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Original

Comparative study of classical and other music on fear extinction in rats

Estudo comparativo entre música clássica e outras músicas sobre a extinção do medo em ratos

Estudio comparativo de la música clásica y otras músicas sobre la extinción del miedo en ratas

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Resumo

Introdução. A exposição à música é benéfica para a aquisição e consolidação da memória. No entanto, pouco se sabe sobre o efeito da música na extinção da memória. **Método.** Analisamos a influência da exposição à Sonata K448 de Mozart e a outros estilos musicais na extinção da memória do medo em ratos. Ratos Wistar machos foram expostos a diferentes estilos musicais ou sons ambientais desde o período gestacional até a vida adulta. No 39º dia do experimento, os animais foram submetidos ao treinamento de condicionamento aversivo por meio de estimulação elétrica para induzir a memória do medo. Do 67º ao 71º dia, os ratos passaram por testes de extinção do medo, nos quais retornaram à caixa de condicionamento aversivo, mas sem a aplicação de estímulos elétricos. No 92º dia, os animais foram novamente colocados na caixa para o teste de recordação, também sem estímulos elétricos. **Resultados.** A partir do terceiro dia de extinção, os grupos condicionados expostos a Mozart e a músicas clássicas apresentaram menor comportamento de *freezing* quando comparados aos grupos expostos à música eletrônica e ao som ambiente. No teste de evocação, o grupo condicionado exposto a Mozart não apresentou diferença significativa em relação ao grupo não condicionado, enquanto os demais grupos condicionados mantiveram níveis elevados de *freezing*. **Conclusão.** Nosso estudo sugere que a exposição a músicas clássicas favorece o processo de extinção da memória do medo. Embora limitado a testes comportamentais, os resultados indicam possíveis implicações positivas para o tratamento de transtornos de ansiedade.

Unitermos. Memória de longo prazo; Memória aversiva; Extinção da memória; Musicoterapia; Efeito Mozart

Abstract

Introduction. Exposure to music is beneficial in acquiring and consolidating memory. However, little is known about the effect of music on memory extinction. **Method.** We analyzed the influence of exposure to Mozart's Sonata K448 and other music styles on fear memory extinction in rats. Male Wistar rats were exposed to different music styles or ambient sounds from pregnancy to adulthood. On the 39th day of the experiment, rats were submitted to aversive conditioning training through electric stimulation to trigger fear memory. From day 67 to 71, rats were submitted to a fear extinction test, during which the animals returned to the aversive conditioning box, but without electric stimulation. On the 92nd day, the animals were returned to the aversive conditioning box without electric stimulation for the recall test. **Results.** Our results indicated that from the 3rd day of the fear extinction test onwards, the conditioned groups exposed to Mozart and classical songs showed less freezing behavior when compared to the groups exposed to Electronic and Ambient sound. On the recall test, the conditioned group exposed to Mozart did not show a significant difference compared to the unconditioned group, while the other conditioned groups showed higher freezing behavior. **Conclusion.** Our study suggests that exposure to classical songs favors the process of extinction of fear memory. The study was limited to behavioral trials, and possible mechanisms underlying the observed behavioral phenomena were not addressed. Despite this limitation, it is plausible to consider the positive implications of these results for anxiety disorders.
Keywords. Long-Term Memory; Fear memory; Memory extinction; Music therapy; Mozart effect

Resumen

Introducción. La exposición a la música es beneficiosa para la adquisición y consolidación de la memoria. Sin embargo, se sabe poco sobre su efecto en la extinción de la memoria. **Método.** Se analizó la influencia de la exposición a la Sonata K448 de Mozart y a otros estilos musicales en la extinción de la memoria del miedo en ratas. Ratas Wistar machos fueron expuestas a distintos estilos musicales o sonidos ambientales desde el período gestacional hasta la adultez. En el día 39 del experimento, los animales fueron sometidos a un condicionamiento aversivo mediante estimulación eléctrica para inducir la memoria del miedo. Del día 67 al 71, se realizaron pruebas de extinción, en las que regresaban a la caja de condicionamiento sin estímulos eléctricos. En el día 92, fueron nuevamente colocados en la caja para la prueba de evocación. **Resultados.** A partir del tercer día de extinción, los grupos condicionados expuestos a Mozart y música clásica presentaron menor comportamiento de congelación que los grupos expuestos a música electrónica y sonidos ambientales. En la prueba de evocación, el grupo condicionado con Mozart no mostró diferencias significativas frente al grupo no condicionado, mientras los demás grupos sí mantuvieron niveles elevados de congelación. **Conclusión.** El estudio sugiere que la exposición a música clásica favorece la extinción de la memoria del miedo. Aunque limitado a pruebas conductuales, los resultados apuntan a posibles beneficios en el tratamiento de trastornos de ansiedad.
Palabras clave. Memoria a largo plazo; Memoria aversiva; Extinción de la memoria; Musicoterapia; Efecto Mozart

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INTRODUCTION

The learning process is understood as a set of cellular and molecular changes in the central nervous system as a result of the exposure – repeated or not – to a particular event or environmental stimulus, causing remodeling in the neural circuits[1-4]. As a result of these neural remodeling, several changes in the physiological mechanisms of synaptic transmission occur, supporting changes in behavioral responses that characterize the memory process[1,5-8]. The memory process can be constituted in different phases, namely: acquisition, consolidation, persistence and extinction of the acquired information[1,7-10]. Currently, several studies demonstrate that the initial phases of this process, acquisition and consolidation, are well-established in the literature[11-14]. However, the literature referring to later phases, mainly about extinction, is still scarce. Extinction is the process that represents a new form of learning in which one learns to no longer express a behavior previously related to a previous memory[15]. This mechanism does not involve the erasing of original memories, but only the attenuation of the behavioral responses that are expressions of a memory[16].

Throughout history, different societies and people have used music as a treatment for several pathological conditions. In ancient Greece, for example, music was used as therapy for the mentally disabled. In the post-World War II period, music therapy was used in conjunction with conventional medical therapy for the treatment of wounded or disabled soldiers[17-20]. In addition, recent studies demonstrate that the use of music therapy has been applied by several sectors of medical clinic, such as arterial hypertension[21-23], cardiovascular diseases[24-26] and cases of fibromyalgia[27-29], showing promising results in the treatment of these conditions.

In this context, in recent years, the influence of exposure to music on learning and memory has been studied. On the literature, several studies have sought to elucidate the effects of classical music (Mozart) in the early stages of memory[11-14,30]. A group of rats exposed to Mozart from gestation to the 60th day of life achieved superior performance in the food maze test compared to other groups, not exposed to music, and exposed to a different musical style[11]. With the focus on another exposure window, another study used two groups exposed to music from the 3rd week of life, until the 70th day[12]. In this study, the group exposed to Mozart performed the route through the food maze more quickly and with fewer errors than the group exposed to Beethoven. 28-day-old rats exposed to music for 30 days were used and, after the Morris water maze test, it was found that the group exposed to Mozart performed better than the control group, not exposed to music[13]. In the inhibitory avoidance test for short-term memory, there were no significant differences in the escape latency between the group exposed to Mozart and the control group not exposed to music[14]. However, in molecular analysis to quantify BDNF (Brain-derived neurotrophic factor) in the hippocampus, a higher value was found among the group exposed to Mozart compared to the control group, which may indicate a greater future development of learning and memory capacity among the group exposed to Mozart.

Regarding the models used in the literature to study the influence of exposure to music on learning and memory, the focus is mainly on explicit spatial memory models, especially for food maze[12] and for Morris water maze[13,30]. The reposts from studies approaching music effects on aversive conditioning memories are still scarce.

In most of the articles currently in the literature regarding this issue, the periods of development of the animal exposed to music are observed in isolation, giving priority to specific periods of the animal's life[12-14,30]. In their research, they observed the influence of exposure to music from the animals' 2nd week of life[12,14]. Another study analyzed the effects on adulthood - after 28 days of life[13]. They observed the effects from birth - without analyzing the gestational period[30]. On the other hand, addressed the entire period, from pregnancy to adulthood, being one of the only sources of research on the influence of exposure to music on memory and learning to widely address all stages of animal development[11]. This demonstrates a scarcity of articles referring to this line of study.

Furthermore, in the current literature, most works focus on analyzing the influence of exposure to music on explicit memory models[11-13,30-33]. Seeking another approach, this study proposed to analyze the effect of exposure to classical music (Mozart's Sonata) on memory process, focusing on scarcely studied aspects like implicit memory (aversive conditioning), as well as the extinction phase.

METHOD

Animals and Experimental Design

For the present study, 20 rats were initially used, with an average of 3 to 4 months, female, pregnant, of the Wistar strain, from the vivarium of the Faculty of Medicine of Itajubá. After delivery, all male products from pregnancies were separated, and were randomly divided into 5 groups: Conditioned Mozart (n=24), Conditioned Classic (n=15), Conditioned Electronic (n=15), Conditioned Ambient (n=15), Unconditioned (n=8). The selection of male rats is due to the possible difference in behavior, which may be influenced by sex hormones in female rats. The animals had free access to water, and to the commercial feed of the Purina® brand "ad libitum" and were kept in plastic cages in a 12-hour light-dark cycle with 05 animals of the same group per cage. All procedures followed the rules of the Ethics Committee on the Use of Animals (CEUA) of the Faculty of Medicine of Itajubá (FMIT) (protocol number: 2018032).

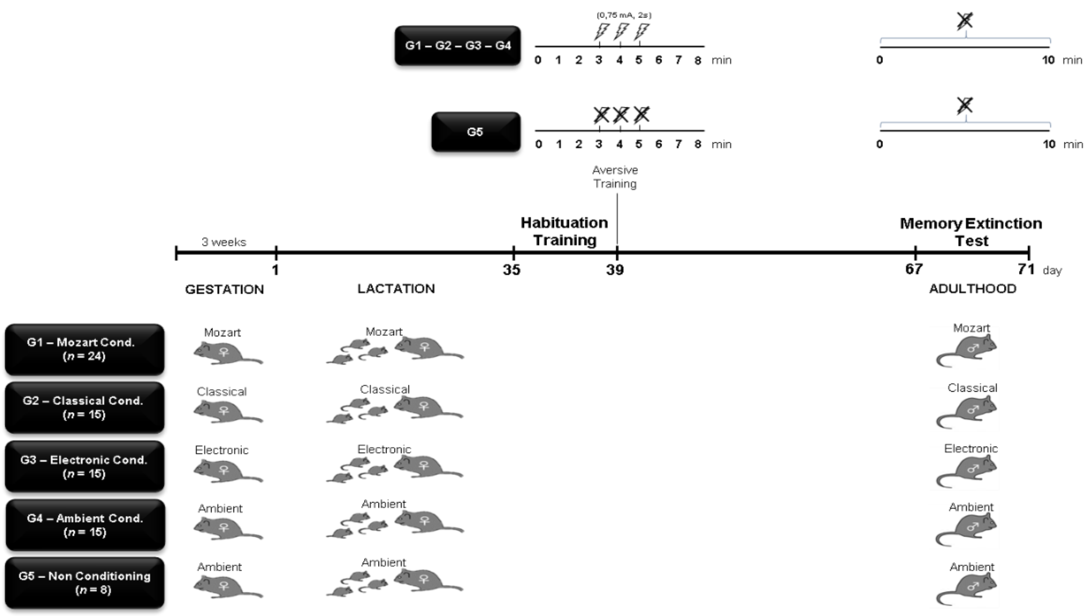
Sample size determination and group allocation

The experimental design involved exposure to music from gestation through adulthood. Due to biological variability in litter sizes and the prior criterion of selecting only male offspring to control for hormonal influences, small differences in the final number of animals per group were observed. To avoid unnecessary increase in animal use, the allocation followed ethical principles of reduction, and no additional matings were performed solely for sample balancing. Statistical analyses applied (repeated-measures ANOVA with Greenhouse-Geisser correction, Bonferroni and Tukey post-hoc tests, Levene's test for homogeneity of variances, and Kolmogorov-Smirnov for normality) are robust to mild sample size imbalances. Literature in similar fear-conditioning paradigms suggests that groups with ≥8 to 10 animals are sufficient to detect behavioral differences exceeding 20% in freezing behavior. In our study, observed differences surpassed this magnitude with strong statistical significance (p<0.001), supporting the adequacy of the sample size.

Behavioral Procedures

The behavioral procedures were inspired by Matsuda *et al*.[31] but adapted to our study design, with Figure 1 presenting an original illustration by the authors.

Figure 1. Experimental design.



Definition of musical styles and acoustic characterization of the tracks

The definition of musical compositions used in this experiment was based on evidence from scientific literature, specific acoustic properties of each genre, and behavioral hypotheses related to memory modulation and neuroplasticity.

Mozart: The Sonata K.448 by Wolfgang Amadeus Mozart was selected based on the Mozart Effect, whose studies demonstrate positive effects on spatial learning, cognition, and BDNF expression[11,34]. The piece has a duration of 24 minutes, a tempo of 60–80 BPM, predominant frequencies ranging from 400 to 4,000 Hz, and is performed in D Major.

Classical: To assess whether the effects observed with Mozart could be generalized, other classical compositions with similar characteristics (moderate tempo, structured harmony, predominance of mid frequencies) were included: High Mountain and Flowing Water (Xiang Sihua): 7min, 200–4,000Hz, 50–70BPM, A Major; The Butterfly Lovers (He Zhanhao & Chen Gang): 26min, 200–3,500Hz, 60–80BPM, variable tonality with passages in D Major; Kinderszenen (R. Schumann): 4min, 300–4,000Hz, 60–90BPM, C Major; Minuets and Preludes (J. S. Bach): 37min, 400–4,000Hz, 60–100BPM, variable tonality.

Electronic: The electronic tracks were selected for their contrasting acoustic properties — high rhythmic repetition, strong pulses, synthesized sounds, and predominance of low frequencies (20–200Hz). The included tracks were: Off the Hook (Hardwell & Armin van Buuren): 4min, 30–10,000Hz, 130BPM, B Minor; Bangarang (Skrillex): 4min, 20–12,000Hz, 120–150BPM, C Minor; Delirious (Boneless) (Steve Aoki): 5min, 50–10,000Hz, 128BPM, variable tonality; Tremor (Dimitri Vegas & Martin Garrix): 5min, 30–12,000Hz, 140BPM, C Major; Cobra (Tony Junior): 4min, 50–10,000Hz, 128BPM, E Major.

Within each experimental group, the tracks were played sequentially in a predefined order, restarting the cycle at the end of each sequence, in continuous repetition (loop) throughout the daily exposure period. All tracks were played at 320 kbps quality, with an intensity of 60–70 dB, for 10 continuous hours daily (9:00 p.m. to 7:00 a.m.), during gestation, lactation, and post-weaning periods.

Exposure to music during pregnancy

Initially, 20 female rats were separated, with an average of 3 to 4 months, pregnant, of the Wistar strain. These were exposed to music from the moment of mating until the birth of the pups. Thus, they were divided into 5 groups: Mozart, exposed to Mozart's Sonata K.448 (n=4); Classic, exposed to a sequence of classical songs (n=4); Electronic, exposed to a sequence of electronic songs (n=4); Ambient and Control, both groups exposed to the ambient sound of the vivarium (n=4).

In each group, 4 female rats were used and kept in individual cages, receiving the musical class corresponding to each group, with intensity between 60 to 70 dB, for 10 hours a day, from 9:00 pm to 7:00 am throughout the gestation period[11,12,34,35].

Exposure to music in the puerperium

After birth, the offspring were kept in individual cages with their respective mother, according to the 5 groups. Mozart, exposed to Mozart's Sonata K.448 (n=4 females + their offspring); Classic, exposed to the classic sequence (n=4 females + their offspring); Electronic, exposed to the electronic sequence (n=4 females + their offspring); Ambient, exposed to ambient sound (n=4 females + their offspring); and Control, also exposed to ambient sound (n=4 females + their offspring). They were exposed to music from the moment of birth until the 28th day.

In each group, 4 rats and their offspring were used, receiving the musical class corresponding to each group, with an intensity of 60 to 70 dB, for 10 hours a day, from 9:00 pm to 7:00 am during the entire breastfeeding period [11,12,34,35].

Exposure to music in adulthood

After the puerperium period (28 days), the male rats of each offspring were separated from the mother and randomly selected from each of the 5 groups. These were exposed from the 28th to the 72nd day to the same music that was given to the mother during the mating and breastfeeding phase. Being divided into: Conditioned Mozart, exposed to Mozart's Sonata K.448 (n=24), Conditioned Classic, exposed to the classic sequence (n=15); Conditioned Electronic, exposed to the electronic sequence (n=15); Conditioned Ambient, exposed to ambient sound (n=15), and Unconditioned, exposed to ambient sound (n=8).

In each group, 5 animals were separated per cage, which received their respective songs, with an intensity of 60 to 70 dB, for 10 hours a day, from 9:00 pm to 7:00 am[11-13,34,35].

Habituation Training

The animals were placed from the 35th to the 38th experimental day, individually, for 10 min daily sessions, in an experimental chamber (measures 71x42s36 cm, with yellow lighting, acrylic floor and sides). This procedure aims to control the interference of behaviors related to the novelty of the new environment during the training sessions[8,31].

Aversive Conditioning Training

On the 39th day, the rats from groups Conditioned Mozart, Conditioned Classic, Conditioned Electronic and Conditioned Ambient were trained in an Aversive Conditioning Model in a box different from the habituation box, with specific dimensions (30x30x15cm), lighting red, metallic floor and side. They remained in this box for 8 minutes. In the first three minutes, they received no stimulus. Then, they received foot shock (0.2s of 0.75mA) at

3min, 4min, 5min intervals. The rats in the Unconditioned group were also placed in the box with red lighting, however, they did not receive any aversive stimulus[31].

The behavior record was based on two distinct behaviors: Freezing: recorded every time the animal has immobility of the head, immobility of the body, eyes wide open, rapid breathing and absence of other observable behaviors[30,31]. No Freezing: Any behavior presented by the animal that is different from the immobility of the head, immobility of the body, eyes wide open, rapid breathing and absence of other observable behaviors. These behaviors include exploring the environment, locomotion, maintenance, among other behaviors[32].

All the freezing behavior raw data (FBRD, in seconds) for each animal was transformed to percentage, by using the following formula: (FBRD*100)/600 seconds, in which 600 seconds were the duration of the whole session. The percentage data was subjected to statistical analysis, detailed in the topics "Analysis of Behavioral Data" and "Statistical analysis".

Fear Extinction Test

After Aversive Conditioning Training, the animals remained for 28 days in the vivarium. On the 67th experimental day, 5 consecutive days of the Fear Extinction Test began. The Extinction Test was performed by exposing the animal to the same box as the Aversive Training, for 10 minutes, without any presentation of a stimulus. All animals in the 5 experimental groups passed the Extinction Test[31].

The behavior record was performed according to the topic “Aversive Conditioning Training”.

Recall Test

After the 5th day of the Fear Extinction Test, the animals remained for 21 days in the vivarium. On the 92nd day, the Recall Test started. The test was performed by exposing the animal to the same box as the Training and Extinction Test, for 2 minutes, without any presentation of a stimulus. The 5 experimental groups passed the Recall Test[31].

The behavior record was performed according to the topic “Aversive Conditioning Training”.

Analysis of Behavioral Data

The recordings of the Aversive Conditioning Training, Extinction Test and Recall Test sessions were recorded, stored and transcribed to record the behaviors, using the program EthoLog 2.22. The reliability of the data was systematically assessed by comparative analysis of the review of the recordings and transcriptions by two observers – main and control – in addition to a third analysis, by a third individual, if the difference between the first two analyzes was greater than 10%.

Statistical analysis

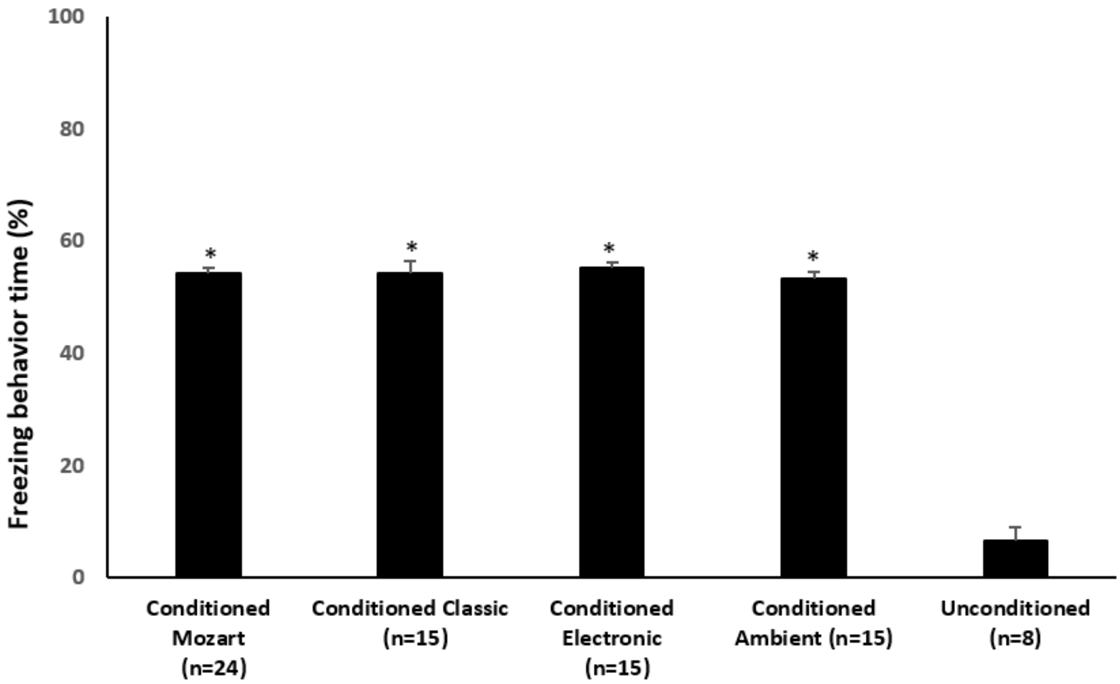
Statistical analyses were performed in IBM SPSS Statistics version 22. All the freezing behavior raw data (FBRD, in seconds) for each animal was transformed to percentage data, by using the following formula: (FBRD*100)/600 seconds, in which 600 seconds were the duration of the whole session.

The percentage data of the freezing behavior time as submitted to Kolmogorov-Smirnov and Levene tests to assure its parametric distribution. The data regarding the freezing behavior time on fear extinction is presented as percentage mean ± standard error of the mean (SEM). Data related to the training and recall sessions was analyzed with a One-way ANOVA, with groups as the independent variable, followed by Tukey tests in case any significant effect was found. Data related to the five days test sessions was analyzed with repeated measures ANOVA, with the percentage of freezing behavior time as the dependent variable, days as the within-subjects factor and groups as the between-subjects factor. Given that the Mauchly's sphericity test was significant ($\chi2(9)=46.09$; $p<0.001$), the Greenhouse-Geisser correction was applied ($\varepsilon=0.737$). To investigate the temporal relationship throughout the days, repeated follow-up contrasts were used. Complementary (post-hoc) analyzes were performed by using the Bonferroni test to evaluate possible significant effects detected among groups. Significance was considered as $p<0.050$.

RESULTS

There was a significant difference between groups during the training session ($F(4,76)=122.80$; $p<0.001$). The four Conditioned groups did not present any difference among each other (Conditioned Mozart mean±SEM =54.30±1.07%, Conditioned Classic =54.30±2.16%; Conditioned Electronic =55.15±1.02%, Conditioned Ambient =53.34±1.09%; all $p>0.980$), but they all showed significant higher freezing behavior time when compared to Unconditioned group (mean ± SEM =6.65±2.36%; all $p<0.001$; Figure 2).

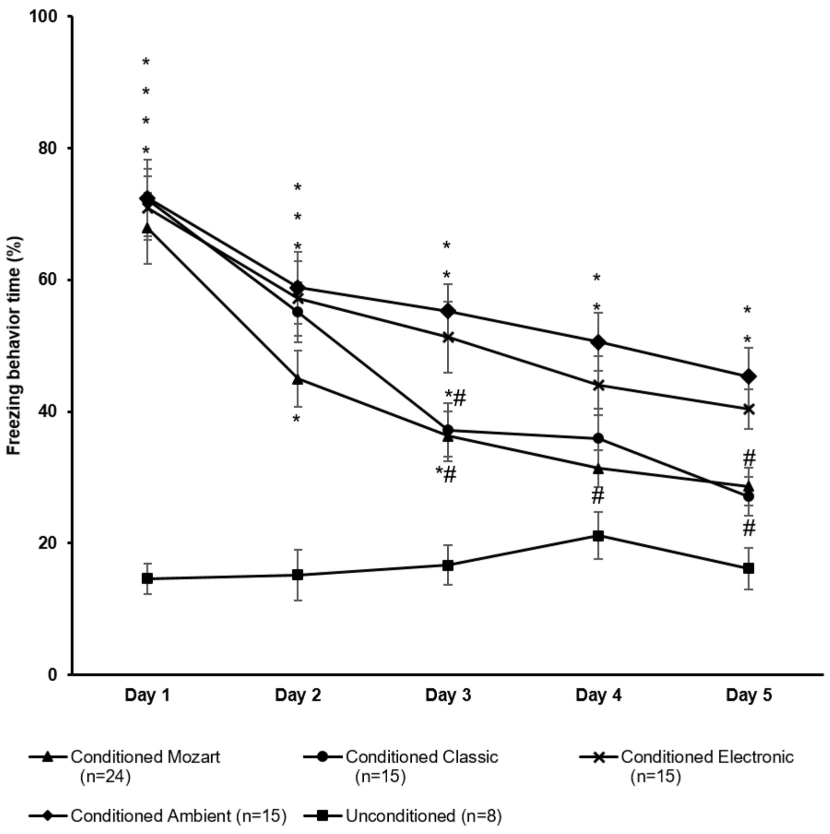
Figure 2. Freezing behavior time (%) of the training session, showing a significant difference between groups ($F(4,76)=122.80$; $p<0.001$).



* p≤0.001, in relation to the Unconditioned group.

Figure 3 shows the mean ± SEM of the five groups' freezing behavior time percentage during the five days of the fear extinction sessions. There was a significant main effect of days ($F(2.95,212.38)=52.68$; $p<0.001$). The follow-up contrast between day 1 vs. day 2 $F(1,72)=37.22$; $p<0.001$), day 2 vs. day 3 ($F(1,72)=20.82$; $p<0.001$), and day 4 vs. day 5 ($F(1,72)=8.64$; $p=0.004$) were significant, showing a decrease on the freezing behavior time over time.

Figure 3. Means (SEM) of the freezing time behavior percentage of the groups during the 5 days of the Extinction Test.



* All ps<0.04, in relation to the Unconditioned group. # All ps<0.02, in relation to the Conditioned Electronic and Conditioned Ambient groups.

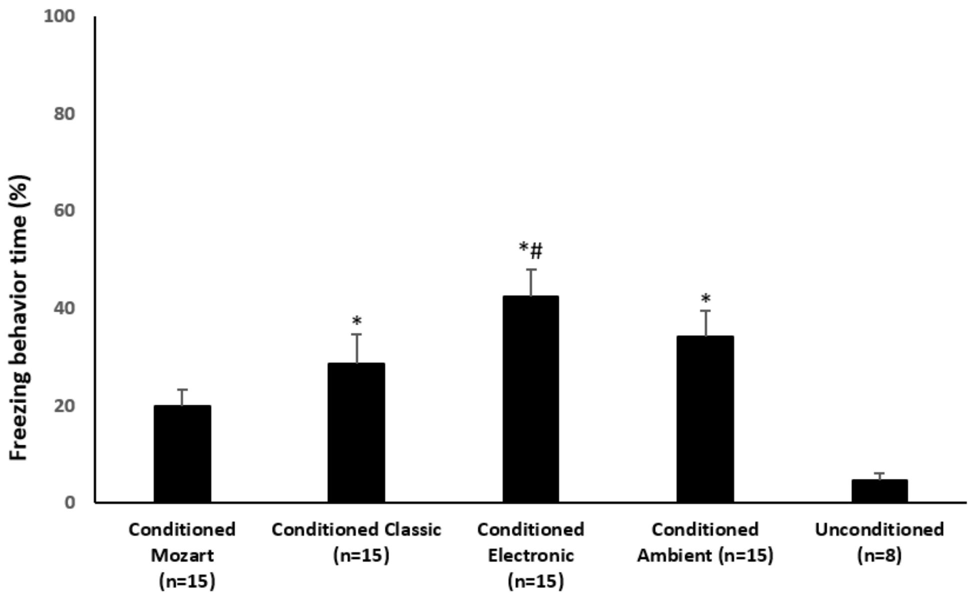
There was a significant group main effect ($F(4,72)=12.35$; $p<0.001$). On the days 1 ($F(4,72)=12.40$; $p<0.001$), 2 ($F(4,72)=7.98$; $p<0.001$) and 3 ($F(4,72)=8.43$; $p<0.001$), the four Conditioned groups showed higher freezing behavior time than the Unconditioned (all $p<0.05$). Besides, on the days 1 and 2, the four Conditioned groups were not significant different from each other ($0.376 \leq p \leq 1.000$). However, on the day 3 onwards, the Conditioned Mozart freezing time was lower when compared to the Conditioned Ambient (all $p \leq 0.010$). The Conditioned Classic also showed lesser freezing time than the Conditioned Ambient on the day 3 ($p=0.005$) and on the day 5 ($p=0.004$), while the Conditioned Ambient and Conditioned Electronic still showed significant higher freezing time when compared to the Unconditioned (all $p \leq 0.015$), both Conditioned Mozart and Conditioned Classic did not show differences when compared to the Unconditioned (all $p \geq 0.244$). Both Conditioned Mozart and Conditioned Classic groups showed a similar pattern to each other overtime ($p=1.000$).

There was a day versus groups interaction ($F(11.80,212.38)=4.23$; $p<0.001$), meaning that the fear extinction process was distinct among groups over time. The follow-up contrast between groups*day 1 and day 2 ($F(4,72)=2.69$; $p=0.038$) and between groups*day 2 and day 3 ($F(4,72)=4.07$; $p=0.005$) were significant. As seen in Figure 3, these results show that the freezing behavior time decrease for both Conditioned Mozart and Conditioned

Classic groups is significantly faster than for the Conditioned Ambient and Conditioned Electronic.

Finally, there was a significant difference between groups in the data of Recall Session ($F(4,63)=6.26$; $p<0.001$; Figure 4). The Conditioned Mozart (mean ± SEM =19.81±3.49%) did not show difference when compared to Unconditioned (mean ± SEM =4.69±1.48%; $p=0.373$), but present lower freezing time than the Conditioned Electronic (mean ± SEM =42.44±5.63%; $p=0.015$). The Conditioned Classic (mean ± SEM =28.65±5.93%), Conditioned Electronic and Conditioned Ambient (mean ± SEM =34.08±5.36%) groups had higher freezing time behavior than Unconditioned (all $p≤0.042$).

Figure 4. Freezing behavior time (%) of the recall session, showing a significant difference between groups ($F(4,63)=6.26$; $p<0.001$).



*ps<0.05,in relation to the Unconditioned group. #p=0.015, in relation to the Conditioned Mozart.

DISCUSSION

To the best of our knowledge, this is the first study to address the effects of exposure to music (Mozart effect and classic sequences) on the process of extinction of the contextual fear memory. Our results demonstrated that the groups exposed to classical music presented greater efficiency in the process of extinction of aversive memory. Such a result can be seen from the analysis of the freezing behavior data from Fear Extinction Test, which shows that rats exposed to Mozart's Sonata K.448 and the classical music sequence presented a faster extinction process compared to the group exposed to the ambient sound - mainly from days 2 and 3, and remaining with the statistical difference to the Conditioned Ambient until the last test day. These findings are in line with studies demonstrating the positive effects of exposure to classical music on different memory types and phases[13,36-40]. In addition, the results about the Recall Test were also able to demonstrate beneficial effects, and it is possible to verify that the animals exposed to Mozart, when compared to the other conditioned groups, were able to remain with less freezing time, even when exposed once again to the aversive box 21 days after the end of the Extinction Test. Due to the fact that this is an unprecedented methodology, currently, there are no previous studies related to such data in the scientific literature.

Several studies have shown that exposure to Mozart's Sonata can generate benefits for short- and long-term memory[11,13]. Rats exposed to Mozart achieved greater efficiency in the process of temporo-spatial learning, when compared to other groups exposed to Philip Glass compositions and ambient sound[11]. Comparable results regarding spatial learning, however, they also demonstrated benefits to long-term spatial memory, suggesting that Mozart's Sonata has a positive effect on memory consolidation[13]. In contrast, our results demonstrated that exposure to different musical classes did not interfere in the process of consolidating aversive memory, since none of the types of music affected the capacity of formation of this memory. Such a statement can be seen by observing the Freezing Time data of the 1st day of the Extinction Test, in which all the conditioned groups demonstrated statistically similar Freezing Time. Thus, it is plausible to consider that such a memory process is specifically related to aversive conditioning, since, from an evolutionary point of view, fear memory is a physiological defense response, being beneficial and necessary for the individual's survival in nature[2,41,42]. Interestingly, another study found a similar result, demonstrating that on the 1st day of the Fear Extinction Test, there was also no significant difference between the conditioned groups submitted, respectively, to physical exercise and physical inactivity[2]. Moreover, the authors found that physical exercise was able to accelerate the process of extinction of fear memory, similarly to the present study. Thus, our study was the first to find that Mozart's Sonata is also capable of generating such an effect. In addition, our results suggest that this effect is not specific to Mozart's Sonata but is also seen from exposure to other classical compositions. Therefore, it is plausible to conjecture that the memory extinction process is involved with complex molecular mechanisms, which may be triggered by an unspecified sound frequency, common to classical compositions. Furthermore, it is possible to consider that the classical compositions sound characteristics and physical activity can activate neuronal circuits in a similar way to promote memory extinction[12]. This issue can be addressed in future studies.

Such findings related to the process of extinction of the fear memory may have direct clinical implications for several psychiatric conditions, mainly on anxiety disorders. According to the American Psychiatric Association, Post-traumatic Stress Disorder (PTSD) currently affects about 3.5% of the adult population in the United States[43]. Regarding panic disorders, they estimated that the prevalence of this condition in the general adult population would be around 3%[44]. In addition, they found through meta-analysis that the incidence of mental disorders is closely related to the increased risk of suicide in these patients[45]. It is known that the isolated drug treatment for psychiatric comorbidities has its limitations, and, because of this, it is necessary to combine other associated therapies[46,47]. In this context, music therapy has been shown to be an important adjuvant tool in the treatment of these pathologies. Patients with PTSD undergoing adjuvant music therapy obtained benefits related to the reduction of anxiety symptoms and the improvement of sleep quality[48]. In this context, the results of our study suggest that exposure to classical music can accelerate the process of extinction of aversive memory, which can directly benefit patients with PTSD. In addition, the data related to the Recall Test suggest that frequent exposure to Mozart can stabilize the picture in the long term, even after new re-exposures, preventing new relapses.

Another important point to be considered is that this is the first study to evaluate the effects of other musical styles on the process of extinction of aversive memory. As already mentioned, they found that rats exposed to Mozart, obtained a much better performance in the spatial memory test, when compared to another group, exposed to Phillip Glass[11]. They sought another approach, comparing the effects of exposure to other classical music, such as the composition "Fur Elise", by Beethoven, in relation to Mozart[12]. Again, it was evidenced that the group exposed to Mozart obtained superior performance in the tests of explicit memory, when compared to Beethoven. In this same work, the authors, based on the "Columnar principle of Mountcastle"[12,49,50], suggests that Mozart's music can stimulate different synaptic transmission pathways in the animal cortex, through specific auditory patterns and symmetries of the composition. Our study, in turn, demonstrated that exposure to electronic music resulted in freezing time expression like the group exposed to ambient sound, thus presenting a process of extinction of memory statistically less efficient than groups exposed to classical music and Mozart. In addition, animals exposed to electronic music maintained a statistically superior freezing time in the recall test, when compared to the group exposed to Mozart. Thus, electronic music could not accelerate the extinction of the fear memory. This result reinforces that the memory extinction process is triggered by unspecified sound frequencies, common to classical compositions. Thus, we can suggest that the neural plasticity and behavioral changes seen in so many works are not caused exclusively by the famous Mozart effect, but by a frequency that other classical compositions also operate.

Therefore, after our unprecedented study, it can be concluded that rats exposed to Mozart and other classical music obtained positive effects on the aversive memory extinction process. On the other hand, it was verified that the exposure to electronic music obtained a result like the ambient sound, being, for this reason, less efficient than classical music for the extinction process. Furthermore, we concluded, through the Recall test, that exposure to Mozart was able to maintain such beneficial effects in the long term.

The present study was limited to behavioral trials and possible mechanisms underlying the observed behavioral phenomena were not addressed. Despite this limitation, it is plausible to consider the positive implications of these results for medical practice. Our data supports the consideration of music therapy as an adjuvant clinical approach for patients with PTSD, panic disorder, among other neuropsychiatric pathologies, contributing thus to a more efficient process of extinction of fear and its stabilization over time preventing new recurrences or relapses. Therefore, based on our study, additional clinical and animal studies are essential for a better understanding of the effects of classical music on anxiety disorders, as well as its adoption as potential therapy. It is noteworthy that clinical data corroborated by animal studies bypass placebo effects and cultural bias from human studies, reinforcing classical music clinical recommendations to approach anxiety disorders.

CONCLUSION

Our study showed that exposure to electronic music led to freezing time responses similar to those observed in the group exposed to ambient sounds, indicating a statistically less efficient memory extinction process compared to the groups exposed to classical music and Mozart. Consequently, it is reasonable to speculate that the memory extinction process involves complex molecular mechanisms, potentially triggered by unspecified sound frequencies common to classical compositions.

It can be concluded, after our unprecedented study, that rats exposed to Mozart and other classical music obtained positive effects on the aversive memory extinction process. Furthermore, the recall test demonstrated that this benefit was not only temporary but maintained in the long term. In this context, music therapy has been shown to be an important adjuvant tool in the treatment of neuropsychiatric disorders. If the exposure to music is sustained, there is the possibility to stabilize the gain in fear memory extinction process, avoiding new relapses even after re-exposure to the aversive stimulus.

Clinical data reinforced by animal studies are an alternative for human studies, which are influenced by cultural biases. In this sense, it is evident that allying those different sources of information provides a solid recommendation to approach neuropsychiatric disorders with classical music therapy.

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