The Power of Classic Music to Reduce Anxiety in Rats Treated with Simvastatin

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A B S T R A C T

Introduction: This study was designed to investigate the effects of music in Wistar rats after sub-chronic treatment of simvastatin.

Methods: The rats were orally administered with either simvastatin or saline (controls). After 4 weeks of drug treatment, the rats were selected for behavioral studies. The rats were exposed to music 24 hours before behavioral tests (Mozart's piano sonata, KV361, Largo).

Results: The experiments suggest that exposure to chronic treatment with simvastatin (1 or 10 mg/kg/day) reduced anxiety levels in rats when associated with Mozart music in an elevated plus-maze.

Discussion: These results indicate that music can serve as an effective adjuvant in rats treated with simvastatin, and that this species could potentially be used in other preclinical models utilizing musical interventions.

Introduction

Soothing effects of music on human psychology and behavior are well known (Koelsch, 2010). Classical music also appears to influence the behavior and/or physiology of captive animals in a manner suggestive of enhanced well-being (Chikahisa et al., 2007; Cruz et al., 2010). In addition, other studies show that music leads to positive behaviors (Wells & Irwin, 2008) and physiological benefits (Núñez et al., 2002; Sutoo & Akiyama, 2004; Nakamura et al., 2007). The value of auditory enrichment has been studied in a variety of species, including birds (Reed et al., 1993), cattle (Uetake et al., 1997), horses (Houpt et al., 2000), dogs (Wells et al., 2002) and primates (Wells et al., 2006). Many of these studies report changes in the behavior and/or physiology of animals exposed to music recordings, radio broadcasts or ecologically relevant sounds; but, to our knowledge, there is not scientifically documented literature data concerning music influence on reduced anxiety in rats treated with simvastatin.

Clinically, simvastatin have been widely used to reduce serum low density lipoprotein (LDL) cholesterol by inhibiting the rate-limiting enzyme, hydroxymethylglutaryl-coenzyme reductase. In addition, evidence show that simvastatin reduce the risk of ischemic heart disease events and cerebrovascular stroke, and have potential applications in multiple sclerosis, traumatic brain injury, Alzheimer's disease, and anxiety (Sett et al., 2011; Swindle et al., 2011; Tramontina et al., 2011;

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Wang et al., 2009; Zhang et al., 2011). Despite growing evidence for the role of simvastatin in central nervous system diseases, there is relatively a little knowledge of their direct psychoneurological impacts on the central receptors and its association with the behavioral effects.

Several lines of evidence have demonstrated that chronic administration of simvastatin does not change the cholesterol level in brain tissue and plasma of rodents (Mok et al., 2006; Selley, 2005; Schoonjans et al., 1999; Wang et al., 2009). It is reasonable to hypothesize that the simvastatin effects in central nervous system are possibly via a central mechanism independent of hypocholesterolemic properties. Interestingly, on human being studies simvastatin indeed lowered plasma cholesterol (Vega et al., 2003). The contradicting findings may result from the different cholesterol metabolisms in human being and rodent. We speculated that it was poor cholesterol-lowering effect by statins in rodents (Schoonjans et al., 1999), unaffected the genes directly involved in cholesterol synthesis (Johnson-Anuna et al., 2005).

Most studies on animals have been focused on the anxiolytic-like behavior under drug treatment, but the behavioral consequences of music exposure have received much less attention. The present study was designed to investigate the anxiety activities in rats after chronic treatment of simvastatin, and its association with Mozart music, as part of environmental enrichment in captive Rattus norvegicus.

2. Materials and Methods

2.1. Subjects

A total of sixty male Wistar albino rats (Rattus norvegicus), genetically heterogeneous, 3 to 5 months of age, weighing 220 to 310 g, were obtained from the animal house of the Regional University of Blumenau. After arrival in the sectorial animal house of the laboratory, these animals were housed in groups of five per opaque plastic cage (50×30×15 cm), with wood shaving bedding and wire mesh tops, under a standard light cycle (12-h light/dark phase; lights on at 07:00 h), in a temperature-controlled environment (23±1ºC), sound level of 50 dB, and the relative humidity was 55±10%. During the light and dark phase, the rats were exposed to a light intensity of approximately 500 and 0.025 lux, respectively. These lux values were chosen because they were the closest values (in our laboratory) possible to natural daytime and nighttime light. During the whole experimental period, the animals received commercial chow for rodents (Nuvital, PR, Brazil) and filtered tap water ad libitum. At irregular intervals the room was visited for an average of once every 2 or 3 days for cleaning cages, placing food and water, and so on. Animals were acclimatized to the animal housing facilities for at least 1 week before starting the experiments. The experiments reported in this article were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Experimental Protocols

The rats were further divided into two groups: silence and music. They were randomized with ten rats per subgroup orally administered with either simvastatin (1 or 10 mg/kg/day by oral gavage, Merck) or saline (controls). After 4 weeks of drug treatment, the rats from each subgroup were selected for behavioral studies. The rats were exposed to music 24 hours before behavioral tests, and also during the tests. Music (Mozart's piano sonata, KV361, Largo, 8:35 min duration) was continuous and repeated in a compact disc player (Cruz et al., 2010). The loud speaker had a frequency range of 100-16000 Hz. The silence room was exactly the same as the room in which music were played but here there was no sounds except for ambient noises like as that produced by the air conditioner. The sound level of the silence group was 50 dB (ambient noise) and music 65-75 dB in the home cages and in the behavioral apparatus.

2.3. Behavioral Tests

The animals were submitted individually to the elevated plus-maze and open-field tests. All behavioral procedures were conducted during the light phase (between 8:00 and 12:00 h), when rodents are less active, in a sound-isolated room. To minimize possible circadian influences on rats, experimental and control observations were alternated. The observer stayed in the same room, 1 m or so away from the elevated plus-maze and open-field (Pellow et al., 1985; Prut & Belzung, 2003).

2.3.1. Elevated Plus-Maze Test

The apparatus consisted of two open arms (50 x 10 cm) and two enclosed arms (50 x 10 x 40 cm) arranged in such a way that the two arms of each type were opposite to each other, and a central platform (10 x 10 cm). The maze´s height was 50 cm and the tests were conducted under dim red light (44 lx). Animals were exposed for 5 min to the red light in their own home cages before the testing procedure. Next, they were placed individually on the central platform of the plus-maze facing an open arm. During a 5 min test period the following measurements were recorded by observer: the time spent on the open arms, the number of entries in the open arms, the time spent on the closed arms, the number of entries in the enclosed arms and risk assessment. Risk assessment is measures comprised for time spent in the head-dipping (exploratory movement of head/shoulders over the side of the maze), and stretched attend postures (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion). Thus, the closed arms and center platform were designated as "protected" areas (i.e., offering relative security) and the "time protected" for head-dipping and stretched attend postures calculated as the time of these behaviors displayed in or from the protected area. The measures no need of that reflects anxiety-like levels in this test are the percentage of entries into open arms versus closed arms and the percentage of time spent in the open arms versus closed arms. We also included ethologically derived measures related to the defensive pattern of risk assessment behavior, which has been proven very sensitive to changes in anxiety (Pellow et al., 1985).

2.3.2. Open-Field Test

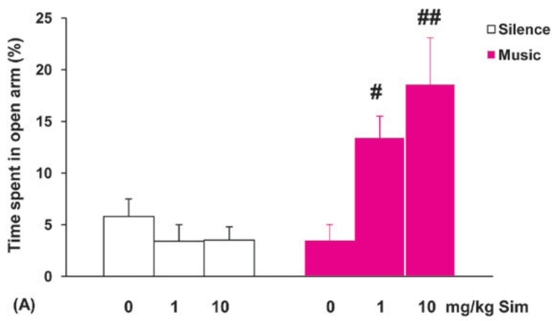
The open-field consisted of a black circular box (60 cm in diameter and 50 cm high). Each rat was placed in the central area and allowed to freely explore for 5 min. The tests were conducted under dim red light (44 lx). In the open-field test, we recorded total time spent locomotion (locomotor activity refers to the movement from one location to another) and immobility (time that the animal remained completely immobile, often in a crouching posture, with eyes wide open and irregular respiration). The time spent rearing and grooming was also recorded. A rearing episode was recorded whenever the rat stood on its hind paws with the body at an angle with the floor greater than 45°. A grooming episode was defined by repetitive movements of front paws or mouth on the fur (Prut & Belzung, 2003).

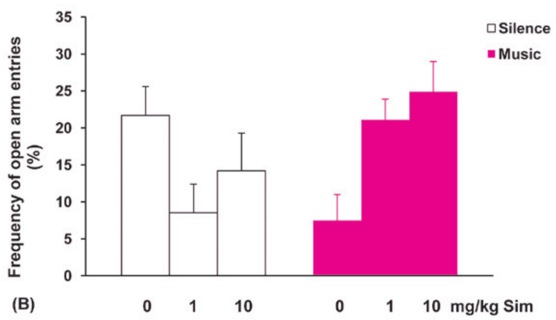
2.4. Statistical Analysis

The data were reported as means ± SEM and were analyzed statistically by analysis of variance (ANOVA) followed by the Newman-Keuls post hoc test. Differences were considered to be significant when p <0,05.

3. Results

Results (F = 7.597; p<0,001) revealed significant differences between subgroups on the % time spent by rats in the open arms of the elevated plus-maze. Music-exposed rats showed increased time in open arms under orally administered with either simvastatin (1 or 10 mg/kg) (p<0,05 and p<0,01; respectively) versus subgroups silence and music (simvastatin 0 mg/kg; Figure 1A). However, there was no significant difference in the subgroups, when considering the % frequency of open arm entries (F = 2.348; p>0,05; Figure 1B). Music exposure significantly decreased the % time spent by rats in the enclosed arms in simvastatin dose of 1 or 10 mg/kg versus subgroup control (F = 9.434; p<0,001; Figure 1C). Considering the % frequency of enclosed arm entries, analysis of variance reveals no significant difference between the subgroups (F = 1.815; p>0,05; Figure 1D). In case of the % risk assessment time, there were significant differences between groups (F = 4.642; p<0,01). The experimental group music, increased the time spent in the risk assessment in simvastatin dose of 1 or 10 mg/kg and saline versus subgroup silence (simvastatin 0 mg/kg; p<0,05; Figure 1E).





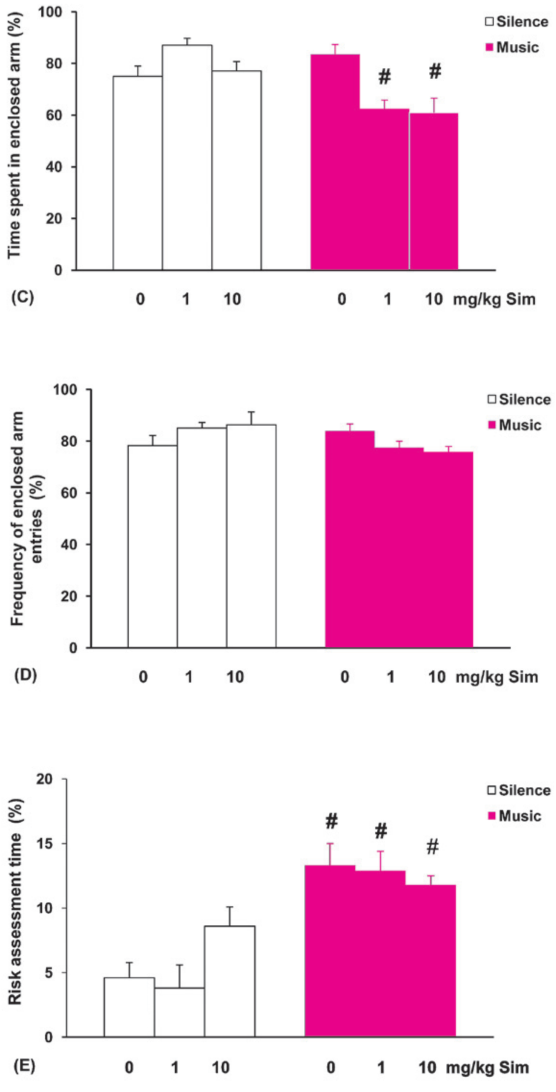


Figure 1. Effect of simvastatin (1 or 10 mg/kg/day) on the anxiety behavior of rats under each sound conditions (silence or music) in the elevated plus-maze. (A) % time spent in open arm; (B) % frequency of open arm entries; (C) % time spent in enclosed arm; (D) % frequency of enclosed arm entries; (E) % risk assessment. Vertical lines expressed as means ± SEM. Bars represent the mean of groups of 10 rats. #p <0,05; ##p <0,01; ANOVA followed by Newman-Keuls test.

Analysis of variance revealed significant differences between subgroups on the % time spent by rats in the locomotion of the open-field ($F = 4.015$; $p<0{,}01$). Music exposure significantly decreased the % time spent locomotion to 10 mg/ kg simvastatin body weight versus subgroup control ($F<0{,}01$; Figure 2A). However, there was no significant difference in the subgroups, when

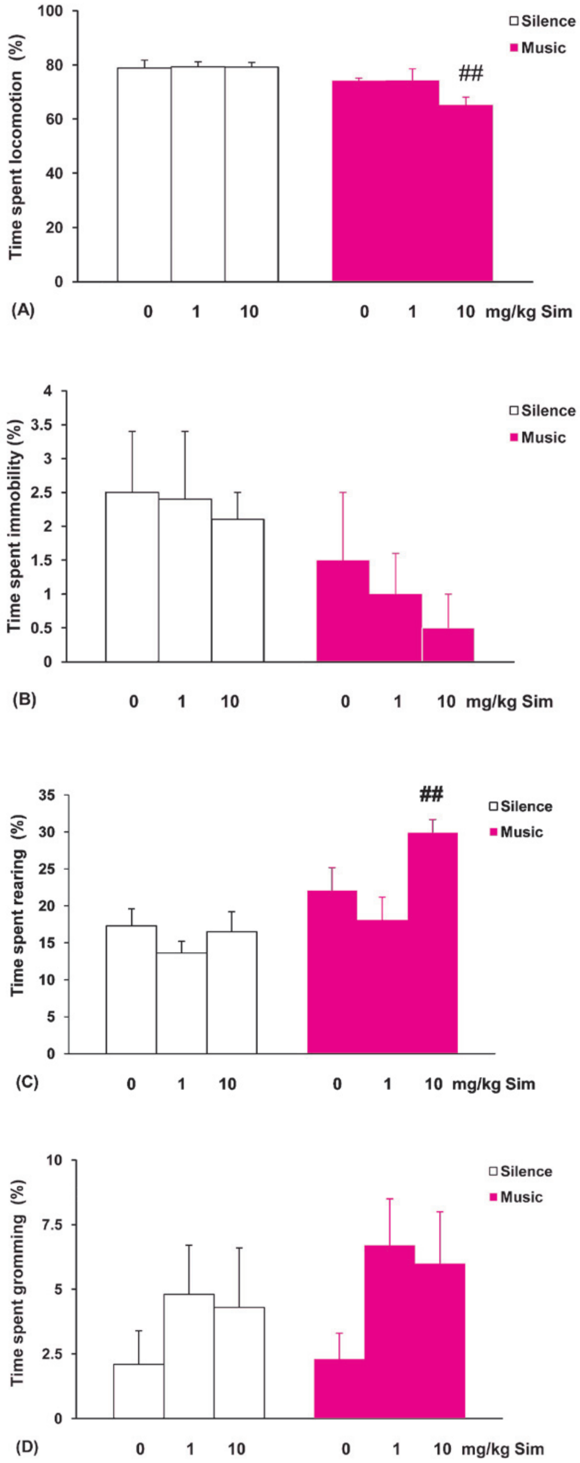


Figure 2. Effect of simvastatin (1 or 10 mg/kg/day) on the anxiety behavior of rats under each sound conditions (silence or music) in the open-field. (A) % time spent locomotion; (B) % time spent immobility; (C) % time spent rearing; (D) % time spent grooming. Vertical lines expressed as means ± SEM. Bars represent the mean of groups of 10 rats. ##p <0,01; ANOVA followed by Newman-Keuls test.

considering the % time spent immobility ($F = 1.138$; $p>0,05$; Figure 2B). In case of the % time spent rearing, there were significant differences between subgroups ($F = 7.492$; $p<0,001$). The experimental subgroup to 10 mg/kg simvastatin, increased the time spent in the rearing compared to subgroup control ($p<0,01$; Figure 2C). There was no significant difference in subgroups, when considering the % time spent grooming ($F = 1.017$; $p>0,05$; Figure 2D).

4. Discussion

The results of experiment suggest that exposure to sub-chronic treatment with simvastatin reduced anxiety levels in rats when associated with Mozart music. The exposed to music combined with simvastatin increased time spent in the open arms of the elevated plus-maze (Figure 1A). The elevated plus-maze is a widely used animal model of anxiety that is based on two conflicting tendencies; the rodent's drive to explore a novel environment and it's aversion to open spaces. Thus anxious animals will spend most time in the closed arms while less anxious animals will explore open areas longer (Pellow et al., 1985). In addition, the experimental group music increased the time spent in the risk assessment in simvastatin dose of 1 or 10 mg/kg and saline (Figure 1E). These ethological elements, which include stretched attend postures (SAP) and head-dipping, have been linked through factor analysis to risk assessment, directed exploration, and displacement activity, respectively (Cruz et al., 1994). Furthermore, pharmacological studies have shown that the incorporation of such measures in plus-maze scoring not only reduces the likelihood of false positives and negatives (Rodgers & Cole, 1993), but also enhances the sensitivity of the model to novel anxiolytic (Setem et al., 1999). These results strongly indicate that simvastatin treatment and/or music results in an improved coping with aversive situations, thus, leading to a reduced anxiety level. These effects of simvastatin were abolished in the absence of music.

The differences in anxiotypic behavior expressed by these animals are not limited to their performance on the elevated plus-maze. The novel environment is an established measure of general anxiotypic behavior, and levels of locomotion and rearing in this paradigm can be used as indices of an anxiety-like state in rats (Prut & Belzung, 2003). The music combined with 10 mg/kg simvastatin reduced time spent locomotion in the open-field (Figure 2A), indicating reduced in exploratory activity. However, the music combined with 10 mg/kg simvastatin increased the time spent in the rearing in open-field (Figure 2C). In this context, it has also been proposed that changes observed in the time spent in the rearing rather reflect changes in the level of locomotor activity (Lister, 1990). It is probable that both of them are indices of rearing behavior and both of them depend on the level of anxiety and the level of locomotor activity (Lister, 1990). These results indicate that simvastatin treatment and music reduces anxiety-like behaviors in one animal test of anxiety, without a significant change in total activity levels.

Consistent with our notion, several human studies showed that there were no association between statins and anxiety and depression (Harrison & Ashton, 1994; Muldoon et al., 2000). However, in a long term human study Young-Xu indicated that a progressive, cumulative reduction in the levels of depression and anxiety for patients with coronary artery disease was observed over a chronic clinical management of statin use and this reduction was likely independent of the statins' cholesterol-lowering effect (Young-Xu et al., 2003). The reasons for the contrasting outcomes may mainly result from their different methodology and follow-up periods. For example, in Young-Xu's study, the average follow-up time was four years and the longest one was about seven years; however, the follow up periods of contrary outcome studies were much shorter. Clinical reports also indicate that music can be an effective treatment for a multitude of disorders. Music therapy has shown promising results in treating anxiety, chronic stress, pain, sleep disorders, autism, depression, psychosis, post-traumatic stress disorder, and as an adjunct therapy for addiction (Bernatzky et al., 2011; Chan et al., 2010; Chan et al., 2011; Gold et al., 2009; Polston et al., 2011; Wan et al., 2010). In addition, the music can enhance medical therapies and can be used as an adjuvant with other anxiety-management programs to increase the effectiveness of those therapies.

There is a need to conduct more studies, which replicate the designs used in the existing studies that met the inclusion criteria, on the level of efficacy of music listening on the reduction of anxiety symptoms for a more accurate meta-analysis of the findings and reflect with greater accuracy the significant effects that music has on the level of anxiety symptoms. However, while musical appreciation is well documented in the human population, there is still significant disagreement in the literature about whether this is confined to the human species or extends to other members of the animal kingdom. Considering the enormous potential that music therapy offers, there is a growing need to develop preclinical models.

The first evidence, indicating the effects of simvastatin treatment on N-methyl-D-aspartate (NMDA) receptor binding density in the brain and reveals possible NMDA antagonist-like effect, which provides an exciting and potential paradigm to ameliorate anxiety deficits (Wang et al., 2009). This study showed that compared with the saline group, treatment of male Sprague–Dawley rats with simvastatin at a high dose (10 mg/kg/day) produced a significant longer travelled distance and higher average velocity in an open-field arena suggesting the hyperlocomotive activity; whilst increased time travelled in the open arms in elevated plus-maze was also observed, reflecting reduced anxiety-like behavior (Wang et al., 2009). However, our studies showed contrary results on treatment of male Wister rats with simvastatin. Different rat strains as well as differing test conditions have a major impact on the outcome of this animal test for anxiety (Rex et al., 2004). But, our results of experiment suggest that exposure to sub-chronic treatment with simvastatin reduced anxiety levels in male Wistar rats when associated with Mozart music. The present study is the first to show that when a statin is combined with music have anxiolytic-like effects in the elevated plus-maze. A possible mechanism mediating these effects could involve modulation of NMDA receptor (Wang et al., 2009; Xu et al., 2009). These results indicate that music can serve as an effective adjuvant in rats treated with simvastatin, and that this species could potentially be used in other preclinical models utilizing musical interventions.

In summary, the exposure to music of Mozart reduced anxiety levels only in Wistar rats sub-chronically treated with simvastatin in a manner suggestive of enhanced well-being. Such investigations could eventually inform us how music might be used more effectively in our therapeutic initiatives. Work on such interesting problems has barely begun.

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