The Effect of Music Tempo on Spatial Learning and Memory in Developing Mice

By
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April 4, 2016



Allegheny College
Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____
Pledge

Approval by the Senior Thesis Committee:

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Dr. Mumme

_____
Dr. Lundberg

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ABSTRACT

Music is a powerful influence that has effects on the body such as change in heart rate and inducing fear or relaxation. It also affects memory and task performance. Spatial learning of novel environments is one aspect to memory that can be influenced by music. Previous studies have demonstrated that exposure to classical music can increase neural plasticity, a process known as the "Mozart effect." Due to music's complexity, there are many variables that could potentially contribute to the relationship between music and memory, one being music tempo. I examined the effect of different music tempos on short-term and long-term spatial memory in four treatments groups of mice. The study consisted of a control group not exposed to music, and three treatment groups exposed to fast, normal or slow tempos of Mozart's Sonata K. 448. Exposure was 4 hours per day, 3 days per week over a 4-week period. At 5 weeks old, twice a week for 3 weeks, each mouse was tested using two mazes: a Morris water maze to test for long-term spatial learning and memory and an 8-arm radial arm maze to test for short-term working memory. All mice showed significant improvement with each trial in both mazes over the course of the study. Music treatment, however, had no significant effect on maze performance, and mice in all four treatment groups showed comparable levels of improvement over time on both spatial tasks. While music tempo is one variable of music that could potentially affect memory, my study was unable to demonstrate an effect of music tempo on either short-term or long-term spatial memory.

INTRODUCTION

The brain is the most complex organ, made up of billions of nerve cells that continue to grow throughout one's life. As it grows, pathways and connections are built between the nerve cells, called synapses (Bales 1998). These synapses are used to make neural connections to what we see or hear and form them into memories. This neural plasticity allows the brain to modify its organization based on new information (Wan and Schlaug 2010). Plasticity is the underlying factor in normal development such as learning and memory, which can include behaviors and personality (Wan and Schlaug 2010). Neural connections are also related to spatial and temporal reasoning and certain stimuli can activate these connections (Bales 1998). Music's affect on brain development can provide the brain with pathways needed for other cognitive tasks.

Music has always had a powerful influence on the human body, physiologically and psychologically. Physically, music has been found to affect stress levels, lower blood pressure and affect heart rates. It has also been shown to alter signaling pathways at the molecular level in mice (Chikahisa et al. 2006). Psychologically, music has been used to relax or excite and enhance learning performance. Music has the ability to positively affect people with neurological diseases (Takahashi & Matsushita 2006). Music and specific sounds have the ability to induce fear based on the sound being played. Sounds that create fear tend to have a smaller stimulus effect, whereas the sound of joyful music has the ability to enhance brain activity (Koelsch et al. 2013). The complexity of music itself offers higher brain enhancement (Bales 1998).

Spatial learning is one aspect of learning performance that can be increased with the addition of a musical stimulus. Spatial learning is the part of memory that is responsible for recording information about one's environment and spatial orientation. Spatial memory is essential to locate food and orient to sounds that are specific to location (Yamamoto & Shelton,

2009). Effective music exposure leads to improved children's performance in specific tasks (Bade et al. 1989). Even more so, another study found that children, when exposed to music before the age of 12, had increased spatial-temporal reasoning (Chan, Ho & Cheung 1998). For learning performance, a study found that prenatal exposure to music enhanced learning performance of adult mice (Chikahisa et al. 2006). Overall, exposure to music has successfully enhanced spatial learning.

Classical music is a genre of music that has produced positive learning effects. While there is little known regarding specific neural mechanisms behind the enhancement of spatial abilities following music exposure, insight to the relationship can be found within the Trion model of neural network organization (Rauscher et al. 1998). The Trion model is the basis for the Mozart effect which has been studied with its relationship to spatial-temporal learning (Rauscher et al. 1998). Listening to Mozart for a short period of time has shown to enhance spatial learning in mice (Chikahisa et al. 2006). The Trion model proposes that musical stimulus can strengthen neural firing patterns which are used when completing spatial-temporal tasks (Rauscher et al. 1998). There has been controversy over the effectiveness of the Mozart effect because some laboratories have been unsuccessful in reproducing the same positive results. One study, for example, argued that the rats used were deaf to most of the notes in the sonata (Steele 2006). However, exposure to Mozart's music early in life can increase the brain-derived neurotrophic factor (BDNF) concentration in the hippocampus in rats (Bade et al. 1989). BDNF plays a key role in how easily the brain can adapt and learn how to effectively complete new challenges, which makes them grow more quickly and develop stronger connections (Ratey & Hagerman 2009). Such results have led to other experiments that have focused on early exposure to Mozart's sonata and the effects on mice or rats in their perinatal period (Chikahisa et al.

2006). The Trion model and the Mozart effect strongly suggest that neural plasticity conforms to an individual experience, and there is a neurophysiological connection between music exposure and enhancement in spatial learning based on those individual experiences (Rauscher et al. 1998). While the Mozart effect has not consistently given positive results, it has created a basis for future research on the effect of music.

Music affects those who listen to it differently. In humans, a specific piece of music may have a deeper meaning for one person than another. However, for animals it is different. Carp are strongly affected by the presence of music waves in water. These fish in turn have slower growing rates and higher anxiety with the music present (Papoutsoglou et al. 2007). Broiler chicks grow more rapidly in the presence of music (Gvaryahu et al 1989). Dairy cows are more willing to go into a milking station if music is played (Uetake et al. 1997). It is even said that plants grow faster when talked to or music played, which is why many greenhouses tend to play classical music (Qin et al. 2003). Mice are social animals and have many similar qualities to humans, which is why they are so commonly used to study the effects of music.

Sound travels at different wavelengths and these different wavelengths can have an effect on the auditory complex in the brain. Sound comes in the form of sound waves which are captured by the outer ear and directed to the eardrum. The sound waves then vibrate the eardrum and move to the cochlea, where hairs are located that send an electrical signal to the brain. In the brain, the sounds are categorized by sound pitch, loudness and location. When the signals enter the brain, the auditory complex processes the signals by adjacent neurons that react to specific pitches and interpret them. The sound signals are processed in different regions of the auditory complex on both sides of the brain. The lobes then move the signal to the amygdala which is then sent to the hippocampus (Koelsch et al. 2013). The hippocampus is responsible for spatial

orientation, navigation and forming memories. In the hippocampus, this is where spatial information is processed and applied, creating the association for the memory to be formed and stored.

Music comes in different forms, for example tempo and key, which are based on different beats and wavelengths. Therefore, each musical piece has specific qualities that can affect task and learning performance. Different musical pieces create a different sense of arousal in a person's mood (Ting 2009). This arousal can increase performance to a higher level because of the influence on mood, which creates optimal learning conditions. However, these arousal levels can differ. While there is an optimal level of arousal, there can also be under arousal and over arousal which may cause deterioration of performance and memory (Hallam et al. 2002). Music tempo has been correlated with the ability to perform tasks, and specifically fast music tempo has been shown to increase spatial reasoning as well as produce positive effects on mood (Husain et al. 2011). Therefore a tempo paced too slow or too fast may lead to deterioration of memory or inability to retain new information.

Music has the ability to decrease stress and improve spatial learning performance. The tempo of music also plays a role in how a task can be performed. The developmental stage of mice is crucial to learning and brain growth. Thus introducing music at an early age may affect learning behavior in adulthood. Based on the Mozart effect and other studies, early exposure to Mozart's music can cause a change in spatial learning. Many other studies have tested mice exposed to music in the perinatal period and following birth, but not solely after birth (Chikahisa et al. 2006). I hypothesize that exposing mice to a Mozart sonata at different tempos after birth will affect their spatial learning performance. Specifically, I propose that a faster-paced tempo will enhance and accelerate their spatial learning response, allowing the mice to process

information about their environment faster. Other studies have exposed their research subjects to music while testing (Chikahisa et al. 2006 & Ting 2009), however, I believe that because my mice will be exposed to long listening periods for the experiment until the start of testing, they will have been adequately pre-conditioned and will not need music played during testing.

My experiment focuses on how exposure to music of varying tempos during development affects spatial memory, as measured by the time it takes the mice to complete two mazes. The mazes were used to test long-term versus short-term spatial learning memory and help determine whether memory benefits from a specific type of music tempo exposure during development.

METHODS

Animal Housing and Handling Conditions

For this study, four pregnant female laboratory mice (*Mus musculus*), brown wild type B6, were obtained from the Allegheny College mouse colony. After the four female mice were placed into four separate cages, they delivered their offspring on 9/30/2015, 10/2/2015, 10/4/2015 and 10/15/2015. The four females and their offspring (19 total) were randomly assigned to one of four treatment groups described in the next section (Table 1). Juvenile mice were weaned between 3-4 weeks old (Figure 1), at which time the sex of the mice was determined. Weaned mice from each litter were then segregated into separate cages by sex to prevent inbred sexual behavior. All mice were fed daily, cages were washed and changed every 3 days and water changed at least once per week as mandated by the Animal Review Committee at Allegheny College. All mice were held in a room at 23° Celsius with a 12:12 photoperiod.

Treatment Groups

The mice were randomly assigned to one of four groups: three music temp groups and one group with no music. The first group contained 7 juvenile mice from a single litter that were

exposed to fast music (F+), the second group contained 3 juveniles who were exposed to normal music (Nm), the third group contained 6 juveniles who were exposed to slow music (S-) and the fourth group contained 3 juvenile mice who were not exposed to music as the control (Ct). Attempts were made to acquire mice of the same age and number for each group. However, due to the unpredictability of the number of offspring and gestation periods, there was uneven distribution for all four groups (Table 1).

Table 1: The date of birth for each litter, the litter size and the randomly assigned treatment group for each litter.

Date of Birth	Litter Size	Males	Females	Treatment
9/30/2015	3	3	0	Nm
10/2/2015	6	2	4	S-
10/4/2015	7	5	2	F+
10/15/2015	3	1	2	Ct

Music Conditioning Timeline

Music exposure started 7 days after offspring birth, when young mice begin to develop hearing (Figure 1, Table 1). The music was played 3 days per week with 4 hours of exposure each, a total 48 hours in the 4-week time period. The mice were 5 weeks old when music exposure stopped (Figure 1, Table 2). Music exposure occurred at the same time for all 3 groups. Due to possible music cross-over exposure, the mice exposed to music were placed into separate rooms during the stimulus period in order to avoid sound crossover and keep the control mice from being exposed. Control mice were handled and treated the same as the treatment groups and were also put into a separate room while the other mice were being exposed to music. The mice were only exposed to music during specific stimulus period in the evening hours.

Three hand-held MP3 players were connected to two PC multimedia speakers in each room and the mice were exposed to their group's specific music tempo. In order to reduce environmental disturbances, such as alternative noise patterns that may be present with students attending classes, the music was played during the evening hours between 1700-2100 hours. Testing began when the mice were 6 weeks old and continued for 3 weeks, with two trials per maze each week (Figure 1). Music treatment ended once the mazing testing began.

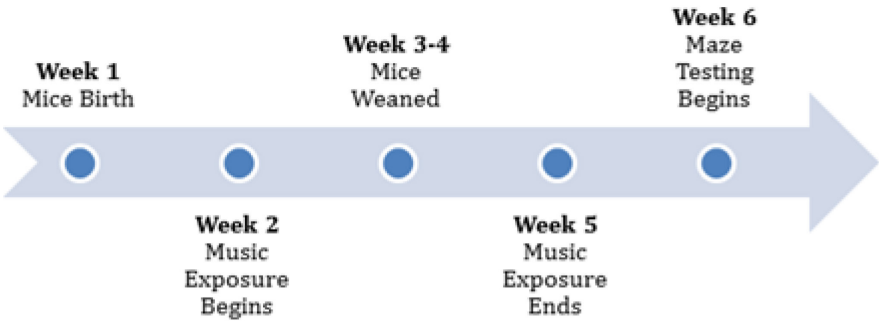


Figure 1*:* A pictorial representation of the timing of experimental treatments and testing.

Musical Stimuli

All music groups were exposed to Mozart's Sonata, K.448. The three treatment groups were exposed to different music tempos, each group with a differing tempo of: normal tempo (120 beats per minute), 40% slower (72 bpm) and 40% faster tempo (168 bpm).

The music tempo was altered using the music editing software package Audacity, http://www.audacityteam.org, by selecting Effects>Change Tempo. Music tempos were created with the selected tempo rates of 40% slower, normal tempo and 40% faster on separate tracks. Although mice hear on a different frequency than humans (Fay 1988), the pitch of the three playback takes were not altered because mice would be able to hear many of the musical frequencies played.

Table 2: Starting and ending dates of music exposure per treatment group.

Treatment Group	Start of Music Exposure	End of Music Exposure
Nm	10/7/2015	10/30/2015
S-	10/9/2015	11/1/2015
F+	10/11/2015	11/3/2015
Ct	N/A	N/A

Testing Protocol

Testing began when the mice were 6 weeks old (Table 3). No music was played during maze testing which lasted for 3 weeks. Two mazes were used to test mice memory and spatial learning: the Morris water maze and the radial arm maze. Each mouse was tested in both the Morris water maze and the radial arm maze twice per week for each maze. Data were collected over 3 weeks, for a total of 6 trials.

Table 3: Starting test dates and ending test dates for each treatment group.

Treatment Group	Start Testing Date	End Testing Date
Nm	11/13/2015	12/4/2015
S-	11/15/2015	12/6/2015
F+	11/17/2015	12/8/2015
Ct	11/25/2015	12/15/2015

To test for longer-term spatial learning and memory, the Morris water maze was used. The maze was a 17-gallon, multi-purpose steel metal tub with a diameter of 2 feet and a platform with a diameter of 3.5 inches (Figure 2). The tub was filled with 30° Celsius water until the water just covered the platform a half of an inch. Four spatial cues (triangle, square, star, and

circle) were set up around the tub, equal distance from each other. The mouse was first placed on the platform in the water for approximately 10 seconds so it could use the spatial cues in relativity to the platform's location. The mouse was then placed in the water at the starting position which was always to the left of the triangle special cue (red star in Figure 2). A total of three timed trials were conducted for each mouse, and then averaged to count as one trial, until all mice were tested. The mice had to swim until the platform was found to stand on. The amount of time it took for the mice to find the platform determines their spatial learning performance. If the mice could not find the platform after 60 seconds or appeared to be in distress, they were taken out of the maze and the maximum time of 60 seconds was recorded. Signs of struggle included no attempts in swimming or the head of the mouse was becoming submerged.



Figure 2: Picture of the Morris water maze and what the mice were expected to do.
http://www.nature.com/nrn/journal/v9/n7/fig_tab/nrn2420_F2.html

To test for short-term memory, the 8 arm radial arm maze was used. The maze consisted of a center platform with eight spokes radiating out from a center point (Figure 3). Before testing began, the mice were given 15- minute habituation periods for 3 days so they could familiarize with the maze and to reduce stress of being placed into a new environment. In each habituation period there were 5 mice or fewer in the maze at a time based on sex and treatment group. Chocolate treats were also scattered throughout the maze during this time to encourage exploration. Eighteen hours before each trial, the mice were deprived of food to increase maze exploration. When testing began, one mouse was placed in the maze. The end of each arm contained a chocolate treat, for a total of 8 treats. The mouse was placed in the center of the maze for ten seconds of acclimation before the octagonal wall was lifted so the mouse could freely explore the maze. To successfully complete the maze, the mouse needed to go down each arm only once and eat all treats. If the mouse entered an arm after already eating the treat in that arm, it was scored as an error. Another way the mice could receive an error was entering an arm again, even if the treat was not eaten. An arm was considered to have been entered when all four feet crossed the line at the base of the arm and the central platform. The mouse needed to use short-term memory and spatial cues to remember which arms it has already visited. There were spatial cues at the end of each arm (circle, triangle, star, square, rectangle, ellipse, star, diamond). The mouse was given a total of 5 minutes to complete the maze. If the mouse ate all the food in the maze the time was stopped and recorded. If treats still remained after 5 minutes, 5 minutes was recorded as the total time in the maze. In between each mouse being tested, the maze was cleaned to prevent odor cross-over which was a possible distraction to the mice.

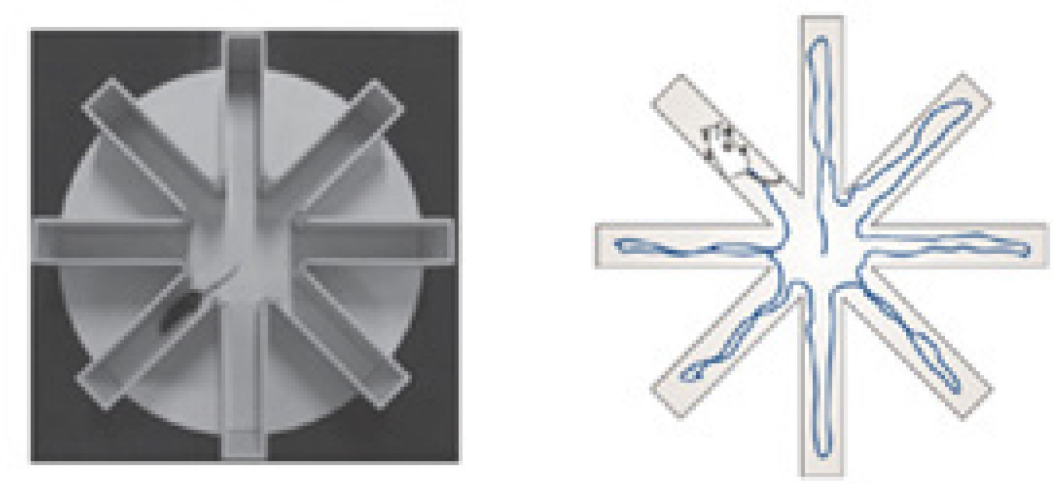


Figure 3: Picture of the 8-arm radial arm maze and what the mice were expected to do. *http://www.nature.com/nrn/journal/v9/n7/fig_tab/nrn2420_F2.html*

Statistical Analysis

Data were collected to determine if the music exposure reduced the average amount of time for the mice to complete the Morris Water Maze. For the radial arm maze, data were also collected to determine if music exposure increased short-term memory by recording the number of treats eaten by the mice compared to the total amount of arms entered, and the number of arms reentered after the treat has already been eaten. The analysis determined if musical stimuli improved maze performance by comparing the control group (no music) to the groups with music and also comparing the three music treatment groups. The statistical package JMP Pro 12.0 was used to create a general linear mixed model with treatment and trial number (time) used as fixed effects and mouse identity as a random effect. A p-value less than 0.05 was considered significant.

RESULTS

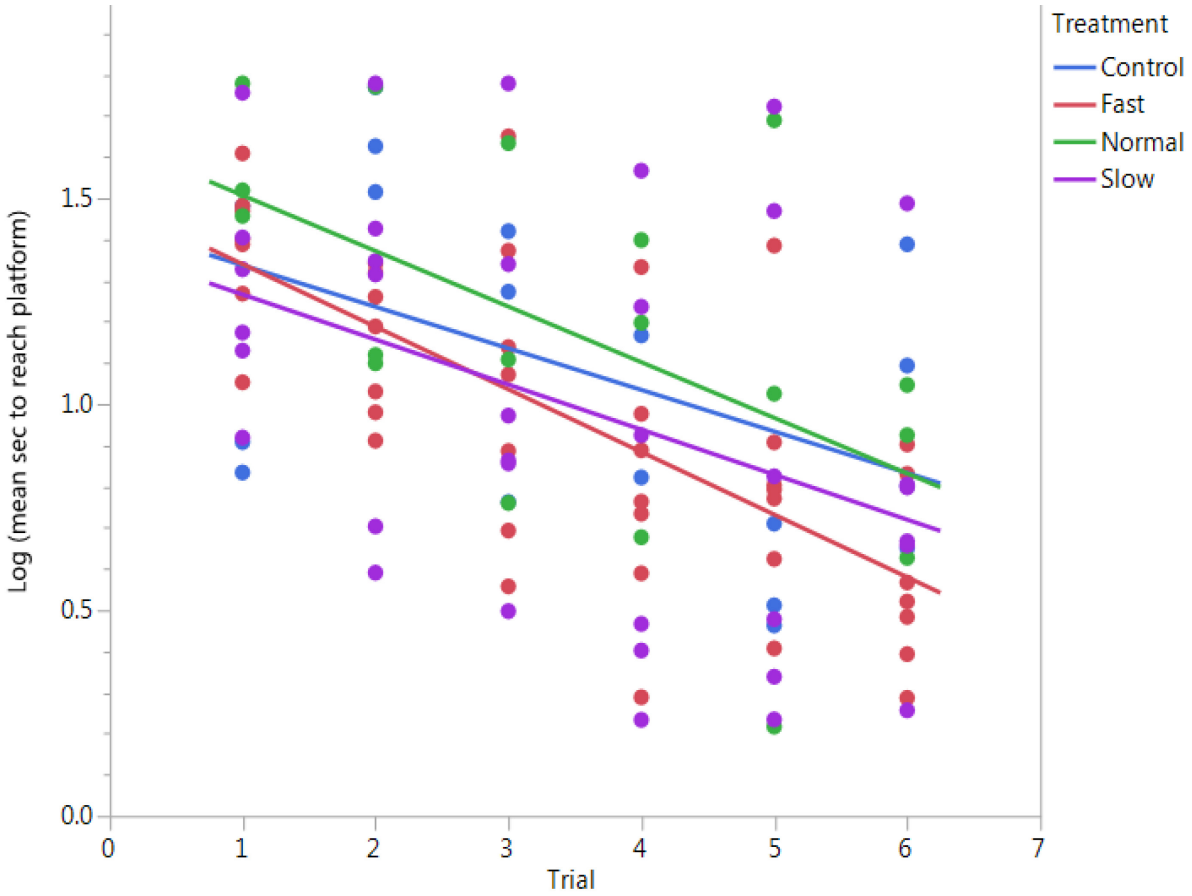


Figure 4: The time it took the mice in each group to reach the platform in the Morris water maze test over 6 trials. The log was taken to normalize the data. Lines represent the linear regression equations for each group.

Morris water maze:

In the Morris water maze experiment all treatment groups improved significantly in average time to reach the platform (Table 4). However, there was no significant effect of treatment; all treatment groups improved at nearly the same rate (Table 4, Figure 4). The F+ and S- groups had similar timing when reaching the platform (Figure 5). The Ct group was slightly higher than S- and F+ and the Nm groups had an overall higher time to reach the platform than the other three groups (Figure 4). Average across all trials, the treatment groups had similar time performance in reaching the platform (Figure 5).

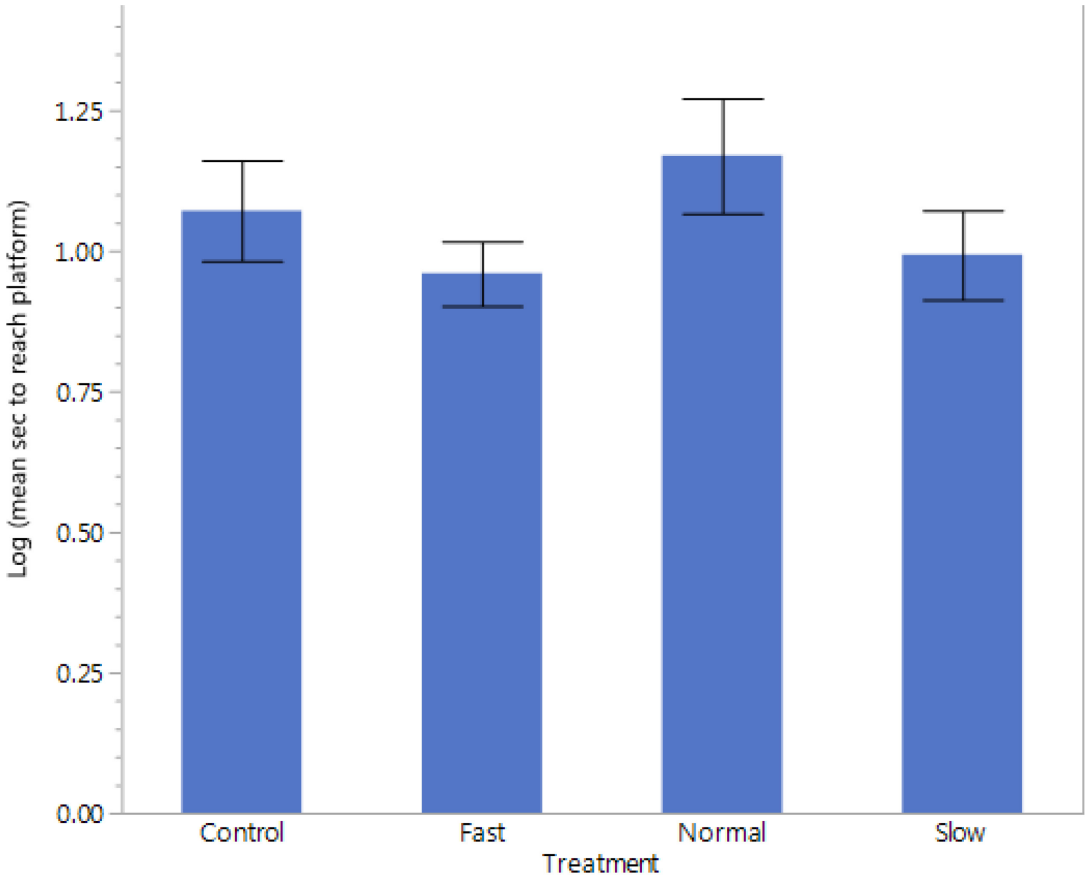


Figure 5: The time in seconds that it took for the mice in each treatment group to reach the platform averaged across all trials. The log was taken to normalize the data. Error bars represent the standard error.

Table 4: Fixed effect tests for the Morris water maze data.

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	3	3	14.92	0.6686	0.5844
Trial	1	1	92.15	47.2876	<.0001*
Treatment*Trial	3	3	92.13	0.4709	0.7033

8-arm radial arm maze:

The top graph of Figure 6 represents the number of times an arm was re-entered after the treat was already eaten. Because re-entries constitute an error, I expected that re-entries would decline over time. However, neither treatment (Table 5) nor trial number (Table 5) had a

significant effect on arm re-entries. The F+, S- and Nm group all had similar performances and showed slight improvement over time. However, the number of re-entries by mice in the control group increased over time (Figure 6, top).

The bottom graph of Figure 6 represents the number of treats eaten relative to the total amount of arms entered. Because this measure is an index of overall maze performance, I expected that the number of treats eaten per arm visited would increase over time. On average, all four treatment groups increased over time and did not keep visiting arm from which they had already removed the treat (Figure 6). While there were no significant differences among the treatment groups (Table 6), there was a significant effect of trials (Table 6).

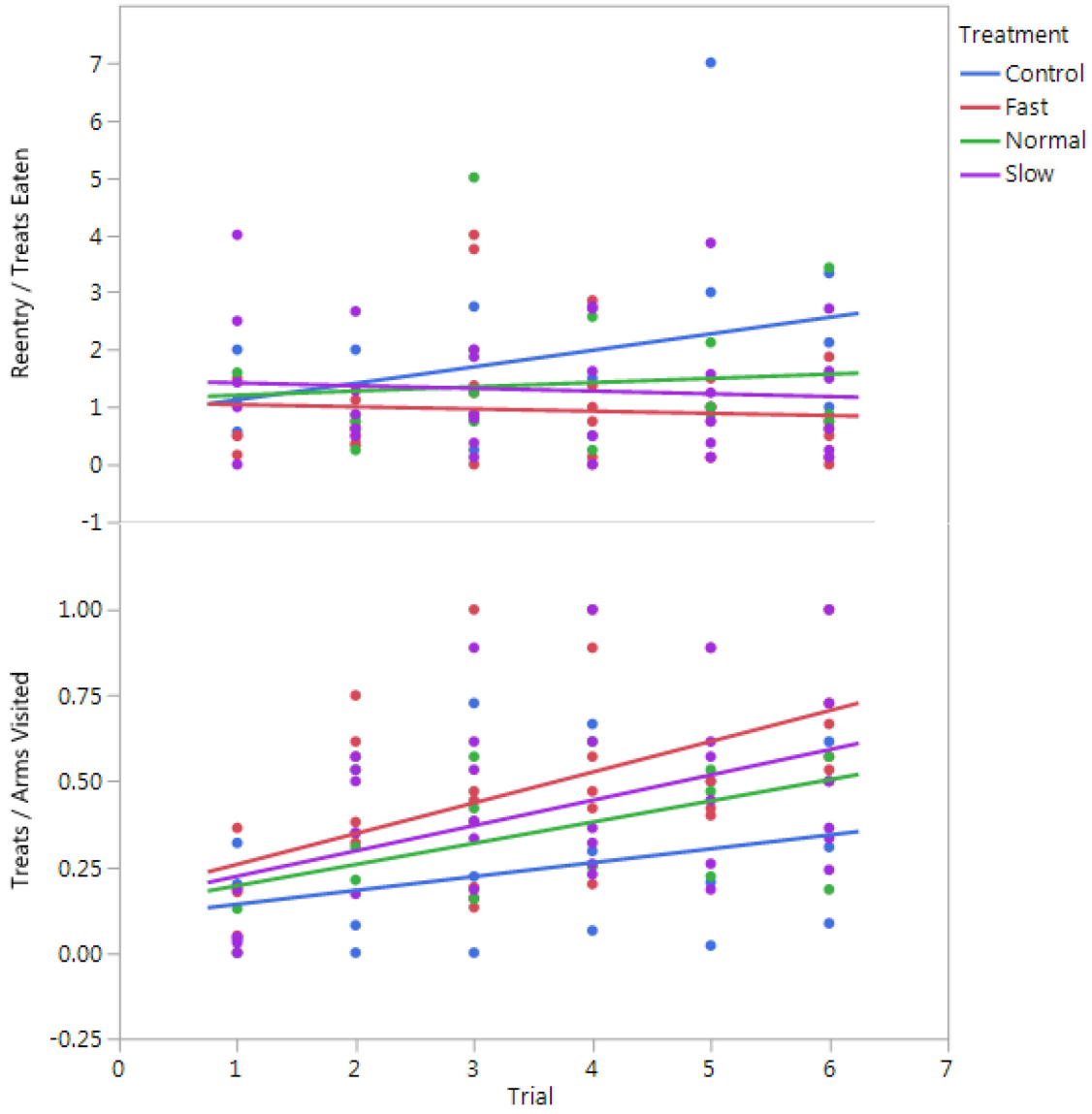


Figure 6: Top: The number of arms re-entered after a treat was already eaten per trial for each group. Bottom: The number of treats eaten compared to the total number of arms visited per trial.

Table 5: Fixed effects tests for radial arm maze data on reentry/treats eaten (see Figure 6, top).

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	3	3	15.17	1.0677	0.3920
Trial	1	1	82.64	0.3903	0.5338
Treatment*Trial	3	3	82.63	1.1102	0.3498

Table 6: Fixed effect tests for radial arm maze treats/arm visited (see Figure 6, bottom).

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Treatment	3	3	15	1.5158	0.2511
Trial	1	1	91	35.4414	<.0001*
Treatment*Trial	3	3	91	0.8947	0.4471

DISCUSSION

The purpose of my study was to determine how different tempos of music affect spatial learning and memory in four groups of mice: a control group with no music exposure (Ct), normal tempo music (Nm), fast tempo music (F+) and slow tempo music (S-). Originally, I hypothesized that mice exposed to Mozart's Sonata K.448 at different tempos would show differences in spatial learning performance, and that music at a faster-paced tempo would produce the greatest enhancement of spatial learning and short-term memory.

My results, however, showed that music tempo, overall, did not have a significant effect on spatial learning and memory in either the radial arm maze test or the Morris water maze test. In both mazes, all mice showed improvement in performance over time and completed the mazes at comparable rates of improvement (Figure 4 and 6, bottom). The only measure that suggested a possible effect of music exposure was arm re-entries per treat eaten from the radial arm maze (Figure 6, top). The musical groups (F+, Nm, S-) were overall consistent in not re-entering an arm once a treat was eaten. However, the control group (Ct), had an increase in the number of arms that were entered again once the treat was already eaten, possibly due to a lack of music exposure. The mice that were exposed to music may have had less anxiety than the Ct mice, which caused them to re-visit more arms.

Although I found no significant effects of music in my study (Tables 3-6), mice did show significant improvement over time in both the Morris water maze test (Table 4) and the number of treats eaten per arm visited in the radial arm maze (Table 6). This result indicates that mice demonstrated spatial learning and memory, and were more efficient in completing the maze with each successive trial.

Music can create physiological responses in both humans to animals. In humans, a study found that fast tempo music of 120-130 beats per minute (bpm) increased anxiety, while slow music of 50-60 bpm decreased anxiety (Edworthy and Waring 2010). Another study in humans found that when 13 undergraduate students were exposed to no music, excited music or sedative music, the sedative and no music induced relaxation while the excited music showed increased relaxation only as the number of conditioning session increased (Iwanaga et al. 2005). With the excited music, it took more times to create a relaxation period almost automatically because the participant needed to adjust to the excited state (Iwanaga et al. 2005). In animals, music caused increased vocalization behavior and increased stress response in pigs (Cloutier et al. 2000). These studies demonstrate that music affects those who listen to it in different ways and may either be beneficial or cause increased anxiety and stress. However, not all studies have found a consistent relationship between music, memory or physiological responses.

One aspect that may explain the inconsistency between tests is the Mozart effect. Many studies use Mozart as the main form of music choice because of its significance in creating a memory response. However, it is possible that Mozart effect may not be as pronounced as once thought, especially in mice. Generally, positive findings of the effect have been found in "enjoyment arousal" (Jenkins 2001). However, in experiments involving animals, there has not been a way to quantify the enjoyment and music appreciation as the basis of increased memory

(Jenkins 2001). This could be due to plasticity in humans that forms with an experience, which is later enhanced by music for spatial learning (Rauscher et al. 1998). A study found that rats were deaf to 69% of the sonata and the portions heard by the rats would have most likely been distorted, meaning the Mozart Effect may not have had any effect on the rats learning performance (Steele 2006). The music for this experiment was chosen to see if music tempo had an effect on memory, but it is possible that the music selected may have been perceived by my mice as it was by the rats studied by Steele (2006). Other studies using human participants used music that varied in genre, such as rock, or a different classical music piece, which may have triggered different responses. However, the effect of music tempo change has not been studied as much, which is why Mozart's Sonata K.448 was chosen to maintain consistency with other studies.

While many studies focus on how music affects the body or memory, there are fewer studies that focus on how music tempo can affect spatial learning and memory. It is virtually unknown what features of music, such as key or tempo, are essential in mice effective learning patterns (Yang et al. 2012). In studies that focus on music tempo, there are many differing results. . One study found that after 44 undergraduate students at Florida State University were exposed to fast or slow paced music. The work completed by the students showed no difference in quantity or quality between the music exposure groups, meaning no significant results were found (Mayheld and Moss, 1989). The same authors found, however, that slow-paced music may have caused relaxation while the rock music may have made the students felt more rushed, causing them to work faster (Mayheld and Moss, 1989). The students reported they felt more distracted with the rock music, which created higher stress and anxiety, but the students ultimately performed better (Mayheld and Moss, 1989). Another study ran a memory retention

test on 30 participants who were exposed to fast tempo, slow tempo or no music (Jurkovic et al. 2013). Other variables such as heart rate and respiration were examined. The study found no statistical significance of music tempos on memory retention (Jurkovic et al. 2013). There were also no large variations between the experimental groups; further supporting that music tempo may have had no effect on memory retention in their study (Jurkovic et al. 2013). The varying results in regards to music tempo could be correlated to the different techniques and methods used in testing and the variability of music itself.

In order to test the music effects on the mice in my study, two mazes were used, the radial arm maze and the Morris water maze. While the radial arm maze tested short-term memory, the Morris water maze tested for longer-term spatial reference memory and recall. Spatial learning is the ability to use memories and environmental cues those memories can be used at a later through short-term and long-term memory (Nadel and Hardt, 2011). The mazes allowed the mice to access the memories regarding the maze layout in order to locate the treats. Contrary to my hypothesis, music did not have a significant effect on the spatial reference memory or recall in any of the treatment groups. All groups in the Morris water maze completed the maze at the same relative speed and the F+ group did not have significantly faster completion of the mazes. However, all treatment groups showed a significant decrease in time per trial, indicating they were able to use the spatial cues and long-term memory to improve with each trial. In the radial arm maze, there was no significance between the treatment groups and the F+ group did not have the fastest time of completion. Instead, the groups all improved per trial at relatively the same speed (Figure 6, bottom). While many studies focus on human participants, the results of my study are supported by the other studies that did not find significant effects of music tempo on appropriate memory tests.

While the results from this study did not show a significant relationship between music tempo and memory recall, there were also many methodological variables that could have affected the outcome. For this study, the litter sizes and sex ratios were very different for the mice assigned to the four treatment groups. The differences in litter size meant that there was unequal representation between the treatment groups (Table 1). The other factor that may have affected the results was the sex ratios (Table 1). Each group had a different number of females versus males which may have had an effect. A study found that male mice placed in a radial arm water maze had fewer working memory and reference memory errors than females (Gresack and Frick 2003). While it is possible that there were no sex difference in my study, it may have affected the results. However, it is hard to determine if there is an effect of sex because the litter sizes were so different which contributed to the difference of sex ratios between treatment groups.

Another decision that I made for this study was to not expose the mice to music during maze testing. This was done to see if early developmental exposure to music altered memory performance later in life. Studies have found that exposure to music early in the developmental stages will lead to better spatial learning and overall memory later in life (Chikahisa et al. 2006). However, it has been found that music can be distracting during task performance (Mayfield and Moss 1989). It has also been recently found that an increase in neurological activity in young infants exposed to music does not produce permanent changes (Underwood 2016). Neural pathways can be stimulated and formed early in life but as the music stops, over time the pathways can weaken (Underwood 2016). There needs to be repeated stimulation to build the neural pathways (Underwood 2016).

In my study the beats per minute for the slow and fast treatment groups were chosen based on a previous study by Ting (2009). Many studies do not define how the music tempos are changed and there is not a defined answer to which tempo may be best for learning in mice. Originally, it was believed that a music tempo could create an over or under-arousal, which can either promote or interfere with spatial learning and memory. Research on humans indicates that music tempo may need to be in a specific range for memory enhancement (Ting 2009). The study was performed on humans and found that in order to get results in memory testing, the tempos should be in the range of 60 bpm (slow), 120 bpm (normal/optimum), and 165 bpm (fast). These findings provided me with experimental justification for increasing and decreasing Mozart's Sonata K.448 by 40% for the fast and slow groups. However, Ting (2009) found that the optimal music tempo for increased memory was 120 bpm and the 60 bpm and 165 bpm groups were over and under-aroused. Because I did not find such an effect in mice, the mice may have a different range of bpms that optimally stimulate memory and learning.

Finally, I elected not to change the Hz frequency. While mice do hear at a different frequency than humans, frequency was not experimentally adjusted due to the tempo already being changed. It has been found that when rats have early exposure to music, that then influences the functional development of the primary auditory complex (Rauscher 2006). This then affects the auditory threshold which can change based on the music that is played (Rauscher 2006). In my study, the mice were exposed to music 7 days after birth when their hearing develops, allowing the auditory complex to develop with the music they were exposed to. Therefore change in frequency was not a concern.

My investigation raises new questions while leaving many questions to still be answered. Numerous variables exist within music and changing music tempo is another factor that could

generate further studies. The experimental variables of my study include sample size of the treatment groups, music tempo and the types of mazes used. Other mazes that could be used that test the same type of memory as the radial arm maze include the Y-maze or the Elevated Plus Maze. Morris water maze variations include the Barnes Maze or the Oasis Maze.

For my study, I manipulated music tempo. While the Mozart effect is a prominent aspect in music and memory research, it may not have necessarily been the best piece to play, compared to other classical music or other music genres that have been studied. The genre of music and length of exposure could have caused an effect. The first month of mice brain development is equivalent to two years in human life (Whiteus et al. 2014). Therefore mice were exposed to music for a total of 48 hours over a 4 week period, which may not have been long enough to reinforce the neural connections between music and learning tasks. While the protocol in my study was modeled after similar research, the variables mentioned above could have potentially affected the results. Research on the effect of music tempo on memory has been studied more in humans; therefore using mice may have been a limitation or a new factor that can be studied further. Ways to see how exactly music tempo may affect the brain could be to examine the physiology and morphology of the brain, specifically the auditory complex and the hippocampus. Both areas may have changed or developed differently from the music exposure. Although I found no significant effects of music exposure on maze performance, my findings do not necessarily suggest that there were no effects on neural development. Other aspects that could be investigated are the stress response of the mice during testing by looking at feces production, or weighing the mice to determine if certain music tempos increased anxiety causing weight decrease. While my study did not produce significant results, other studies have suggested that music tempo can have an effect on memory and learning in some situations. It may just be a

matter of finding the right balance of experimental conditions to produce repeatable and significant results.

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