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By Syania M O, Hermanto T J & Mudjiani B

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Result: There were significant differences of the number of neurons between Mozart (71,96 ± 14,44) with Jazz (41,06 ± 7,65), Blues (40,92 ± 6,36), and Rock group (31,31 ± 8,19) with p=0,000.

Conclusion: The number of neurons in the cerebrum of *Rattus norvegicus* offsprings exposed to Mozart compilation during pregnancy proved to be higher than Jazz, Blues, and Rock compilations.

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GJMR-E Classification: *NLMC Code: WQ 240*



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Syania M O [α], Hermanto T J [σ] & Mudjiani B [ρ]

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I. Introduction

At least there are seven phenomenon/awareness that lead to this effort, i.e. modify the structure and function of the fetal brain to have more neurons, more glia cells and more dendritic density as follow 1. The need for the next better generation, especially with the better brain and its breakthrough[1-14] 2. The findings from Gardner on multiple intelligences[15] 3. The revelation that brain growth and development start in the womb[16-20] 4. The environment enrichment by M. Diamond, including analysis of the Einstein brain[21-23] 5. *The findings of Tomatis:* brain growth and development needs sound and music[24-26] 6. Rauscher and Shaw, findings[27-31] 7. The FOAD and DOHAD hypothesis[32-33]. There were already series of studies in Surabaya that analyzed this effort in what kind of (musical) stimulation, the orders, duration, gestational age, what kind of nutrition – with the dependent variables: BDNF, numbers of neurons, glia cells, dendritic density and neuronal apoptotic index. This team also had analyzed the influence of frequency, color, intensity, beat of various compilations including traditional, jazz, rock, blues and pop music compilations and its influence on some plants [34-48].

In this study, Mozart compilation during pregnancy were compared to Jazz, Blues and Rock compilations to more deeply understand the neurophysiology impact of musical exposure to the structure and the function of the fetal brain.

II. Materials and Methods

This research was an experimental laboratory study with a single-blind randomized post-test only control group design. The sample size was calculated using the Federer formula: for each group was seven. The subjects were pregnant healthy *Rattus norvegicus*, weighing 130-180 grams and never giving birth. The subjects were divided into 4 groups randomly, which are the treatment groups that were exposed Mozart, Jazz, Blues, and Rock compilations. All exposure carried out in day 10 of pregnancy until delivery at day 19, for 1 hour in a dark atmosphere (represent the night atmosphere) with an intensity of 60 dB and at of 25 cm distance from the exposure box. At cesarean delivery, two offsprings with the heaviest weight were taken in each group, sacrificed by chloroform, weighed, and the brain were prepared and then stained by Hematoxylin-Eosin methode. The number of neuron cells calculated by 1000x magnifying microscope: 5 right and 5 left hemispheres. Statistical analysis was chosen accordingly. The study was conducted in the Animal Cages and Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, during March-May 2019 after ethical clearance.

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Fig.1: The Exposure Box

III. Results

a) *Offsprings Birthweight*

Table 1: Rattus norvegicus offsprings birthweight

Groups	N		Mean ± SD
	Mother	Offspring	
X1	5	10	4,42 ± 1,15
X2	7	14	4,78 ± 0,42
X3	6	12	4,11 ± 0,81
X4	7	14	4,62 ± 051

Note: X1 (Mozart) X3 (Blues)
X2 (Jazz) X4 (Rock)

b) *The Number of Neurons in Cerebrum Cortex*

The number of cerebrum neuron cells was known by a dark blue cell nucleus, then the preparation was counted by 1000x magnifying microscope 10 visual fields consisting of 5 right and 5 left hemispheres. There were 6 preparations that could not be counted because 4 cortex of Mozart group had meningitis and 2 cortex of Blues group were not found.

Table 2: The Number of Neurons in Cerebrum Cortex

Groups	N Preparation	Mean ± SD
X1	10	71,96 ± 14,44
X2	14	41,06 ± 7,65
X3	12	40,92 ± 6,36
X4	14	31,31 ± 8,19

Note: X1 (Mozart) X3 (Blues)
X2 (Jazz) X4 (Rock)

This table shows the average number of neurons of the *Rattus norvegicus* offsprings in cerebrum cortex. The highest mean value was in the Mozart group (X1) followed by Jazz, Blues, and Rock (X2, X3, X4) groups.

Visualization of the distribution of the number of neurons will give better impression that shows the highest number of Mozart group.

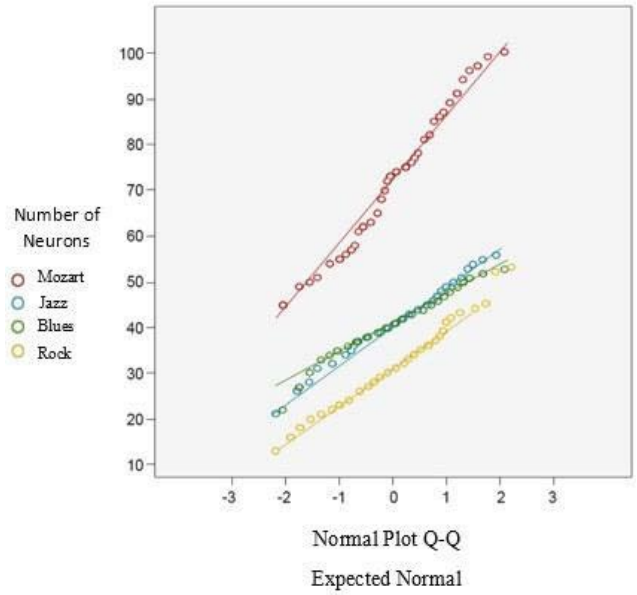


Fig. 2: Scatter Diagram of the Number of Neurons in the Cerebrum Cortex

Table 3: Post-Hoc Test of the Number of Neurons in the Cerebrum Cortex

Groups		P Value	
	X2	X3	X4
X1	0,000*	0,000*	0,000*
X2	-	0,938	0,000*
X3	-	-	0,000*

Note: X1 (Mozart) X3 (Blues)
X2 (Jazz) X4 (Rock)

This table shows the results of the analysis with post-hoc LSD test: Mozart group with Jazz, Blues, and Rock groups shows the value of p = 0,000 (p <0.05), which means that there were significant differences. Analysis of Jazz group with Blues group showed the value of p = 0.938 (p <0.05) which means there was no significant difference. Analysis of Jazz group with Rock group shows the value of p = 0,000 (p <0.05) which means that there was a significant difference. Furthermore, the analysis of the Blues group with the Rock group shows the value of p = 0,000 (p< 0.05) which means that there was also significant difference.

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Table 4: Analysis of the Number of Neurons in the Right and Left Hemispheres

Mean ± SD			P	
Groups	Right	Left	Right	Left
X1	70,80 ± 15,75	73,12 ± 13,46	0,177	0,200
X2	39,34 ± 7,37	42,77 ± 7,64	0,200	0,200
X3	39,92 ± 6,01	41,92 ± 6,66	0,200	0,200
X4	31,46 ± 6,78	31,17 ± 9,49	0,200	0,101

Note: X1 (Mozart) X3 (Blues)
X2 (Jazz) X4 (Rock)

This table shows that the average number of neurons were not much differences between right and left hemispheres even the number a little bit higher in the left one.

IV. Discussion

Developing countries with more than 100 orders in Human Development Index (HDI) should have a program to increase their levels, and education is one of the single solution of choice.[49] Education needs – best education program and brain capacity: multiple biopsychosocial potencies. These countries can only catch up higher HDI rank not by better education program which is very expensive but through a breakthrough program related to the brain structure and function.[50] We believe in "From Neurons to Nations" premise and the idea of environment enrichment which have been mentioned in the introduction.

Environment enrichment by combination of certain musical stimulation and nutrition during pregnancy have been studied here in the last twenty years. This program in line with the Harvard program which address the influence of early sensory exposure to the brain growth and development; but not with Brain Decade premise which address mostly the brain disease.[17] We understand that medicine especially Obstetrics and Maternal Fetal Medicine deal with pregnancy, birth and puerperium period, also their diseases, not with fetal brain growth and development. Only in pediatrics there is subspecialties in Growth and Development.

Our standard operating procedure is default Mozart compilation which is exposed one hour in duration, at night 8-11 PM, start 20 weeks of pregnancy or day 10, 65 dB, 25 cm distance. This procedure has been used for almost twenty years with the same results in variables studied (BDNF, synaps, number of neurons, glia, dendritic density).

There have been many studies regarding the effect of music on brain development. For example, Kuhlmann, Mariana, Tomatis, Campbell, Kendrick and other research groups. They assume that different types and genres of music have different influences. Music with classical genres has a positive effect on listeners, while rock music has a negative effect on listeners.[24,26,51-53] The statement is proven in studies with plant and animal subjects. On the subject of plants, researcher Dorothy Retallack started the determination by using the control variables of light, temperature, and air it turns out some types of plants with rock music exposure for four weeks stopped growing and damaged.[54] While research with animal subjects was conducted by researchers Harvey Bird and Farleigh Dickinson. The two experts conducted their research on mice as experimental animals given exposure to Strauss music from Strauss, voodoo drum rhythms, and then noiselessly measured their ability to complete the game 'maze'. The results showed that the rats given drum exposure had difficulty completing the game. Other evidence was carried out by Hermanto et al., with the title "The Influence of 11 Mozart Compositions during Pregnancy to The Perinatal Outcome and BDNF Umbilical Cord Blood". The purpose of this study was to compare BDNF levels in infants exposed to Mozart music while in the womb and without exposure. It turned out that there were significant differences at the BDNF level in the two groups.[9]

Based on several studies conducted by experts, shows that the pre-natal period is the most amazing period in the fetal development phase. This phase does not only depend on genetics, but environmental factors also play an important role in the development of the functional capacity of the body's organs. So it can be said that during pregnancy is the right time in preparing the potential for fetal intelligence early on. The studies of Brent Logan, Rene van de Carr and Beatriz Manrique show the existence of environmental interference on fetal intelligence. They discovered the premise of "stimulation induced morphological changes" which means that the structure of the brain is formed by external stimuli.[56-58]

Music exposure given during prenatal has an influence on the process of proliferation, migration, differentiation, myelination, synaptogenesis, and apoptosis of brain cells. The human brain is one of the most complex organ systems. Cellularly, the nervous system consists of two types of cells, namely neuron and glia cells. These two cells work in harmony so that the brain's commutation ability goes well. Fetal neuron cells stop proliferating until 32 months of gestation, while glia cells can proliferate until post-natal. The formation of these two cells can be stimulated through exposure to music during pregnancy. The more the number of neuron cells formed, it is hoped that intelligence will increase.[4,12]

This research is a continuation of a series studies with the same aim in preparing a smarter generation from the womb. This research was conducted to find out the differences in the number of neurons in the brain of new born *Rattus norvegicus* in the cerebrum between those who were exposed to Mozart, Jazz, Blues, and Rock compilations during pregnancy.

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In this study we compared musical exposure of 4 kinds of western music compilations, we also compared with traditional Indonesian music and religious music compilations in other studies.

The mechanism/s how the music affect/s the brain are not completely understood. It is correlated with BDNF, different in frequency, color, beat, timing, and duration have detected probably explain in parts the mechanism.

We can find that differences in analyzing the frequency and major minor proportion.

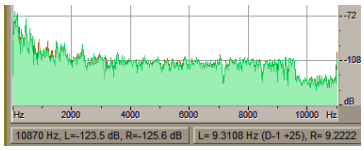
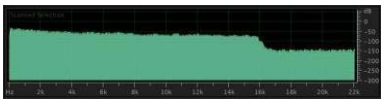
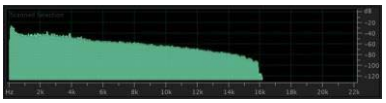
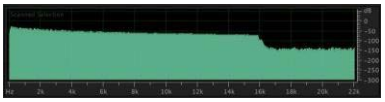
Mozart	
Jazz	
Blues	
Rock	

Fig. 3: The Different Frequency of Mozart, Jazz, Blues and Rock Compiltions. Mozart Shows the Lowest Frequencies

This research was carried out since the 10th day pregnancy of *Rattus norvegicus* due to ear formation in *Rattus norvegicus* was complete at 9-10 days of gestation. Ernawati in her study said that there was no significant difference in the apoptotic index of Mozart's music exposure at the beginning of pregnancy compared to 10 days of gestation. This is possible because the stimulus in the form of sound is received through the ear, then these mechanical waves are converted into electrical pulses and transmitted to the auditory cortex through the auditory nerve when the ear is fully formed. So that the provision of music stimuli will begin to affect after the ear is formed and functioning and begin to form synapses in the 20-24th weeks of pregnancy or equivalent to the 10th day of pregnancy in mice.[59]

Music stimulation during pregnancy is reported to improve fetal brain development, increase spatial-temporal abilities in newborn mice, and trigger rapid progress in motor abilities such as sitting and walking in infants. Research conducted by Kim et al., found that prenatal noise exposure resulted in stunted growth, decreased neurogenesis in the hippocampus, and disruption of spatial abilities in newborn rats. In contrast, prenatal music exposure can improve neurogenesis in the hippocampus and spatial ability in newborn mice. [60-61]

Brains that grow in a stimulus-rich environment have thicker cortex, larger nucleus neuron cells and more glia cells. Brain neurons that grow in there have more dendritic sites, allowing more synapses to form. Rees also stated that the number of cells undergoing apoptosis depends on synapses, the more synapses the less apoptosis occurs. The richer the neuron cells with more dendritic sites, the more synapses are formed so that the number of cells undergoing apoptosis will also be reduced. Brains that grow in a stimulus-rich environment will experience less apoptosis, thereby increasing brain capacity.[20] Ismudi in his study compared 3 Mozart music compilations consisting of several random song sequences and analyzed using computer software "Cool Edit Pro 2.0". As a result of the three compilations, compilation 1 has a high frequency tone, a wider amount of energy area, and regular intensity. Ismudi believes that these characteristics are an effective composition for stimulating neurons. While Mozart 2 and 3 compilation tends to have irregular fluctuations in intensity, so that it will produce an amount of energy that changes in every seconds. In that study showed that exposure to Mozart 1 music compilation during rat pregnancy produced the lowest apoptotic index in neuronal cells of newborn mice compared to Mozart 2 compilation, compilation 3, and control. For this reason, compilation of Mozart 1 according to Ismudi is a standard Mozart compilation which forms the basis of research and application of Mozart's music exposure during pregnancy as a prenatal stimulation.[37]

The results of our study in the four groups showed the average number of neurons in the cerebrum in the Mozart group compared to the Jazz, Blues, and Rock groups. The average number of neurons of Mozart group is higher among others. Meanwhile, the average number of neurons in the Jazz group is higher than Blues, and Rock. And the average number of neurons in the Blues group is higher than Rock. In the cerebrum, the mean number of neurons in Mozart was 71.96 ± 14.44, in Jazz 41.06 ± 7.65, in Blues 40.92 ± 6.36, and in Rock 31.31 ± 8.19.

We also counted the average number of neurons in right and left hemisphere. It was done by looking at 10 fields of view (consisting 5 fields each hemisphere). The highest average number of neurons was found in the left hemisphere compared to the right hemisphere in the Mozart, Jazz, and Blues Group. Otherwise, in the Rock group the average number of neurons in the right hemisphere was higher compared to the left hemisphere. Based on the theory, right and left brain have different functions and communicate each other through a band of nerves. Left-brain has specialization in language and logic, meanwhile right-

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brain has specialization in creativity and intuition. Commonly in adults, we believed that music is processed in the right-brain. One of the right-brain function is for controlling the ability to play instrument with ease, recognize a song from melody, and play it back upon "hearing it". In this study, we know that it is different if we look from the number of neurons in each hemisphere of *Rattus norvegicus* offsprings.[62,63] The probable cause is still unknown.

In the analysis of variance using the ANOVA test, there was a significant difference in the data of the number of neurons in the cerebrum between all groups ($p < 0.05$). That means there are significant differences in the number of neuron cells in the cerebrum of *Rattus norvegicus* offsprings.

Furthermore, Post-Hoc LSD (Least Significant Difference) analysis was performed to determine all possible differences in the comparison of each group. If $p < 0.05$, there was a significant difference. Table 3 shows the results of the Post-Hoc LSD test. The comparison of each group shows that there were significant differences with the p value <0.05, between the Mozart group with the Jazz, Blues, and Rock groups with a value of $p = 0.000$, $p = 0.000$, and $p = 0.000$ ($p<0.05$).Analysis was also carried out between groups, the results obtained from the analysis of the Jazz with the Blues groups showed a value of $p = 0.938$ ($p < 0.05$) which means no significant difference between the number of neurons in the Jazz and Blues groups. Analysis of the Jazz with the Rock groups showed the value of p = 0,000 which means that there was a significant difference between the number of neurons in the Jazz and Rock groups. Furthermore, the analysis of the Blues group with the Rock groups showed the value of p = 0,000 ($p<0.05$) which means that there was a significant difference between the number of neurons in the Blues and Rock groups.

From this study it can be concluded that Mozart exposure gives the highest number of neurons compared to other western music exposure which are Jazz, Blues, and Rock with significant differences. In addition, Jazz exposure gave a higher number of neurons compared to Blues and Rock with a significant difference from Rock, but didn't make a significant difference from Blues. Exposure to Blues compilation gave a higher number of neurons than Rock with significant differences. This might be caused by every compilation (music) has multidimensional properties and attributes of perception that affect the apoptosis index of brain cells. Other studies conducted by Kauser et al., and Sanyal found that giving a prenatal stimulus with music that has regular rhythm can improve postnatal spatial ability and also memory function, but giving music with complex rhythms and arithmetic noises has the opposite effect. [64-67]

Study conducted by Sanyal et al., about the effect of music and noise on changes in the number of neuron and glia cells in several brain areas of newborn chicks. The results showed an increase in the number of neuron cells in the brain area studied due to music stimulation. Music stimulation will have an effect on increasing neurogenesis or decreasing brain cell death. [68]

If traced from several literature reviews, Mozart has a frequency of around 8000 Hz, different from Jazz, Blues, and Rock which has a frequency of 15 000Hz. Campbell suspected that Mozart's stimulation in general can affect neurochemical changes in which the clarity, majesty, rhythm, melody and high frequency which are able to stimulate the creative and motivational areas of the brain so as to calm its listeners, improve concentration, memory and spatial perception. The results of the Borner et al., 2000 study revealed that Mozart K488 increased the activity of the dorsolateral prefrontal cortex, occipital cortex and cerebellum compared to piano music of the 1990s and Beethoven. The same thing is supported by Ningsih in her research which shows Mozart is better than the music of Beethoven and Chopin. This opinion was proven from the calculation of the number of neurons in the cerebrum and cerebellum of *Rattus norvegicus* offsprings exposed by Mozart's music during pregnancy proved to be higher than those exposed to the music of Beethoven, Chopin, and not exposed to music and there were significant differences. [26,46]

Research conducted by Kirchberger and Russo comparing the dynamic range of various music stated that Classical and Jazz music has a wider dynamic range than Rock, Rap, Schlager, and Pop music. Dynamic range is the ratio between the lowest and highest volume of an instrument. Narrow dynamic range can make hearing fatigue for the listener.[69]

We recognize that the slow tempo can make an individual feel calmer and lighter. Classical music with a tempo of 60x / minute has been proven to reduce anxiety levels and also improve memory. While Jazz music is estimated to have a tempo of about 240x / minute.[65] Based on How Music Affects Us and Promotes Health, Mozart's Classical Music has the ability to activate brain areas in processing information.[70]

Research conducted by Poikonen et al., comparing Event Related Brain Potential measured through EEG produced by stimulation of Jazz and Rock music, it was found that Jazz music activates the brain more strongly than Rock music. Whereas Jazz music with Blues music has almost the same characteristics in terms of frequency. The differences also come from the characteristic of music, Jazz music has a distinctive character that is blue notes, improvisation, polyrhythms, syncopation, and shuffle notes, while Blues music tends to represent feelings of sadness, somber, and depressed.[71]

This shows that Mozart proved to be better than western music such as Jazz, Blues, and Rock music

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according to the results of our research supported by the theory of Hermanto that the brain needs energy and the best energy is sound. The type of season that can help increase the number of brain cells is classical music from Mozart with a frequency of 5000-8000 Hz where the frequency is not too high so it is considered suitable for the fetal environment during pregnancy.[8-9]

V. Conclusion

The number of neurons in the cerebrum of *Rattus norvegicus* offsprings in the Mozart exposure was higher than the Jazz, Blues, and Rock with significant differences.

VI. Research Excellence

This research is the first study conducted by comparing the differences in the number of neurons to four types of Western music, namely Mozart, Jazz, Blues, and Rock compilation.

VII. Research Novelty

This research was conducted by looking at 10 fields of view of *Rattus norvegicus* offsprings neuron cell preparations, including in 5 fields of the right and 5 fields of the left hemispheres of the *Rattus norvegicus* cerebrum. So it can be known which part of the brain is thought to have a higher influence in thought processes and intelligence.

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