ORIGINAL ARTICLE :

Default sequence** of Mozart's compositions during pregnancy gave higher dendritic density in the cerebrum and cerebellum of *Rattus norvegicus* offsprings compared with reversed sequence and control

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**Arranged and patented by Hermanto TJ

ABSTRACT

Objectives: This study aimed to analyze the differences in dendritic density of cerebellum and cerebellum of offsprings *Rattus norvegicus* between default and reversed sequence Mozart compositions during pregnancy
Materials and Methods: Experimental study randomized post test only control group design using pregnant *Rattus norvegicus*. After ethical clearence, animal subjects were divided into three groups: control group, and the treatment group that were given exposure to default and reversed sequence of Mozart's compositions from gestation day 10. On day 19-21 we sacrificed the rat, prepared offsprings' brain by silver impregnated staining, counted by 400x magnifying microscope and used statistical analysis accordingly
Results: In the cerebrum there was a significant difference in dendritic density between default group, reversed group and no exposure with p=0.003 and p=0.000. In the cerebellum there was also significant difference in dendritic density in the reversed group compared with default sequence and in the default Mozart group with the control group, with p=0.000 and p=0.000. However, there was no significant difference between control group and reversed group with p=0.109 and p=0.077
Conclusion: The density of dendrites in the cerebrum and cerebellum of *Rattus norvegicus* offsprings exposed to Mozart's compositions during pregnancy with default sequence was higher than that in those receiving reversed order and without exposure. There were no significant differences between the density of the cerebrum and cerebellum dendrites between groups exposed to reversed sequence of Mozart's composition and those without exposure.

Keywords: Mozart; pregnant, *Rattus norvegicus*; dendritic density

ABSTRAK

Tujuan: Menganalisis perbedaan kepadatan dendrit cerebrum dan cerebellum *Rattus norvegicus* baru lahir yang mendapat paparan komposisi musik Mozart urutan baku, urutan terbalik dan tanpa paparan dalam rahim.
Bahan dan Metode: Penelitian analitik eksperimental laboratoris dengan *randomized post test only control group design* menggunakan hewan coba *Rattus norvegicus* sebagai model perlakuan. Sesudah lolos kelaikan etik, Kelompok hewan coba dibagi tiga kelompok yaitu kelompok kontrol, kelompok paparan Mozart Urutan baku, kelompok urutan terbalik. Setelah lahir, dilakukan preparasi termasuk pengecatan dengan impregnansi perak dan analisis kepadatan dendrit serebrum dan serebelum tikus baru lahir dengan pembesaran 400 kali. Kemudian dilakukan uji komparasi kepadatan dendrit
Hasil: Pada cerebrum didapatkan perbedaan bermakna pada kelompok Mozart urutan terbalik dengan Mozart urutan baku dan pada kelompok Mozart baku dengan kelompok tanpa paparan, dengan nilai p=0,003 dan p=0,000. Pada cerebellum didapatkan perbedaan bermakna antara kelompok urutan baku dengan urutan terbalik dan kelompok kontrol, dengan nilai p=0,000 dan p=0,000. Namun tidak didapatkan perbedaan bermakna antara kelompok kontrol dengan kelompok urutan terbalik dengan nilai p=0,109 dan p=0,077.
Simpulan: Kepadatan dendrit di cerebrum dan cerebellum *Rattus norvegicus* baru lahir yang mendapatkan paparan musik Mozart selama kebuntingan dengan urutan Mozart baku lebih tinggi dibanding dengan urutan terbalik dan tanpa paparan. Tidak didapatkan perbedaan bermakna kepadatan dendrit cerebrum dan cerebellum antara kelompok urutan terbalik dan tanpa paparan.

Kata kunci: Mozart, bunting; *Rattus norvegicus*; dendritic density

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INTRODUCTION

Various types of efforts have been made to obtain optimal intelligence that is seen from the ability of an individual to solve problems or create something new. One effort is to provide education as early as possible, even in utero. In the uterus, brain cells of the fetus undergo proliferation, migration, synaptogenesis and apoptosis. Efforts that can be done during pregnancy is to provide adequate nutrition and stimulation, in addition to the characteristics of the parents who play a role in the potential intelligence of the fetus. Sound is one of the fetal growth factors on the grounds that there are always sounds in the uterus, including heart rate, intestinal peristalsis and mother's voice. Because the ears of the fetus can hear perfectly since the age of 18 weeks, the communication pathway between the fetus, mother and the outside world has been obtained through sound, even compositions.[1]

Many studies of Mozart's compositions have found that the compositions has a significant influence on improving memory and learning ability in normal children, even children with epilepsy. Cellularly, it has been proven that Mozart's exposure affects more cell numbers than exposure to pop and gamelan songs. Research on the intelligence of the fetus in the uterus in rats showed that more brain cell proliferation occurred in rat fetuses exposed to compositions than controls. Compositions stimulation is a cheap, effective and rational means. Therefore, further research needs to be developed to find the right formula to provide compositionsal stimulation to the fetus and the effect of nutrition on dendrite density.[2]

The brain component, the cerebrum, which is the largest part of the brain, has a major role in motor, sensory, intellectual processes, memory, and information processing. The other component, the cerebellum, was previously thought to have only cognitive functions. As time goes by, several discoveries show that cerebellum has more complex functions, such as attentional control, emotional control, and social skills. In addition to the cerebrum and cerebellum, another role is needed to connect neurons in the brain. The role of dendrite with its complexity illustrates the number of connections that a neuron can receive in the brain. Neurons that do not have dendrites will not be able to receive impulses because the area to receive impulses is limited and dendrites function as cell expansion of neurons results in increased surface area without increasing brain volume.[3] A previous study has shown that the more dendritic sites in neuron cells, the more synapses can be formed, so that the number of cells undergoing apoptosis will also decrease. Therefore, the brain that grows in a more stimulus-rich environment will have a higher density of dendrites and better synapse density, thus increasing brain capacity.[4]

This study was a continuation of a series of studies to educate in utero fetuses with the aim of identifying the influence of Mozart's composition in reversed order on the expression of BDNF of the *Rattus norvegicus*. In 2002, Hermanto made a formula for providing in utero stimulation using a patented default sequence of Mozart compositions. This combination of compositions stimulation has been included in the Republic of Indonesia Minister of Health Regulation no. 97 of 2014 known as brain booster. Then Xing et al. (2015) stated that reversed Mozart composition had a negative effect on rat performance. Elements in compositions are very instrumental in determining Mozart's combination choices, be they frequencies, intensities, beats, melodies, and energy areas. Our analysis with the Cool Edit Pro 2.0 software on default Mozart composition had a frequency range under 10000 Hz with an intensity of 35-130 db, while the reverse sequence Mozart had a frequency range of 75-15,000 Hz with an intensity of 35-130 db. With more or less the same frequency and intensity, we intended to identify whether reversed Mozart composition sequence gave a different effect on the level of dendrite density of *Rattus norvegicus* compared to that receiving default sequence and receiving no exposure, or there possibly there were other factors involved.[4,5]

MATERIALS AND METHODS

This study was an experimental analytic study with a single blind design randomized post test only control group design, involving *Rattus norvegicus* as experimental animals. After ethical clearence, the study sample was divided into three groups. The treatment group was *Rattus norvegicus* receiving default and reversed Mozart compositions composition for 1 hour at night. The control group was *Rattus norvegicus* who were not exposed to Mozart's composition. The study was conducted at the Experimental Animal Cage and Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, during October-December 2016.

The inclusion criteria were healthy pregnant *Rattus norvegicus*, 2 months old, weight 120-150 grams, and and *Rattus norvegicus'* offsprings. The dropout criteria were *Rattus norvegicus* that was ill at the time of treatment, intrauterine fetal death, or was born before the 19-day pregnancy.

The sample size was calculated using Federer formula, and the number of samples per group was obtained as

many as 9. Of each pregnant *Rattus norvegicus* mother, 2 offspringss with the heaviest weight were taken. Once born, the *Rattus norvegicus* offsprings was sacrificed using chloroform, weighed, the brain was taken, prepared and stained with silver impregnation. Dendrite density of each sample was calculated. The groups were compared using statistical tests accordingly. Ethical eligibility was obtained from the Research Ethics Commission, Faculty of Veterinary Medicine, Universitas Airlangga.

RESULTS AND DISCUSSION

Subject characteristics

Table 1. Characteristics of *Rattus norvegicus* mothers based on gestational age

Gestational age	Control		Default Mozart		Reversed Mozart	
	∑	%	∑	%	∑	%
19 days	2	22.2%	1	11.1%	3	33.3%
20 days	5	55.6%	6	66.7%	5	55.6%
21 days	2	22.2%	2	22.2%	1	11.1%
Total	9	100%	9	100%	9	100%

Table 1 shows that the highest percentage of *Rattus norvegicus*' gestational age was 20 days; 55.6%, control group and 66.7%, in group receiving default Mozart composition and group receiving reversed Mozart composition 55.6%. The *Rattus norvegicus* mothers used in this study were chosen based on body weight ranging from 120-150 grams, then we randomized 9 samples to each group.

Table 2. Characteristics of *Rattus norvegicus* mothers based on body weight before pregnancy

Body weight (gram)	Control		Default Mozart		Reversed Mozart	
	∑	%	∑	%	∑	%
120-130	-	-	-	-	-	-
131-140	2	22.2%	3	33.3%	4	44.4%
141-150	7	77.8%	6	66.7%	5	55.6%
Total	9	100%	9	100%	9	100%

Table 3. Mean dendritic density/10 µm in *Rattus norvegicus* offspringss' brain

Groups	Cerebrum	Cerebellum
Default Mozart	7.21	8.63
Reversed Mozart	5.65	5.51
Control	3.62	4.03

Table 3 shows higher dendritic density in the the cerebrum and cerebellum of group receiving default Mozart composition of 7.21 and 8.63, compared with group receiving reversed Mozart composition and control group. Table 4 shows mean *Rattus norvegicus* mothers' and offspringss' body weights in each group.

Table 4. Mean and default deviation of *Rattus norvegicus* mothers' and offspringss' body weight control and treatment groups

Groups	Mothers' bodyweight (gram)		Offsprings' bodyweight (gram)	
	Mean	S/D	Mean	S/D
Control	144.89	± 5.84	5.46	± 0.43
Default Mozart	141.89	± 4.62	5.54	± 0.52
Reversed Mozart	142.11	± 6.56	5.53	± 0.38

We performed homogeneity tests and obtained mothers' body weight of 0.149 and offspringss' bodyweights of 0.77 and 0.93. The p value was > 0.05, indicating that the bodyweight of *Rattus norvegicus*' mothers and offspringss had the same variant.

Analysis of the results on cerebrum

The Shapiro Wilk test was carried out first as a normality test for dendritic density in each group. If the results had p>0.05 in all three groups, the distribution of data was regarded as normal. The results of normality test of dendritic density in the cerebrum showed normal data distribution (p>0.05) and statistical analysis was continued with ANOVA test.

Table 5. Mean and default deviation of dendritic density and normality in cerebrum

	Mean and SD		Shapiro wilk
	Mean	S/D	P
Control	3.62	± 1.60	0.712
Default M.	6.78	± 0.94	0.690
Reversed M.	5.02	± 1.29	0.093

Table 5 shows mean density of dendrites in the cerebrum. Data with normal distribution with p> 0.05 in dendrites showed higher mean in group with default sequence Mozart composition (6.78 + 0.94) compared to the the group with reversed Mozart composition (5.02 + 1.29) and control group (3.62 + 1.60).

Table 6. Anova test on dendrite densities

Groups	Dendrit Density (Mean)	P value
Between Groups	23.139	0.000
Within Groups	1.826	

Table 6 shows the results of Anova test on the cerebrum dendritic density of with p=0.00 (<0.05) indicating significant difference in dendritic density between

groups with default Mozart composition, reversed Mozart composition, and without exposure.

Table 7. Post-hoc LSD test on cerebral dendritic density of groups receiving default, reversed, and no Mozart composition

Groups	Groups	P value
Control	Default M.	0.000
	Reversed M.	0.109
Default M.	Reversed M.	0.003

Table 7 shows the results of the analysis with post-hoc LSD test on cerebral dendrite in groups receiving default, reversed and no Mozart composition with normal data distribution. The default Mozart group was significantly higher than the group without exposure with p=0.000 (p <0.05) and also from the group with reversed sequence, with p=0.03 (p<0.05). In contrast, the group with reversed Mozart composition was not significantly different from the group without exposure with p=0.109 (p>0.05).

Analysis of results in cerebellum

As with the cerebrum, data on the cerebellum were first tested with Shapiro Wilk as normality test for dendrite density in each group. The results with p>0.05 in three groups showed normal data distribution. Normality test result of dendrite density in the cerebellum showed normal data distribution (p> 0.05) and we analyzed further using ANOVA test.

Table 8. Mean and default deviation of dendrite density and normality in the cerebellum

	Mean and SD		Shapiro wilk
	Mean	S/D	P
Control	4.42	± 1.22	0.923
Default	8.96	± 1.98	0.156
Reversed	5.77	± 1.33	0.055

Table 8 shows mean density of dendrites in the cerebellum. Data with normal distribution in dendrites shows higher mean group receiving default Mozart composi-tion (8.96 $\pm$ 1.98) than the group with reversed composition (5.77 $\pm$ 1.33) and the control group (4.42 $\pm$ 1.22).

Table 9. Anova test for dendrites density in the cerebellum

Groups	Dendrite Density (Mean)	P value
Between Groups	48.885	0.000
Within Groups	2.413	0.000

Table 9 shows the results of ANOVA test on cerebral dendritic density with a p value of 0.00 (<0.05) which shows a significant difference in dendrite density between groups receiving default, reversed, and no Mozart composition.

Table 10. Results of post-hoc LSD of cerebellum dendritic density in groups receiving default, reversed, and no Mozart composition.

Groups	Groups	P value
Control	Default M.	0.000
	Reversed M.	0.077
Default M.	Reversed M.	0.000

Table 10 shows the results of analysis using post-hoc LSD test on cerebellar dendrite density in groups receiving default, reversed, and no Mozart composition with normal data distribution. The group with default Mozart composition was significantly higher than the group without exposure, with p=0.000 (p <0.05) and also with reversed sequence with p=0.000 (p<0.05). Again, reversed sequence group was not significantly different from the group without exposure, p=0.077 (p> 0.05).

This study was conducted to compare the dendritic density in the cerebrum and cerebellum against exposure to default sequence, reversed sequence and no exposure for one-hour at night after the 10th day pregnancy. This was to prove that the sequence of the composition given also has an important role in the exposure of Mozart's composition. This premise: the sequence of musical compositions influence the function and structure of fetal brain, almost never discussed in western literature.

Other variables had been studied by Hermanto TJ *et al* since 2002 found that combination of certain nutrition including DHA supplementation and Mozart's composition during pregnancy increased 1. the number of brain neurons and glia in offsprings rats higher than gamelan, dangdut compositions, and no exposure. 2. serum BDNF levels in offsprings rats/ mice. 3. BDNF level in umbilical cord blood serum 3. Synapsin, TrKB, MTORC1. And also decreased neuronal apoptotic index.

Compared with so called “Mozart effect”, our approach has several differences ie 1. Just for fetus in the womb not for adults 2. Always in combination with nutrition 3. Exposed in the night only, for one hour, since 20 weeks of pregnancy until delivery of the baby 4. with certain sequence of compositions. We do believe that fetal environment enrichment during pregnancy increase the psychosociobiology potency (intelligences) that representated by number of neuron and glia and also the quality of synaps. The fetal environment enrichment during pregnancy through combination of certain auditory stimulation(Mozart compositions in ordered sequence) and enriched nutrition give higher neurotropic factors(BDNF, Synapsin) that influence the neuronal – glia – synaps proliferation and apoptosis. This fetal environment enrichment replaces the insults in fetal programming stated in FOAD(Fetal Origins of Adult Disease) by David Barker and DOHaD (Developmental Origins of Health and Disease) hypothesis by Peter Gluckman – Mark Hanson. The usage of Mozart compositions is based on Tomatis – Campbell premise that Mozart compositions are rich in frequency 5000 – 8000 Herzt which is highly energize the brain, major dominance and sinusoidal pattern in vizualizations of frequency analysis.Our previous study, with two other different Mozart compositions(by Thomas Verny and Don Campbell) during pregnancy showed lowest neuronal apoptotic index in default sequence group[1,2,4,5,6,17,18]

The spine dendrite is an important structure for synapse transmission, where the exchange of neurotransmitters between presynaptic and postsynaptic elements occurs in an area called post-synaptic density (PSD). PSD consists of receptors and signaling systems associated with synapse transmission. Increased dendrite density increases the area of synapse transmission, thereby increasing the effectiveness of transmission and synapse formation. In vitro research with the administration of exogenous BDNF in rat hippocampus culture showed an increase in the number and complexity of neurons and increased dendrite density.

In his research, Chaudhury (2009) showed that the density of dendrites in chicken hippocampus that received compositions stimulation during concoction was higher than that of chicken receiving sound stimulation similar to their parents and that of the control. Another study by Bose et al. in 2010 showed that mice that received sound stimulation had a higher density of dendrites than the group of deaf and control mice.[13]

A study conducted by Xing et al. (2015) showed that, while Mozart K.448 had a positive effect on cognitive, Mozart's reversed version had negative effect on rat's performance. Until now we have not found studies on Mozart composition that are given in reverse order. This study was conducted using randomized post-test only control group design to obtain optimal results by sealing the code on the object glasses during the counting of each brain cell and dendrite spine density. The code was unsealed only after the examination was completed so the examiners and researchers did not know the results of the previous examination.

From several studies on Mozart composition, a study conducted by Marzban et al. (2011) showed that BDNF levels in the hippocampus of offsprings rats that received Mozart's composition exposure increased significantly when compared to rats without exposure.[9] This is also supported by the results of research conducted by Fran Rauscher and Hong et al. on mice stimulated with Mozart's composition compared to mice receiving noise stimulation. They found that mice receiving Mozart's composition stimulus were smarter than mice receiving noise stimulation. Examination of the rats' brain showed increased expression of BDNF, CREB, and synapsin I in mice given with Mozart's composition stimulation compared to control. BDNF is a nuerotropin that affects the growth of dendrite through the mechanism of BDNF mediated signaling via the TrkB receptor, thereby giving rise to dendritic spine growth. Research in mice showed that exposure to Mozart's composition during pregnancy can increase the density of dendrites in the cerebrum and cerebellum in offsprings *Rattus norvegicus*.[10,11,12]

The research on the characteristics of the cerebrum and cerebellum that we performed obtained higher dendritic density in group with default sequence of Mozart composition when compared with group receiving reversed Mozart composition and without exposure. Dendrite density in the reversed sequence Mozart group had a higher value than in group without exposure. In the cerebrum mean dendrite density in group with default Mozart sequence was 6.78, in reversed Mozart composition group it was 4.68 and in control group 3.62, whereas in the cerebellum of the group with Mozart default the dendrite density was 8.98, in group with reversed order it was 5.77 and in control group 4.42. Anova test showed a significant difference in dendrites density in three groups, both in the cerebrum and cerebellum with p=0.000.

Comparison of groups with default Mozart composition and non-exposed groups in cerebrum (p=0.000) and cerebellum (p=0.000) showed significant dendritic density. On the contrary, there was no significant difference between groups with Mozart's reversed sequence and without exposure in cerebrum (p=0.109) and cerebellum (p=0.077). This study showed that

exposure to default sequence of Mozart composition during pregnancy gave the highest results of dendrite density compared to no exposure and reversed Mozart composition with significant differences. However, comparison between no exposure and reversed Mozart composition groups did not show significant difference. The results of the statistical analysis above are in accordance with the initial hypothesis that dendritic density in the cerebrum and cerebellum of newly born *Rattus norvegicus* receiving exposure to Mozart's composition during pregnancy with default sequence is higher than that in reversed order and without exposure.

Compositions has a multidimensional nature that consists of eight attributes with various levels of abstraction, tone level, rhythm (organizational perception of sound events in time), tone/timbre, tempo, meter/key, level of loudness, melody, energy area and spatial location. Until now the exact mechanism of musical exposure to the brain especially to the fetal developing braing still unknown. The evidence showed maybe through the BDNF pathway.

Analysis using Cool edit pro 2.0 software showed that Mozart's composition in default sequence and reversed sequence has almost the same frequency and intensity. However, changing the order will change the placement of the melody, where the intro became the cauda and vice versa, so the brain accepts it differently. The resonant energy area is also found to be smaller in reversed sequence of Mozart composition. This shows that the possibility of energy produced is also smaller than in default Mozart composition.[5]

Comparison of dendritic density in the cerebrum and cerebellum showed no difference between reversed and no exposure groups. It is rather weird that the reversed sequence gave no differences and also different comPared with Ismudi findings

CONCLUSION

Dendritic density in the cerebrum and cerebellum of *Rattus norvegicus* offsprings that exposed to Mozart's composition during gestation period with default sequence was higher compared with reversed order and those without exposure. Dendrite density of the cerebrum and cerebellum in *Rattus norvegicus* offsprings did not differ significantly between those with reversed sequence and those without exposure. This study confirmes the influence of environment enrichment through combination of musical stimulation and enriched nutrition to the number of neuron, glia, dendritic density and BDNF and highlights the importance of sequence of Mozart compositions. In the future, hopefully the curative highlights in nowadays medical service enriched by preventative medicine which we do believe use music as one of the prescription. And also the fetal brain growth and development and its intelligences become one the main topic on the first thousand days and maternal fetal medicine/ obstetrics domain

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