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SHORT ARTICLE

Auditory Stimulation with Mozart Sonata k-448 and Heavy Metal Music Improves Short-Term Memory in Rats

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Abstract:

Objectives: We aimed to assess the effect of both classical and heavy metal music on short-term and long-term memory.
Methods: Male rats were separated into three groups: Mozart (n=14), rats were exposed to Mozart´s K-448 piano sonata; Heavy Metal (n=15), rats were exposed to Psychosocial (band: Slipknot), and Control (n=12), rats were exposed to ambient sounds in an acoustically isolated room. The animals were exposed to the music for 8 hours per day, between 10:00 pm to 06:00 am, with an intensity of 50-75 dB, for 61 consecutive days. Twenty-four hours after the final day of adjustment to the enclosure the animals were individually exposed for 5 minutes in the enclosure to explore two identical objects. Ninety minutes after the object recognition training session, the animals were submitted to the short-term memory test and twenty-eight days after the object recognition training session, the animals were submitted to a long-term memory test.
Results: There was no modification amongst the three groups during the object recognition training. Regarding the short-term memory test both Mozart (U=33.00, p=0.016, FDR-corrected) and Heavy Metal (U=44.00, p=0.033, FDR-

corrected) groups revealed a higher percentage value of significance compared to the control group. In contrast, there was no significant change in relation to the long-term memory test.
Conclusion: Mozart Sonata K-448 and heavy metal music achieved beneficial effects on the short-term memory in rats.

Keywords: Memory, Memory, Long-Term, Memory, Short-Term, Music, Rats.

Introduction

Neuroplasticity may be defined as the capability of the central nervous system (CNS) to remodel itself in response to external stimuli [1]. This process triggers behavioral, cellular and molecular alterations in the CNS, resulting in memory formation [2]. Memory may be classified according to its duration, specifically short-term and long-term memory [3]. Short-term memory refers to the storage for a limited period (seconds to a few minutes, up to three hours) of small amounts of information, regardless of the transcription of messenger ribonucleic acid (mRNA) and protein synthesis. Then again, long-term memory refers to the storage over a prolonged period of time (hours, days and occasionally years) of large amounts of information [4, 5, 6].

Music may induce behavioral, cellular and molecular alterations in different animals [7, 8, 9, 10, 11] and humans [12, 13, 14]. These effects are established in peripheral tissues, given that music can cause physiological changes of heart rate, respiratory rate and blood pressure [15]. Nonetheless, the effect of music is not solely restricted to the peripheral tissue, but also interacts with CNS functions [16]. For instance, it was revealed that music improves mental functions while Pauwels et al. [17] described beneficial effects of music on anxiety and pain. A recent review highlighted the interventions of music on behavior, neurochemistry, immunology, brain structure and physiology in rodents [18]. As a main finding, the authors reported that music has positive effects, which are consistent with studies in humans.

Additionally, the effects of music on the CNS may also be realized on both learning and memory processes [8, 18, 19, 20] reinforced that music improved learning and spatial memory in animals. Several studies displayed the beneficial impact of classical music in the initial stages of memory [11, 21]. Mice that were exposed to Mozart's Sonata K-448 for 8hrs/day (30 days) [8] had a lower escape latency compared to the control group when submitted to the Morris's aquatic maze. Also, a study observed that rats exposed to Mozart's Sonata K-448 for 12hrs/day (60 days) exhibited an improvement of the short-term memory when compared to the control group [20]. Nonetheless, others had difficulty to replicate Rauscher et al., 1993 data [20].

The object recognition model consists of sessions during which rats explore two identical objects, followed by a test where the old object is presented along with another unknown one (new object) [22]. Several animal models have been applied to identify these possible behavioral alterations caused by exposure to music [1, 20, 23]. Numerous studies have revealed the effect of classical music (particularly the Mozart effect) on animal behavior in explicit memory models expressly by applying the food maze [1, 8, 20, 23] and the Morris's aquatic maze [8]. Yet, there are no reports concerning the music effect on the object recognition model, the possible impact on the long-term memory, nor a plausible effect of other music styles besides the classical.

On the other hand, there is little attention on the possible influence of heavy metal music on brain activity. Greenberg et al. [24] made an interesting association between musical preferences and cognitive styles. A very recent study evidenced that brain regional activity and functional integration was associated with sensation seeking in heavy metal music and classical music lovers [25]. Nonetheless, after searching on Pubmed/Medline database, we did not find any investigation regarding the effect of heavy metal music genre on memory.

We assume, this is the first study in which the possible effect of both classical music and heavy metal on short-term and long-term memory has been estimated in rats submitted to the recognition object model. On the basis of previous research literature, we project that the animals exposed to music would exhibit an improved performance over the control group.

Methods

Animals

We assessed 53 male Wistar rats of mean age of 28 days and masses between 120 to 180 grams, from the Faculty of Medicine of Itajubá. The animals had free access to water and nourishment (Purina®) and were set aside in plastic cages for a 12-hours dark-light cycle, with 5 animals of the same group per cage. The present study was approved by the local Animal Ethical Committee (No. 013/15).

Behavioral procedures

The animals were split into three groups: Mozart (n=14), Heavy Metal (n=15), and Controls (n=12). They were either exposed to music (according to their respective group) or to ambient sounds in analogous rooms, acoustically isolated to avoid the interference of external sounds. Figure 1 illustrates the study experimental design.

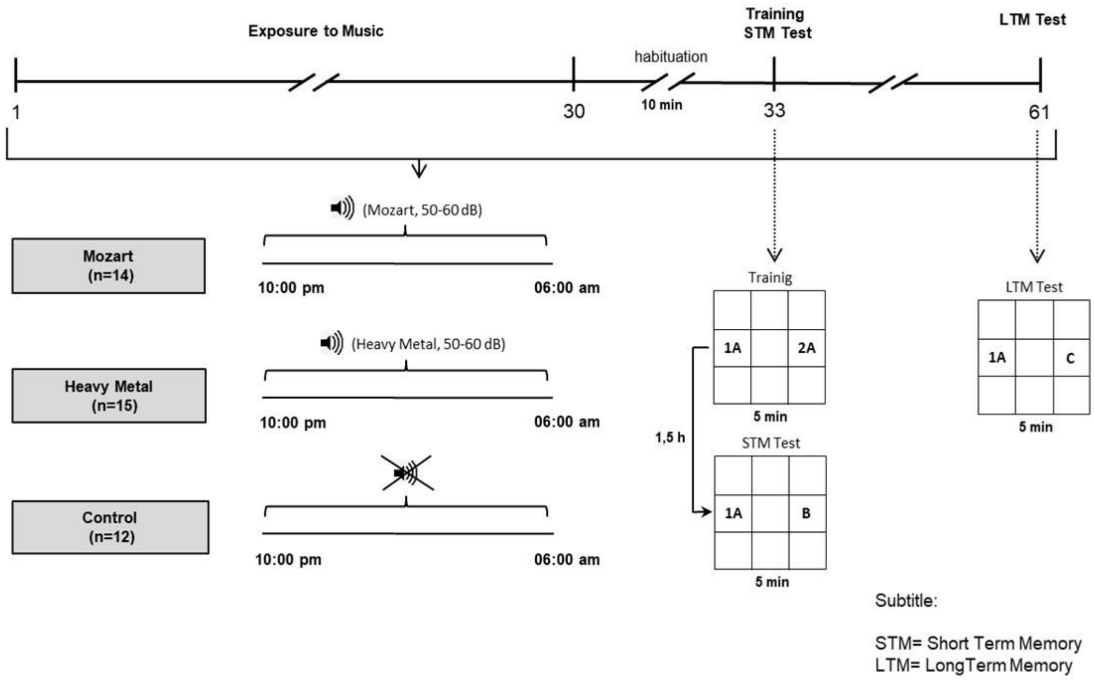

Figure 1: Study experimental design

Exposure to music

The Mozart group was exposed daily to Mozart´s K-448 piano sonata, frequently needed to examine the Mozart effect [25, 26], 8 hours a day, between 10:00 pm to 06:00 am, with an intensity of 50-75 dB, for 61 consecutive days. The Heavy Metal group was exposed daily to the music Psychosocial (band: Slipknot), 8 hours a day, between 10:00 pm to 06:00 am, with the identical intensity and for the same number of consecutive days. The Control group was exposed to ambient sounds with no music in the same acoustically isolated room (˜50 dB). Ambient sound included rats noise during their maintenance in the cage and air conditioning (Split Inverter 9000 BTUs LG Dual Inverter, Sao Paulo, Brazil) to control temperature [8].

Apparatus and stimuli

Music was played through a CD player apparatus (Philco Ph61, Sao Paulo, Brazil), which remained by the animals cage and equivalent pressure level was measured via a decibel meter (Sound meter – Decibel(dB), Seremban, Negeri Sembilan, Malaysia).

Familiarization with the enclosure

The animals were positioned for 10 minutes for 3 consecutive days to explore the enclosure (40.5 cm by 40.5 cm by 30.0 cm), but in the absence of the objects. This procedure was intended to familiarize the animals with the location where the subsequent tests would be completed [22, 26].

Object recognition training

Twenty-four hours after the final day of familiarization with the enclosure, the animals were individually exposed for 5 minutes in the enclosure to explore two identical objects (object A1, a cube, and A2, a ball). The objects were positioned in two opposing corners, 6 centimeters distant of the container walls [26].

Short-Term memory test

Ninety minutes after the Object Recognition Training session, the animals were submitted to the Short-Term Memory Test, where they explored the same training enclosure for 5 minutes in the presence of a familiar object (A1, a cube) and a new one (object B, a cylinder). All procedures were in accordance with Matsuda et al. [28].

Long-term memory test

Twenty-eight days after the Object Recognition Training session, the animals were submitted to the Long-Term Memory Test for 5 minutes, during 5 consecutive days [28, 29]. In these tests, the animals explored the same training enclosure in the presence of a familiar object (A, a cone) and a new one (object C, a triangle). This protocol followed Matsuda et al. (2014) study [28].

We recorded the following variables: the time spent to distinguish the familiar object in the same previous position (object A); the time spent to distinguish the new object at a first instant and the time spent to distinguish the familiar one at a second instant (objects B, C). The objects were both made of plastic, had the same texture, color and size, but alternate forms. The results were exhibited according to the exploratory preference. The recognition index was calculated for each animal by the formula [B,C/(A+B,C)]×100 where A is the time spent to explore familiar object A and B, C is the time spent to explore the new objects B, C.

Statistical analysis

The results regarding the object recognition training session are presented in absolute values (Mean ± Standard Error). This datum was submitted to Kolmogorov-Smirnov (normality) and Levene (homogeneity of variance) tests, to assure its parametric distribution. To verify possible differences in the mean times of exploration between the A1 and A2 objects for each group (Mozart, Heavy Metal and Control), the data were inspected with the independent t test.

The results correlated to the behavior of object exploration are presented as an exploratory preference [26]. For this purpose, the recognition index for each animal was calculated using the ratio TB/(TA+TB,C)×100, where TA is time spent exploring familiar object A and TB,C is time spent exploring novel objects B,C. The index data were submitted to the Kolmogorov-Smirnov and Levene tests, which identified violations concerning the parametric distributions. So, the datasets were analyzed using non-parametric tests, and all experimental results are presented as median [lower (25%) and upper (75%) quartiles]. Kruskal-Wallis tests were completed to compare the values amongst the three groups during the following phases of the study: a) short-term memory; and b) long-term memory. Matching (post-hoc) assessments were performed via the Mann-Whitney test to evaluate possible significant effects detected through Kruskal-Wallis. To lessen the chance of false-positive occurrences, inferences were made at the level $p<0.05$ ($<5\%$) after applying the FDR (false discovery-rate) correction to multiple comparisons [30]. In the case of single comparisons, values of $p<0.05$ ($<5\%$) were considered significant.

We calculated effect size through Cohen's d in order to measure the magnitude of difference for significant difference. Large effect size was considered for Cohen's d $>$ 0.8 and medium effect size for values between 0.8 and 0.6.

Results

Figure 2 displays the time of the object exploration behavior for the A1 and A2 objects, during the object recognition training, for the following groups: Mozart (Fig. 2A), Heavy Metal (Fig. 2B), and Control (Fig. 2C). There was no adjustment amongst the three groups during the training phase: Mozart group (A1=2.62 ± 0.37 s. A2=2.48 ± 0.43 s; t_{26}=0.24. p=0.82); Heavy Metal group (A1=1.32 ± 0.29 s. A2=1.57 ± 0.28 s; t_{28}=0.62. p=0.54); Control group (A1=2.11 ± 0.59 s. A2=2.85 ± 0.75 s; t_{22}=0.78. p=0.45).

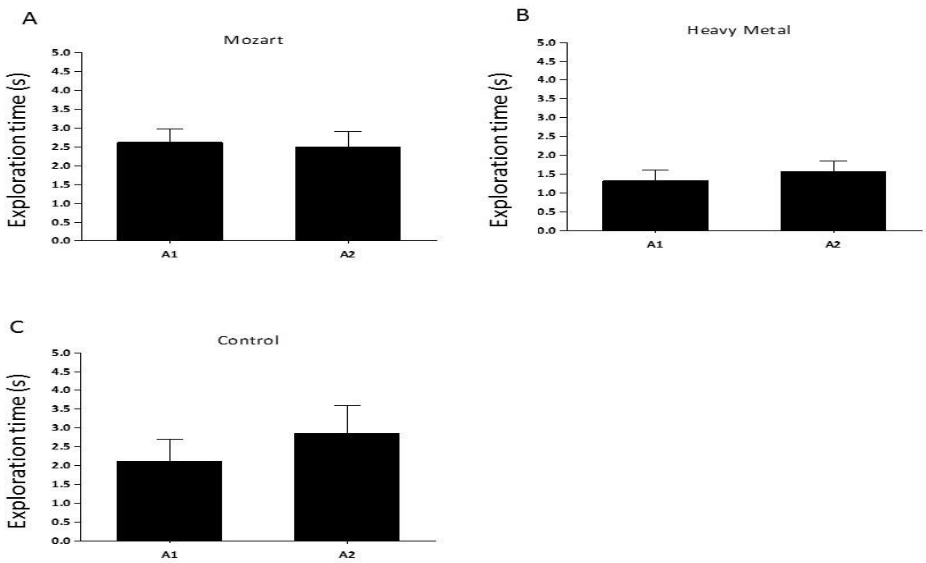

Figure 2. Mean + standard error of the time of the object exploration behavior, for the objects A1 and A2 during the object recognition training for the Mozart group (n=14, Fig 2A), Heavy Metal group (n=15, Fig 2B) and Controls (n=12, Fig 2D). Independent t-test analysis did not show significant difference among the three groups (p>0.05).

Figure 3 displays the time of the object exploration behavior for the A1 and B objects, during the test performed 90 minutes after the object recognition training (short-term memory test), for the Mozart, Heavy Metal and Control groups. A Kruskal-Wallis analysis exhibited differences amongst groups (H(2)=7.98; p=0.019). The median time between the Mozart (77.94% [60.92%-100.00%]) and Heavy Metal (65.52% [62.27%-100.00%]) groups did not reveal significant differences (U=97.50, p=0.747). Yet, when compared to the Control group

(56.81% [49.00%-69.62%]), both Mozart (U=33.00, p=0.016, FDR-corrected, Cohen's d=1.31) and Heavy Metal (U=44.00, p=0.033, FDR-corrected, Cohen's d=1.04) groups presented a higher percentage value of exploitation and level of significance.

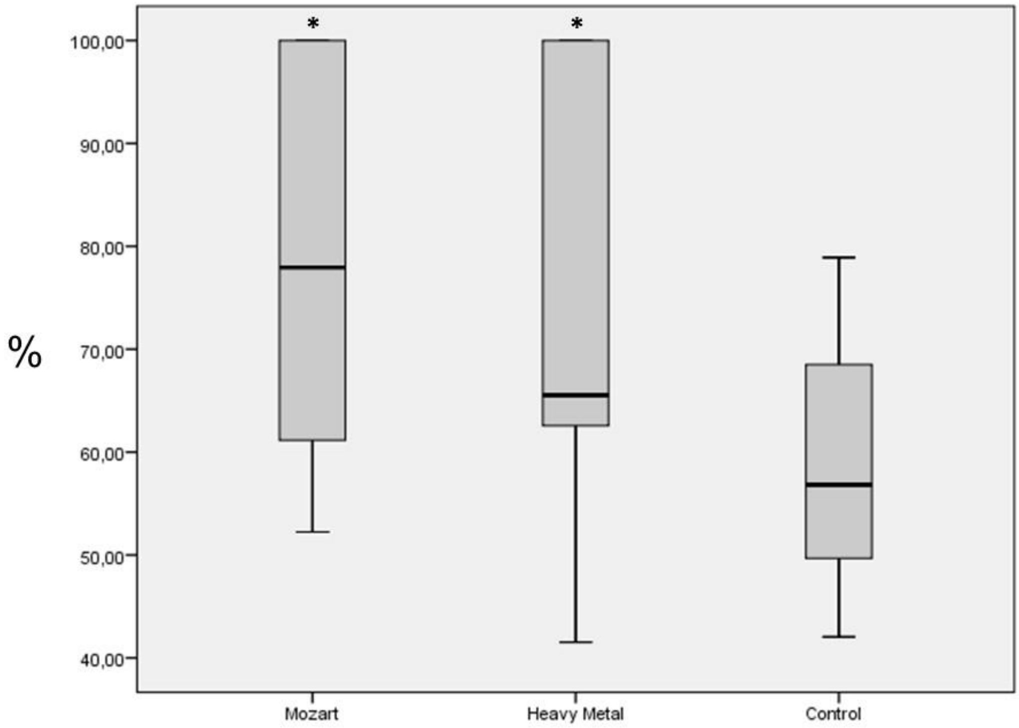

Figure 3. Boxplot of the percentage time of the object exploration behavior for the A1 and B objects during the short-term memory test for the groups Mozart (n=14), Heavy Metal (n=15) and Control (n=12). A Kruskal-Wallis test showed differences among the groups (H(2)=7.98; p=0.019). Post-hoc analysis showed that both Mozart (U=33.00;p=0.016; FDR-corrected) and Heavy Metal (U=44,00; p=0,033; FDR- corrected) groups had higher percentage time when compared to Controls.

Figure 4 illustrates the time of the object exploration behavior for the A1 and C objects, throughout the test performed 28 days after the object recognition training (long-term memory test), for the Mozart, Heavy Metal and Control groups. A Kruskal-Wallis analysis was unable to show variances amongst groups (H(2)=1.21; p=0.545) (Mozart=75.76% [62.92%-84.19%]. Heavy Metal=66.90% [00.00%-100.00%]. Control=58.45% [13.19%-76.96%]).

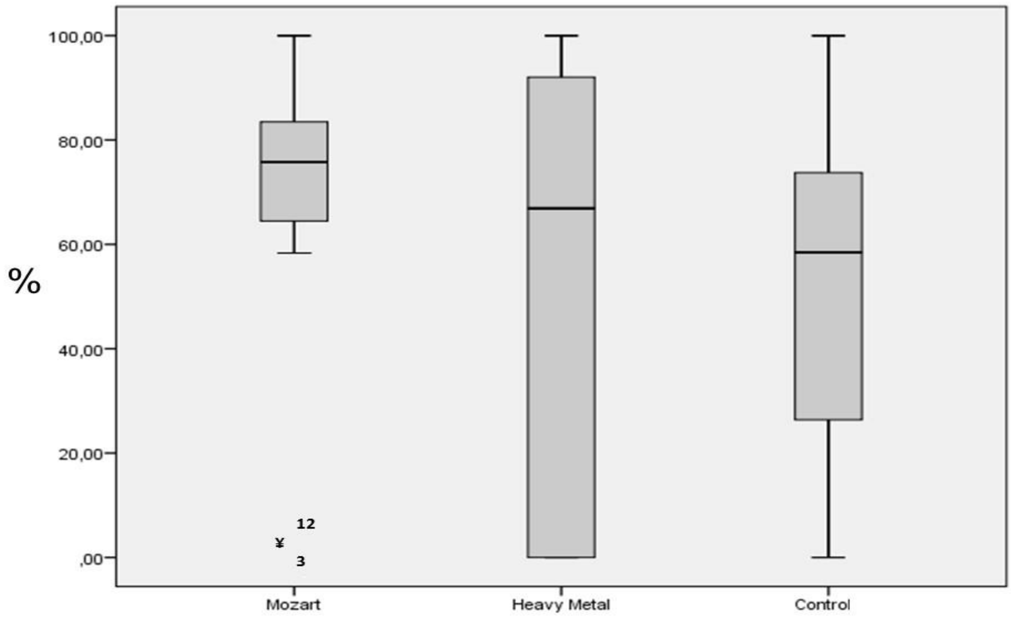

Figure 4. Boxplot of the percentage time of the object exploration behavior for the A1 and c objects during the long-term memory test (28 days) for the groups Mozart (n=14), Heavy Metal (n=15) and Control (n=12). A Kruskal-Wallis test showed no differences among the groups (H(2)= 1.21; p=0.545) (Mozart=75.76% [62.92%-84.19%]. Heavy Metal=66.90% [00.00%-100.00%]. Control=58.45% [13.19%-76.96%]). all p>0.05. ¥means outliers.

Discussion

To the best of our knowledge, this is the first study to explore the Mozart effect on both short-term and long-term memory phases in the new object recognition model. Besides, this is the first investigation to assess the possible impact of the heavy metal music on memory. When verified 90 minutes following training (short-term memory), both Mozart and heavy metal groups revealed a level of significance for the new object, when compared to the control group. Then again, when verified 28 days after the training (long-term memory), the groups were unable to reveal any significant statistical difference.

Several studies have revealed possible musical effects on peripheral tissues. Mostly, music can trigger physiological changes in the heart and respiratory rates, in addition to blood pressure [15, 31]. This music effect appears not to be only restricted to the peripheral tissue; it also interacts with the functions of the CNS. Sack [16] exhibited that music improves psychological cognitive functions. In 2014, Pauwels and colleagues described the beneficial effects that music has on anxiety and pain [17]. Furthermore, the effect music has on the CNS are also correlated to learning and memory processes [8,9, 20].

Consistent with the research literature, our results confirmed a beneficial Mozart effect on the short-term memory in the new object recognition test. Rauscher et al. [32] submitted rats that had been exposed to the Mozart's Sonata K-448 for 60 days to a multiple T-maze. The researchers observed that the Mozart group presented an improvement in the short-term memory when compared to the control group [8] submitted mice that had been exposed to Mozart's Sonata K-448 for 30 days to the Morris water maze. These animals presented lower escape latency three days after the training session, when equated to controls [8].

We assume this is the first study to assess the Mozart effect in different memory phases, predominantly on the long-term memory. Our main result highlighted a beneficial effect on the short-term memory. Though, we did not find any positive impacts on the long-term memory. The animals in the Mozart group were unable to achieve statistical difference on exploration behavior when compared with the control group. These results may suggest that the Mozart effect was unable to induce neuronal alterations required to establish the long-term memory. So, the Mozart effect may be restricted to the short-term memory.

Hitherto, animal studies have principally been shown displaying the Mozart effect (Sonata K-448) in memory [11]. Extra studies have also investigated possible influences of different classical composers and/or music modalities in behavioral alterations related to learning and memory processes. When submitted to a T-maze, mice that had been exposed to Mozart (Sonata K-448) for 10 weeks presented improvements in short-term memory compared to animals that had been exposed to the Beethoven´s Fur Elise [23]. Rauscher et al.[20] reported that rats that had been exposed to minimalist music (a Philip Glass composition) for 60 days were unable to achieve positive impacts in memory when compared to the control group. Yet again, this is possibly the first investigation of the impact of the Heavy Metal on memory. We revealed a beneficial effect on the short-term memory in the Heavy Metal group in contrast to the control group. Furthermore, this positive effect was unable to achieve statistical differences with regards to the Mozart group, signifying that both may cause positive impacts on the short-term memory. But, we did not observe any consequence of the Heavy Metal on the long-term memory (beyond 28 days).

The Mozart effect K-448 or classical music has been studied in animal models of both implicit and explicit memories. To evaluate implicit memory, Lee and co-workers submitted rats that had been exposed to relaxed classic music for 2 weeks to a step-dawn avoidance task. The animals were unable to achieve significant differences to the control group when examined two days following this training session [3]. Yet, the explicit memory has been greatly reported in the research literature, in distinct models [8, 11, 20, 23]. While Raucher [32] and Auon [23] used a T-maze to examine the Mozart effect, Meng [8] and Xing [1] stated beneficial Mozart effects in animals submitted to a water maze. Despite everything, this study is the foremost that employed the new object recognition model to examine music effects in animals. We revealed a beneficial Mozart impact on short-term memory specifically for this model.

According to our results, we may propose physiological mechanisms to explain the benefic effects of classical music on short-term memory. Xing et al. [11] investigate the effects of Mozart K.448 on learning behavior in developing rats through the classical Morris water maze task. As a main conclusion, the authors reported that music improved the spatial memory, which may be associated with increased brain-derived neurotrophic factor/Tropomyosin receptor kinase B in the dentate gyrus and dorsal hippocampus. In this line, previously, autistic rat pups were exposed to comfortable classic music for one hour once a day beginning postnatal day 15 and maintained until postnatal day 28. Music intervention improved hippocampal cell proliferation[33]. Taken together, we suggest that music has significant influence on hippocampus function and neurotransmission.

On the other hand, there is scarce literature on the effect of Heavy Metal music genre on memory. Considering that the rats evaluated by us do not have music preference, we may hypothesize that auditory stimulus is an important variable to be investigated. Very recently, Hampton [34] highlighted evidences that indicated positive influence of auditory stimulus in the brain. A recent study reported that an auditory gamma entrainment using sensory stimuli improved hippocampal function. The authors observed that auditory stimulus increased amyloid-β peptide uptake by microglia and also increased reactive astrocytes number [35].

We did not perform histological analysis to verify whether specific brain areas are influenced by music. Further studies are encouraged to do it.

The present study provide important support for clinical application of music. We evidenced that Mozart Sonata K-448 and heavy metal music presented positive influence on short-term memory in rats. In this context, we encourage future studies with the intention to develop procedures for music therapy in patients with cognitive impairment, including Alzheimer disease and dementia for example.

Conclusion

In summary, our results demonstrate that both Mozart Sonata K-448 and heavy metal music revealed beneficial effects on the short-term memory in the new object recognition test, when compared to the control group. Still, these music modalities did not exert any change on the long-term memory. Thus, this study contributes to and complements the current research literature, demonstrating that both Mozart and heavy metal music interfere with the explicit memory in new object recognition. Effects of music appear to be limited to the short-term memory, perhaps modulating a series of molecular and cellular events required for the fine regulation of neuronal plasticity vital for memory consolidation.

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