impact of music on all the data collected during the 10-day experiment. We then extended our analysis within each session to study the impact of music throughout the daily exposure period and explore the potential implementation of a habituation phenomenon.

3. Results

3.1. The Overall Impact of Music

3.1.1. Expression of Stress Generated by the Different Categories of Stimuli

The stress behaviors displayed by individuals during the trials were statistically different between conditions for the visual and sudden stimuli (p = 0.006 and 0.007 respectively) but not for the auditory stimuli (p = 0.4) (see **Table 2**). When comparing conditions for the visual stimuli, the intensity of the reaction was lower for the music condition than for the no-music condition (permutation test, $p < 0.001$), the other comparisons being non-significant (music vs. noise and no-music vs. noise conditions, p = 0.1, post-hoc permutation test). For the sudden stimuli, differences were found between the noise condition and the two other conditions (post-hoc permutation test, music vs. noise, p = 0.004; no-music vs. noise, p = 0.04) while music and no-music conditions did not differ (permutation test, p = 0.2). In the noise condition, horses were more reactive to stimuli that appeared suddenly.

3.1.2. Cardiac Physiology

There was a significant difference between the mean heart rates of individuals during the test and post-test phases (p = 0.005 and 0.001 respectively) depending on the condition, but not for the pre-test phase (p = 0.9) (see **Table 3**). Only the music condition differed from the two other conditions during the test and post-test phases (permutation test, music vs. no-music p_{test} = 0.02 and $p_{post\text{-}test}$ = 0.04; music vs. noise $p_{test} < 0.001$ and $p_{post\text{-}test}$ = 0.005). The mean heart rate was significantly lower in the music condition compared to the non-music condition or the pink noise condition.

3.2. Evolution of Music Impact on Stress Behavior Across Days

To study the potential habituation to music, the behavioral variables (occurrence of stress behaviors and response intensity score) were analyzed day after day.

3.2.1. Visual Stimuli

The evolution of the mean occurrence of stress behaviors generated by the visual stimuli across days is presented in **Figure 2**. The expression of behavioral stress responses only differed between experimental conditions for days 2, 4, and 7 (see **Table 4**). Post-hoc comparisons showed that on days 4 and 7, horses in the music condition expressed significantly fewer stress behaviors than those in the no-music condition (permutation test, day 4: p = 0.01; day 7: p = 0.01). For day 2, stressful behaviors tended to decrease in the music condition compared to the no-music condition (permutation test, p = 0.06). Moreover, there was no difference between the music and noise conditions (permutation test, day 2: p = 0.9, day 4: p = 0.8, and day 7: p = 0.5). This result could reflect an attenuation of the individual's sound environment by the pink noise. Indeed, fewer stress behaviors were observed in the noise condition than in the no-music condition on days 2 and 4 (permutation test, day 2: p = 0.03 and day 4: p = 0.01) and a trend was observed on day 7 (permutation test, day 2: p = 0.06).

3.2.2. Sudden Stimuli

The stress response to sudden stimuli (umbrella, balloon, and flags) across days is presented in **Figure 3**. The only difference in the expression of stress occurred on day 4 (permutation test, p = 0.04, see details in **Table 4**). Post-hoc comparisons showed that the response intensity scores tended to decrease in the music condition vs. the no-music condition (permutation test, p = 0.06, see **Table 4**). Moreover, there was no statistical difference in the expression of stress behaviors between music and noise conditions (permutation test, p = 0.7) or between noise and no-music conditions (permutation test, p = 0.08).

Table 2: Means and standard deviation (±) of stress responses according to the conditions corresponding to the occurrence of stress behaviors for visual stimuli and the response intensity score measured for auditory and sudden stimuli. The significance level was set at 0.05.

	Nb of horses	Visual	Auditive	Sudden
Music	6	6.5 ± 12.5	17.0 ± 20.0	14.2 ± 12.6
No music	6	13.3 ± 24.3	13.2 ± 13.7	16.6 ± 11.8
Pink noise	6	9.6 ± 28.9	15.3 ± 12.5	21.8 ± 15.6
p value		0.006	0.4	0.007

Table 3: Means and standard deviation (±) of the heart rates (in beats per minute) of the individuals during the different test phases and according to the conditions. The significance level was set at 0.05.

	Nb of horses	Pre-test	Test	Post-test
Music	12	35.4 ± 4.2	47.8 ± 8.8	35.8 ± 4.0
No music	12	35.5 ± 3.4	50.8 ± 12.1	37.5 ± 3.5
Pink noise	12	35.6 ± 4.4	50.2 ± 10.0	37.2 ± 3.5
p value		0.9	0.05	0.001

Table 4: Means and standard deviation (±) of stress responses from day 1 to day 10 according to the conditions corresponding to the occurrence of stress behaviors for visual stimuli and the response intensity score measured for auditory and sudden stimuli. The significance level was set at 0.05. Only significant differences are reported.

		Visual			Sudden
	Nb of horses	Route 2	Route 4	Route 7	Route 4
Music	6	3.0 ± 4.6	3.3 ± 3.0	5.3 ± 4.7	10.1 ± 6.7
No music	6	6.1 ± 6.6	13.2 ± 20.6	16.4 ± 25.5	27.0 ± 19.5
Pink noise	6	3.0 ± 2.2	3.0 ± 5.8	6.7 ± 9.7	11.5 ± 7.2
p value		0.03	0.002	0.01	0.04

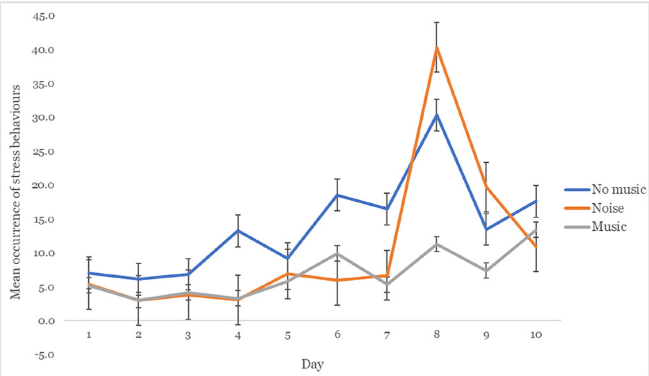

Figure 2: Evolution of the expression of stress behaviors (mean occurrence weighted by the duration of the trial) in response to visual stimuli in the different conditions day after day. Permutation tests, * $p < 0.05$, ** $p < 0.01$.

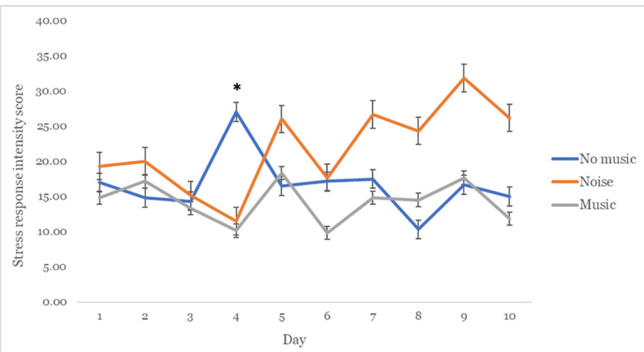

Figure 3: Evolution of the expression of stress behaviors (mean occurrence weighted by the duration of the trial) in response to sudden stimuli in the different conditions day after day. Permutation test, * $p < 0.05$.

4. Discussion

4.1. Benefits of Music Diffusion

In the overall analysis, our results reveal a calming impact of music on the behavioral and physiological components (i.e., heart rate) of horses. This effect is reflected by a decrease in the expression of stress responses of horses to visual stimuli when music was played compared to the absence of music. Interestingly, intermediate levels of stress were observed in case of pink noise, as the behavioral expression in the pink noise condition did not differ from the music or the no-music conditions. Such a gradient of efficiency between the conditions, going from the no-music, the pink noise to the music condition, suggests that the calming effect of music could be explained both by its auditory (such as the pink noise) and musical nature (unlike the pink noise). The auditory stimulation provided by music or pink noise could have distracted the horses from the environmental stimuli (here the visual ones). However, the fact that the behavioral expression differed from the no-music condition only in case of music indeed suggests that the musical features of the music could have an additional calming impact on the horses. The fact that lower heart rates during and after the tests were only observed in the music condition reinforces this hypothesis.

These results confirm previous observations concerning the ability of music to alleviate horses' reactions in a stressful context [16–18] and other studies in non-stressful conditions [8–11]. Moreover, horses faced with a stressful situation had a lower heart rate in the music condition compared to those in the non-music or noise conditions, supporting the hypothesis of a calming effect of music as already found in rodents [7]. Furthermore, we can hypothesize that the beneficial effects of music on a stress state have a long-lasting impact since the effect continued after exposure to the stressful situation.

As regards the difference in responses to sudden stimuli between individuals in the music and no-music condition, the results indicate that music had no effect on the response of individuals to unexpectedness. However, we observed a higher expression of stress for individuals within the noise condition. An explanation could be that pink noise at constant intensity is a monotonous sound that could reduce the alertness of the horse. If this is indeed the case, the horse's reaction when confronted with a sudden stimulus would be stronger than reactions in the no-music condition.

4.2. Evolution of the Effects of Music over Days

The three conditions were tested on routes that were different across days. The calming effect of music was not observed every day, but significant differences were observed between music and no-music conditions on day 4 and day 7 specifically. This result suggests that no habituation occurred until after at least seven exposures. Furthermore, the stress response curves for the visual stimuli had a sawtooth progression that was present for all conditions **(Figure 2)**, showing that we succeeded in limiting habituation to the stressful situation. It is however important to note that music and noise conditions led to similar responses. Conversely, the stress response to sudden stimuli seems to decrease with each exposure, suggesting habituation to this category of stimuli. While some individuals faced the stimuli despite their apprehension, others adopted a strategy of sensory subtraction, i.e., they stood still during the walks and turned away (in terms of their gaze, their attention, and sometimes even their entire body) from the stimulus that the experimenter was inviting them to encounter. As the variations in heart rate due to the presentation of the different stimuli were punctual and the strategies adopted by the horses in response to stress were multiple, a more targeted analysis of the heart rates at the precise moments of the presentation of each stimulus should therefore help us to clarify our question of the impact of music on the individual's state of stress and identify learning phenomena such as habituation that could be involved as a consequence of its use.

5. Conclusion and Perspectives

This study showed that the use of music did not interfere with awareness of and response to environmental noise, which is crucial in case of real danger. The lack of a calming effect in the specific case of auditory stressors could be explained by the fact that auditory stressors might have directly disrupted the perception of the auditory features of the music or pink noise because they are all perceived via the same sense (i.e., hearing). Indeed, as auditory stressors were sudden and intense sounds – such as the sound of a sports motorbike engine or a car horn – they were probably perceived and processed as a priority by the horse in order to face the potential threat they represent. In other words, the attentional focus might have switched from the music or pink noise to the auditory stressors.

The stressful situation we chose was leading the horses along a track on which they were subjected to unexpected and potentially stressful stimuli designed to simulate real situations encountered by domestic horses. Indeed, in their daily life, domestic horses are confronted with the sudden appearance of objects of all shapes and colors; they may come across cars that have varying levels of noise and speed with drivers who may honk their horns or dogs that may show aggression and bark, etc. **[12–14]**. All of these situations may lead to the expression of fear, and to flight responses that cause many accidents involving both the horse and humans in the vicinity. Thus, our results can contribute to making human-horse interactions safer by limiting the stress of horses within their environment.

Regarding habituation to music for managing daily stress, we have shown that this tool continues to reduce the behavioral expression of stress even after seven consecutive exposures. However, a clear significant effect was not found every day. It could be explained by slight variations in the intensity of the stimuli perceived by the horse, especially as our experimental situations were not designed to be highly intense but mild, making day-to-day comparisons less powerful than the global analyses discussed earlier. It is also possible that the use of various music tracks could have had a higher and more consistent effect. Moreover, continuous playing of music in the living environment has been shown to lose its calming effect over time **[11]**. It suggests that playing music continuously throughout the day, as often seen in stables, for instance, might be counterproductive. Altogether, those elements that playing music could indeed be used to mitigate the negative impact of acutely stressful situations, especially when they possess a visual component. However, to keep the music's efficiency, it should be used parsimoniously to target time-limited events. Further investigation is needed to understand the underlying perceptual and emotional mechanisms of the impact of music on horses and to ensure safe and consistently efficient use on the field.

Supplementary Materials

Supplementary Material 1 includes pictures depicting the evolution of the "framed surface" stimulus in the different routes, ranging from the 1st version (on the left) to the 3rd version (on the right). **Supplementary Material 2** provides definitions of the various behaviors.

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Authors' Contributions

M.V., O.P., and C.E. designed the experiments; C.E. and M.C. conducted the experiments; C.E., M.V., O.P., and O.A. discussed the results and interpretations; O.P., C.E., and M.V. wrote the manuscript.

Data Availability

Data are available upon request from the authors.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

Ethical Approval

All the experiments undertaken in this study complied with the ethical standards of French research practices. The authorization number is AL/25/09/18/02 for the Regional Ethical Comity for Animal Experimentation (CREMEAS). Neither procedure adversely affected the horses in the short term or for the overall period of the study. Additionally, the authors confirm that the study has followed the guidelines of the Declaration of Helsinki.

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