VIRTUTIS INCUNABULA

Vol. 06, No. 1 (2019)

Evaluation of the Effects of Musical Styles and Speed on the Anxiety Levels of Stress-Induced Mice (*Mus musculus*)

Kathleya Faith H. Cobing[1], Mary Clarice D. Dajay[1], Carol Anne A. Dagu-ob[1], Edgar Philip T. de Asis[1], Isabel G. Henares[1], Pamela Joy B. Lagansoa[1], and Jude Xerxes M. Herbolario[1]

[1] Senior High School Department, University of Negros Occidental-Recoletos, Incorporated, Bacolod City, Philippines

ABSTRACT

Music has anecdotally been used to affect mental conditions in humans, and literature suggests that they affect animal physiology and biochemistry similarly to humans. This study subjected mice to selected musical compositions in three genres to test their effects on anxiety levels. Researchers exposed three groups of three mice to classical, pop, and hard rock music with one control group to determine the effect of music on the anxiety level of mice. After a 5-day conditioning period for all groups, researchers subjected each mouse to a stress tube model for 10 minutes. Each mouse was then exposed to one musical genre and tested on an elevated plus-maze. Anxiety was measured in frequency of open arm entry and percent duration in open arms. Results showed that exposure to classical music led to the most significant decrease in anxiety levels and hard rock music to the most significant increase. Pop music did not affect anxiety in mice compared to a control. After examining the difference between classical and hard rock music, researchers identified that multiple contrasting tempos affected performance in the elevated plus maze. The tempo of the classical music piece was altered to double and quadruple its original tempo, and results showed that faster tempos increased anxiety levels and created the gap between classical and hard rock music. Future studies should explore pulsation and rhythmic pattern variances that may contribute to the phenomenon.

Keywords

anxiety, music, mice, elevated plus-maze, neuropsychology, experimental research, Philippines

INTRODUCTION

Music as an art of sound expresses ideas and emotions in significant forms through the elements of rhythm, melody, harmony, and color. Exposure to music has been shown to strengthen inherent neural firing patterns that are as well utilized by spatial-temporal tasks (Rauscher, 2006). Although neuropsychology continues to explore the role of musical processes in brain function, music has already been proven to affect brain plasticity to positively impact the neural networks of musicians and allow a

better understanding of brain development on non-musicians (Peretz & Zatorre, 2005).

Music can reduce anxiety. Niehues da Cruz et al. (2011) demonstrated that mice treated with an anxiolytic drug and exposed to classical music exhibited lower anxiety levels than those treated with the drug alone. This effect demonstrates the potential for human applications, as humans derive various physiologic and psychological benefits from listening to music (Howell et al., 2003). Music is already strategically used to manage the anxiety of people waiting to be attended to in office and medical settings for short amounts of time (Cooke et al., 2005) and control the perceptions of pain among patients during or immediately after a minimally invasive procedure (Angioli et al., 2014).

Although listening to music is a complex process for the brain and requires several stimulations on areas of the brain involving perception, memory, emotion, and performance (Peretz & Zatorre, 2005), music has even been shown to improve memory and cognition in mice models. The changes in physiology, cognition and brain chemistry, and morphology induced by music have been studied in mice, providing evidence that music may affect the behavior of animals similar to humans (Alworth & Buerkle, 2013). Classical music, in particular, influences the physiology and behavior of captive animals (Chikahisa et al., 2007). Classical music has been demonstrated to improve the welfare of laboratory mice (Falkenhorst, 2013). Stephens (2010) demonstrated that mice exposed to classical and rock and roll music had 80% and 45% better memory performance, respectively, compared to control (silence) mice. Rats exposed to Mozart completed mazes more rapidly (Rauscher et al., 1998), suggesting that music can improve spatial learning. Kour et al. (2012) proved that classical Indian instrumental music is a cost-effective, easily reachable remedy in relieving the negative effects of stress-induced cognitive impairments.

Literature suggests that music induces neuropsychological effects. Behavioral changes can be observed when music is subjected; past studies also indicate that music can reduce anxiety. Humans derive psychological and physiological benefits from listening to music, including reduced anxiety, pain relief, and decreased stress measures such as blood pressure and heart rate. However, little is known about the effect of each of these genres and its potential for environmental enrichment in biomedical research laboratories (Howell et al., 2003). This study was designed for modulating the general impression that music has positive effects on human cognition and gives a general background on what music does to a specific state of mind, such as anxiety. This research establishes a distinction on the neuropsychological effects of the varying genres of music as measured by the performance of mice on the elevated plus maze test.

Furthermore, this study was conducted to evaluate the impact of exposure to music on the anxiety level of mice, as applied to humans. Mice (*Mus musculus*) exposed to music are observed through a device called the plus-maze that served as the medium in which their behavior can be observed after being exposed to varying genres of music (Walf & Frye, 2007). Rhythmic pulsations, tempo, and melody are among the most observed contrasts between the genres of music, and researchers accounted for these variables in providing a post-experiment. In addition, researchers manipulated the tempo of the music after the determination of the musical genre with the most significant reduction in stress indicators among test subjects to contrast results and give an underlying understanding of the phenomenon.

METHODOLOGY

The study was conducted for a total of four weeks, including the preparation of the materials necessary,

the regulation of the venue for the experiment, and the conditioning of the test subjects. The most important parameter to be used in this study is the frequency of the open arm entries and the duration spent on the open arm. Other behavioral scores such as closed arm entries, duration on the closed arm entries, central square entries, grooming, rearing, urinating, and defecating are recorded as well but do not significantly affect the results and findings of the study. This study is limited to giving the validated and interpreted results of the performance of the mice exposed to the three genres and does not extend to explaining the technicalities in the genres chosen to explain the phenomenon.

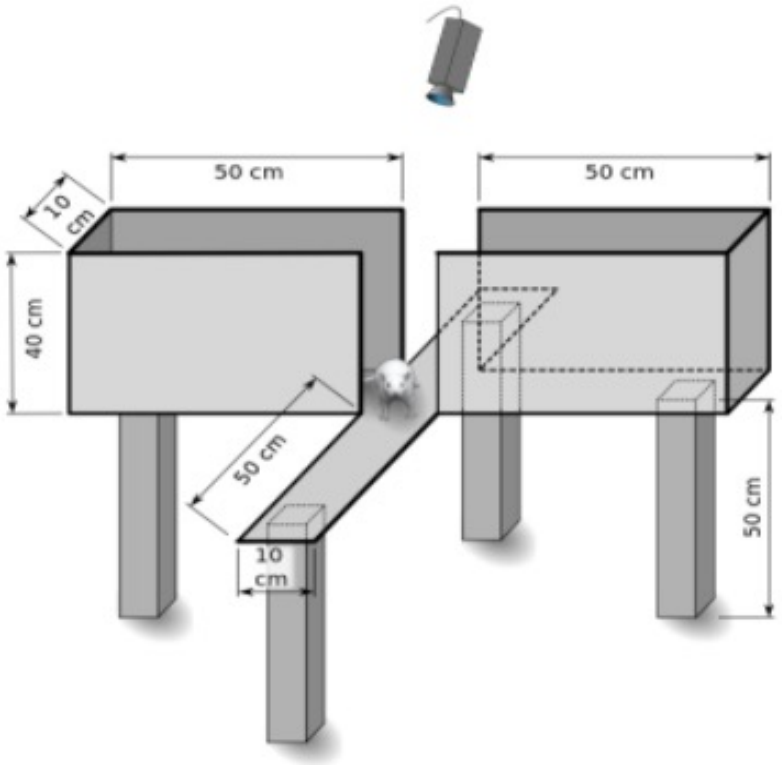
Materials

The master list for all the components used for the completion of the study presents 28 male *Mus musculus* (16 for the initial experiment and 12 for the tempo test), the Animal Housing (5 mice cages, sawdust bedding, water dispenser, food container), Elevated Plus Maze, Sound Level Meter, Sound System (speakers, laptop, and downloaded songs) and two Cameras (placed on each arm of the maze).

Animal Housing

Test subjects were 28 sexually mature male common laboratory white mice (*Mus musculus*) (Lister, 1990), sexually mature, 2 to 5 months old during the duration of the experiment, each weighing between

Figure 1
Elevated plus maze dimensions.



15 and 30g. Mice were housed in groups of six in a cage measuring 250cm^2 with 2-cm deep bedding (Australian Animal Research Review Panel, 2012) and kept in a 12-hour light/dark cycle (O'Reilly et al., 2006). Food and water were provided for each cage ad libitum (Hatori et al., 2012). Mice were conditioned for seven days after transferring to housing prior to experimentation. As per regulation, the minimum floor area of the housing cage should be 250cm^2 (AARRP Guideline, 2012). In addition, the bedding should be 2cm deep in minimum to allow the mice to dig or burrow. To minimize the time of intervention or disruption in the daily routines of the mice, a conditioning period of seven days after transferring the mice to their housing before the experimentation period allows them to get accustomed to the new environment. In addition, this period avoids unprecedented anxiety for the mice, which can affect the study results.

Apparatus: The Elevated Plus Maze

The elevated plus maze was used to measure the anxiety level of the mice (Dawson & Tricklebank, 1995; Handley & Mithani, 1984; Montgomery, 1955; Walf & Frye, 2007). Mice are placed at the junction of the four arms of the maze, facing an open arm, and a video-tracking system records entries/duration in each arm. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior (Walf & Frye 2007).

It consists of two open (50x10 cm) and two closed (50x10x40 cm) arms that originated from a single platform (10x10) to form the plus sign (Tiwari et al., 2012). The plus maze was raised 50 cm from the floor to the central platform by support on each arm of the maze. Four fluorescent lights were placed 100 cm above the maze and were arranged like a cross to illuminate the area. A camera was placed vertically directly above the maze to record mice activity.

This study also considered the efficacy of the elevated plus maze to observe anxiety levels. This plus maze is a widely used behavioral assay for mice and has been validated to assess the anti-anxiety effects of brain regions and mechanisms after being treated under external environmental exposures or pharmacologically altered drugs that result in underlying anxiety-related behavior (Carobrez & Bertoglio, 2005; Mulder & Pritchett, 2004). In addition, Montgomery (2007) described the elevated plus maze as a simple method for assessing the anxiety responses of rodents.

The anxiety behavior of rodents can be assessed based on the ratio of time spent on the open arms and the time spent on the closed arms. Other behavioral assays assess anxiety responses based on the presentation of stimuli such as electric shock, food/water deprivation, loud noises, exposure to predator odor, and others to produce a conditioned response. The elevated plus maze depends upon rodents' proclivity toward dark, enclosed spaces and an unconditioned fear of open spaces (Dere et al., 2002).

Face validity of the elevated plus maze has been observed as the basis for anxiety reaction (Hogg, 1996). The elevated plus maze has face validity or the ability to appear to measure what it is supposed to measure. In this plus maze, the anxiety or fear of open spaces/heights of rodents are measured (Carola et al., 2002). Because open arms are avoided, and rodents spend the majority of the time in this task in the closed arms of the maze (Carobrez & Bertoglio, 2005).

Sound System

Speakers were put on 122-cm stools from the animal cages, and the sound pressure level (SPL) from the cages was within 65-70 decibels (Rauscher et al., 1998). A sound level meter was used to confirm the SPL.

Stress-Induction Protocol

To induce stress on the mice, each mouse was subjected to a stress tube (Zimprich et al., 2014) consisting of a 50-mL syringe tube with a cap that replaced the plunger. The movement was further restricted using a hole where the tail of the mice slipped out (Kim & Han, 2006). The mice were left undisturbed under an opaque box (25 × 12 × 8.5 cm) for 30 minutes. After the restraint period, the mice were transferred to their house cage and were then exposed to music.

Exposing the Test Subjects to Music - Song Selection

This study used three musical genres adapted from Ginocchio (2008). Classical music is music derived from the Western Classical tradition of composition and performance that commonly uses an orchestra, piano, and highly defined voices. Pop music is characterized by simple, catchy, and memorable melodies generally accompanied by acoustic guitar and occasionally with a synthesizer, lending a generally lighter and less stressful quality of sound that is radio-friendly. Finally, hard rock music is blues-based music characterized by strong riffs and hooks with major keys and chordal fifths instead of full chords, heavy bass and drums, and the distortion of guitar and vocal sounds.

Four house cages containing the test subjects were numbered accordingly and were assigned a music genre. Test subjects in each cage were exposed to one assigned music genre for 10 minutes. "Allegro con spirito of Mozart's Sonata for Two Pianos in D. Major (K. 448)" was used for the classical music group (Rauscher et al., 1998). "Relax" by Frankie Goes to Hollywood and "Slave to the Rhythm" by Grace Jones (Warner, 2017) was used for the pop music group. "Back in Black" by AC/DC and "Cyanide" by Metallica (Stephens, 2010) were used for the hard rock group. The control group was not exposed to any music after the stress-induction protocol but was kept in the same environment as other home cages. Researchers based the song selections on the previously studied matter about the learning competencies of mice when exposed to Allegro con spirito of Mozart's Sonata for Two Pianos in D. Major (K. 448) (Rauscher et al., 1998). For Pop music, researchers have based it on the songs produced by Trevor Horn, the most successful British pop record producer of the early 1980s (Warner, 2017). As for hard rock, researchers have based it on its definition provided by Ginocchio (2008).

Manipulating Tempo

This experiment was done once the most effective music genre to alleviate stress was determined. Twelve mice were purchased, divided into two groups, six each – one group exposed to classical music with the tempo doubled, and the other on classical music with the tempo quadrupled. The same methodology as for the course of the experiment was observed.

Behavioral Testing and Data Gathering

The subjects were put individually into the elevated plus maze for five minutes during the dark phase (09:00 - 17:00 h), the period wherein rodents are most active (Prut & Belzung, 2003). Each test subject was placed at the central platform of the maze, facing an open arm. The test lasted five minutes, during which an observer seated 1 m away from the maze recorded the mouse's behavior on the maze. The percentage of open arm entries and percentage open arm time were used as indices of anxiety. Risk assessment behaviors were also observed, such as frequency of head dipping, stretch attend posture, rearing, grooming, and urination and defecation (Bindra & Thompson,

1953; Hall, 1934).

Mice are naturally afraid of open and elevated areas; they tend to enter less in the open arms and have a shorter amount of time spent on the open arms as compared to the results obtained in the closed arms when they are allowed to explore the maze within the allotted time (Pawlak et al., 2012; Pinheiro et al., 2007). Conversely, less agitated mice would spend a longer period at the open arms and have a higher frequency of open arm entries (Blanchard, 2001).

Data to be analyzed in this research study is termed as "behavioral scores" (Anseloni & Brandão, 1997; Espejo, 1997), which include: a.) Open arms entry - the number of times all four paws of the test subject were in the open arm zone b.) Closed arms entry - the number of times all four paws of the test subject were in the open arm zone, c.) Open arm and closed arm duration - the amount of time the test subject spent on each arm, d.) Center square entries - the frequency in which all four paws entered the center square, and the risk assessment behaviors, a.) Head dipping - the number of times the mouse lowered its head towards the floor (over the open arms' sides) b.) Rearing - the number of times the mouse stands on its hind legs or leans against the walls of the maze with its front paws, c.) Grooming, d.) Urinating, and e.) Defecating. Simultaneously, a video camera placed above the maze records the whole procedure. A mouse was considered to enter the arm when all four paws are on the arm. The plus maze is cleaned thoroughly after every trial using damp and dry towels.

Calculations/Statistics

Duration and frequency of both open arm and closed arm entries are recorded. This parameter is used for both the difference in genre set up and was measured in four replicates, while six replicates for each group were used for the tempo manipulation set-up. The average of the frequency the mice entered the open arm and the average of the open arm time are used as the standards for measuring anxiety (Tiwari et al., 2012). The anxiety behavior of rodents can be assessed using the ratio of time spent on the open arms to the time spent on the closed arms (Pellow et al., 1985). The elevated plus maze relies on the rodents' proclivity toward dark, enclosed spaces and an unconditioned fear of open spaces (File et al., 1994; Walf & Frye, 2007).

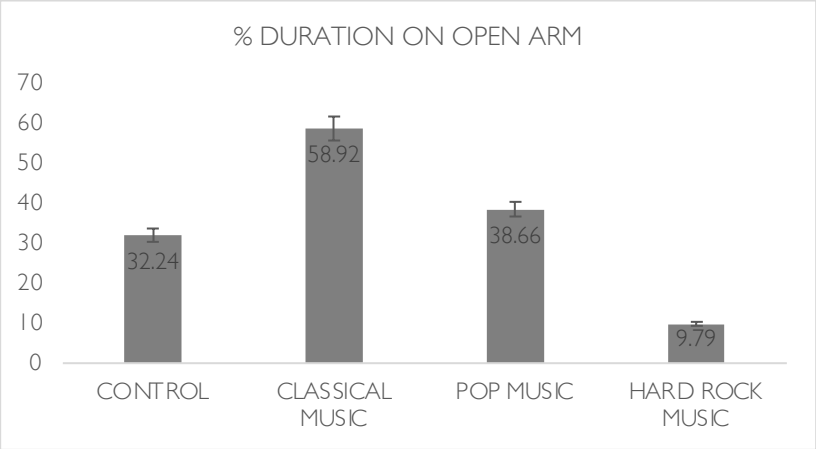
All values were given in mean±SEM. The data gathered was analyzed using the Independent Samples T-Test. An alpha level of 0.05 was used to define statistical significance.

RESULTS AND DISCUSSION

The mice's open arm entries were significantly more frequent when exposed to classical music, slightly recurrent when exposed to pop, and significantly shorter and less frequent when exposed to hard rock. Results showed that music with a slower tempo caused the mice to be less anxious and, therefore, willing to venture outside of their instincts. The duration of the mice on the closed arm was also significantly higher when the subjects were exposed to hard rock. This implies that hard rock music caused the mice to feel exceedingly anxious compared to their normal behavior, as shown in the control group. The heightened anxiety of the mice was most likely caused by the high tempo and spontaneity of the hard rock genre. However, for classical music, subjective anxiety was significantly decreased because of the slow tempo and calm music applied to their environment. Classical music has already been proven to lower the heart rate and systolic blood pressure of those exposed to stress. This can lead to decreased activity of the adrenal glands, and it can allow the subjects to return to their normal functions before their exposure to the stressor (Knight & Rickard, 2001).

The percent duration on open arms was significantly

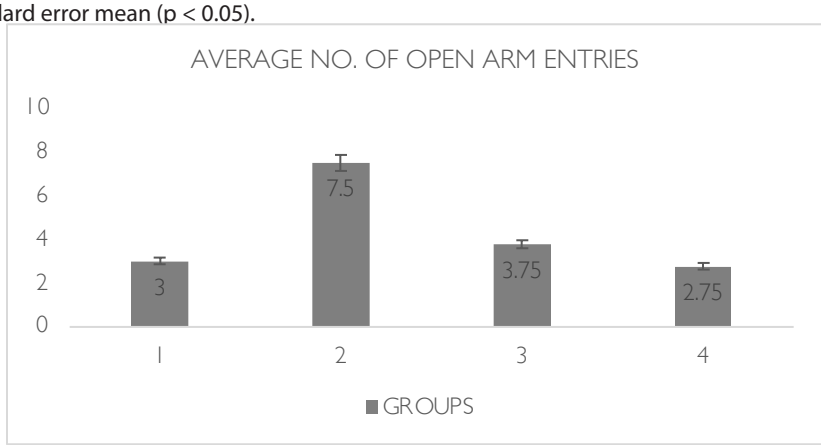
Figure 2
Percent of time spent on open arm. Y values calculated from the average duration on both closed arm and open arm entries. Results presented with standard error mean ($p < 0.05$).



higher when the mice were exposed to classical music. This means that compared to the control group and other music genres, classical music made the largest impact on lowering the anxiety level of the mice. However, exposure to hard rock music caused the lowest percent duration on open arms. Its percentage is less than one-third of the control group's percent, meaning that hard rock music led to a significantly increased anxiety level in the stress-induced mice; fast-paced music like hard rock is more stimulating than the other genres and, as a result, acts as a stressor that increases anxiety but may also act as a significant distractor (Mayfield & Moss, 1989). As for pop music, the percent duration was significantly higher compared to hard rock music and slightly higher than the control group's percent duration.

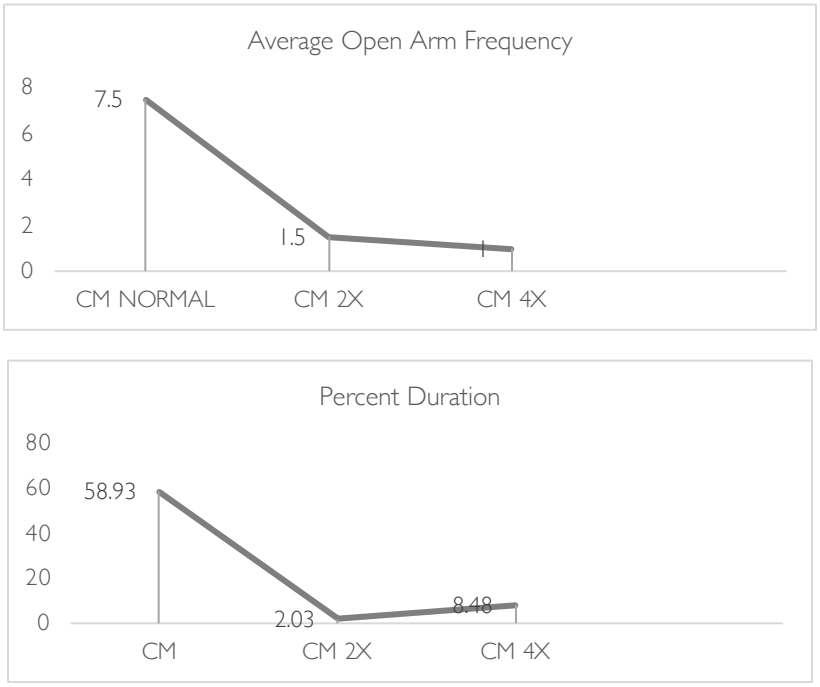
The results showed that mice exposed to classical music have more open arm entries and that their duration is longer than other mice exposed to another music genre. This experiment reflects similar results

Figure 3
Average Number of Open Arm Entries. Group 1= Control, 2=Classical Music, 3=Pop Music, 4= Hard Rock Music. Results presented with standard error mean ($p < 0.05$).



Vol. 06, No. 1 (2019)

Figure 4
Average Number of Open Arm Entries. Group 1= Control, 2=Classical Music, 3=Pop Music, 4= Hard Rock Music. Results presented with standard error mean ($p < 0.05$).



in the study done by Kour et al. (2012), which further supports that classical music can lower anxiety in mice more than other genres can. The potential applications of therapeutic treatment involving the use of classical music are already feasible; Mozart's Serenade No. 10 or "Gran Partita" has demonstrably reduced anxiety levels in rats treated chronically with simvastatin, suggesting that classical music is an effective adjuvant in behavioral treatments (Niehues da Cruz et al., 2011). This property of classical music is also supported by controlled behavioral studies of chimpanzees exposed to classical music; of note is the immediate soothing effect it has on one subject in Howell et al.'s study (2003).

Potential human applications of the ability of classical music to alleviate stress may be limited by personal preference or general age-related preference; school-age children do not generally favor classical music over more popular styles in pop and punk rock (Ginocchio, 2008). The waning popularity of classical music may affect stress alleviation in humans since musical preference can have significant effects on stress alleviation, although even unfamiliar classical music still produces physiological benefits on people (Mornhinweg, 1992), suggesting that classical music could continue to have stress-alleviating properties despite pop and other genres being more familiar to younger listeners.

A significant decrease in the frequency of open arm entries and duration on the open arms was observed in mice exposed to classical music played at double and quadruple its original speed. The duration of mice exposed to classical music with normal tempo is more frequent and yielded longer time compared to time spent in the close arm. The result also means that mice exposed to music with a slower tempo are

less anxious than any mice coming from any other group.

The percent duration on open arms was significantly higher when the mice were exposed to classical music at normal speed. This result further supports the observation that classical music made the largest impact on lowering the anxiety level of the mice. Classical music played two or four times its normal speed produced greatly decreased the percent duration on the open arm. There was no significant difference between the results from the group exposed to music twice its normal speed and the group with music played four times its normal speed.

The degree of decline in the open arm frequency between the classical group with the normal tempo and the groups with altered tempo shows a significant difference in the results. This observation supports the hypothesis that the varying tempo generally affects the disposition of the stress-induced mice, affecting anxiety and cognitive function as in the state of situational arousal (Holbrook & Anand, 1990); it supports a strong psychological and physical relationship between the perceived activity and increasing music tempo. Situational arousal shifts to the right with increasing tempos. The results support the claim that music tempo can increase agitation in the subjects exposed and that increasing speeds of music correlate with an increase in excitement or anxiety.

Absent other stimuli, music has already shown largely visible motor and emotional effects, although its perception is largely internal. Human applications of tempo regulation are often observed in the culture and behavior at different musical venues, and it can be vastly beneficial as a stimulating agent among patients that require increased stimulation (Sacks, 2006). Although the current study focuses on anxiety-alleviating properties of select genres of music, it has also shown that stimuli from music can be controlled through the manipulation of tempo; this property can be used therapeutically as necessary, using preferential musical genres to stimulate patients at higher tempos or relax patients at lower tempos. Patients with coronary heart disease reportedly actively choose music at slower tempos and without abrupt changes for sedative purposes, although clinical trials continue to investigate how musical genres and tempos can affect the reduction of stress among patients (Bradt et al., 2013). One pediatric emergency department reportedly observed the alleviation of anxiety among its patients and visitors using music at 60 to 70 bpm (Holm & Fitzmaurice, 2008) – which is slightly but not significantly faster than the current study's tempo for classical music at 58 bpm – supporting this study's results on the stress-alleviating properties of music at slower tempos. The stimulating effects of different tempos of music are also observable on task performance. Mayfield and Moss (1989) found that playing music at a pace near the average heartbeat of a person relaxes workers but significantly reduces their working output.

CONCLUSION AND RECOMMENDATIONS

Mice had significantly more entries into and spent significantly more time on the open arms of the elevated plus maze when exposed to classical music, which significantly decreased as the tempo of the music increased. On the other hand, hard rock music significantly increased anxiety levels, while pop music did not alter anxiety levels in any significant manner compared to silence. Music with low rhythm can reduce anxiety among stress-induced mice, which may have human implications. Music has a great role in alleviating and increasing anxiety levels, and that tempo can indeed be a factor that affects the study results.

It is important to consider the accuracy of stress-induced inducement to mice to have a baseline basis as to how equally the stress is distributed. The

researchers highly recommend exploring different stress-inducing processes like the use of stress drugs or exposure to a certain laboratory activity fit for mice and other similar test subjects. There are also different genres to consider. Future studies can explore other genres and subgenres of music that this study did not test. More emphasis may be given to the physiological aspect of this behavior, like hormone production, brain activity, and other bodily functions. Researchers also recommend the exploration of other factors aside from tempo that affects mice behavior. This could further explain how some music genres are generally good at alleviating stress levels, and some further induce stress.

This study only examines the stress- and anxiety -alleviating properties of music to the differences in genre and the tempo of music played on animal models. However, since the language in music may affect the perception of human listeners and change how the musical content – regardless of genre or tempo – affects human listeners, researchers also recommend that future studies should explore mental health statuses on human participants.

REFERENCES

Alworth, L. C., & Buerkle, S. C. (2013). The effects of music on animal physiology, behavior and welfare. *Lab Animal*, *42*(2), 54–61. https://doi.org/10.1038/laban.162

Angioli, R., De Cicco Nardone, C., Plotti, F., Cafà, E. V., Dugo, N., Damiani, P., Ricciardi, R., Linciano, F., & Terranova, C. (2014). Use of Music to Reduce Anxiety during Office Hysteroscopy: Prospective Randomized Trial. *Journal of Minimally Invasive Gynecology*, *21*(3), 454–459. https://doi.org/10.1016/j.jmig.2013.07.020

Anseloni,V.,&Brandão,M.(1997).Ethopharmacological analysis of behaviour of rats using variations of the elevated plus-maze. *Behavioural Pharmacology*.

Australian Animal Research Review Panel (AARRP). (2012). *Guidelines for the Housing of Mice in Scientific Institutions*. AARRP. https://www.animalethics.org.au/__data/assets/pdf_file/0004/249898/Guideline-22-mouse-housing.pdf

Bindra, D., & Thompson, W. R. (1953). An evaluation of defecation and urination as measures of fearfulness. *Journal of Comparative and Physiological Psychology*, *46*(1), 43–45. https://doi.org/10.1037/h0057952

Blanchard, D. (2001). Mouse defensive behaviors: Pharmacological and behavioral assays for anxiety and panic. *Neuroscience & Biobehavioral Reviews*, *25*(3), 205–218. https://doi.org/10.1016/S0149-7634(01)00009-4

Bradt, J., Dileo, C., & Potvin, N. (2013). Music for stress and anxiety reduction in coronary heart disease patients. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD006577.pub3

Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience & Biobehavioral Reviews*, *29*(8), 1193–1205. https://doi.org/10.1016/j.neubiorev.2005.04.017

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behavioural Brain Research*, *134*(1–2), 49–57.

Chikahisa, S., Sano, A., Kitaoka, K., Miyamoto, K.-I., & Sei, H. (2007). Anxiolytic effect of music depends on ovarian steroid in female mice. *Behavioural Brain Research*, *179*(1), 50–59. https://doi.org/10.1016/j.bbr.2007.01.010

Cooke, M., Chaboyer, W., & Hiratos, M. A. (2005). Music and its effect on anxiety in short waiting periods: A critical appraisal. *Journal of Clinical Nursing*, *14*(2), 145–155. https://doi.org/10.1111/j.1365-2702.2004.01033.x

Dawson, G. R., & Tricklebank, M. D. (1995). Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends in Pharmacological Sciences*, *16*(2), 33–36. https://doi.org/10.1016/S0165-6147(00)88973-7

Dere, E., Topic, B., De Souza Silva, M. A., Srejic, M.,

Frisch, C., Buddenberg, T., & Huston, J. P. (2002). The graded anxiety test: A novel test of murine unconditioned anxiety based on the principles of the elevated plus-maze and light–dark test. *Journal of Neuroscience Methods*, *122*(1), 65–73. https://doi.org/10.1016/S0165-0270(02)00274-1

Espejo, E. F. (1997). Structure of the mouse behaviour on the elevated plus-maze test of anxiety. *Behavioural Brain Research*, *86*(1), 105–112. https://doi.org/10.1016/S0166-4328(96)02245-0

Falkenhorst, O. (2013). *Is Mice Behaviour Influenced by Music?* [Thesis, Szent Istvan University].

File, S. E., Zangrossi, H., Sanders, F. L., & Mabbutt, P. S. (1994). Raised corticosterone in the rat after exposure to the elevated plus-maze. *Psychopharmacology*, *113*(3–4), 543–546. https://doi.org/10.1007/BF02245237

Ginocchio, J. F. (2008). Fifth-grade listeners' music style preferences: A ranking of contemporary popular music styles and comparison to Leblanc, 1979. *Contributions to Music Education*, 9–21. https://www.jstor.org/stable/24127160\

Hall, C. S. (1934). Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. *Journal of Comparative Psychology*, *18*(3), 385–403. https://doi.org/10.1037/h0071444

Handley, S. L., & Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *327*(1), 1–5.

Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E. A., Gill, S., Leblanc, M., Chaix, A., Joens, M., Fitzpatrick, J. A. J., Ellisman, M. H., & Panda, S. (2012). Time-Restricted Feeding without Reducing Caloric Intake Prevents Metabolic Diseases in Mice Fed a High-Fat Diet. *Cell Metabolism*, *15*(6), 848–860. https://doi.org/10.1016/j.cmet.2012.04.019

Hogg, S. (1996). A review of the validity and variability of the Elevated Plus-Maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior*, *54*(1), 21–30. https://doi.org/10.1016/0091-3057(95)02126-4

Holbrook, M. B., & Anand, P. (1990). Effects of tempo and situational arousal on the listener's perceptual and affective responses to music. *Psychology of Music*, *18*(2), 150–162. https://doi.org/10.1177/0305735690182004

Holm, L., & Fitzmaurice, L. (2008). Emergency department waiting room stress: Can music or aromatherapy improve anxiety scores? *Pediatric Emergency Care*, *24*(12), 836–838.

Howell, S., Schwandt, M., Fritz, J., Roeder, E., & Nelson, C. (2003). A Stereo Music System as Environmental Enrichment for Captive Chimpanzees. *Lab Animal*, *32*(10), 31–36. https://doi.org/10.1038/laban1103-31

Kim, K.-S., & Han, P.-L. (2006). Optimization of chronic stress paradigms using anxiety- and depression-like behavioral parameters. *Journal of Neuroscience Research*, *83*(3), 497–507. https://doi.org/10.1002/jnr.20754

Knight, W. E. J., & Rickard, N. S. (2001). Relaxing Music Prevents Stress-Induced Increases in Subjective Anxiety, Systolic Blood Pressure, and Heart Rate in Healthy Males and Females. *Journal of Music Therapy*, *38*(4), 254–272. https://doi.org/10.1093/jmt/38.4.254

Kour, H., Ravishankar, R., & Goudar, S. (2012). An experimental study to evaluate the effect of instrumental Indian classical and western music therapy on learning and memory in stress induced young rats. *IOSR Journal of Pharmac*, *2*(4), 29–32.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacology & Therapeutics*, *46*(3), 321–340. https://doi.org/10.1016/0163-7258(90)90021-S

Mayfield, C., & Moss, S. (1989). Effect of Music Tempo on Task Performance. *Psychological Reports*, *65*(3_suppl2), 1283–1290. https://doi.org/10.2466/pr0.1989.65.3f.1283

Montgomery, K. C. (1955). The relation between fear induced by novel stimulation and exploratory drive. *Journal of Comparative and Physiological Psychology*, *48*(4), 254–260. https://doi.org/10.1037/h0043788

Mornhinweg, G. C. (1992). Effects of music preference and selection on stress reduction. *Journal of Holistic Nursing*, *10*(2), 101–109.

Mulder, G. B., & Pritchett, K. (2004). The elevated plus-maze. *Contemporary Topics in Laboratory Animal Science*, *43*(2), 39–40. https://www.ingentaconnect.com/content/aalas/jaalas/2004/00000043/00000002/art00011

Niehues da Cruz, J., Delwing de Lima, D., Delwing Dal Magro, D., & Geraldo Pereira da Cruz, J. (2011). The Power of Classic Music to Reduce Anxiety in Rats Treated with Simvastatin. *Basic and Clinical Neuroscience*, *2*(4), 5–11. http://bcn.iums.ac.ir/article-1-173-en.html

O'Reilly, K. C., Shumake, J., Gonzalez-Lima, F., Lane, M. A., & Bailey, S. J. (2006). Chronic Administration of 13-Cis-Retinoic Acid Increases Depression-Related Behavior in Mice. *Neuropsychopharmacology*, *31*(9), 1919–1927. https://doi.org/10.1038/sj.npp.1300998

Pawlak, C. R., Karrenbauer, B. D., Schneider, P., & Ho, Y.-J. (2012). The Elevated Plus-Maze Test: Differential Psychopharmacology of Anxiety-Related Behavior. *Emotion Review*, *4*(1), 98–115. https://doi.org/10.1177/1754073911421374

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*(3), 149–167. https://doi.org/10.1016/0165-0270(85)90031-7

Peretz, I., & Zatorre, R. J. (2005). Brain Organization for Music Processing. *Annual Review of Psychology*, *56*(1), 89–114. https://doi.org/10.1146/annurev.psych.56.091103.070225

Pinheiro, S. H., Zangrossi-Jr., H., Del-Ben, C. M., & Graeff, F. G. (2007). Elevated mazes as animal models of anxiety: Effects of serotonergic agents. *Anais Da Academia Brasileira de Ciências*, *79*(1), 71–85. https://doi.org/10.1590/S0001-37652007000100010

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33. https://doi.org/DOI: 10.1016/s0014-2999(03)01272-x

Rauscher, F. H. (2006). The Mozart Effect in Rats: Response to Steele. *Music Perception*, *23*(5), 447–453. https://doi.org/10.1525/mp.2006.23.5.447

Rauscher, F., Robinson, D., & Jens, J. (1998). Improved maze learning through early music exposure in rats. *Neurological Research*, *20*(5), 427–432. https://doi.org/10.1080/01616412.1998.11740543

Sacks, O. (2006). The power of music. *Brain*, *129*(10), 2528–2532. https://doi.org/10.1093/brain/awl234

Stephens, T. J. (2010). *The Effects of Classical and Rock Music on Memory*. http://csef.usc.edu/History/2010/Projects/J0631.pdf

Tiwari, D., Nagar, H., Dwivedi, G., Tripathi, R., & Jena, J. (2012). Evaluation of anti-anxiety activity of Plectranthus amboinicus (Lour.) on rats. *Asian J Pharm Clin Res*, *5*(4), 110–113. https://www.researchgate.net/profile/Jitendra-Jena/publication/236368108_Evaluation_of_anti-anxiety_activity_of_Plectranthus_amboinicus_Lour_on_rats/links/0deec51a4fde081306000000/Evaluation-of-anti-anxiety-activity-of-Plectranthus-amboinicus-Lour-on-rats.pdf

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322–328. https://doi.org/10.1038/nprot.2007.44

Warner, T. (2017). *Pop Music Technology and Creativity: Trevor Horn and the Digital Revolution*. Routledge.

Zimprich, A., Garrett, L., Deussing, J. M., Wotjak, C. T., Fuchs, H., Gailus-Durner, V., de Angelis, M. H., Wurst, W., & Hölter, S. M. (2014). A robust and reliable non-invasive test for stress responsivity in mice. *Frontiers in Behavioral Neuroscience*, *8*, 125. https://doi.org/10.3389/fnbeh.2014.00125