ABSTRACT

The Effect of Music on Biomolecule Production and Listening Satisfaction

Jessica Fan

Director: Thomas D. McGrath, Ph.D

Listening to music is enjoyable, but can it do more for the human body than satisfy the ears? This thesis examines the effects of music with a literature review through a biological lens, focusing on how listening to and producing music can affect levels of cortisol, beta-endorphin, oxytocin, and various components of the immune system, as well as what effects these species have on the body. Additionally, it includes a survey exploring how tuning frequency affects listening satisfaction and how one's area of study (specifically, science or music) may influence one's preference. Continued support for tuning to 432 Hz despite the current tuning standard of 440 Hz inspired this investigation into the physical qualities of music and its effects on the listener. The results of this survey were inconclusive, perhaps because the sample size was not large enough and the ratio of science to music majors was not representative.

APRROVED BY DIRECTOR OF HONORS THESIS:

____

Dr. Thomas McGrath, Department of Chemistry and Biochemistry

APPROVED BY THE HONORS PROGRAM:

____

Dr. Elizabeth Corey, Director

DATE: _______________________

THE EFFECT OF MUSIC
ON BIOMOLECULE PRODUCTION AND LISTENING SATISFACTION

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

Jessica Fan

Waco, Texas

May 2022

TABLE OF CONTENTS

TABLE OF FIGURES

INTRODUCTION

Bridging Music and Science

Music is a universal love among mankind. The auditory experience of listening to music can produce a wide range of mental and physical changes beyond one's awareness. Perhaps listening to one's favorite upbeat song will lift one's mood, or a relaxing piece will physically ease one's tension. Music can also bring people together in a way that transcends the barriers of different languages or cultures; it "is intricately intertwined with every human experience at any age, it can touch a person as a whole (i.e., the body, mind, heart, and soul) just like 'a mysterious artery'"(1). Why is that so? What is it, scientifically, that underlies the human reaction to music? Are there qualities of music that can be altered to affect our emotional or physiological response? These are the questions that inspired this exploration of how the body responds when listening to music; specifically, what molecules and processes contribute to our response, and if tuning frequency affects our enjoyment of music.

The species that will be investigated are cortisol, beta-endorphin, oxytocin, and components of the immune system (specifically immunoglobulins, cytokines, and lymphocytes). The reason that these were chosen in particular is because they are related to the physical effects that may occur when listening to music: cortisol is involved in regulating the body's stress response, which music is often said to relieve; beta-endorphin, commonly associated with physical activity, inhibits pain and may contribute to music's feel-good effects; oxytocin is involved in social and emotional interactions, which are also significant for music; and the immune response may be activated as a result of a positive response to music. Through an analysis of the levels of these molecules when listening to music, it will be possible to investigate the source of the body's response.

CHAPTER ONE

Background: Mechanisms of Production and Action of Biomolecules

Cortisol

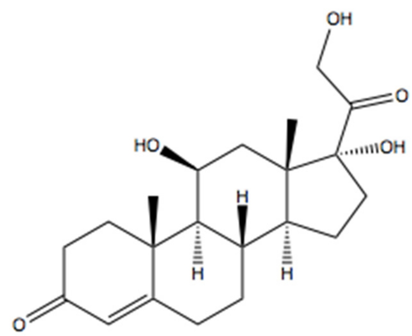


Figure 1. Chemical Structure of Cortisol.

When the body encounters a stressor, one part of its response is the cerebral cortex's activation of the hypothalamus-pituitary-adrenal (HPA) axis. The hypothalamus secretes corticotropin-releasing hormone (CRH) and vasopressin, which causes subsequent release of adrenocorticotropic hormone (ACTH) from the pituitary gland. This then signals to the adrenal cortex to produce glucocorticoids, including cortisol (Fig. 1) (2). The hormone is synthesized from the steroid cholesterol (3) in the zona glomerulosa of the cortex. Hormone levels can be regulated through feedback loops that may act at several points in the pathway to production (adrenal gland, hypothalamus, frontal cortex) which return cortisol levels to homeostasis (2). After an acute stressor, cortisol levels are at their highest about 20-25 minutes after its occurrence and may take an hour to recover to baseline levels. Additionally, it is important to note that cortisol levels respond not only to strong emotional stressors. Even momentary negative moods can cause an acute increase in cortisol. Levels also rise and fall regularly over time, peaking in the morning and decreasing throughout the day (4). The amygdala, which is associated with the fear response, can also activate the HPA axis to prepare for the body to respond to a threat causing fear. Other systems that respond to stress and are associated with the HPA axis include the autonomic nervous system, systems involving inflammatory cytokines, and metabolic hormones (2).

Cortisol can affect the body in several different areas, including the nervous, immune, cardiovascular, respiratory, reproductive, musculoskeletal, and integumentary systems. All of these organs have glucocorticoid receptors that allow cortisol to act upon them. It is important to detail cortisol's effect on the immune system; it induces apoptosis of T-cells promoting inflammation, suppresses B-cell antibody production, and reduces migration of neutrophils during inflammation (3). Cortisol can also have long-term effects that affect basal cortisol levels; chronic stress may temporarily elevate cortisol's basal levels, but after some time, drop it to hypocortisolemic levels and ultimately cause low basal cortisol. This effect can last for years, especially if the cortisol-altering events occur early in childhood (4). Cortisol's influence impacts processes throughout the body, both immediately and longitudinally.

Beta-endorphin

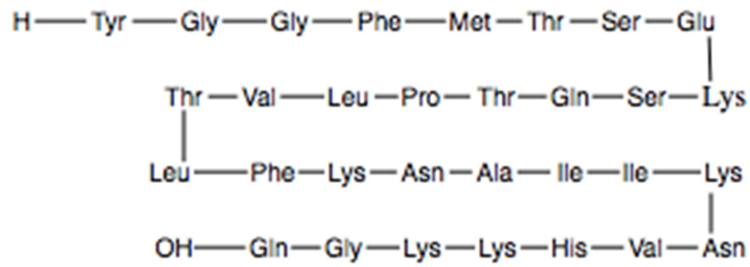


Figure 2. Amino Acid Sequence of β-Endorphin.

Beta-endorphin (Fig. 2) is an opioid peptide composed of 31 amino acids that acts as a neurotransmitter in the central nervous system. It is produced from the cleavage of proopiomelanocortin (POMC) protein to the hormone beta-lipotropin, which is then cleaved into the opioid beta-endorphin. This process occurs in neurons in the hypothalamus, pituitary gland, and brainstem (5). Beta-endorphin levels can be affected in several different ways. Exercise, or physical activity, is a more well-known method of producing endorphins, causing an acute increase in the opioids and the subsequent rewarding physiological response (6,7). The body's stress system is another pathway through which beta-endorphin levels can be increased; CRH stimulates the production of POMC-derived peptides (including beta-endorphin) from the hypothalamus. It has also been suggested that beta-endorphin is regulated by the pituitary gland in a similar way as the stress hormone adrenocorticotropic hormone (ACTH) (8). In addition, dopamine influences the basal opioid tone in the nucleus accumbens of the basal ganglia (5).

Beta-endorphin has several effects on the body. As mentioned earlier about exercising, it plays a role in shaping behavior through positive reinforcement (7). As an opioid, it increases dopamine release by inhibiting gamma-aminobutyric acid (GABA), which blocks dopaminergic neurons, resulting in more open dopamine production. Beta-endorphin also has long-established anti-nociceptive effects, and further research has revealed its role in decreasing feelings of distress and mediating human memory revision during heightened emotional states. In addition, it can factor into stress-related disorders, obesity, diabetes, and the immune response. Its part in psychiatric diseases such as anxiety-related disorders is less defined, but studies have found that acute stress is often accompanied by higher levels of beta-endorphin and anxiety-related behaviors are related to lowered beta-endorphins (5). This molecule's role in the body is diverse and complex, resulting in a variety of pathways in which it may both influence and be influenced.

Oxytocin

Oxytocin (Fig. 3) is a peptide composed of nine amino acids that is involved in several different biological and chemical processes (9). It is produced in the paraventricular nuclei of the hypothalamus and released by the pars nervosa of the posterior lobe of the pituitary gland (10). There are also theories that the heart may be a source of oxytocin production (11). Oxytocin is autoregulated by self-produced peptides, auto-receptors, and calcium. When oxytocin binds to specific oxytocin receptors (OTR) on oxytocin-producing cells, it causes an influx of calcium and an increase in calcium release from intracellular calcium stores (10). This causes exocytosis of the hormone and further release, producing the positive feedback mechanism in oxytocin release. When there are mutations present within the OTRs, oxytocin is unable to enact this cascade, and psychiatric disorders may arise such as depression, mood disorders, and autism spectrum disorders (9). Oxytocin is released during specific events related to social bonding, such as parturition and lactation. Inhibitory control is exhibited by opioids (10).

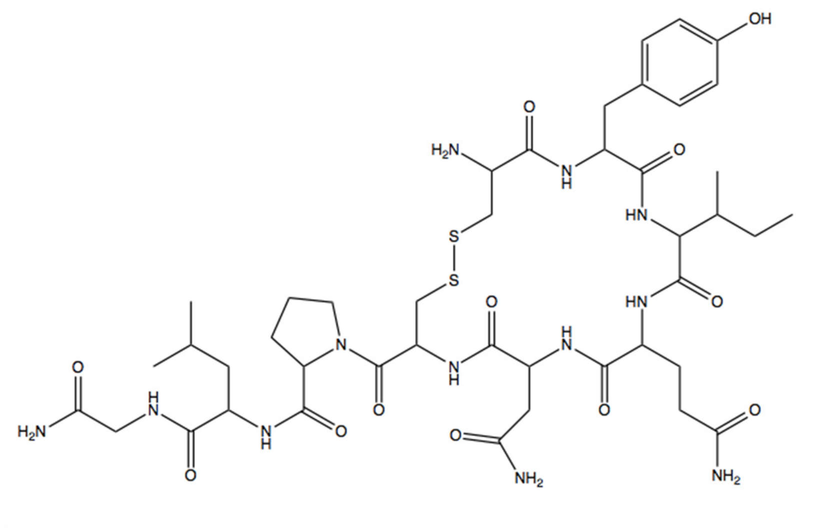


Figure 3. Chemical Structure of an Oxytocin Molecule.

Oxytocin affects a wide range of processes connected to social behavior, including perception, cognition, recognition, intimacy, and anxiety (9,12). Besides its function in parturition and lactation, the peptide binds significantly within the brain, specifically in the amygdala of the limbic system (12). It inhibits neurons here that are associated with the fear response, decreasing anxiety in response to stress in social interactions (9,12). Oxytocin binding to the caudate nucleus can cause similar effects on anxiety levels as well as affect learning, memory, and feedback processing. It may also bind to other OTRs in the brain with various effects on social interactions. These can include trust, emotion recognition, mating, pair-bonding, adult-infant attachment, and psychological disorders such as autism and obsessive-compulsive disorder. Under stress conditions, oxytocin acts as a hypophysiotropic hormone, increasing adrenocorticotropic hormone (ACTH) levels secreted from the anterior pituitary (10). It also regulates HPA activity under stress, reducing cortisol secretion; this may be the reason that some studies have found oxytocin to be able to change neural connections and stimulate social cohesion during stressful situations (9,10). Outside of the central nervous system, oxytocin acts on a number of different organs in surprisingly different ways. It can slow the onset of osteoporosis through influencing osteoblasts and osteoclasts, making it a potential treatment for skeletal loss due to pregnancy and lactation or post-menopause. In the pancreas, it can either cause an increase in glucagon and glucose levels (if peripheral, circulating) or insulin (if central, likely injected). In the heart, the peptide can affect cardiac cells, altering atrial natriuretic peptide (ANP) release, estrogen receptor expression, and cardiac differentiation. Oxytocin can also influence cell differentiation outside of the heart and act as a growth factor, indicating a potential role in cancer. Interestingly, it can either impair or promote neoplastic cell proliferation depending on the location (10). While oxytocin has diverse functions within the body, its actions have been well-studied and are more clearly defined in literature.

Immune System

Figure 4. Immunoglobulin, Basic Structure.

The immune system is incredibly broad and complex, with several specific components and processes that function to defend the body from disease. As such, a more general overview including relevant parts of the human body's immunity will be included in this section.

Most immune cells come from the bone marrow. This production starts early in life and continues throughout one's lifetime. There are a large variety of cells involved with immunity, which can broadly be divided into innate and adaptive immunity. The innate branch responds to naturally dangerous motifs present on molecular structures; immune cells bind to these patterns and either immediately destroy the hazard or send out signals to other phagocytic cells like neutrophils and macrophages (13). These cells can then present the threat to the other branch of immunity, adaptive immunity. Innate immunity targets several different threats, such as viruses, parasites, and fungal infections, in a fast and non-specific manner. One important cell involved is the natural killer (NK) cell, which destroys cells that do not present a major histocompatibility complex (MHC) I molecule. Every nucleated cell in the human body should express MHC I on its surface, so a lack of this molecule is a major warning sign for the immune system.

If the threat is not contained by the innate immune response, adaptive immunity provides the body's secondary response to the pathogen. This branch can be further divided into two sections, humoral and cell-mediated immunity. Both branches' responses begin after innate antigen-presenting cells (APCs) ingest pathogens and present digested pieces of them (antigens) on their surface with MHC I and II molecules (13). In cell-mediated immunity, cytotoxic T-lymphocytes (or T-cells) are stimulated by the APCs and directly attack the dangerous cells. Helper T-cells can also stimulate cytotoxic T-cells after being exposed to APCs using special signaling molecules called cytokines. They also differentiate into memory T cells, which serve as a source from which helper T-cells can quickly be produced in the future. In humoral immunity, B lymphocytes (or B-cells) are stimulated by the antigen, then activated by helper T-cells. The B-cells will then differentiate into plasma cells, which produce antibodies, or memory B cells, which remain in the body long-term and allow a faster and more robust antibody response the second time the antigen is encountered. Antibodies, or immunoglobulins (Ig), are antigen-specific and coat pathogens in a process called opsonization. They are divided into five different classes (A, D, E, G, and M), with IgA being most pertinent to the studies referenced in Chapter 3. IgA is the primary immunoglobulin in secretions, including saliva, intestinal secretions, urine, tears, and other mucosal fluids (14).

An important component of immune system communication and coordination is cytokines. Cytokines are produced by one cell to signal to another, and they are involved in cell regulation and growth (13). There are a great variety of cytokines involved in the immune system, so a more specific focus on relevant cytokines will benefit the aim of this paper. Cytokines are produced by a number of different immune cells (such as helper T-cells, as mentioned earlier) and can act in a pro-inflammatory manner, or anti-inflammatory, or both (15). Examples of pro-inflammatory cytokines include interleukin 2 (IL2), interferon gamma (IFNɣ) and tumour necrosis factor alpha (TNFα) (16). These generally act to induce the immune system to action. In opposition to these effects are anti-inflammatory cytokines, such as interleukin 4 (IL4) (16). These cause suppression of the immune system, inhibiting its actions. Some cytokines can also act in both pro-inflammatory and anti-inflammatory ways, like interleukin 6 (IL6) and interleukin 17 (IL17), depending on the source, target, and phase of the immune response of the cytokine (15,16). With the intricacies of the immune system's organization and function, cytokines are essential to the performance of a healthy immune response. Their levels can indicate a general upregulation or downregulation of the immune system, which can be utilized to examine the immune system's level of activity.

CHAPTER TWO

The Effect of Music on Biomolecule Production

Cortisol

Music is often said to be relaxing or soothing, making it an appropriate subject for the study of whether it reduces physiological stress. Several studies have investigated the effects of music on cortisol, whether through listening to music or engaging in production of music, with varying results.

Many findings indicate that cortisol levels are decreased after listening to music. In 2009, Nakayama found that when terminal cancer patients in palliative care listened to music, their cortisol levels decreased significantly (1). This study only involved 10 patients, most likely because it was conducted in Japan, which has a relatively undeveloped palliative care system. Miluk-Kolasa et al found similar results among subjects awaiting a surgical procedure (17). Patients who listened to a one-hour music session while waiting for the procedure had significantly reduced cortisol levels while listening when compared to the control group. Another study done on patients awaiting surgery was conducted by Leardi et al. several years later in 2007 and found comparable results. Listening to music both before and during surgery caused a significant decrease in cortisol levels for patients who listened to music versus those who listened to the regular sounds of the operation (18). Interestingly, this study also found that the type of music made an impact on how strongly the music affected the subject; self-selected music caused more of a decrease in cortisol than experimenter-selected music, and for self-selected music this effect continued after the surgery as well.

There are also several studies that found conflicting results, finding either no relationship between cortisol levels and listening to music or increased cortisol levels after listening to music. Qiu et al. found that cortisol levels were not significantly different for infants that listened to music when undergoing painful procedures when compared to infants that underwent surgery without music. In fact, the control group that underwent surgery without music had a more significant decrease in cortisol (19). Additionally, Koelsch et al. discovered similarly that when comparing a group that listened to a variety of music and a control group that did not listen to music immediately after exposure to a stressor, the group that listened to music had higher cortisol levels than the control group (20). Knight & Richard also found that listening to relaxing music during stressful tasks did not have any effect on cortisol levels (21). These seemingly conflicting results regarding cortisol levels in response to music can be explained by the interaction between the immune system and stressors. All of these studies involved the participants being directly exposed to a stressor. One of the body's responses to an acute stressor is to increase immune function (20), so it may be argued that the increased levels of cortisol actually reflect a higher preparedness of the body's immune system to fend off the "challenge" that the stressor will present. This argument also suggests that cortisol levels are not always directly related to the level of stress an individual experiences.

In engaging with music, cortisol levels are affected differently when comparing rehearsal with performance. Beck et al. found that while rehearsing music, cortisol decreased significantly (an average of 30%). However, performing music was often associated with an increase in cortisol levels. After choral performance, choral singers had a cortisol level increase of 37% (14). Fancourt found similar results for subjects singing in choirs. Among people caring for someone with cancer, people who cared for cancer patients that passed away, and cancer patients who sang in various choirs in a non-performance setting, cortisol levels significantly decreased after singing for one hour (8). Another study analyzed cortisol levels in a very specific instance of performance: singing lessons, in which the musician performs for an instructor for feedback on potential improvements. Grape et al. discovered that cortisol levels increased after a 30-minute singing lesson, regardless of whether the singer was an amateur or a professional (22). This suggests that the nervousness associated with a performance setting may cause stress and therefore an increase in cortisol that offsets any reduction that might have occurred after singing. The relationship between cortisol and engagement with music can vary based on the specific setting of engagement, so it would be beneficial for future studies to explore this connection further.

Beta-Endorphin

As previously mentioned, beta-endorphin is an opioid associated with feelings of reward. This may initially imply that listening to or engaging with music would cause an increase in beta-endorphin; however, its relationship with the HPA axis complicates this relationship so that it is not a simple direct correlation. Further complicating this understanding is the scarcity of studies on this relationship.

One study indicates that beta-endorphin levels increased after listening to music. Qiu et al. found that for premature infants undergoing daily painful procedures, infants that listened to music during their procedures through a form of nonpharmacological treatment called combined music and touch intervention (CMT) had a significant increase in beta-endorphin levels; the control group that had no music during their procedures did not (19). In contrast, other studies have found a decrease in beta-endorphin in relationship to listening to or engaging with music. In addition to cortisol, Fancourt et al. studied how singing was related to beta-endorphin levels. The results showed that singing caused a decrease in beta-endorphin (8); however, given the parallel between beta-endorphin levels and the body's stress response, this may have been due to the reduction in stress (indicated by lower cortisol levels in the same study) in response to singing. Chanda and Levitin came to a similar conclusion in their review of musical neurochemistry, finding that beta-endorphin was reduced with musical interventions (23).

With these results, one of two things can be argued. First, one may suggest that beta-endorphin should decrease after listening to or engaging with music. This particular relationship is indicated by both Fancourt et al. and Chanda and Levitin. The study done by Qiu et al. (19), which found the opposite effect of music on beta-endorphin, may have come to its findings due to the touch portion of CMT, as CMT did not investigate the impact of music alone. Second, the conflicting results may indicate a more complex relationship between beta-endorphin and music. Given the shortage of research in this area, it is more likely that this is the case. The actions of beta-endorphin and some of its effects are still unclear, and it is possible that these pathways need to be explored in more detail before further studies can be conducted on the relationship between beta-endorphin and music.

Oxytocin

Oxytocin is associated with a multitude of social interactions and feelings of bonding or attachment. Several studies have analyzed the relationship between this peptide and listening to or engaging with music, with the majority of them concluding that oxytocin levels increase with musical interaction.

The results of these studies were similar regardless of whether subjects listened to or engaged with music (both through practice and performance). In terms of listening, Nilsson found that for patients who were undergoing open heart surgeries, those who listened to music during bed rest had significantly increased oxytocin levels while the control group, with no music during bed rest, actually exhibited a reduction in oxytocin over time (24). Seltzer et al. reached a similar result in their study, discovering that hearing "motherese," a music-like linguistic sound to children (23), caused a significant increase in oxytocin levels for children who experienced a social stressor (25). This study also highlighted that the sound of "motherese" alone caused a similar response in oxytocin levels to combined sound and touch, confirming that the auditory experience alone caused these results. Research has found that after engaging with music, oxytocin levels also tended to increase. Grape et al. found that after a 30-minute singing lesson, subjects experienced a significant increase in oxytocin levels, regardless of their status as an amateur or professional singer (22). Another study by Yuhi et al. investigating oxytocin levels in school children after a group drumming recital found the same relationship between oxytocin and musical engagement (26).

Several studies have suggested the opposite correlation between oxytocin and musical interaction. In fact, Yuhi et al. also analyzed oxytocin levels when subjects transitioned from free activities to drumming practice sessions, which resulted in decreased oxytocin (26). However, this result may be explained by the nature of children being less pleased when required to participate in something versus playing with others in the activities of their choosing. Another study by Fancourt et al. found a decrease in oxytocin levels after subjects sang in choir for an hour (8). These results are in contrast to most findings on oxytocin's relationship to music but may be explained by the non-performance setting of their subjects' musical engagement. As mentioned earlier, because of the more relaxed form of musical production, cortisol levels decreased among the singers in the study. This downregulation of the stress response was also associated with a decrease in beta-endorphin; therefore, with the positive correlation between oxytocin and beta-endorphin, the decrease in oxytocin after singing is to be expected as well. It is difficult to say which one of oxytocin or beta-endorphin caused the reduction in the other, but this study only further highlights the complexity of the relationships between these molecules and a need for further investigation.

Immune system

Studies have analyzed various components of the immune system's response to both listening to and performing music. Several of them used salivary IgA as their measure of immune function because of IgA's role as a "first line of defense" against infection. Charnetski et al. found that listening to 30 minutes of "smooth jazz" music caused a 14.1% increase in IgA levels, while non-musical and absent auditory stimulus conditions led to lower increases (7.2%) and even decreases in IgA concentration, respectively (27). Knight & Rickard found a similar result when analyzing IgA levels in participants subjected to a cognitive stressor. Those who prepared for the stressor in the presence of music (Pachelbel's Canon in D Major) had a higher increase in IgA compared to those who prepared in silence; in fact, music enhanced baseline IgA levels even without the presence of a stressor (21). Chanda & Levitin also refer to several studies that found an increase in IgA levels after subjects listened to music. One outlier study found no significant difference between surgery patients who listened to music and those who did not (23). However, this may have been due to the effects of the local anesthetic utilized for the operations, which might have interfered with normal biological processes. Ultimately, most research investigating this relationship concurs in its finding that IgA levels increase when listening to music. Music performance caused similar effects in IgA levels. Beck et al. found that after both rehearsal and performance conditions, choral performers had significant increases (150% and 240%, respectively) in IgA levels compared to their baseline levels (14). This drastic increase may also be related to the respiratory system's involvement in singing, which is likely to introduce foreign bodies to the body through the repeated inhalation and therefore trigger a heightened IgA response.

Research has also analyzed the response of several types of lymphocytes in response to music, including T lymphocytes, B lymphocytes, and natural killer (NK) lymphocytes. Leardi et al. studied patients in surgery and found that NK cell counts decreased in groups that listened to music during surgery, while increasing in the control group without music. T lymphocytes (including suppressors and helpers) and B lymphocytes did not show a significant difference between the music and control groups (18). Considering that higher levels of stress are associated with an increase in NK lymphocytes (18), this result is to be expected assuming that music lowered patient stress levels. Several studies have also investigated the effect of music performance on these lymphocytes. Bittman et al. studied group drumming, documenting an increase in NK cell activity in those participating in group drumming activities when compared to baseline levels (28). This suggests an increase in immune system activity. Koyama et al. conducted a similar investigation on recreational music making, finding evidence of significant increases in T cells (helper and memory T cells) in elderly adults over 65 while finding no significant increase in younger adults under 65 (29). This age-related difference may be due to the effects of aging on baseline lymphocyte numbers. In addition, Wachi et al. found that recreational music making changed NK cell activity by decreasing NK cell counts in participants with higher baseline levels and increasing counts in participants with lower baseline levels (30). Based on these studies, it is clear that the relationship between music listening or performance with the immune system is complex. However, they continue to indicate the efficacy of musical interventions in modulating immune system function.

One final aspect of the immune system that has been studied is cytokines. As noted previously, there are different types of cytokines: pro-inflammatory, anti-inflammatory, and both pro- and anti-inflammatory. All three types can be affected both by music listening and performance. Koelsch et al. found that after an acute stress test, those who underwent the recovery period while listening to music had a higher cytokine-induced cortisol response than those who recovered with only a non-musical acoustic stimulus (20). Because short term stress and stress hormone levels have immuno-enhancing effects, the music group's higher cortisol levels indicates that immune activity was also higher during the recovery. The study found that cytokine IL-6, which has pro- and anti-inflammatory action, reached the highest levels about 1 hour after the stress test. However, there was no effect on TNFα, another cytokine with a pro-inflammatory profile. This may have been because IL-6 was acting in an anti-inflammatory manner to calm the body's heightened immune response to the stressor, while TNFα's pro-inflammatory action was not needed. Chanda and Levitin also cite evidence that cytokine IL-6 is affected by music listening. Both critically-ill and healthy patients who passively listened to music had decreases in IL-6 levels (23). Because of IL-6 activity's dual nature, one may suggest that it was also acting in an anti-inflammatory manner in this study, and the cytokine decreased because participants listening to music felt relaxed and had no flare up in immune activity.

Music production has also been found to affect cytokine levels. Fancourt et al. studied cytokines IL2, IFNɣ, TNFα, IL4, IL6 and IL17 in choir singers across several groups. They found that IL17, IL2, TNFα, and IL4 levels increased significantly after singing. IL17 and IL4 can both act to be anti-inflammatory, so their increased levels may have been to control higher immune activity. However, IL2's and TNFα's increases in their pro-inflammatory action indicate that the body also prepared to encounter foreign antigens through singing. Interestingly, there was no significant difference in IFNγ and IL6 levels. The balance between these various cytokines highlights the complex fine-tuning required within the immune system to keep the body healthy. Koyama et al. also found that recreational music making caused an increase in IFNγ and IL6 levels for elderly adults (65 years of age and older) (29). Because IL6 has both anti- and pro-inflammatory actions while IFNγ is pro-inflammatory, this may indicate either a balance between two opposing actions to regulate the immune system or a coordinated effort to increase immune activity. Wachi et al. also analyzed IFNγ gene expression levels for subjects participating in recreational music making versus other non-musical leisure activities such as reading. They found that IFNγ gene expression was reduced for the music group (30). With IFNγ's pro-inflammatory actions, this suggests that recreational music making was relaxing and that the immune system had no need to activate. The results of these studies further emphasize the intricate harmony between cytokines and the complex role of the immune system.

CHAPTER THREE

Effect of Music Frequency on Listening Satisfaction

Music can be tuned to different frequencies, which may influence the perceived pitch one hears when listening to it. This frequency is measured in Hertz (Hz). The modern international standard of tuning is 440 Hz (musical note A4), which was officially established in 1975 (31). However, today there are still several proponents of using 432 Hz to tune. These include both musicians and research institutions, such as New Age musicians and the Schiller Institute, who cite biological and even mathematical reasons for their choice (31). This continued support for 432 Hz has garnered attention from several researchers curious about the effects of listening to music tuned to an alternate frequency.

Several studies have investigated the biological response to music tuned at 432 Hz. There already exists a popular consensus that this frequency is generally more likeable, peaceful, and harmonious, and several studies have confirmed this perception. For example, Renold found that when an audience listened to the same piano concert tuned to both 440 Hz and 432 Hz, people thought the music was better and were more attentive when the piano was tuned to 432 Hz (31). Calamassi and Pomponi came to a similar conclusion while comparing 440 Hz and 432 Hz tuning, with participants more focused on and satisfied with their music after listening to it at 432 Hz (31). Other studies have examined the physiological effects of listening to music tuned to 432 Hz. Aravena et al. found that for patients awaiting a tooth extraction, those who listened to 432 Hz music had significantly lower cortisol levels than patients listening to 440 Hz music and patients with no music (32). This indicates that 432 Hz music helped to lower patients' stress levels. Dubey et al. found similarly that participants who fell asleep while listening to 432 Hz music had a higher alpha wave energy during their sleep than participants who did not listen to music (33). Alpha waves indicate relaxation of the mind (33), suggesting that 432 Hz music had an effect on the brain even as participants were asleep. These studies show that music's tuning frequency may affect not only people's attitude toward the music, but also their biological processes.

These findings inspired this investigation into the question of whether tuning frequency truly affects the listener's impression of the music. A survey was conducted to examine students' listening satisfaction when listening to short music clips tuned to 440 Hz and 432 Hz. Participants were presented with three questions that asked them to select which music clip they preferred between two clips of the same music tuned to the two different frequencies. They also had the option to indicate that they did not have a preference, "no preference/difference heard." Additionally, the survey considers how music perception may vary depending on the area of study that a student is majoring in; specifically, whether they study music, science, or another area ("other"). A list of the survey questions is provided in Appendix A.

Between February and March 2022, 57 students voluntarily and anonymously completed the survey. Of these, 42 students studied science and 10 students studied music. 5 students indicated "other" as their area of study and provided further detail about their major. These responses for the selection "other" and the distribution of students' areas of study are provided below in Figures 5 and 6.

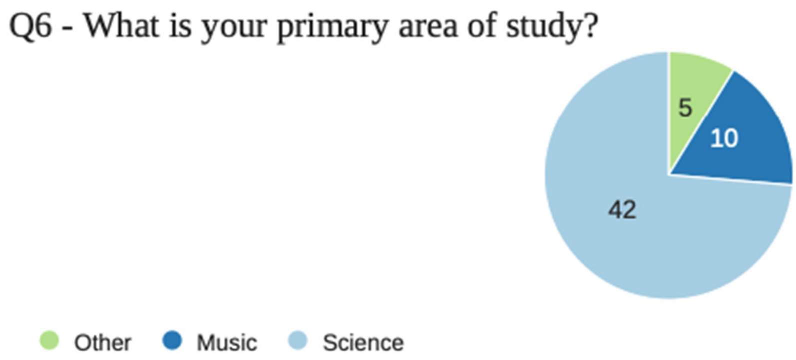


Figure 5. Number of Participant Responses for each Area of Study.

Q7 - If you selected "other" for your primary area of study, please specify.
Biology
Education
Engineering
Humanities
I'm both science and music!!

Figure 6. Participant Responses for Area of Study That Selected “Other.”

Students were presented with three questions asking them to choose between 440 Hz and 432 Hz tuning. Answers were randomized for each question. Each question had a different style of music in its clips. The first question included music that was digitally created: “Setakamuy” (Blanju Remix) by Tomoyuki Sakakida. Among music majors, 5 of the students (about half) selected the clip tuned to 432 Hz, while the rest selected either the 440 Hz clip or had no preference/heard no difference. Among science majors, 21 students (about half) selected the clip tuned to 440 Hz, and the rest selected either the 432 Hz clip or had no preference/heard no difference. The results for question one are summarized below (Fig. 7 and 8).

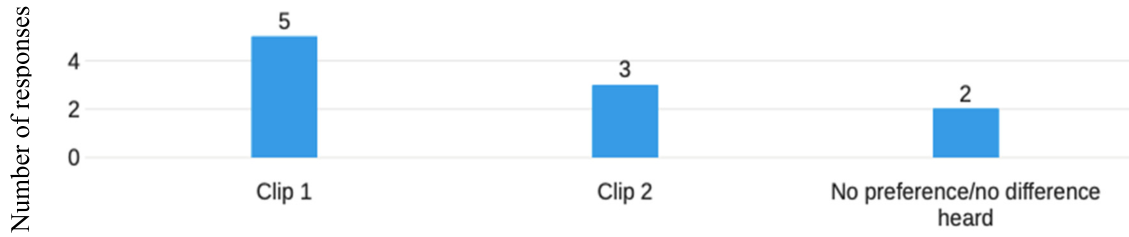


Figure 7. Music Major Responses to Question One; Clip 1 is Tuned to 432 Hz.

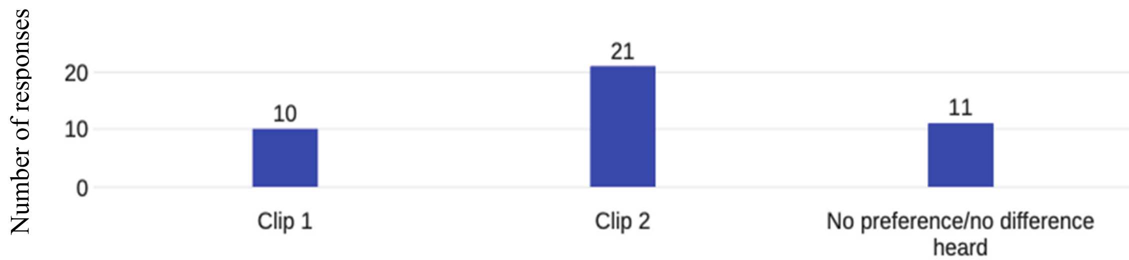


Figure 8. Science Major Responses to Question One; Clip 1 is Tuned to 432 Hz.

In the second question, the music was a clip from the Bach Cello Suite No. 1 Prelude. All of the music majors with a preference selected the clip tuned to 440 Hz; only 2 students did not have a preference/heard no difference. Among science majors, students with a preference were divided almost evenly between the 440 Hz clip and the 432 Hz clip. Only 7 students had no preference/heard no difference. The results for question two are summarized below (Fig. 9 and 10).

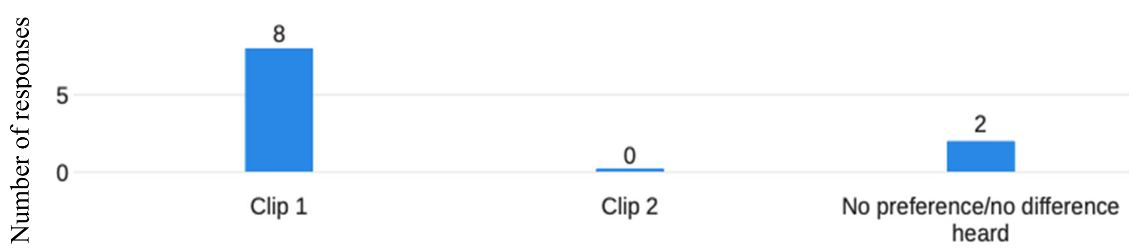


Figure 9. Music Major Responses to Question Two; Clip 2 is Tuned to 432 Hz.

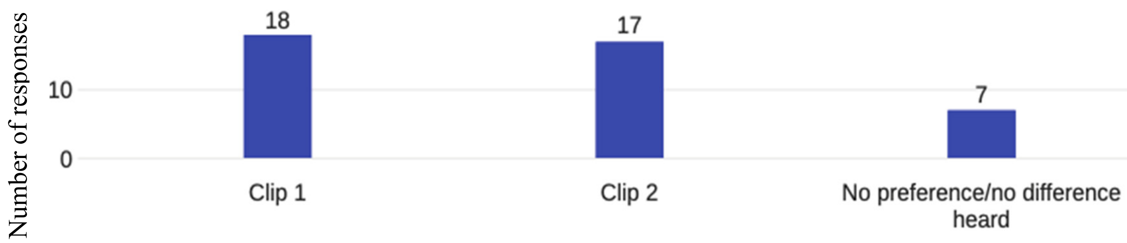


Figure 10. Science Major Responses to Question Two; Clip 2 is Tuned to 432 Hz.

For question three, the music was a clip from "Thriller" by Michael Jackson. For both music and science majors, students were almost evenly divided between all three options. The results for question three are shown below (Fig. 11 and 12).

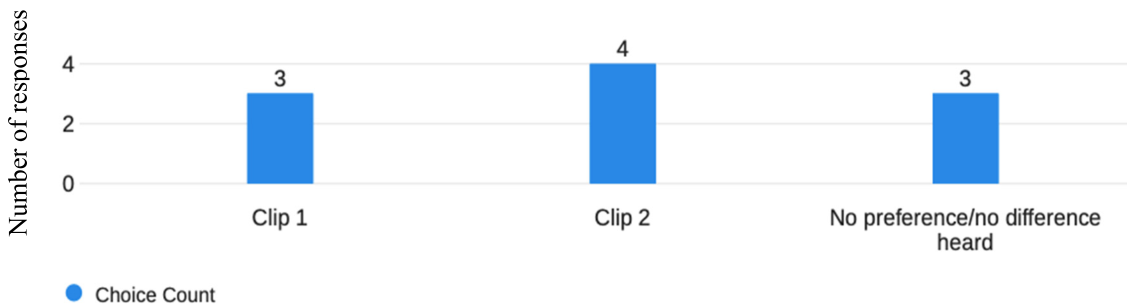


Figure 11. Music Major Responses to Question Three; Clip 1 is Tuned to 432 Hz.

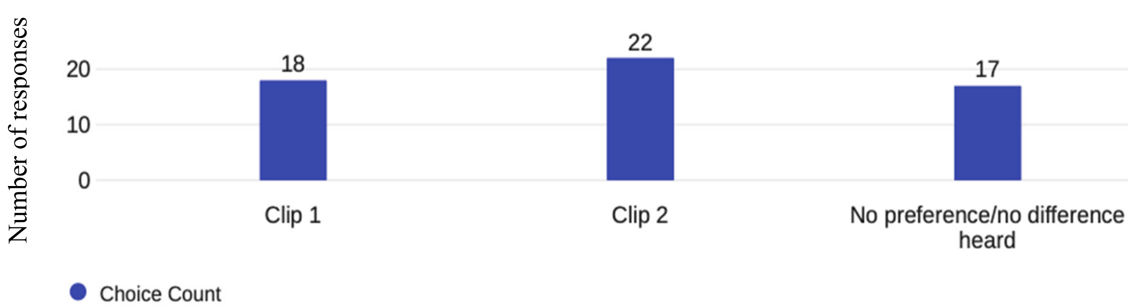


Figure 12. Science Major Responses to Question Three; Clip 1 is Tuned to 432 Hz.

Discussion of Results

The results for each question varied greatly and do not fully indicate a preference for either 440 Hz or 432 Hz. For question one, only 2 more music students selected the 432 Hz clip than the 440 Hz clip. In addition, almost the same number of students selected "no preference/no difference heard" as the 432 Hz clip. In contrast, over double the number of science majors selected the 440 Hz clip instead of the 432 Hz, and more students had no preference/heard no difference than preferred the 432 Hz clip. Because music students preferred the 432 Hz clip slightly more while science students preferred the 440 Hz clip, these findings do not suggest that either tuning frequency is favored.

For question two, the results for music students were interesting because nobody selected the 432 Hz clip as their preference. This finding may be related to the music utilized in this question; the Bach Cello Suite No. 1 Prelude is a popular and highly recognizable piece among classical music, and it can be assumed that music students frequently hear it tuned to 440 Hz. That may have caused the 432 Hz clip to sound amiss, leading to all students with a preference selecting the 440 Hz clip. Only 2 music students had no preference/heard no difference. On the other hand, science majors were nearly evenly split between the 440 Hz and 432 Hz clips (18 and 17 students, respectively). Only 7 students had no preference/heard no difference. These findings highlight the influence of one's area of study on their selection, especially if the piece is familiar to them. In addition, this effect may be greater for those who study music because they listen to and analyze music much more frequently. Further research into the effect of familiarity on music listening satisfaction may be required.

For question three, the results were also intriguing because for both science and music majors, there was a near equal distribution between all three choices. Although the music utilized in this question is considered popular across the general public, this expected recognizability did not have the same effect on listening preferences as the music in question two did. This may have been caused by several different points. It may have been related to the key signature of the music; “Thriller” is in a minor key while the Bach music is in a major key. It also may have been because “Thriller” includes a vocal part while the other music pieces did not. Whatever the cause may be, the results are unclear in suggesting a preference for either 440 Hz or 432 Hz.

It is important to consider the factors that may affect the validity of this survey’s results. Firstly, the sample size was rather small with only 57 students. Additionally, within the sample, the pool of science students was disproportionately large compared to the music student pool (42 students and 10 students, respectively). Finally, the primary purpose of differentiating area of study was to compare the preferences of music and science students, which is the reason the analysis of results only compared these two majors. However, there were two students who selected “other” as their area of study that studied one of these areas or both. They are not included in the results analysis above because of their selection, but their data may have provided further insight into the findings of this survey. Ultimately, the results do not indicate a preference for either 440 Hz or 432 Hz tuning.

CONCLUSION

Music's effects on biomolecule production are complex, but research is increasingly unveiling this relationship. Music affects cortisol, beta-endorphin, oxytocin, and the immune system in unique, but interdependent ways. There may be other molecules, chemicals, or processes in the body that are also affected by music (and may even play a role in the above biomolecules' responses to music) but have yet to be discovered.

This highlights that the connection between music and science is one that needs to be strengthened. Although there do exist many studies investigating the effect of music on the body, the results are not always consistent. The differing conditions under which participants were studied and biomolecule data was collected may also contribute to this inconsistency. Future studies may benefit from examining music's effects in more similar conditions. In addition, the chemical and biological intricacies of several molecules and their actions within the body may require further research to better understand music's effect on them, especially concerning the interactions between them.

In the future, if a greater understanding about how music affects the body is achieved, music could be more widely integrated into care of the body. This could be through something as simple as everyday wellness practices or as significant as medical care. Music may have the potential to heal, and understanding its connection with human biology can help unlock it.

APPENDIX

Survey Questionnaire and Responses

Baylor University

This survey is being conducted by Jessica Fan for completion of a thesis within the Baylor Honors Program. As part of this study, you will be asked to listen to short audio clips and complete the questionnaire regarding your preferences on which music you prefer. Please ensure you are in an environment conducive to listening. The survey should take no longer than 5 minutes to complete.

Your responses will be anonymous. There are no risks associated with this study. By selecting yes, you consent to participate in the study and agree that the purpose of this research has been satisfactorily explained to you. You understand you are free to discontinue participation at any time if you so choose and the researchers will answer any questions that may arise during the course of research.

Survey will be open until February 28th, 2022 or until we have received a satisfactory number of responses. Please contact Jessica Fan (Jessica_Fan1@baylor.edu) or Dr. Tom McGrath (Tom_McGrath@baylor.edu) if you have any questions.

I have read the above and give my consent to participate in this study.

- ◯ Yes
- ◯ No

Figure A.1. Consent Question for Survey.

Baylor University

Please listen to about 30 seconds of each of the short audio files within the links below, then select the one you find more enjoyable to listen to. You do not need to listen to the entire clip.

Clip 1

Clip 2

- ◯ Clip 1
- ◯ Clip 2
- ◯ No preference/no difference heard

Figure A.2. Listening Section, Question One.

Clip 1 music source: https://soundcloud.com/musicin432hz-freeyourmind/432-hz-sample-track?utm_source=clipboard&utm_medium=text&utm_campaign=social_sharing

Clip 2 music source: https://soundcloud.com/musicin432hz-freeyourmind/440-hz-sample-track?utm_source=clipboard&utm_medium=text&utm_campaign=social_sharing

Please listen to each of the short audio files within the links below, then select the one you find more enjoyable to listen to.

Clip 1

Clip 2

- ◯ Clip 1
- ◯ Clip 2
- ◯ No preference/no difference heard

Figure A.3. Listening Section, Question Two.

Clip 1 music source: https://musopen.org/music/3998-cello-suite-no-1-in-g-major-bwv-1007/, under “Recordings”: “Cello Suite No.1 in G major, BWV 1007” by Colin Carr]

Clip 2 music source:

https://www.youtube.com/watch?v=DREroaTAk4g&ab_channel=JoyfulSounds

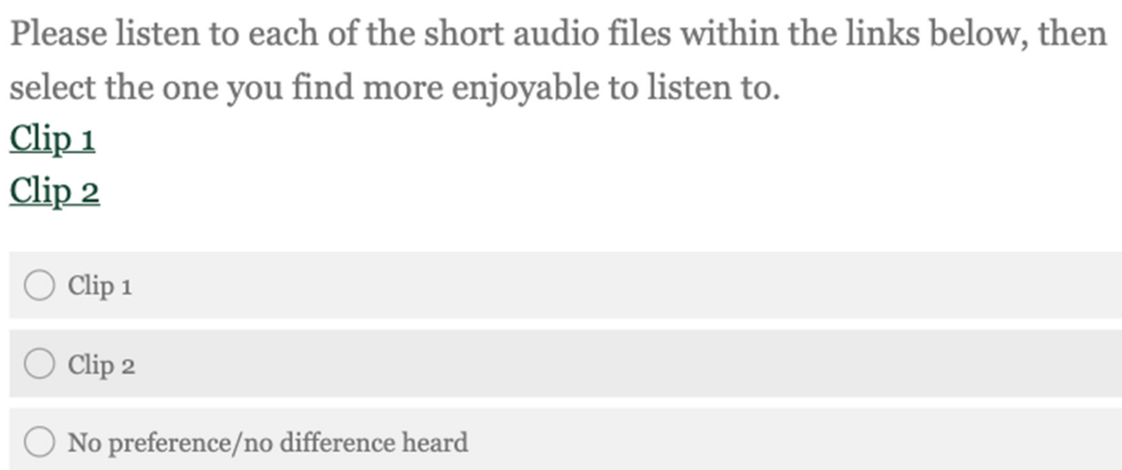


Figure A.4. Listening Section, Question Three.

Clip 1 music source:

https://www.youtube.com/watch?v=bZW7e95uz6Q&ab_channel=0liR

Clip 2 music source:

https://www.youtube.com/watch?v=xIx_HbmRnQY&ab_channel=TimeForLyrics


Baylor University

What is your primary area of study?

- Science
- Music
- Other

Figure A.5. Demographics Section, Question One.

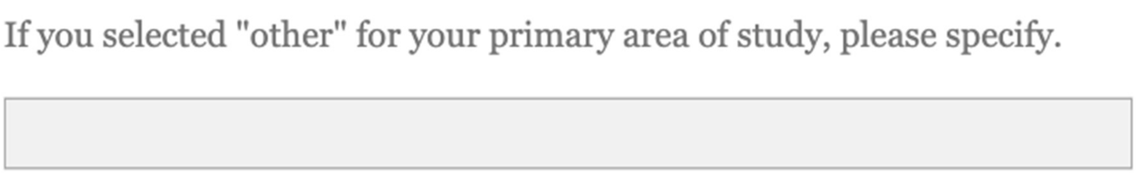
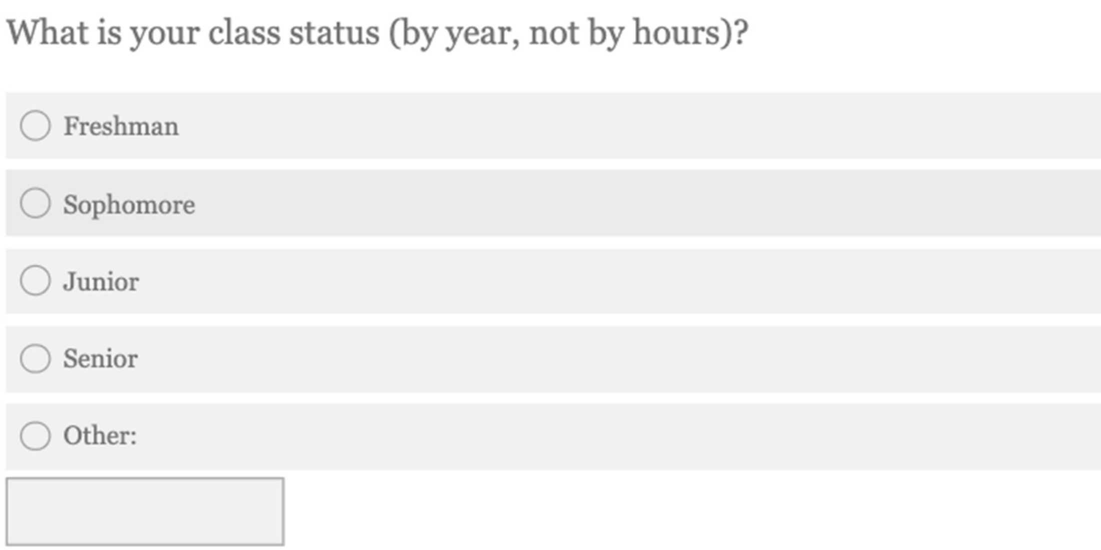

If you selected "other" for your primary area of study, please specify.

Figure A.6. Demographics Section, Question Two.


What is your class status (by year, not by hours)?

- Freshman
- Sophomore
- Junior
- Senior
- Other:

Figure A.7. Demographics Section, Question Three.

How do you describe yourself?

- ◯ Male
- ◯ Female
- ◯ Non-binary / third gender
- ◯ Prefer to self-describe
- ◯ Prefer not to say

Figure A.8. Demographics Section, Question Four.

Baylor University

We thank you for your time spent taking this survey.
Your response has been recorded.

Figure A.9. End of Survey Message.

REFERENCES

1. Nakayama H, Kikuta F, Takeda H. A Pilot Study on Effectiveness of Music Therapy in Hospice in Japan. J Music Ther. 2009 Jun 1;46(2):160–72.

2. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 2009 Jun;10(6):434–45.

3. Thau L, Gandhi J, Sharma S. Physiology, Cortisol. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [cited 2021 Sep 24]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK538239/

4. Adam EK. Emotion—Cortisol Transactions Occur Over Multiple Time Scales in Development: Implications for Research on Emotion and the Development of Emotional Disorders. Monogr Soc Res Child Dev. 2012;77(2):17–27.

5. Merenlender-Wagner A, Dikshtein Y, Yadid G. The β-Endorphin Role in Stress-Related Psychiatric Disorders. Current Drug Targets. 2009 Jun;10(11):1096-1108.

6. Hildebrandt T, Shope S, Varangis E, Klein D, Pfaff DW, Yehuda R. Exercise reinforcement, stress, and β-endorphins: An initial examination of exercise in anabolic–androgenic steroid dependence. Drug Alcohol Depend. 2014 Jun;139:86–92.

7. van Ree JM, Smyth DG, Colpaert FC. DEPENDENCE CREATING PROPERTIES OF LIPOTROPIN C-FRAGMENT (ß-ENDORPHIN) : EVIDENCE FOR ITS INTERNAL CONTROL OF BEHAVIOR. Life Sciences. 1979;24(6):495-502.

8. Fancourt D, Ockelford A, Belai A. The psychoneuroimmunological effects of music: A systematic review and a new model. Brain Behav Immun. 2014 Feb;36:15–26.

9. Jones C, Barrera I, Brothers S, Ring R, Wahlestedt C. Oxytocin and social functioning. Dialogues Clin Neurosci. 2017 Jun;19(2):193–201.

10. Viero C, Shibuya I, Kitamura N, Verkhratsky A, Fujihara H, Katoh A, et al. REVIEW: Oxytocin: Crossing the Bridge between Basic Science and Pharmacotherapy. CNS Neurosci Ther. 2010 Jul 7;16(5):e138–56.

11. Jankowski M, Hajjar F, Kawas SA, Mukaddam-Daher S, Hoffman G, McCann SM, et al. Rat heart: A site of oxytocin production and action. Proc Natl Acad Sci U S A. 1998 Nov 24;95(24):14558–63.

12. Heinrichs M, von Dawans B, Domes G. Oxytocin, vasopressin, and human social behavior. Front Neuroendocrinol. 2009 Oct;30(4):548–57.

13. Nicholson LB. The immune system. Essays Biochem. 2016 Oct 31;60(3):275–301.

14. Beck RJ, Cesario TC, Yousefi A, Enamoto H. Choral Singing, Performance Perception, and Immune System Changes in Salivary Immunoglobulin A and Cortisol. Music Percept Interdiscip J. 2000;18(1):87–106.

15. Borish LC, Steinke JW. 2. Cytokines and chemokines. J Allergy Clin Immunol. 2003 Feb 1;111(2):S460–75.

16. Fancourt D, Williamon A, Carvalho LA, Steptoe A, Dow R, Lewis I. Singing modulates mood, stress, cortisol, cytokine and neuropeptide activity in cancer patients and carers [Internet]. 2016 [cited 2020 Oct 7]. Available from: http://ecancer.org/en/journal/article/631-singing-modulates-mood-stress-cortisol-cytokine-and-neuropeptide-activity-in-cancer-patients-and-carers

17. Miluk-Kolasa B, Obminski Z, Stupnicki R, Golec L. Effects of music treatment on salivary cortisol in patients exposed to pre-surgical stress. Exp Clin Endocrinol Diabetes. 1994;102(02):118–20.

18. Leardi S, Pietroletti R, Angeloni G, Necozione S, Ranalletta G, Del Gusto B. Randomized clinical trial examining the effect of music therapy in stress response to day surgery. Br J Surg. 2007 Jul 16;94(8):943–7.

19. Qiu J, Jiang Y, Li F, Tong Q, Rong H, Cheng R. Effect of combined music and touch intervention on pain response and β-endorphin and cortisol concentrations in late preterm infants. BMC Pediatr. 2017 Dec;17(1):38.

20. Koelsch S, Boehlig A, Hohenadel M, Nitsche I, Bauer K, Sack U. The impact of acute stress on hormones and cytokines, and how their recovery is affected by music-evoked positive mood. Sci Rep [Internet]. 2016 Mar 29 [cited 2021 Mar 17];6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4810374/

21. Knight WEJ, Rickard NS. Relaxing Music Prevents Stress-Induced Increases in Subjective Anxiety, Systolic Blood Pressure, and Heart Rate in Healthy Males and Females. J Music Ther. 2001 Dec 1;38(4):254–72.

22. Grape C, Sandgren M, Hansson L-O, Ericson M, Theorell T. Does singing promote well-being?: An empirical study of professional and amateur singers during a singing lesson. Integr Physiol Behav Sci. 2002 Jan;38(1):65–74.

23. Chanda ML, Levitin DJ. The neurochemistry of music. Trends Cogn Sci. 2013 Apr;17(4):179–93.

24. Nilsson U. Soothing music can increase oxytocin levels during bed rest after open-heart surgery: a randomised control trial. J Clin Nurs. 2009;18(15):2153–61.

25. Seltzer LJ, Ziegler TE, Pollak SD. Social vocalizations can release oxytocin in humans. Proc R Soc B Biol Sci. 2010 Sep 7;277(1694):2661–6.

26. Yuhi T, Kyuta H, Mori H, Murakami C, Furuhara K, Okuno M, et al. Salivary Oxytocin Concentration Changes during a Group Drumming Intervention for Maltreated School Children. Brain Sci. 2017 Nov 16;7(12):152.

27. Charnetski CJ, Brennan FX, Harrison JF. Effect of Music and Auditory Stimuli on Secretory Immunoglobulin a (IGA). Percept Mot Skills. 1998 Dec 1;87(3_suppl):1163–70.

28. Bittman BB, Berk LS, Felten DL, Westengard J, Simonton OC, Pappas J, et al. COMPOSITE EFFECTS OF GROUP DRUMMING MUSIC THERAPY ON MODULATION OF NEUROENDOCRINE-IMMUNE PARAMETERS IN NORMAL SUBJECTS. Alternative Therapies. 2001 Jan;7(1):38-47.

29. Koyama M, Wachi M, Utsuyama M, Bittman B, Hirokawa K, Kitagawa M. Recreational Music-Making Modulates Immunological Responses and Mood States in Older Adults. J Med Dent Sci. 2009;56:79-90.

30. Wachi M. Recreational music-making modulates natural killer cell activity, cytokines, and mood states in corporate employees. Med Sci Monit. 2007 Jan 30;13(2):CR57-70.

31. Calamassi D, Pomponi GP. Music Tuned to 440 Hz Versus 432 Hz and the Health Effects: A Double-blind Cross-over Pilot Study. EXPLORE. 2019 Jul;15(4):283–90.

32. Aravena PC, Almonacid C, Mancilla MI. Effect of music at 432 Hz and 440 Hz on dental anxiety and salivary cortisol levels in patients undergoing tooth extraction: a randomized clinical trial. J Appl Oral Sci. 2020;28:e20190601.

33. Dubey P, Kumar Y, Singh R, Jha K, Kumar R. Effect of music of specific frequency upon the sleep architecture and electroencephalographic pattern of individuals with delayed sleep latency: A daytime nap study. J Fam Med Prim Care. 2019;8(12):3915.