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Kaitlin O'Toole *Coastal Carolina University* 

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## Of Mice and Mozart: Testing Spatial Reasoning and Memory of Rats \$2012\$

#### BY KAITLIN O'TOOLE BIOLOGY/PSYCHOLOGY

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Of Mice and Mozart: Spatial reasoning and memory of rats

#### Abstract

An increase in spatial-temporal reasoning has been documented in the presence of Mozart's sonata K.448; this enhancement of physical and psychological activities is called the "Mozart Effect" (Hughes 2001). This effect has been recorded in humans and animals alike, and its influence reaches a myriad of applications including calming wild animals to increasing test scores in college students to lowering high blood pressure. Using a cheeseboard apparatus to test spatial reasoning in rats, this experiment recorded the results from maze tasks completed in the presence of sonata K.448 compared to trials completed in silence. Results showed a significant difference between their task completion time when comparing the music and silence treatments. In the presence of Mozart music, rats completed the maze faster and therefore more efficiently. This evidence can be utilized to improve conditioning techniques in captivity and at rehab centers, as well as bolster support for the debated Mozart Effect and new Cheeseboard maze apparatus.

#### Introduction

Music is a connective thread that reaches through time and culture. Consequential benefits of the music of Mozart in particular have been researched and documented with increasing popularity. The effects of Mozart's music seem to be wide spread, whether it be touching an aboriginal people that have never come into contact with the music before or producing health benefits in the brain and circadian rhythmic functions of the body (Lemmer 2008). Mozart's music has reportedly helped ease depression symptoms, Alzheimer's and Parkinson's diseases, high blood pressure, and increase learning and spatial reasoning (Hughes 2001). The music itself is highly rhythmic in structure, and it is presumed that the body's response to this repeating structure of sound synchronizes bodily rhythms like the cardiovascular and respiratory systems (Lemmer 2008). This is similar to what a mantra, meditation, or repeated prayer can physically do to the body (Lemmer 2008). The specific enhancement of physical and psychological activities that are linked to listening to Mozart is called the "Mozart Effect" (Hughes 2001).

The Mozart Effect has been documented in humans and animals alike, often with similar results. Mice and rats are the most commonly studied animals for much of scientific research, as is the case when studying the Mozart Effect. Even though mice have a higher hearing range than humans (800 kHz to 115.4 kHz), Chikahisa et al. found that music played for lab mice at a standard range for human hearing (20 Hz to 20 kHz) still had an effect on the test subjects (2006). This experiment tested if the Mozart Effect could start early in prenatal development and help later in solving a spatial memory maze task (Chikahisa et al. 2006). Three test groups were assigned: a Mozart sonata, white noise, or silence was played during the night when mice were active during pregnancy, for sixty days after birth, and continued until the last days of the maze trials were completed with the newborn mice (Chikahisa et al. 2006). All groups succeeded in lowering the maze-running time throughout the trials, meaning that the mice were learning the maze itself, but the Mozart group had significantly fewer errors than the silence or white noise groups (Chikahisa et al. 2006). Music group mice had higher protein levels in their cortex and hippocampus, suggesting that Mozart music enhances these proteins that help in spatial memory tasks (Chikahisa et al. 2006). This study concluded that continued exposure to Mozart music—from prenatal stage through adulthood—increases brain activity and spatial reasoning and memory skills in mice (Chikahisa et al. 2006). Another possibility for the results, however, states that the music provided an enriching environment for the growing mice and their abilities were a direct reflection of the nurturing environment rather than the science of brain and music functioning together (Chikahisa et al. 2006).

Similar encouraging results were found after exposing mice to Mozart's music for one month compared to musically naive mice (Meng et al. 2009). Mice in the music treatment showed increased

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gene expression in both the hippocampus and cortex compared to the mice in the silent treatment (2009). This is further evidence suggesting that the brain is more stimulated when Mozart's music is present, the stimulation allowing the increase in spatial memory to come about. Interestingly, not only did spatial memory improve for the mice in the music treatment, but an increase was also shown in fear-motivated learning compared to the non-musical mice (Meng et al. 2009), suggesting that the Mozart Effect can reach beyond just improving spatial memory to improve learning and memory in general.

Lemmer found the Mozart Effect to be more pronounced in changing cardiac output in hypertensive rats compared to normotensive rats (2008). Unlike the previous experiment, Lemmer presented the music in periods of light and dark and monitored heart rate and blood pressure during both testing periods (Lemmer 2008). Neither white noise nor the music of another composer, Ligeti, made a significant difference to heart rate or blood pressure in any of the rats (Lemmer 2008). During the light period—the rats' resting phase—Mozart music significantly decreased cardiac output in hypertensive rats by decreasing heart rate (Lemmer 2008). The normotensive rats, however, showed no changes in cardiac output in any of the experiments (Lemmer 2008). This study suggests that Mozart music may produce a therapeutic effect in hypertensive patients, whose body and cardiovascular system appear in rats to be more sensitive to change than normal rats; the organized tone, pitch, and other rhythmic qualities of Mozart's music, was again hypothesized to be the reason for the soothing affects in the cardiovascular system (Lemmer 2008).

Spatial memory in human participants has been shown to improve after listening to Mozart's music as well. Compared to listening to increasingly repetitive music and a short story read on tape, only Mozart's music improved spatial memory in college students (Rauscher, Shaw, and Ky 1995). Interestingly, it has been determined that a positive or negative impression about the Mozart Effect will not affect the level of spatial memory increase that occurs because of the music (Stnading, Verpaelst,

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and Ulmer 2008). These findings suggest that the Mozart Effect is indeed true, no matter the preconceived notion of the effect on participants. Jausovec and Habe (2003) issued a "visual oddball task" to students in a psychology class under two conditions—listening to Mozart's sonata K. 448 or in silence. Sonata K. 448 is often used in experiments testing for the Mozart effect. An EEG was recorded for each participant while they were completing the tasks, and Mozart's music significantly increased brain waves and activity in the left hemisphere (Jausovec and Habe 2003). Although brain activity increased while listening to music during the task, the music did not significantly aid in faster or more correct answers (Jausovec and Habe 2003). It was hypothesized increased brain activity in the music trials connected different areas of the cortex, therefore increasing activity and potential in thinking processes; however, since the brain had more stimuli to take in when music is playing it was proposed that the positive effects ended up evening out with the silent trials because of the increased brain power needed to intake all of the stimuli (Jausovec and Habe 2003). Overall, the experiment concluded that the Mozart effect can be explained by the coupling of brain areas which allows faster processing, but the effect could have not been seen in this task because of its simplicity (Jausovec and Habe 2003). It will be interesting to see if animals can handle an increased stimuli load including listening to music and performing an unrelated task.

Similarly, college students were found to show an increase in spatial memory after listening to Mozart's music compared to completing a spatial task in silence (Jones, West, and Estell 2006). Since this was somewhat unsurprising, the researchers went a step further to see if the actual increase in spatial memory was due to arousal in the brain based on the presence of music (2006). Music increases brain activity—as discussed in the previous paragraph—and arousal as well as positive feelings; this increase in arousal and mood was hypothesized to be the reason that spatial memory increased while listening to Mozart, an occurrence referred to as the arousal effect (2006). The researchers did in fact find that in college students, self-reported arousal and emotions increased while listening to music and

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were significantly responsible for the increase in spatial memory (2006). Jones and Estell found similar results when testing the arousal effect on high school students (2007). While listening to Mozart's music, high school students scored better on spatial memory tasks and were reported to be significantly more awake and aroused (Jones and Estell 2007).

Jausovec performed another experiment to see how the Mozart effect could be related to learning traits and priming (Jausovec, Jausovec, and Gerlic 2006). Participants were equalized in traits such as emotional states, personality, and intelligence because all of the tested traits are connected to different brain activity (Jausovec, Jausovec, and Gerlic 2006). The same Mozart sonata was used in three of the experimental groups in which music was listened to before, after, or before and after the task (Jausovec, Jausovec, and Gerlic 2006). A last experimental group was the control group that did not involve any music, and this group scored significantly lower on task performance than all of the other groups (Jausovec, Jausovec, and Gerlic 2006). Furthermore, all conditions where subjects were exposed to music experienced a benefit in its performance (Jausovec, Jausovec, and Gerlic 2006). Brain activity was found to decrease in complexity of active waves when music was present, mostly observed right after the music played in the first "1000 to 3000ms of problem solving" (Jausovec, Jausovec, and Gerlic 2006). Less complex brain wave patterns that were recorded from listening to Mozart's music was a benefit because more intelligent and creative people are known to have "less complex EEG patterns" (Jausovec, Jausovec, and Gerlic 2006). Finally, males had a higher level of success than did females (Jausovec, Jausovec, and Gerlic 2006). The final conclusions show that Mozart music has benefits in the two stages of the learning process—priming and consolidation (Jausovec, Jausovec, and Gerlic 2006). Although positive effects were seen for all music trails, only the group that listened to music during both phases of learning displayed the lower levels of brain waves in their EEG's (Jausovec, Jausovec, and Gerlic 2006). This suggests that the Mozart effect has different levels it can reach depending on the presentation during tasks.

Spatio-temporal reasoning increases when Mozart is played, and helps humans and mice alike in successfully completing spatial tasks. Therefore, rats should learn to run a maze course faster in the presence of Mozart's music than without it, much like students perform spatial memory tasks better while the music is playing in the background. The classic spatial-reasoning testing tool in the world of rat research is the water maze, but it is hardly the ideal option. Since mice and rats are not forced to swim in their everyday lives, they can hardly be considered "natural" swimmers; it therefore stands to reason that putting them in a testing environment in which swimming is necessary to find a goal and record findings on cognition is not the best choice available (Karl et al. 2011). A new maze model called the cheeseboard maze is considered the dry version of the water maze, providing the same spatialreasoning test while keeping the rats dry and therefore more comfortable and less stressed, hopefully providing more reliable results (Karl et al. 2011). Within this cheeseboard, visual stimuli around it still need to be controlled; one study noted that a spacious room with little visual stimuli is best for luring the rats into maze exploration and successfully completing tasks (Violle et al. 2009). Since the previously mentioned study found that rats pay attention to the room they are placed in during experimental trials, it would stand to reason that the orientation of the open-faced cheeseboard might affect results, but the exact opposite was actually supported in another study. Cressant and Granon found that orienting their cross-armed maze in different directions had no significant impact on their animals' results (2003). Utilizing a cheeseboard maze in a controlled environment in the presence of Mozart's music should therefore increase spatial reasoning and allow rats to learn the maze path faster than learning it without music present.

If this succeeds, the idea can be transferred to other areas of animal training from teaching animals in zoos how to target feed or prepare for blood samples to research studies utilizing mazes, such as a task in which canines had to find the correct path based on decreasing amounts of food present in each path (Macpherson and Williams 2010). The positive effect of music may even reach to animals that live in water; it has been found that music will increase growth in common carp, even in the absence of light (Popoutsoglou et al. 2007). Carp restricted to a dark-only cycle did not grow, but carp in a darkonly cycle with music playing grew just as well as the normal light-dark cycle (2007). It was therefore concluded that music acts as an anti-stress agent in these carp; growth was better and brain waves were calmer when music was present, indicating low stress levels independent of light and dark cycles presented to the fish (2007). From aquariums to zoos to rescues, therefore, in any scenario playing Mozart's music should allow animal training and rehab to be faster and easier, saving resources like time and money.

#### Methods

#### Participants

Eighteen female, white rats (*Rattus norvegicus*) were obtained from Coastline Pet Supply in Myrtle Beach, SC. The rats were about 120 days old at time of delivery, meaning that they were fully weaned and developed in early adulthood. Sixteen rats were used for experimental trials, and two extra rats acted as substitutes if needed. The rats were housed in groups of four (and the two substitutes) in a temperature/humidity-controlled room; fresh water and food was provided and cages were cleaned as needed. After initial setup they were allowed to acclimate to the surroundings for one week.

#### Apparatus

A cheeseboard maze apparatus was utilized for this experiment, provided by Coastal Carolina University. Eighteen small craters were drilled into a larger circular wooden board to make the cheeseboard maze. After sheening the maze with polyurethane to make a clean and comfortable surface for the rats to walk on, the maze was introduced to the rats before trials by placing them on it and allowing them to explore. To encourage rats to run the maze, a primary reinforcement of food was provided at the finish line—which could be moved to any of the holes in the maze. Mazes are common tools used to measure spatial memory in mice and rats, and although this apparatus is new, it has been shown that the Cheeseboard and traditional Water Maze are equally efficient at providing spatial memory results; the main conclusion suggested that most spatial memory tests for mice and rats are equivalent and provide similar results (Lopez at al. 2010). Since the Mozart Effect is often linked to studying anxiety in the brain, Walf, Koonce, and Fry's study using a maze task to measure stress activity in rats further justifies using a maze to determine another factor of the Mozart Effect (2008). A maze is also an appropriate test of spatial memory based on results showing that animals use sequential spatial queues to find a goal (Tamara, Timberlake, and Leffel 2010).

#### **Experimental Trials**

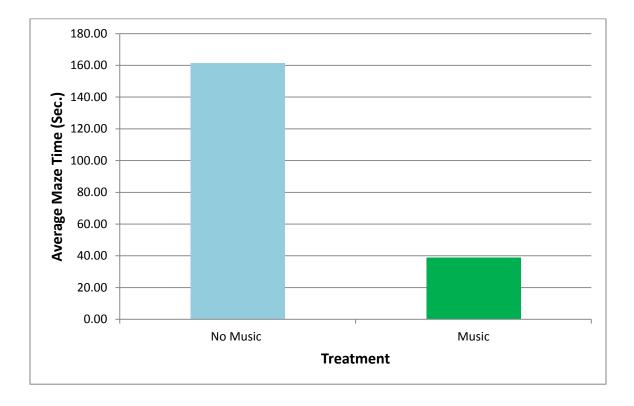
Each rat ran two timed trials in the maze: one in silence and one in the presence of Mozart's sonata K. 448. The trials were alternated so half of the rats ran the silence trials first and half ran the music trials first to ensure that no carry-over was associated with learning the maze. Since the Mozart Effect has been seen to only last ten minutes, there would be no "learning lag" based on previous trials. Rats were given breaks between trials so fatigue and stress would not be an issue. If the rats exceeded four minutes in the silence trials or one minute on the music trials, the trials ended at these times and the rats were placed back in their home cages. If the rats found the finish line within the time constraints, the trial was considered a success. All times were recorded and observations were taken for data analysis.

#### Analysis

A t-test was used to compare the average times between all of the silent treatments and all of the music treatments to determine if a significant difference exists between the two. A standard alpha level of .05 was used for determination of significance.

#### Results

Most of the rats finished the maze in the presence of music faster than in the silence treatment or did not complete the task in either treatment. Nine of the rats finished succeeded in completing the task faster in their music trials; five of the rats timed out of their trials for both experimental treatments; only two of the rats completed their maze task faster during the silence treatment compared to the Mozart treatment. Average completion time (in seconds) was lower for the Mozart trials than the silence trials: the mean time to completion for the silence trials was 161.44 seconds while the music trials were completed in an average of 38.99 seconds (Graph 1). A one-tailed t-test assuming unequal variances shows a significant difference in the completion time between the silence and music treatments (p=0.000001).



Graph 1. Mean maze completion time in the silence and Mozart music treatments.

#### Discussion

Although the rats completed the maze significantly faster in the presence of Mozart's sonata, therefore providing further evidence for the Mozart Effect, further attempts at this experiment would improve this evidentiary support. A large portion of the rats never completed their task in any experimental trial, this could be fixed by providing the rats with more time to equilibrate to and learn the maze task. Even allowing the rats to become more familiar with the apparatus itself before starting to complete trials; this may decrease stress or confusion the rats may be faced with in the maze since many of the rats relieved themselves on the maze, suggesting that some stress was associated with the initial placement in the maze.

Because of time constraints, only one trial in each experimental treatment was completed per rat. The number of rats allowed the small number of trials to hold significance, but more trials with more rats would be yield the optimal results.

Budget did not allow for appropriate sound system to play the sonata for trials, the music was played off of an iPhone4. Similar studies have played test music and noises via a stereo or laptop computer; a similar sound system that could project the music in a better way could produce more reliable results.

Rats were kept in groups of four and were always with the same rats and were often seen sleeping together. Stress could have increased when the rats were taken away from their cage-mates and placed in a solitary maze. Alternating cage mates could therefore change results in a similar trials.

Trials were run in the afternoon and the mice were kept on a normal sleeping cycle with an uncurtained window in their living room. This means that although rats are usually nocturnal, they had sun or other natural weather shining in on them with uncovered windows while they would normally be

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sleeping. This could have affected their stress levels and results while running the maze (again, when they would have normally been sleeping). With a larger budget, curtains and an automatic light timer would have helped this experiment. Overall, the experiment should be repeated with an increased amount of trials using more advanced audio equipment and in a light-controlled room.

Although the experimental conditions could be tweaked to achieve more reliable results, the sonata significantly decreased maze completion time, adding to the support for the Mozart Effect as well as the use of the Cheeseboard maze in rat/mice spatial memory studies. Only three of the sited studies were found to use this new maze in similar experiments, the oldest one dating back to only 2010; although this apparatus is in its infancy in the world of research, this experiment provides further evidence for making it the first choice instead of just an alternative to the water maze. Furthermore, each experiment (including this one) used a different configuration and number of "holes" in the cheeseboard and still showed positive results. This means that the Cheeseboard is a tool that can be edited for any particular study without decreasing effectiveness, making it a useful tool.

This was a pioneer experiment in the fact that no other sited research has quantified spatial reasoning increases in rats listening to a Mozart sonata under Cheeseboard maze pressures. In a more general context, it was also the only experiment of its kind that studied the Mozart Effect in rats with purely a memory rather than medical end goal. In other words, much of the research in this topic is dedicated to using the Mozart Effect to treat medical condition such as hypertension as seen in Lemmer's 2008 research. This knowledge sets out to use rats/mice as model organisms for humans and increase medical treatment knowledge for human patients with hypertension, epilepsy, and more. This experiment, however, set out to test an animal's ability to increase spatial memory for training purposes.

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The purpose of testing spatial memory capacity based on the Mozart Effect is significant because it had not been determined if animals could benefit from this effect to the same extent that their human counterparts benefit. In an experiment that proved that this music increased brain activity but not test scores (Jausovec and Habe 2003), it was hypothesized that the effect was not reflected in test scores because the brain was taking in too much stimuli compared to the silent treatment testtakers. In other words, the music participants did have the effect upon them, but it was basically cancelled out in test scores because they were doing more work by taking in the music. Since rats have simpler brains, it was unclear if the effect would resonate in them or if the added stimuli would actually decrease their maze-solving abilities. The later only happened in two trials where the silent treatment was completed faster than the music treatment, and most of the rats performed better in the presence of music. Therefore, this not only supports the Mozart Effect in general, but also the fact that mammalian brains ranging from humans to rats should be able to process this stimuli and still reap the benefits from it.

Since Mozart's Sonata K.448 increased the finishing time of the rats, this knowledge can be transferred to a wide range of animal-related instances ranging from fisheries and wildlife management to training in captivity to veterinary medicine. For example, this sonata can be played in the background during training sessions and should increase the rate at which animals learn their appropriate tasks. As noted in a study concentrating on carp, music acted to alleviate stress on the fish and would be beneficial for veterinary services where the animals are always in a heightened state of alert and stress (Popoutsoglou et al. 2007).

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