

# 16. Mozart music stimulation

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## Mozart music stimulation effect on wistar rats' neurogenesis



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### ABSTRACT

**Introduction:** Optimal brain development is required to achieve optimal health. Therefore, stimulation is needed to achieve optimal development, and one of the stimulations which can be provided is music stimulation. Several prior investigations have been completed to prove the impact of music stimulation on brain development, whose results still showed both pros and cons. This investigation had the intention of determining musical stimulation's impacts on neurogenesis.

**Method:** Thirty 30 days old male Wistar rats were grouped randomly but equally into experimental and control groups, with 15 rats in each group. The experimental group was exposed for 90 minutes twice a day in 30 constitutive days to Mozart music (Mozart Sonata for two pianos K. 448) with sound pressure levels between 60 and 80 dB. After 30 days, the rats were euthanized. The neurogenesis parameters, such as plasma corticosterone level, Brain-Derived Neurotrophic Factor (BDNF) and Insulin-like Growth Factor 1 (IGF-1) concentration, and cerebral cortex thickness, were assessed.

**Result:** Rats with Mozart music exposure had considerably lower plasma corticosterone. They had substantially higher BDNF concentration in the hippocampus compared to the control group (p-value 0.049 and 0.040, respectively). Still, no significant effect was found on IGF-1 concentration and cerebral cortex thickness (p-value 0.148 and 0.094, respectively).

**Conclusion:** The brain development process requires stimulation, and Mozart's music is proven to be an alternative to stimulations provided for brain development.

**Keywords:** BDNF, IGF-1, Cerebral Cortex, Corticosterone, Mozart Music.

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### INTRODUCTION

Growth and development are continuous, starting from the embryonic phase and continuing until after birth. Similarly, a constant interaction between genetic and environmental factors, such as nutrition and stimulation, drives and directs brain development.<sup>1-4</sup> Genetic factors keep making a major contribution to development during the fetal and postnatal periods, in which environmental factors influence brain development in increasingly prominent ways during the later periods.<sup>5</sup> One of the environmental factors that affect brain development is musical stimulation.<sup>6</sup> Listening to classical music like Mozart's music was reported can increase neurogenesis in the brain and increase children's intelligence.<sup>7,8</sup> Several studies using experimental animal models carried out in relation to musical stimulation on brain development displayed still pros and cons results.<sup>9</sup> However, it is still a mystery whether

any specific mechanisms on how music's effects are exerted. Based on previous investigations, the need for an intact auditory pathway must be fulfilled, as the effects of music are displayed only after there are no lesions of the eardrum, cochlea, suprachiasmatic nucleus or auditory cortex.<sup>10,11</sup> The impacts of the specific interval, rhythm, and melody effects could be found in another investigation in which negative effects were exhibited in the spatial performance of rats with the reversed version of the music. In contrast, positive impacts were displayed in the rat performance with the original music version compared to controls. In that study, rhythm became a crucial element.<sup>12</sup>

The focus of the significant impact of music stimulation in brain development is in the improved thickness of the cerebral cortex, as a sign of positive neurogenesis regulation, as the result of music stimulation in lowering the levels

of corticosterone hormone or the stress hormone, extending proteins and growth factors which contribute to neurogenesis process such as Insulin-like Growth Factor 1 (IGF-1) and Brain-Derived Neurotrophic Factor (BDNF).<sup>13,14</sup> Furthermore, adult musicians can increase gray matter density in cortical areas, including cognitive and auditory processing areas, such as the right mid-orbital frontal gyrus, both right and left prefrontal cortexes, posterior cingulate cortex, and cerebellum.<sup>6</sup> Another report showed that music exposure in rats stimulates brain development and neuroplasticity through mechanisms that influence neurogenesis in the hippocampus, neurotrophin synthesis, and glutamatergic signaling.<sup>15</sup> Therefore, this investigation aimed to determine music stimulation effects on neurogenesis in Wistar rats (*Rattus norvegicus*) by measuring its plasma corticosterone levels, hippocampal concentrations in BDNF and IGF-1, and cerebral cortex thickness.

## METHODS

The experimental study was implemented from December 2021 to January 2022 in the Experimental Animal Research Laboratory, Faculty of Medicine, Universitas Brawijaya. The experiments were completed according to the regulations of the local ethical committee for animal experiments in the Faculty of Veterinary Medicine, Airlangga University, with ethics approval number: 2.KE.070.06.2021.

### Rats

Thirty male Wistar rats were utilized in this study and obtained from the Experimental Animal Research Laboratory, Brawijaya University, Malang. Thirty days old rats, with body weights around 80-100 grams, were divided randomly and equally in numbers into experimental and control groups, with 15 rats in each group. Body weight was measured using a digital scale with an accuracy of  $10^{-2}$  grams. We checked the body weight at the study's beginning and end. Body weight was measured to see if there was an effect of Mozart's music on the rats' body weight. Free access to food and water, ad libitum, was provided for each group of rats. The temperature was kept at about 25-28 °C. Rats were adapted to the new environment for seven days, and the group with music treatment would be treated with Mozart music (Mozart Sonata for two pianos K. 448) for 90 minutes, twice a day with the sound pressure levels between 60 and 80 dB during 30 days in a row. Cages need to be placed at a 30 cm distance from the speaker. The music was played from 08.00 a.m. to 09.30 a.m. and from 3.00 p.m. to 4.30 p.m. A separate room was provided for the control group with the same housing conditions without music exposure.

### Outcome Measure

All rats were euthanized after 30 days, then the plasma corticosterone level, the hippocampal concentration of BDNF and IGF-1, and cerebral cortex thickness were measured. ELISA assay was utilized for assessing plasma corticosterone level and hippocampal BDNF and IGF-1 concentration. In this investigation, rat corticosterone was analyzed with CORT ELISA Kit with a catalog number:

E0496Ra; while BDNF ELISA Kit was used to assess Rat Brain-derived neurotrophic factor with a catalog number: E0476Ra; and IGF-1 ELISA Kit was applied in measuring Rat Insulin-like growth factor with a catalog number: E0709Ra. For plasma corticosterone, plasma was generated using EDTA or heparin as an anticoagulant. After mixing for 10-20 minutes, the samples were centrifuged for 20 minutes at 2,000-3,000 RPM. Then, the non-sediment supernatant was collected. For hippocampal content of BDNF and IGF-1, hippocampal tissue was extracted, then the tissues were rinsed in ice-cold PBS (pH 7.4) to remove the blood residue thoroughly and weighed before homogenization. The tissues were minced and homogenized in PBS with the comparison of tissue weight (g): PBS (mL) volume at 1:9 using a glass homogenizer on ice. For additionally dismantling the cells, the suspension was sonicated with an ultrasonic cell disrupter or subjected to freeze-thaw cycles. Then, the homogenates were centrifuged for 15 minutes at 12,000 RPM at 4°C temperature to obtain the supernatant. In addition, the thickness of the cerebral cortex was assessed through histological examination, applying Hematoxylin Eosin (HE) staining. An examination using a micrometer on a microscope was conducted, and the results were recorded photographically by a computer connected to the microscope.

### Statistics

To assess whether the data distribution was completed normally or not, a Shapiro-Wilk test was applied. Meanwhile, a Mann-Whitney test was performed for not normally distributed data, and an Independent T-test was implemented to analyze the comparison between the experimental and control groups in

normally distributed data. IBM SPSS Statistic Version 23 was employed in analyzing the data. A statistical significance was acknowledged if the p-value < 0.05.

## RESULTS

### Rats' Weight Measurement during the study

The rats' body weight measurements were accomplished at the beginning and end of the study. The rats' initial body weights present no normal distribution (Shapiro-Wilk test,  $p < 0.05$ ). In contrast, their body weights at the end of the study have a normal distribution in each group (Shapiro-Wilk test,  $p > 0.05$ ). The initial body weights and study end body weights between control and music-exposed groups exhibit no significant difference (Table 1).

### Measurement of Plasma Corticosterone level, Hippocampal BDNF & IGF-1 Concentration, and Cerebral Cortex Thickness

Based on the measurement results, no normal distribution was displayed in the plasma corticosterone levels (Shapiro-Wilk test,  $p < 0.05$ ), while the hippocampal BDNF and IGF-1 concentration along with the thickness of cerebral cortex showed a normal distribution in each group (Shapiro-Wilk test,  $p > 0.05$ ). Rats with Mozart music exposure display considerably lower plasma corticosterone and significantly higher BDNF concentration in the hippocampus than in the control rats. However, there's no significant effect on IGF-1 concentration and cerebral cortex thickness (Table 2). Measurement of cerebral cortex thickness for the music and control group can be seen in Figures 1 and 2.

**Table 1. The weights of rats during study.**

Group	N	Initial Body Weight (g) <sup>a</sup>		End Study Body Weight (g) <sup>b</sup>	
		Median	p	Mean ± SD	p
Music	15	87	0.884	175.87 ± 24.13	0.882
Control	15	88		186.18 ± 16.54	

Data are presented as N (Number of rats)

<sup>a</sup>Difference between the control and music group is not significant (Mann-Whitney tests)

<sup>b</sup>Difference between the control and music group is not significant (Independent T-tests)

<sup>c</sup>a p-value < 0.05 is considered as statistically significant

**Table 2.** Plasma corticosterone level, hippocampal BDNF & IGF-1 concentration, and cerebral cortex thickness of subject.

Group	N	Plasma Corticosterone Level (ng/ml) <sup>a</sup>		BDNF Concentration (ng/ml) <sup>b</sup>		IGF-1 Concentration (ng/ml) <sup>c</sup>		Cerebral Cortex Thickness (µm) <sup>d</sup>	
		Median	p	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p
Music	15	10,442	0.049*	1.87 ± 0.30	0.040*	96.28 ± 14.20	0.14	2,430.2 ± 392.4	0.94
Control	15	11,486		1.58 ± 0.43		104.39 ± 15.60		2,810.6 ± 15.6	

Data are presented as N (Number of rats)

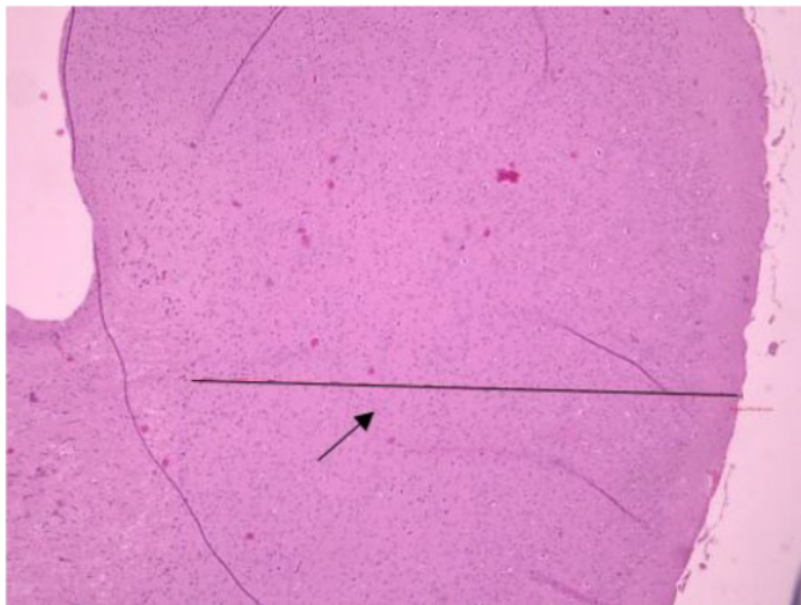
<sup>a</sup>Difference between the control and music group is significant (Mann-Whitney tests)

<sup>b</sup>Difference between the control and music group is significant (Independent T-tests)

<sup>c</sup>Difference between the control and music group is not significant (Independent T-tests)

<sup>d</sup>Difference between the control and music group is not significant (Independent T-tests)

\*a p-value < 0.05 is considered as statistically significant



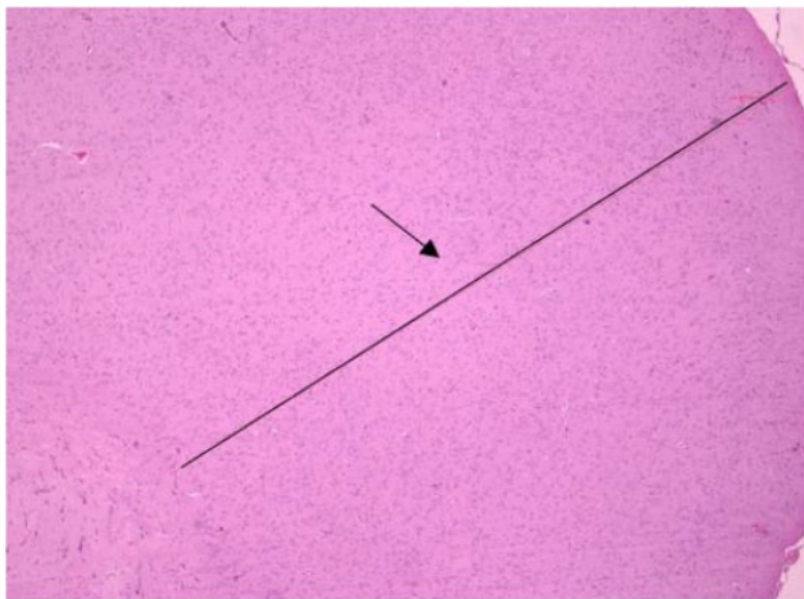
**Figure 1.** Histological picture of cerebral cortex thickness (black arrow) of Wistar rat in coronal section, from the outer layer of the cerebral cortex to the outer border of the white matter in coronal section (HE, 40x magnification), in the music group. Mean cerebral cortex thickness of music group is 2,430.2 µm with standard deviation (SD) 392.4 µm.

## DISCUSSION

The measurement of the rat's initial body weights was completed, and no indication of significant difference between groups is shown from the statistical analyses at the beginning of the experiment. At the end of the study, body weight was also measured, and data analysis showed no significant music effect on rats' body weight. This result is consistent with previous research evaluating the effect of music treatment on

body weight.<sup>16-18</sup> However, it is in contrast with the study result from Puspitawati, which found that the prenatal period of music exposure had a significant influence on weight growth in 7-, 25-, and 35-day old rat-litters in which the music-exposed rats have heavier weights than the control ones.<sup>19</sup> Meanwhile, in this current investigation, music was given in the post-natal period, which might cause a difference in the study results. In this study, rats with Mozart music exposure have

significantly lower plasma corticosterone. The previous study also displayed the significantly declined corticosterone levels after music treatment.<sup>18,20,21</sup> However, a study by Chikahisa *et al.* exhibited different results showing no significant decrease in plasma corticosterone levels in the offsprings upon pre and postnatal music treatments.<sup>16</sup> The role of the autonomic nervous in reducing stress explains the physiological effects induced by the music system resulting in stress reduction. The autonomic nervous system consists of the sympathetic and parasympathetic nervous systems that interact with each other, usually in opposition to maintaining the body's homeostasis. The sympathetic nervous system adjusts the body for any energy use, under stress or in an emergency. In contrast, the parasympathetic one is more active in the resting or normal state. Listening to music can activate the parasympathetic nervous system causing a comfortable and calm effect.<sup>9</sup> Moreover, music is also reported to have the ability to activate the pleasure areas in the limbic system, such as the nucleus accumbency, hippocampus and amygdala, which in turn can produce neuropeptides such as dopamine and endogenous opioids and can lead to reduced corticosterone levels. Dopamine is a neurotransmitter that contributes majorly to pleasure, motivation, learning, mood, and attention. Corticosterone (Glucocorticoids in rats) is an important stress biomarker that regulates the stress response. Glucocorticoids penetrate the brain through the blood-brain barrier, increasing their levels during stressful



**Figure 2.** Histological picture of cerebral cortex thickness (black arrow) of Wistar rat in coronal section, from the outer layer of the cerebral cortex to the outer border of the white matter in coronal section (HE, 40x magnification), in the control group. Mean cerebral cortex thickness of control group is 2,810.6  $\mu\text{m}$  with standard deviation (SD) 15.6  $\mu\text{m}$ .

events. The increased glucocorticoid levels can suppress progenitor cell proliferation in the dentate gyrus.<sup>22-24</sup> The result of the investigation found that music exposure can increase BDNF concentration in the hippocampus in rats. BDNF, known as a molecular mediator of synaptic plasticity, contributes majorly to regulating the structure and function of nerves in the central nervous system. This finding is coherent with some studies that showed music statistically significantly increased levels of BDNF compared to the comparator situations.<sup>7,25,26</sup> However, it differs from a study by Chikahisa et al., which expressed the decreased BDNF concentration in the cortex and no significant change found in mice hippocampus.<sup>16</sup> In the Chikahisa et al. study, receptor tyrosine kinase (TrkB) levels were also investigated. TrkB is the receptor for BDNF. However, BDNF declined significantly, while TrkB inclined in the music treatment group.<sup>16</sup> Thus, this study proposed the possibility of TrkB levels baseline alteration due to the long-term music stimulation, which also may affect BDNF levels in a feedback manner.<sup>16</sup> Insulin-Like Growth Factor 1 (IGF-1) is a member of the family of Insulin-

Like Peptides (ILPs), which is a crucial growth factor in the Central Nervous System (CNS) with pleiotropic action on all major cell types.<sup>27</sup> The insulin peptide superfamily is phylogenetically ancient and fundamental for CNS growth and development. Correspondingly, this study found that the mean of hippocampal IGF-1 levels in the group with music exposure was lesser than in the control group. However, no statistically significant ( $p = 0.14$ ) was displayed when it was statistically analyzed. Perhaps, this result occurred because the music played in the daytime can disrupt the rats' sleep time. Thus, it may also affect the concentration of IGF-1, considering that rats are nocturnal animals. A study by Everson and Crowley is similarly coherent with the possibility that disturbed sleep in rats can lower the production of anabolic hormones such as growth hormone and IGF-1.<sup>28</sup> Cortical thickness is positively associated with general intelligence.<sup>29</sup> In this study, the mean thickness of the cerebral cortex was lower in the group of rats with music exposure compared to the control group. However, when statistically analyzed, the results were not statistically significant ( $p$

$= 0.94$ ). Consistently, a previous study also exhibited that music exposure does not considerably affect the layer thickness of the motor or somatosensory cortex.<sup>14</sup> This result may be related to the lower IGF-1 concentrations of the music-exposed group obtained in this study, causing declines in brain size.<sup>30</sup> This investigation found that Mozart's music can decrease plasma corticosterone and increase hippocampal BDNF concentrations in rats. Stimulation is needed in the process of brain development, and Mozart's music can be an option in providing stimulation for brain development. The limitations of this study are related to the time exposure to Mozart music was not carried out at night, when rats are nocturnal animals, and no tests were carried out to assess the intelligence of the rats. Therefore, it is suggested that exposure to music is played at night and the need for tests to assess the intelligence of rats, for example, the Morris Water Maze test to assess the spatial intelligence of the rats.

## CONCLUSION

Mozart music influences neurogenesis by decreasing plasma corticosterone and increasing hippocampal BDNF concentrations. Mozart music is proven to be an alternative to stimulation provided for brain development.

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## CONFLICT OF INTEREST

All author declares there is no conflict of interest regarding the publication of the current Study

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None.

## AUTHOR CONTRIBUTION

All authors contribute to all phases of this study, from literature searching,

protocol development, research permit, research implementation, data collection and analysis, study result reporting, and journal article writing processes. All authors have approved this article.

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