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Depressive Behavior and Metabolic Alterations in Mice are Musical Style-Dependent

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Abstract. Nowadays, the world population has been affected by two serious psychological disorders, anxiety and depression, but there are few discoveries for new therapies to combat them. Studies have shown that music therapy has its beneficial behavioral effects. Therefore, the aim of the present study it was to investigate the possible effects of two music styles in some lipids and carbohydrate metabolism parameters resulting from behavioral changes related to anxiety and depression. So, mice were used with 30 days of age, divided into 6 groups: G1: saline, G2: Diazepam (DZP), G3: Fluoxetine (FLX), G4: control (no treatment), G5: Rock, and G6: Mozart Sonata. The animals from groups G1, G2 and G3 received treatments by oral route (gavage) for 15 days. The music therapy sessions (2x/day 4 hours/day) occurred in the same period of time at a 65dB frequency for G5 and G6 groups. After being evaluated in spontaneous locomotion, elevated plus maze and forced swimming tests, the animals were euthanized. The lactate, total cholesterol and plasma glucose levels were measured from the blood. No change was observed in spontaneous locomotion test and elevated plus maze. In the forced swimming test animals exposed to Rock showed an increase in immobility time. Furthermore, it was observed an increase in glucose and a reduction in cholesterol levels in the groups exposed to Rock and Mozart, while a decrease of lactate was observed only in group Rock. It was concluded that the auditory stimulus caused by music in mice was able to encourage depressive behavior and alter some lipids and carbohydrate metabolism parameters dependently of the musical style.
Keywords: Anxiety, Depression, Metabolism, Music.

Introduction

In recent years, there was a lot of attention given to the mental health area, since in living conditions that the world's population is today, many variables appear as result of stressful events that can trigger a number of diseases (MARTINS et al. 2012). Anxiety and depression are a major and growing problem for public health because they are common diseases associated with severe symptoms and widely distributed in the population (SUN et al., 2014).

Anxiety is characterized by the presence of irrational fear, excessive and persistent. According to the Diagnostic and Statistical of Mental Disorders, it is classified as generalized anxiety, specific phobia, social phobia, panic disorder, obsessive compulsive disorder and disorder of post-traumatic stress, differing from each other due to the nature of the involvement of its symptoms (CRYAN & HOLMES, 2005).

The depressive disorder has its origin in genetic and biochemical psychological factors (TAMATAM et al., 2012). In this way, the depression is classified as a group of disorders shown in a certain frequency, duration and intensity. It is described in the world and thoroughly by psychiatric manuals in mild, moderate or severe degree, and their varied classification according to the symptoms presented by the patient. It can show mood changes, decreased labor activities, reduced physical energy, as well as feelings of deep sadness, negative views about themselves and others and lack of confidence. In long term, it shows loss of interest in daily activities, headaches and fatigue accompanied by sleep and appetite disturbances, at its highest level, so that these changes can predispose to suicide (ICD 10, 1997). Therefore, it is believed that depression is the main source of mental disability globally, and it is estimated that by 2020, this disorder will be the second cause of health disability (CUNHA et al., 2012).

Therefore, by their characteristics, anxiety and depression share several psychosomatic events

and further stimulate the stress experienced by many patients (SUN et al., 2014). The stressed person may have changes in the metabolism of proteins and carbohydrates, expressing hyper-metabolism due to lipolysis and changes in water balance through the water and sodium retention. In response to acute or chronic stress, there is an increase in blood pressure and heart rate, decreased gastric emptying, decrease in blood flow to the organs attached to the digestive system and mobilization of energy stocks, causing metabolic and homeostatic changes. In summary, the stressors can induce psychological and physical changes that will affect energy production (ALMEIDA et al., 2011).

Faced with these problems, the research looking for treatments for depression and anxiety have become important and necessary for today's society. Among them, there are the synthesized in the laboratory. Because they are chemical substances, they usually act in order to change the unregulated body but there are unwanted effects. The alternatives from natural sources are not always fully exempt of these types of effects (LIMA et al, 2014;. URTADO et al, 2013.). On the other hand, the music has demonstrated its effectiveness in having beneficial effects, contributing to the improvement of cognition, promoting personal harmony, facilitating the psychosomatic balance, as well as the integration and social inclusion (CHIARELLI , 2005).

Based on this assumption, the aim of the present study is to investigate the possible effects of two music styles in some parameters of the metabolism of carbohydrates and lipids due to the behavior of mice subjected to experimental testing of anxiety and depression.

Methods

There were 48 albino male mice, Swiss, 30 days old and initial weight of 28.31 ± 0.68g, obtained from the Central Animal Center of the Federal University of Mato Grosso (UFMT), from the University Campus of Cuiabá, acclimated for two weeks in a vivarium of the UFMT Sinop Campus Vet Hospital, under controlled temperature of 22±2°C, regular light-dark cycle of 12 hours and with feed for rodents in the form of pellets and water *ad libitum.*

After acclimation, animals were randomly separated into 6 groups of 8 mice each one, called G1: saline, G2: Diazepam (DZP-1 mg/kg) G3: fluoxetine (FLX-10 mg/kg); G4: no treatment and handling, G5: Rock (Territory) and G6: Mozart (Sonata for two pianos).

During two weeks, G5 and G6 groups underwent music therapy twice a day (four hours/day) during the period from 06: 00-08: 00 and 12:00-14:00 for the Mozart group and 09:00-11:00 and 15:00-17:00 for the Rock group, at a frequency of 65 decibels at a distance of 10 cm from the stereo for 2 weeks, based on the methodology adapted by Almeida et al. (2011). The groups G1, G2 and G3 were treated daily (2x/day, 100 µL of the respective treatments), orally (gavage). At the end of the last stage of musical sessions, the animals were taken to the behavioral assessment room for carrying out the experimental procedures.

The test consisted of open field in a timber arena (60x60x35 cm) where the floor was divided into nine quadrants of 20x20 cm. It was considered as locomotor activity when the animal crossed with four paws on one of the quadrants, with the assessment made in a period of five minutes (LUCENA et al., 2010).

After the evaluation in the open field, the animals were exposed to the Elevated Maze test, formed by two open arms (50 cm x 10 cm) and two enclosed arms (50 cm x 10 cm x 40 cm) away from 50 cm above the ground, and each type of arm arranged on opposite sides and separated by a platform (10 cm x 10 cm). This equipment measured the number of entries and time spent in the open arms and closed for five minutes (HANDLEY and MITHANI, 1984).

Then, the animals were evaluated in the forced swimming test, which is the most widely used animal model for evaluating the antidepressant activity, which immobility reflects the exhaust failure associated with the possible low mood state during the experimental situation (PORSOLT et al. , 1977a, b). Mice were individually placed in a cylinder (30 cm in diameter and 50 cm height), containing approximately 30 liters of water at a temperature of 23±1°C for five minutes. The first two minutes were considered for habituation to the test. In the last three minutes, only continuous immobility were observed (minimum movements necessary to keep their heads above water and stay afloat).

The devices used for the evaluation of locomotion and anxiety were cleaned with 10% alcohol between each procedure. All experiments occurred in the period from 08 a.m. to 1 p.m., to avoid variations of the circadian cycle. At the end of the experiment, the animals were submitted to inhalation anesthesia in vaporization chamber with isoflurane in above 5 V % concentration for 5 minutes (MASSONE, 2008). Blood was collected by cardiac puncture, centrifuged (4000 rpm/15 minutes) to obtain plasma and anesthetized animals were sacrificed by cervical dislocation. From the plasma, biochemical parameters such as total cholesterol, glucose and lactate using commercial kits were measured (triplicate) (Labtest, Brazil).

Data were expressed as average ± standard error of the average (E.P.M.) and were calculated as percentages (%) of entry into open arms (EBA), and time in the open arms (TBA) according to the formulas: (EBA/EBA+EBF) x100 AND (TBA/TBA+TBF) x100, respectively (PELLOW et al., 1985). Statistical comparisons of the results were performed by analysis of variance (ANOVA) of way and the groups were compared at post-hoc Newman-Keuls test. The accepted probability as indicative of the existence of statistically significant difference was $p<0.05$.

This research was submitted to the Ethics Committee on Animal Use (CEUA) at the University

of Brasilia - UnB by the opinion Number 80243/2013, and was approved within ethical principles and the law.

Results and discussion

In a general context, it is observed that significant behavioral results were observed only for the group that received Rock music, that demonstrate a suggestive behavior of depression, while in the biochemical analysis showed that changes in glucose levels, total cholesterol and lactate were dependent of the musical style.

The open field test, as previously mentioned, is a model used to assess the locomotors activity of the animal and can help in analyzing the results of stimulating or depressant activity of a given compound. The results of this research did not show statistical difference in the treatments proposed (Figure 1).

In the elevated maze test (Figure 2), it was observed that the G4, G5 and G6 showed an increase in the percentage of animals staying time in the open arms, only compared to animals treated with saline controls, DXZ and FXT. When the analysis was performed to its control, without interference from the handling or any kind of treatment, different musical styles did not show significant difference. However, these observed results are not due to any change in the transportation of animals, since the frequency of closed arm entries was unchanged, reproducing the results also observed in the open field test. Thus, the data do not suggest anxiolytic effect presented by these musical styles in mice at least in the period applied for only four days.

Evaluations of metabolites present in plasma are shown in Table 1. In this study, blood glucose of animals that were exposed to music increased when compared to the control group, but there was no statistical difference between the rock and Mozart groups. Cholesterol levels were reduced in the Rock group, with no change in Mozart group. Lactate levels decreased in both groups. As the blood samples from animals were collected after the last test, the forced swimming, it is suggested that the glucose levels were increased because the animals were exposed to stressors. It is possible that the gradual increase in glucose levels has been caused by the release of high blood glucose due to the activation of hepatic glycogenolysis together with gluconeogenesis triggered by the release of adrenaline and glucagon added to the stress neurotransmitters activating the escape mechanisms (BEAR, CONNORS And PARADISO, 2002; NELSON AND COX, 2011).

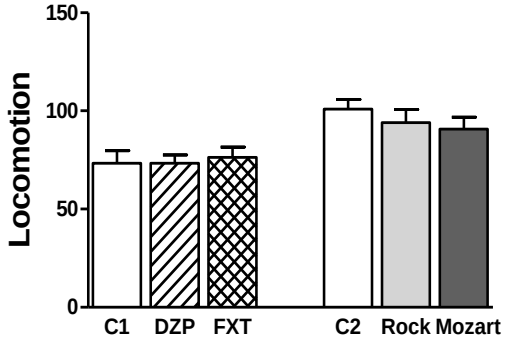

Figure 1 – Music therapy effect in locomotion of mice evaluated in the test of the open field for 5 min. For comparative analysis diazepam (DZP, 1 mg / kg) and fluoxetine (FXT, 10 mg / kg) were used. The results were expressed as the average ± e.pm of 8 animals/group. C1=control group administered with saline, C2=untreated control group. DZP=diazepam, FXT=fluoxetine.

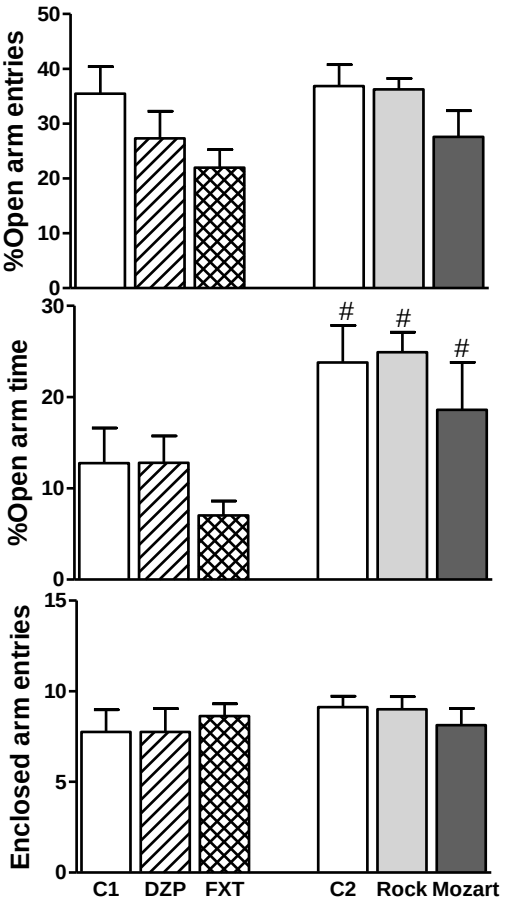

Figure 2 - Music therapy effects of anxiety behavior in mice evaluated in elevated maze test, for 5 min. For comparative analysis Diazepam (DZP, 1 mg / kg) and fluoxetine (FXT, 10 mg / kg) were used. The results were expressed as the average±e. pm of 8 animals/group. Top panel: is the % entries into the open arms; mid panel: represents the % time in the open arms; Bottom panel: is the frequency of closed arm entries. # $p<0.05$ means significant difference compared to control of animals treated with saline, DZP or FXT (ANOVA, Newman Keuls test). C1=control group administered with saline, C2=untreated control group. DZP=diazepam, FXT=fluoxetine.

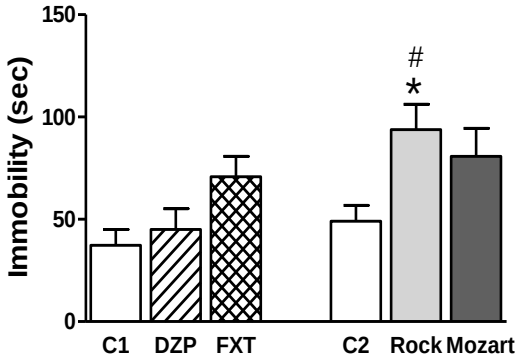

Figure 3 - Music therapy effect on the suggestive of depression behavior of mice evaluated in the forced swimming test for 3 min. For comparative analysis diazepam (DZP, 1 mg / kg) and fluoxetine (FXT, 10 mg / kg) were used. The results were expressed as the average ± e.pm. #p<0.05 means significant difference in its own control 2 (without any treatment); #p<0.05 means statistical difference compared to the control group 1 (treated with saline) (ANOVA, Newman Keuls test). C1=control group administered with saline, C2=untreated control group. DZP=diazepam, FXT=fluoxetine.

The reduction in blood cholesterol levels in the animals of rock group for 14 days may have been due to the fast pace that favored the stress of animals, and therefore probably mobilized cholesterol for the synthesis of the hormone cortisol (BEAR, CONNORS E PARADISO 2002; NELSON AND COX, 2011). However, this hormone was not measured.. In the Mozart group, the cholesterol showed no significant change. In addition, the rock group also had reduced lactate levels, suggesting a confirmation that the animals that were submerged in the water were probably depressed. The Mozart group showed a reduction in lactate levels, but less than in the rock group.

In the forced swimming test, many variables can interfere with the immobility of time, where typically its reduction is accompanied by general activity increase (BOGDANOVA et al., 2013). Data from this study demonstrated that animals that received Rock as music therapy, increased immobility time, evocative response of depressive behavior, as shown in Figure 3.

Table 1. Music Therapy Effect, Diazepam and Fluoxetine in glucose, cholesterol and lactate evaluation of mice previously subjected to experimental models of locomotion, anxiety and depression.

	C1	C2	FLX	DZP	ROCK	MOZART
Glucose	164,3±4,57	170,2±12,96	194,2±17,11	222,6±5,35	239,9±6,62 ^{ab}	253,1±19,48^{abc}
Cholesterol	78,82±1,65	56,81±2,63	64,47±2,28	74,61±2,43	68,89±2,43^{ab}	79,46±0,63^{bc}
Lactate	65,71±2,43	58,38±0,30	55,95±1,26	51,63±2,02	46,74±2,43^{abc}	56,31±2,66^{ad}

Each column represents the average ± S.E.M. experiments with animals (n=6), where ^{a}p<0,05 ≠ CN1; ^{b}p<0,05 ≠ CN2; and ^{c}p<0,05 ≠ FXT; ^{d}p<0,05 ≠ ROCK (Newman-Keuls Test).

Conclusion

Given the results presented, it is concluded that music therapy did not cause changes in mobility patterns and on behavior related to anxiety in the animals tested in the open field and elevated maze, respectively. However, depending on the music style, the metabolic changes involving increased glucose and decrease total cholesterol and lactate may be favoring somehow the behavior suggestive of depression for animals that underwent Rock Territory. Other research involving music therapy on some aspects raised here, are in progress to enable the elucidation of the mechanisms involved in the influence of music in the body.

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