Journal of the American Association for Laboratory Animal Science
Copyright 2018
by the American Association for Laboratory Animal Science

Vol 57, No 6
November 2018
Pages 695–697

Effects of Music Enrichment on Individually Housed Male New Zealand White Rabbits

Jessica L Peveler* **and Debra L Hickman**

The beneficial effect of music has been demonstrated in many species. Although commercially available music CDs intended for use with rabbits are available, these enrichments have not been critically evaluated to determine whether they reduce distress. In this study, we used an aging colony of male rabbits to evaluate how the provision of music enrichment affected the wellbeing of the colony. After 6 mo of music enrichment, the rabbits in the colony demonstrated a significant decrease in fecal cortisol, suggesting that their stress was reduced. Six months after removal of the music enrichment, the rabbits demonstrated a significant increase in fecal cortisol and the heterophil:lymphocyte ratio, suggesting that they were stressed. These findings suggest that music enrichment with a commercially available music discs for rabbits can be used to improve the wellbeing of animals used in biomedical research.

DOI: 10.30802/AALAS-JAALAS-17-000153

Although animals' hearing abilities and perceptions may differ vastly among species, the beneficial effects of music exposure have been demonstrated in many, including humans,[11] dogs,[9] cats,[12,15] pigs,[2] rats,[2,14] mice,[18] chickens,[4] fish,[8] and elephants,[10] and cows.[6] Classical (or similarly modulated) music has been shown to decrease anxiety measures in several species. In humans, 'pleasant' music activates autonomic responses of the hypothalamus, including heart rate and respiration, as well as reward circuits in the brain in absence of any explicit rewards.[11]

Dogs in rescue shelters demonstrate more 'relaxation behaviors' when classical music is played ambiently.[17] Canine patients in a veterinary hospital had a reduction in visual measures of discomfort (restlessness, anxiety, and respiration rates) when they heard harp music.[3] Compared with white noise and minimalist music, rats exposed to Mozart completed a learning maze faster and with fewer errors.[14] A recent review noted many examples of studies where music decreased measures of stress and increased learning and neural reward centers in rodents.[2]

The theory guiding the development of species-specific music involves an attempt at finding an external rhythm (of the music) that most closely matches an animal's internal patterns (circadian rhythms, brain waves, breathing, and heart rates). Proponents of this theory hypothesize that calm frequencies from an appropriate entrainment discharges the nervous system, helping an animal or person relax.[10]

Owners of pet rabbits report positive effects for their pets that have been exposed to commercially available music CDs designed for this species. However, no controlled studies have been reported about the effects of music on rabbit behavior. Because music can be a simple, inexpensive way to enrich laboratory animals, with little anticipated deleterious consequences,[2] we hypothesized that the outcomes would be similar to that of other captive prey species (such as rodents and chickens) and that musically enriched rabbits would demonstrate improved wellbeing, manifested as lower cortisol and stress leukogram levels.

Received: 20 Nov 2017. Revision requested: 22 Jan 2018. Accepted: 15 Mar 2018.
Indiana University School of Medicine, Indianapolis, Indiana
**Corresponding author. Email: JessicaRVT@gmail.com*

Materials and Methods

The Indiana University School of Medicine housed 5 male New Zealand White rabbits (Western Oregon Rabbit, Philomath, OR) for a dietary longevity study. All parts of this study were conducted under a protocol reviewed and approved by the school's IACUC. Rabbits were single-housed, fed a commercial diet (Teklad Global Rabbit Diet 2030, Envigo Madison, WI), daily received blocks of Western timothy or alfalfa hay (Oxbow Timothy and Alfalfa, Omaha, NE) and filtered municipal (Indianapolis, IN) water through an automatic system, and were exposed to a 12:12-h light:dark cycle. Pan liners were changed 3 times each week, and racks were washed weekly. Three conventional racks were rotated through for change-outs (Double Wide Comfort Cage, 18 in. high with 5.2 ft^2 per compartment, Lenderking, Millersville, MD; Euro Rabbit Housing, 17.7 in. high with 5.0 ft^2 per compartment, Allentown Caging, Allentown, NJ; and Six-cage Plastic Rabbit Unit, 18 in. high with 5.1 ft^2 per compartment, Allentown Caging). The colony was known to be positive for *Pasteurella multocida*.

Enrichment consisted of one of a variety of cage toys (Bio Serv, Flemington, NJ), rotated weekly, and a half hour of exercise time in a floor pen 1 d each week. This rabbit colony was known by staff to be aversive to change, especially changes in the housing environment and the presence of new people. Because of their advanced age (approximately 5 y) and dietary study restrictions, these rabbits were individually housed, with few additional enrichment opportunities. Because of these limitations, these rabbits were prime candidates for measuring how enrichment of the macroenvironment can decrease distress. All procedures (including the individual housing of adult rabbits) were reviewed and approved by the IU School of Medicine IACUC prior to initiating the current study.

We evaluated the long-term effects of music by using an A-B-A application of music enrichment. According to their dietary study requirements, monthly physical exams were performed, with blood drawn every 6 mo. Because the rabbits

Table 1. Hematology results (mean, $n = 5$)

	WBC ($\times 10^3/\mu L$)	Heterophils ($\times 10^3/\mu L$)	Lymphocytes ($\times 10^3/\mu L$)	H:L ratio
Baseline	5.1	2280.6	2396.2	0.9
Music enrichment	5.4	2315.2	2523.8	0.9
Music removed	5.4	3380.6	1561.6	2.9
Standard error	0.6	483.2	223.4	0.7

were housed long-term without the benefit of music therapy, the 6-mo blood collection immediately prior to initiation of music enrichment served as a baseline (A phase). Music enrichment was provided for 6 mo, and then the next blood collection was performed (B phase). Rabbits then returned to housing without the benefit of music and were reevaluated at the end of another 6 mo (A). Thus, each rabbit in the study served as its own control before and after music enrichment.

Soothing music from a commercial music disc (Pet Melodies Rabbit Edition, Pet Rhythms Research Institute, played on a model CFD-S50 Personal Audio System, Sony, New York, NY) was provided Monday through Friday, during the workday, generally from 0700 until 1600, according to the *Guide*, which recommends that radios be turned off at night).[7] Volume was set at a comfortable listening level for humans, where one does not need to talk loudly to be heard over the music.

Every 6 mo, blood was drawn from the auricular artery. Approximately 30 min prior to collection, rabbits were sedated with acepromazine (0.3 mg/kg SC) and butorphanol (0.75 mg/kg SC). In addition to these routine samples, we collected feces from the cages for cortisol processing. Feces were collected the morning after physicals and blood samples were performed. Blood was sent to a reference lab (Antech Diagnostics (Fishers, IN)) for CBC analysis, and fecal cortisol was measured by Charles River RADS (Wilmington, MA). Normal reference ranges were provided by the respective labs. Parameters assessed for this study included the total WBC count, total heterophil count, total lymphocyte count, neutrophil:lymphocyte ratio, and fecal cortisol.

Statistical analysis. All statistical analyses were performed by using JMP 10.0.0 software (SAS Institute, Cary, NC). The measured parameters were analyzed by using one-way ANOVA with Tukey posthoc tests applied as needed. Summary data are expressed as mean ± 1 SE, and a P value of less than 0.05 was considered to be statistically significant.

Results

Neither mean total WBC count nor mean total heterophil count differed between treatment groups (WBC, $F_{2,12} = 0.1120$, $P = 0.8950$; heterophils, $F_{2,12} = 1.6747$, $P = 0.2283$). For the total lymphocyte count ($F_{2,12} = 5.4735$, $P = 0.0205$), the mean total lymphocyte count for the rabbits at the end of the study (6 mo after music enrichment ceased) was significantly decreased as compared with baseline ($P = 0.0065$). Overall the heterophil:lymphocyte ratio did not differ as compared with baseline ($F_{2,12} = 2.7246$, $P = 0.1058$), but the mean ratio after music enrichment was greater than before and during music enrichment ($P = 0.0378$). The CBC data are provided in Table 1.

Fecal cortisol levels were well above reference ranges during all 3 phases of this study. When music was provided, fecal cortisol decreased significantly ($P = 0.0024$) from baseline (Figure 1). After music enrichment had been absent for 6 mo, fecal cortisol rose significantly ($P = 0.0466$) compared with levels when music was provided (Figure 1).

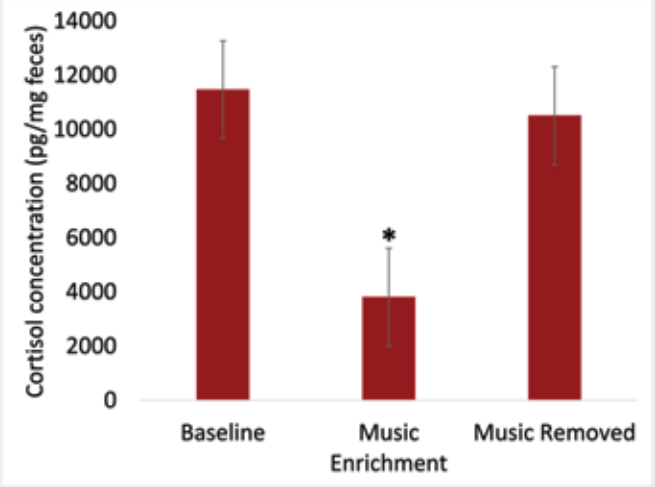

Figure 1. Fecal cortisol levels (mean ± SE; $n = 5$). *, Value differs significantly ($P < 0.05$) from baseline (before enrichment). Laboratory reference range, 156 to 1000 pg/mg feces.

Discussion

Although providing music enrichment might have increased stress in rabbits, a more likely failure of this study was expected to be the inability to capture any effect. Our animal set was small, but the study was strengthened by each animal serving as its own control before and after the music enrichment phase.

CDs were an ideal source for the music in our study because we could carefully control the sounds. Programming from radio stations is an uncontrollable variable, and in basement vivariums (such as ours), radio signals do not penetrate into the rooms. Music was provided on weekdays only to ensure the provision of music enrichment for most of the day; this limitation likely will occur at many institutions. Although the weekend staff might be able to start CDs, no one (because of weekend work schedules) would be available to turn it off at the end of the day. In addition, timers were impractical for our situation, given that CD players frequently are unplugged to allow for cleaning (with a water hose), thus requiring reprogramming each time. Furthermore, timers could be set to power on the stereo, they would be ineffective for starting they playing of a CD and setting the player to the repeat mode. Playing music during weekdays only enabled us to control the amount of music actually provided.

Volume levels are important when considering noise exposure to captive animals; lab animals cannot escape music if they do not find it enriching. Loud noise leads to increases in cortisol as well as adverse reproductive outcomes in several species.[8] Noises created by humans are usually louder and more frequent than the natural noises animals evolved to;[8] music enrichment is sometimes considered to provide benefit as a buffer to mask other foreign sounds. Music that is too loud no longer provides the benefit of 'passive listening,' during which harmonic

patterns increase relaxation and preserve mental energy.[10] In addition to the inherent benefits of soothing music, it can establish a classic Pavlovian response, where animals associate it with positive experiences or environments.[2]

This study suggests that music enrichment benefits rabbits. Although the study population was small and included males only, we were able to demonstrate an effect on cortisol levels when these rabbits were provided soothing music and a reversal when that enrichment was stopped. Whereas measuring stress by using serum cortisol can be confounded by a temporary spike due to handling-associated stress, measuring levels in the feces avoids the effect of handling-associated stress and represents a long-term value.[1,13,16] In addition, our rabbits demonstrated an increase in the heterophil:lymphocyte ratio when the music was removed, suggesting the animals were stressed.[5] All of the rabbits in our study maintained cortisol levels that exceeded the normal reference range, suggesting that the animals were in a state of chronic stress, which not completely ameliorated by music. No obvious other variables were noticed during this study (construction, personnel changes,), but their potential effect cannot be discounted. Acute changes, if present, are unlikely to modulate chronic stress; the correlation of our results with our study timeline gives us confidence in our interpretation. Further investigations into the effects of music on rabbits' physiologic and behavioral wellbeing are warranted.

Rabbits are a complex mammalian species, and each animal responds differently. To best cope with the rigorous demands of a laboratory setting, a comprehensive enrichment strategy would be more appropriate than any single intervention. Still, music enrichment has the benefits of minimal cost, easy application, and lack of demonstrable negative effects that endorse it as worthy first step.

Acknowledgments

We thank Johns Hopkins' Center for Alternatives to Animal Testing for funding this study.

References

1. **Adina B, Bodnariu A, Nichita I, Cristina R**. 2012. Stress in laboratory juvenile rabbits: physiological indicators. Scientific Papers: Animal Science and Biotechnologies **45:** 142–145.
2. **Alworth LC, Buerkle SC.** 2013. The effects of music on animal physiology, behavior, and welfare. Lab Anim (NY) **42:**54–61. https://doi.org/10.1038/laban.162.
3. **Boone A, Quelch V.** 2003. Effects of harp music therapy on canine patients in the veterinary hospital setting. Harp Therap J **8:**4–5, 15.
4. **Dávila SG, Campo JL, Gil MG, Prieto MT, Torres O.** 2011. Effects of auditory and physical enrichment on 3 measurements of fear and stress (tonic immobility duration, heterophil to lymphocyte ratio, and fluctuating asymmetry) in several breeds of layer chicks. Poult Sci **90:**2459–2466. https://doi.org/10.3382/ps.2011-01595.
5. **Davis AK, Maney DL, Maerz JC.** 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol **22:**760–772. https://doi.org/10.1111/j.1365-2435.2008.01467.x.
6. **Godoy M.** [Internet]. 2014. Moo-d music: do cows really prefer slow jams? [Cited: 8 November 2016]. Available at: http://www.npr.org/sections/thesalt/2014/03/06/285314648/secret-life-of-cows-part-deux-milking-mood-music.
7. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
8. **Kight CR, Swaddle JP.** 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol Lett **14:**1052–1061. https://doi.org/10.1111/j.1461-0248.2011.01664.x.
9. **Kogan LR, Schoenfeld-Tacher R, Simon AA.** 2012. Behavioral effects of auditory stimulation on kenneled dogs. J Vet Behav **7:**268–275. https://doi.org/10.1016/j.jveb.2011.11.002.
10. **Leeds J, Wagner S**. 2008. Through a dog's ear: using sound to improve the health and behavior of your canine companion. Boulder (CO): Sounds True.
11. **Menon V, Levitin DJ.** 2005. The rewards of music listening: response and physiological connectivity of the mesolimbic system. Neuroimage **28:**175–184. https://doi.org/10.1016/j.neuroimage.2005.05.053.
12. **Mira F, Costa A, Mendes E, Azevedo P, Carreira LM.** 2016. Influence of music and its genres on respiratory rate and pupil diameter variations in cats under general anaesthesia: contribution to promoting patient safety. J Feline Med Surg **18:**150–159. https://doi.org/10.1177/1098612X15575778
13. **Nemeth M, Pschernig E, Wallner B, Millesi E.** 2016. Noninvasive cortisol measurements as indicators of physiological stress responses in guinea pigs. PeerJ **4:**1–21. https://doi.org/10.7717/peerj.1590.
14. **Rauscher FH, Robinson KD, Jens JJ.** 1998. Improved maze learning through early music exposure in rats. Neurol Res **20:**427–432. https://doi.org/10.1080/01616412.1998.11740543.
15. **Snowdon CT, Teie D, Savage M.** 2015. Cats prefer species-appropriate music. Appl Anim Behav Sci **166:**106–111. https://doi.org/10.1016/j.applanim.2015.02.012.
16. **Wasser SK, Hunt KE, Clarke CM.** 2002. Assessing stress and population genetics through noninvasive means, p 130–144. In: **Aguirre A**, **Ostfeld RS**, **Tabor GM**, **House C**, **Pearl MC**, editors. Conservation medicine: ecological health in practice. New York (NY): Oxford University Press.
17. **Wells DL.** 2004. A review of environmental enrichment for kennelled dogs, *Canis familiaris*. Appl Anim Behav Sci **85:**307–317. https://doi.org/10.1016/j.applanim.2003.11.005.
18. **Ying H, Lan-Wen X, Fei Y, Ping Y.** 2007. The effects of enrichment with music or colorful light on the welfare of restrained mice. Laboratory Animal and Comparative Medicine **27:**71–76.