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# The Effect of Exposure to Music on Spatial Learning and Memory in Rats

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## ABSTRACT

**Introduction:** The increase in spatial learning and memory performance caused by music is called the 'Mozart effect'. Increased NMDA receptor (NMDAR) expression plays a role in this effect. Inhibition of NMDARs reduces Prepulse Inhibition (PPI) % values. The study aims to investigate the relationship between the Mozart effect and the NMDAR expression.

**Methods:** Rats were divided into 6 groups. Three groups listened to white noise (WN) while the other three groups listened to Mozart (M). After the rats were performed in the 8-arm radial maze test, one of the three groups in both sound environments was chosen as the control group and was injected with saline. For the remaining two groups, one was injected with ketamine and the other was injected with MK-801. Then all groups underwent the PPI protocol.

**Results:** It was found that Mozart groups had higher memory errors. The M+MK-801 group had lower PPI% values with 74 dB prepulse compared to the WN+Ketamine group.

**Conclusions:** While the Mozart effect was not observed, on the contrary, a decrease in memory performance was detected. The effect of music on NMDARs may be at levels that do not change PPI values. Considering that parameters like the duration and intensity of music may cause stress, repeating the experiment with different conditions may provide new clues.

**Keywords:** Learning, memory, Mozart effect, music, prepulse inhibition

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## INTRODUCTION

Music is an art form that conveys certain feelings and thoughts through harmonious sounds. Scientists have been investigating the effects of music on learning and memory. In 1993, Rauscher et al. found that listening to Mozart for 10 minutes (min) increased spatial learning performance in students lasting for 10–15 min (1). This finding was called the "Mozart effect" and was widely reported in the media. However, other researchers could not replicate the study, which led to controversy about its scientific validity.

Rauscher et al. then identified an increase in spatial learning performance of children receiving music lessons for 6 months. Thus, it has been shown that the Mozart effect can occur not only in a short time but also in a long time period (2). Studies on rats and mice have also shown that listening to music can improve spatial learning (3,4). Additionally, these studies have found that music can increase levels of hippocampal cell proliferation (5), brain-derived neurotrophic factor (BDNF) (5), dopamine (6), and N-methyl D-aspartate (NMDA) receptor expression (7). The NMDA receptor (NMDAR) is a glutamate-gated cation channel that plays a key role in learning and memory (7,8). Studies have shown that NMDAR expression decreases in the auditory brainstem of rats with early postnatal deafness (8) and in the auditory cortex of rats with early auditory deprivation (9). This suggests that there may be a link between hearing and the NMDAR.

### Highlights

- **The Mozart Effect on spatial learning and memory in rats was investigated.**
- **Only short-term memory impairment was detected in the 8-arm radial maze.**
- **Prepulse inhibition was evaluated in saline, ketamine, and MK-801 treated groups.**
- **The effect of music on NMDARs may be too low to be detected by the PPI test.**

The startle reflex is the contraction of skeletal muscles in response to a sudden and intense stimulus, which may be acoustic (10). Giving a weak pre-stimulus that does not trigger the startle reflex 30–500 ms before a strong acoustic stimulus reduces the intensity of the acoustic startle reflex. This is called prepulse inhibition (PPI) and is a physiological response (10). Decreases in startle intensity in response to repeated stimuli during PPI measurement is a type of learning. As a result, PPI measurements can be used to understand the neural mechanism of learning (11).

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NMDARs play a critical role in learning and memory by both creating hippocampal long-term potentiation and increasing synaptic plasticity (7,8,12). Blockage of NMDARs causes a reduction in PPI. Ketamine and MK-801 are among the most studied non-competitive NMDA antagonists for their effects on PPI (13,14). Both antagonist agents prevent the flow of cations into the cell by binding to pores in the NMDAR ion channel. This impairs learning and reduces PPI% values, even if the drugs are given in low doses that do not affect locomotor activity (15).

This study has two purposes. First, to test the effect of music on spatial learning and memory through the 8-arm radial maze, and second, to indirectly observe the effect of music on NMDAR expression by measuring PPI after administration of NMDA antagonists. Thus, this study will provide new data both on the reproducibility of the Mozart effect and on the change in NMDAR expression using a different non-invasive method that has not been used before in music studies.

## METHODS

### Ethical Issues

Approval for the study was obtained from the Local Ethics Committee of Üsküdar University (date: 15.02.2019; number: 2019-04; topic: ÜÜ-HADYEK 29th committee meeting). Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, which was published and released on 22 September 2010, was followed. Regarding animal welfare, the advanced principles of the 4R guidelines were followed. Experiments were completed at the Üsküdar University Neuropsychopharmacology Application and Research Center (NPARC), Istanbul, Türkiye.

### Drugs and Treatment

In the study, 3 mg/kg ketamine (Sigma Chemical, USA) and 0.1 mg/kg MK-801 (Dizocilpine) (Sigma Chemical, USA) were used in accordance with the literature (16,17). While both drugs were dissolved in 0.9% isotonic NaCl solution (saline) at a dose of 0.1 mL/kg and injected into the related groups, only 0.1 mL/kg of saline was injected into the control groups. All solutions were administered subcutaneously 5–10 minutes before PPI measurements (16,17).

### Subjects and Laboratory

To determine the sample size, power analysis was performed based on similar studies in the literature and expected effect sizes (6,7,9). This analysis showed that; in a study planned with 6 groups, analyzed by one-way ANOVA, with an alpha level of 0.05 and a power of 0.80, a minimum sample size of 5 animals per group would provide sufficient power to detect significant differences. Considering ethical issues to minimize the use of animals in experimental studies, the sample size of each group was determined as n=6. While the Mozart effect was observed in rats that listened to music 12 hours/day in the dark period for 42 days starting from PND14, it was reported that it could not be detected in rats listening to music from PND28 and PND56 (7). Considering these data, 36 14-day-old male Wistar albino rats were included in the study to perform the 8-arm radial maze test on the 42nd day of the study. For the maze experiment in the first phase of the study, rats were randomly assigned to one of two groups according to their sound environment: white noise group (WN) (n=18) or Mozart group (M) (n=18) (Fig. 1). Then, for the PPI test in the second phase, these 2 groups were randomly divided into 3 subgroups according to the drugs used: WN+Saline, WN+Ketamine, WN+MK-801, M+Saline, M+Ketamine, and M+MK-801 (n=6) (Fig. 1). Previously no significant difference occurred in learning and memory performance between groups exposed to silence and white noise (18). White noise was chosen in our study instead of silence as it masks other sounds in the environment. Two different music files were created to play uninterrupted for 12 hours with one containing white noise and the other containing the 08:42 min overture of Sonata K.448 in D major for two pianos (Allegro con spirito) composed by Mozart. The WN group was placed in the room with white noise, while the M group was placed in the room with the Sonata. Rats were housed in plexiglass cages with a 12:12 h light-dark cycle maintained (lights on through 07:00–19:00 h). The sound intensity of white noise was 65 dB, while Sonata's was in the range of 65–75 dB. In order not to disrupt the sleep quality of nocturnal rats, these 12-hour music files were played during the dark period of the day throughout the study. Room temperature was set to 22±2°C and humidity was 60±5%.

### Eight-Arm Radial Maze Task

The 8-arm radial maze consisted of eight arms (57 × 11 cm) produced from black plexiglass. Visual clues were placed around the maze as a reference for the rats to recall the location of the baits. The camera placed on the ceiling was connected to a computer. Rats were placed on the central platform during the experiments and were expected to find baits. Food restriction was applied to the rats to increase their motivation to search for food in the maze. Accordingly, after the rats completed the daily maze test, they were given food, and the food was taken back two hours later. Thus, they were left fasting for 22 hours until the next measurement.

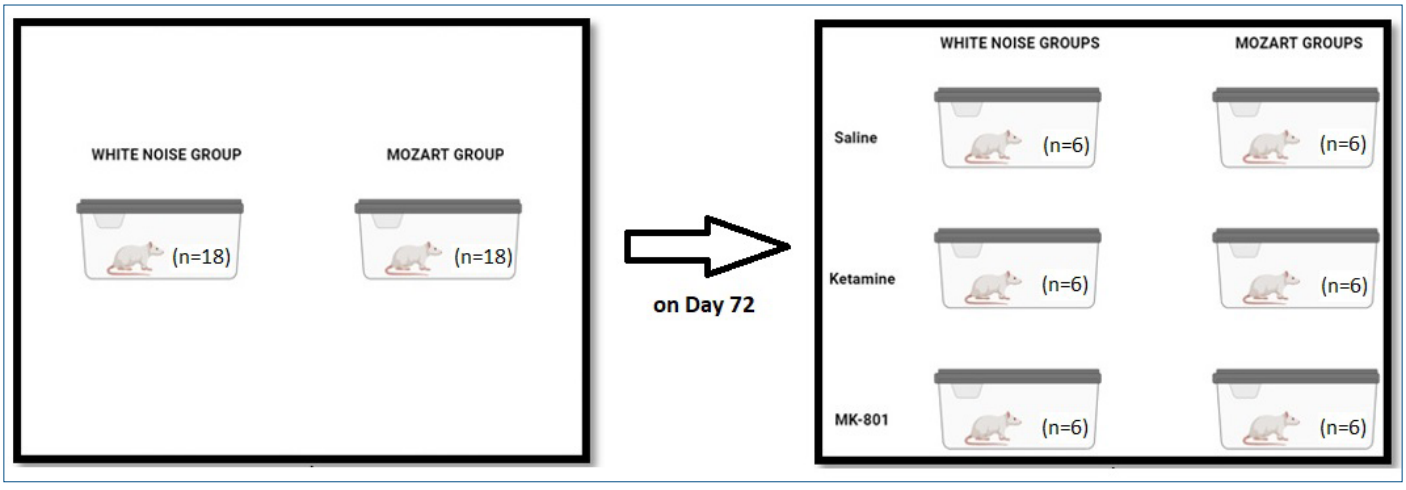


**Figure 1.** Group design

According to the 21-day protocol; Days 1–3 were the habituation phase, Days 4–11 were the acquisition phase, and Days 12, 14, 17, and 21 were the retention phase. During the habituation phase, rats were allowed to move freely through the maze for 10 minutes. On the first day, one bait was placed at the entrance, center, and end points of the arms, and two baits were placed in the middle of the central area. On the second day, baits were only placed in the center and endpoint of the arms. On the third day, baits were only placed at the endpoints of the arms. During the acquisition and retention phases, baits were placed at the endpoints of arms 2, 3, 5, and 7. The sessions ended when rats had consumed all 4 baits or after a maximum of 10 minutes. Thus, rats were habituated to the maze in the habituation phase. In the acquisition phase, it was aimed to learn the location of the baits. Scoring was performed in the retention phase.

Entry into arms 1, 4, 6, and 8 were accepted as 'reference (long-term) memory error (RME)', while repeated entry into arms 2, 3, 5, and 7 after eating the bait were accepted as 'working (short-term) memory error (WME)'. The sum of RME and WME was used as 'total error'and the duration of eating the 4 baits was assessed as 'total time'.

Thus, by leaving 1-, 2- and 3-day gaps between the test days (day 12, 14, 17, and 21), a total of 4 measurements were made on each rat. The results were compared between the WN and M groups. Additionally, data from 4 test days were compared within both groups.

### Prepulse Inhibition of Acoustic Startle Reflex Test

Acoustic Startle Reflex System (SR-Lab San Diego Ins. CA, USA) was used for PPI measurements. This device consists of a soundproof cabin, a cylindrical rat cage placed on a motion sensor system, speakers inside the cabin, and a computer.

According to the procedure by modifying the study of Kayir et al., rats were handled for 10 minutes per day for 3 days and habituated to the injection hold (19). On the fourth day, rats were placed in the cage to habituate to the device for 10 minutes, with no acoustic stimuli. On the fifth day, basal measurements were made by following the procedure in the computer software without any injection. Thus, it was confirmed that the rats were startled by acoustic stimuli, that is, they did not have auditory problems. Baseline measurements were not used as experimental data.

The drugs were administered 5–10 minutes before the main measurements. Measurements began with a 5-minute habituation period, during which a 70 dB background noise was administered. Subsequently, five acoustic stimuli of 120 dB, which cause the startle reflex, were administered. Then, ten consecutive blocks were applied at random intervals of 10 to 30 seconds. Each block consisted of five different acoustic stimuli in which the stimulus sequence was chosen randomly:

**I-** 120 dB intensity acoustic stimulus with 40 milliseconds (ms) duration,

**II-** A prepulse of 74 (basal +4) dB lasting 20 ms, then 100 ms later 120 dB stimulus lasting 40 ms,

**III-** A prepulse of 78 (basal +8) dB lasting 20 ms, then 100 ms later 120 dB stimulus lasting 40 ms,

**IV-** A prepulse of 86 (basal +16) dB lasting 20 ms, then 100 ms later 120 dB stimulus lasting 40 ms,

**V-** Only 70 dB background noise.

After these ten blocks, the five 120 dB stimuli were administered again and the measurements ended. The total measurement duration was about 25 min. The percentage reduction in startle intensity for each of three different prepulses (74.78, and 86 dB) was evaluated as 'prepulse inhibition (PPI)' and was calculated by computer software using the variables specified in Fig. 2.

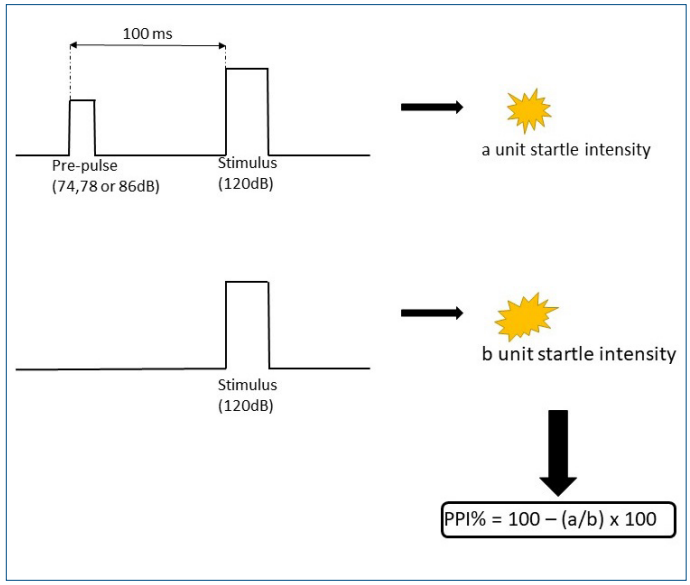


**Figure 2.** Variables of the PPI% formula (PPI: prepulse inhibition).

### Experimental Design

The experimental design is schematized in Fig. 3. As lactation in rats continues until the PND21, 14-day-old male rats included in the study were initially placed in the rooms with female siblings and mothers. Food and water were available ad libitum. Mothers and female siblings were removed from the room on Day 7 of the study. Rats were handled for 10 minutes per day to habituate to human contact on Day 30–32. On Day 33, the habituation phase started for the rats to get used to the PPI device. At this stage, the rats were placed in the cabin and left without operating the device for 10 minutes. Basal PPI measurements of rats were made on Day 34. Basal weights of rats were measured, and food pellets were given only between 10:00–12:00 until day 42 to adjust to the food restriction applied during the maze tests. From this stage, the rats were weighed twice a week and their weights were checked to ensure that they did not fall below 85% of the basal weight. Chocolate cornflakes used as reward baits in the maze test were added to food pellets to ensure the rats recognized the reward baits on Day 41. The 21-day 8-arm radial maze test protocol began on Day 42. Following the final maze measurements, rats were fed ad libitum for 10 days to reach a minimum weight of 200 g for ideal PPI measurements. Ketamine, MK-801, and saline were administered to the related groups on Day 72. Then PPI measurements were made and PPI % values were recorded with prepulse at 74, 78, and 86 dB intensities.

### Statistical Analyses

Normality was checked using the Shapiro-Wilk test. Since the WME, RME, total error, and total time data for the WN and M groups did not show normal distribution, group comparisons in the 8-arm radial maze test were analyzed by the non-parametric Mann-Whitney U test. Comparisons between the 4 test days within groups were analyzed with the Friedman test followed by the Wilcoxon test with Bonferroni correction. Since PPI% values were normally distributed, the comparison between the 6 groups was analyzed by one-way ANOVA followed by Tukey's post-hoc test. P values less than 0.05 were considered statistically significant, and the Bonferroni corrected p-value was set at 0.008. All data were given as the mean ± SEM.

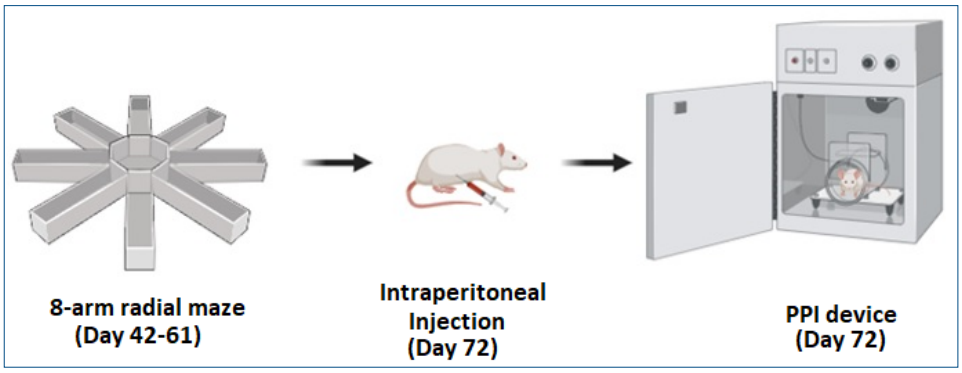


**Figure 3.** Experimental design.

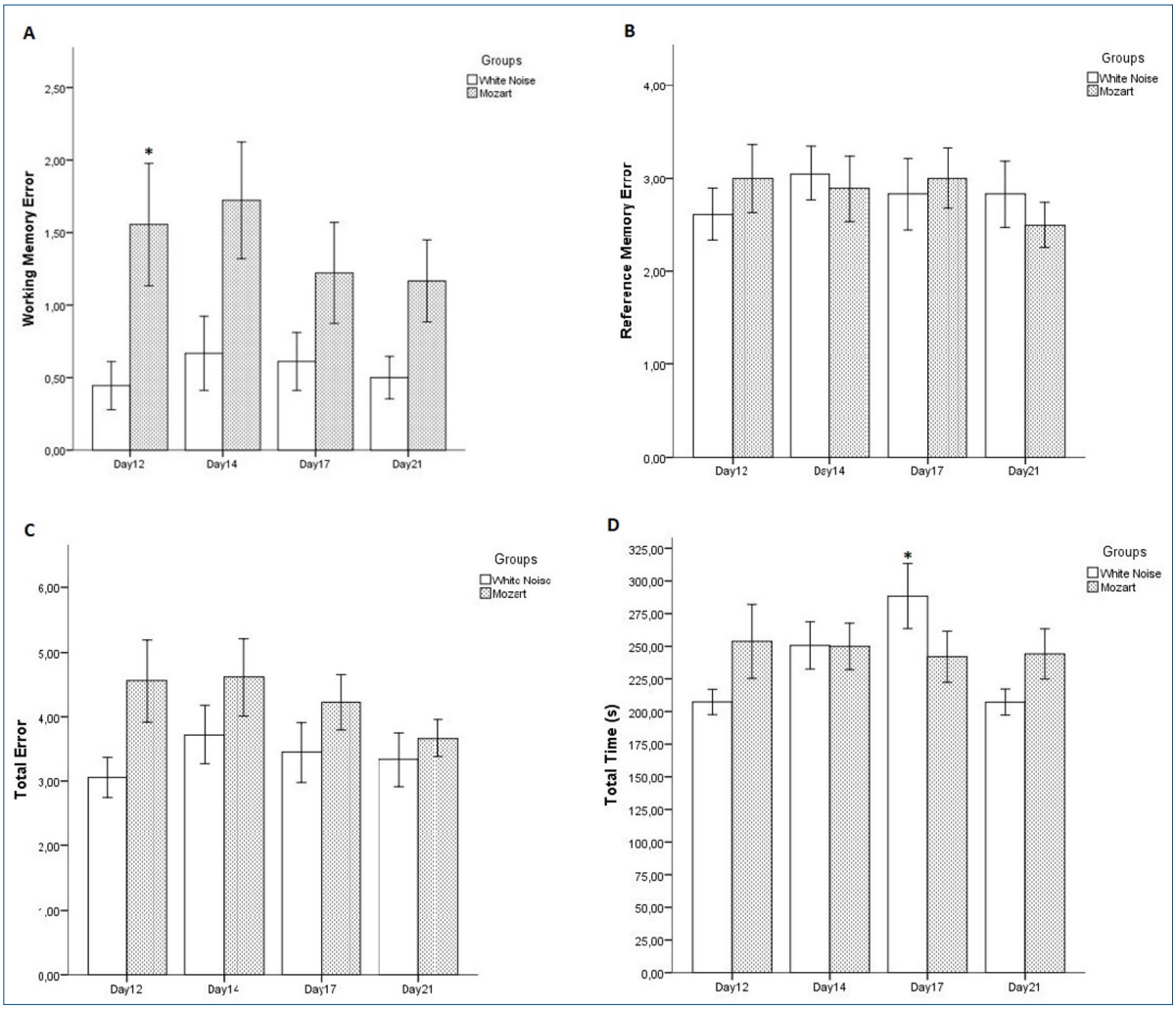


**Figure 4.** a–d. Effect of White Noise and Mozart's Sonata on Spatial Learning and Memory. The data are presented as the mean ± SEM. A: Comparison of Working Memory Error scores in WN and M Groups on 4 different test days. *A statistically significant difference was found between the White Noise and the Mozart groups on Day 12. (p<0.05; p=0.032) B: Comparison of Reference Memory Error scores in WN and M Groups on 4 different test days. C: Comparison of Total Error scores in WN and M Groups on 4 different test days. D: Comparison of Total Time (s) scores in WN and M Groups on 4 different test days. *A statistically significant difference was found between Day 17 with Day 12 and Day 21 for the WN group (p values are 0.001 and 0.002, respectively; p<0.008). (WN: white noise; M: Mozart's sonata)

## RESULTS

On Day 35, basal body weights measured before food restriction were 158±14 g. On Day 62, body weights after the 8-arm radial maze test were 165±18 g. On Day 72, body weights before PPI measurements were 224±24 g. No rats fell below 85% of their basal weight after food restriction. On Day 72, the rats reached the ideal PPI measurement weight (min. 200 g).

On Day 12, the M group had more WME than the WN group (Fig. 4A; p<0.05; p=0, 032). RME, total errors, and total time did not show statistically significant differences between the two groups across all four test days (Fig. 4B-D; p>0, 05). When all groups were compared within each of the four test days, the WN group had significantly longer total time on Day 17 compared to Day 12 and Day 21 (Fig. 4D; p values are

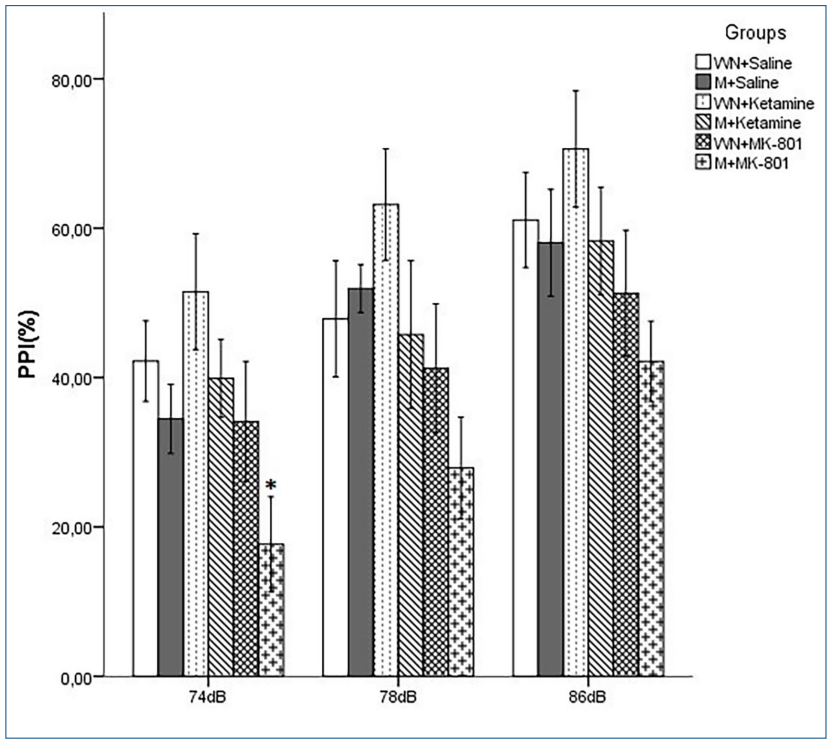


**Figure.** 5. PPI% of WN+Saline, M+Saline, WN+Ketamine, M+Ketamine, WN+MK-801, and M+MK-801 groups at 74 dB, 78 dB, 86 dB prepulse intensities. The data are presented as the mean ± SEM. *A statistically significant difference was found between the M+MK-801 group and WN+Ketamine group for 74 dB intensity (p<0.01; p=0.009; F=3, 121).

0.001 and 0.002, respectively; p<0.008). In terms of WME, RME, and total error, neither the WN group nor the M group had significant differences in four test days (Fig. 4A-C; p>0.05).

For 74 dB the M+MK-801 group had lower PPI% values than the WN+Ketamine group (Fig. 5; p<0.01; p=0.009; F=3, 121). No statistically significant difference was found between groups for 78 dB (p>0.05; p=0.063; F=2, 373) and 86 dB (p>0.05; p=0.142; F=1, 803).

## DISCUSSION

Contrary to expectations, the Mozart effect was not observed. The most notable result is that the WME of the M group was higher compared to the WN group on Day 12. On the other test days, there was no significant difference between the two groups in terms of the measured variables. In their meta-analysis investigating the Mozart effect, Pietschnig et al. stated that the Mozart effect was noticeably higher in the studies of Rauscher and colleagues than in other studies, and that this may be due to the fact that the laboratory features systematically moderated the results (20). In order to observe this effect, laboratory conditions and experimental setups must be planned appropriately (21). Rauscher stated that the music does not necessarily have to be Mozart's K.448 Sonata, and the tests used should be aimed at measuring spatial learning and memory (22). While there are several human studies in the literature in which the Mozart effect could not be observed despite the design being suitable for these conditions (23–25), there are no animal studies. In addition, the mentioned meta-analysis concluded that the observed effect may be related to publication bias and that there is little support for the positive effects on cognitive functions through exposure to Mozart's Sonata K.448 (20).

Steele disputed the Mozart effect, arguing that rats could hear only 31% of the notes in the Sonata (26). Rauscher suggested that the Mozart effect may be related to different musical factors other than notes (27). In another study, it was suggested that the crucial element is not the melody but the rhythm and that animals do not need to hear all the notes (28). However, rhythm theory cannot fully explain why some music does not increase spatial learning test performance.

Stress is another factor to consider that can impair memory. The dB values and exposure times used in our study are consistent with the literature (29). However, exposure to the non-physiological conditions may have caused chronic stress. In addition, while the dB value of the white noise was constant (65 dB), the changing dB values of the Sonata throughout the piece (65–75 dB) may have caused more stress in the M group.

There are studies observing the Mozart effect in Wistar (29), Sprague Dawley (30), Long Evans rats (18), and even in mice (3). Considering that there may be significant differences between different rat species in spatial learning performance, it would be appropriate to repeat our study with other rat species (31).

When the maze test results were compared within the two groups, the only significant difference was the higher total time on Day 17 for the WN group compared to Days 12 and 21. Since this significant difference in total time is not supported by other parameters such as WME and RME, it is difficult to evaluate this result as a difference in memory and learning performance.

The PPI value for the M+MK-801 group with 74 dB prepulse was significantly lower compared to the WN+Ketamine group. No significant difference was detected in the analyses of 78 dB and 86 dB. Additionally, although not statistically significant, the PPI values in the white noise groups generally tended to be higher than the Mozart groups subjected to the same drug conditions. This may be due to a slight decrease in NMDAR expression, as music appeared to reduce short-term memory performance in this study.

Although a trend toward PPI-reduction was observed with MK-801 at all intensities compared to the saline groups, statistical significance was only reached between the M+MK-801 group and the WN+Ketamine group.

Since the M+MK-801 group and the WN+Ketamine group were exposed to different music files, it is not possible to interpret the significance of this in terms of drugs. No PPI deficit was observed in the ketamine groups compared to the saline groups.

Ketamine and MK-801 have affinities to other receptors other than the NMDAR (32). These complex pharmacological properties may account for the lack of PPI-impairing effects of these antagonists. At this point, further studies describing the pathways in which NMDA antagonists are involved are needed.

The lack of significant difference in PPI% values in the paired groups may indicate that exposure to music that impairs short-term memory did not induce a significant difference in NMDAR expression at levels detectable with the PPI device.

In conclusion, the first aim of this study was to test the Mozart effect in rats with the 8-arm radial maze, and the second aim was to observe the effect of music on NMDAR expression with PPI measurement. However, contrary to the literature, exposure to music impaired short-term memory and did not cause a significant difference in PPI% values. The reason for the negative effect may be unpredicted stress in rats with exposure to musical stimuli at non-physiological duration and intensity and different laboratory conditions. Additionally, the probability of publication bias should always be remembered. The complex pharmacological properties of the drugs may play a role in the lack of significant disruption of PPI. Apart from this, the effect of music on NMDAR expression may be too low to be detected by PPI measurement.

The limitations of this study are that molecular analysis of NMDARs was not performed, and possible stress behavior was not measured. Future studies using different types of rats and stress behavior tests such as open field or elevated plus maze may provide a better understanding. Additionally, trying 45–60 dB ranges where the Mozart effect has been demonstrated (4), the use of different NMDA antagonists, and dose studies of antagonists may provide new explanations for the results. Finally, molecular analysis of NMDARs in the prefrontal cortex and hippocampus, which are responsible for short– and long-term memory, will provide data on the mechanisms underlying the Mozart effect. Understanding the effects of music on experimental animals may also provide clues to understanding its effects on human health.

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**Ethics Committee Approval:** Approval for the study was obtained from the Local Ethics Committee of Üsküdar University (date: 15.02.2019; number: 2019-04; topic: ÜÜ-HADYEK 29th committee meeting).

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**Author Contributions:** Concept- HA, BÇ, GÖ; Design- HA, BÇ, GÖ; Supervision- HA, BÇ, GÖ; Resource- HA, BÇ, GÖ; Materials- SHA, BÇ, GÖ; Data Collection and/or Processing- HA, BÇ, GÖ; Analysis and/or Interpretation- HA, BÇ, GÖ; Literature Search- HA, BÇ, GÖ; Writing- HA, BÇ, GÖ; Critical Reviews- HA, BÇ, GÖ.

**Conflict of Interest:** The authors declared that there is no conflict of interest.

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