ORIGINAL ARTICLE:

Effect of prenatal Mozart composition on Brain Derived Neurotrophic Factor expression in cerebrum and cerebellum of *Rattus norvegicus* offspring from Food Restriction 50 model

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ABSTRACT

Objectives: To analyze the difference of BDNF expression on *Rattus norvegicus* offspring FR50 model in cerebrum and cerebellum between Mozart composition stimulation group and control.
Materials and Methods: An analytical experimental study with single blind randomized post test only control group using animal subjects *Rattus norvegicus* FR50 model. This study was conducted at animal laboratory, Faculty of Veterinary Medicine, Airlangga University. Animal subjects were divided into Mozart music stimulation group and control. The BDNF expression was analyzed using comparison test, with significancy p<0,05.
Results: There was no difference of BDNF expression on *Rattus norvegicus* offspring FR50 model between Mozart music stimulation group and control in cerebrum with p=0.495 (mean Mozart group 6.89+2.52 vs control 6.22+1.99), and cerebellum with p=0.146 (mean Mozart group 7.41+2.67 vs control 5.73+2.45).
Conclusions: There was no difference of BDNF expression between Mozart composition stimulation group and control in the cerebrum and cerebellum of *Rattus norvegicus* offspring FR50 model.

Keywords: Mozart music Stimulation; nutrition; food restriction 50%; Brain Derived Neurotrophic Factor (BDNF); *Rattus norvegicus*.

ABSTRAK

Tujuan: Menganalisis perbedaan ekspresi BDNF di cerebrum dan cerebellum *Rattus norvegicus* baru lahir dari induk model FR50 dengan dan tanpa paparan musik Mozart.
Bahan dan Metode: Penelitian analitik eksperimental dengan desain single blind randomized post test only control group menggunakan hewan coba *Rattus norvegicus* model FR50 di kandang hewan coba Fakultas Kedokteran Hewan Universitas Airlangga. Kelompok hewan coba dibagi menjadi kelompok perlakuan dengan paparan musik Mozart saat kebuntingan hari ke-10 dan kontrol tanpa paparan. Ekspresi BDNF dianalisis menggunakan uji komparasi dengan signifikansi p<0,05.
Hasil: Tidak terdapat perbedaan ekspresi BDNF yang bermakna pada *Rattus norvegicus* baru lahir dari induk model FR50 di cerebrum dengan nilai p=0,495 (mean 6,89+2,52 pada kelompok perlakuan dan 6,22+1,99 pada kontrol), begitu pula di cerebellum dengan nilai p=0,146 (mean 7,41+2,67 pada kelompok perlakuan dan 5,73+2,45 pada kontrol).
Simpulan: Paparan musik Mozart selama kebuntingan tidak berpengaruh terhadap perubahan ekspresi BDNF di cerebrum dan cerebellum *Rattus norvegicus* baru lahir dari induk model FR50.

Kata kunci: Mozart music Stimulation; nutrition; food restriction 50%; Brain Derived Neurotrophic Factor (BDNF); *Rattus norvegicus*.

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INTRODUCTION

Until now the functional development of human brain is still being studied, ranging from the influence of the development of brain structure (neuroanatomy) on motor function, perception and cognitive in the childhood to the growth and development of fetal brain in the womb and the factors that influence it. Such study is related to intelligence, which in the medical world is associated with developmental neuroscience and developmental psychology. In this field it is still debatable to date which factor is the most influential, whether nature or nurture.[1-3]

Based on neuroscience, it has been recognized that infant behavior change (neuro-behavior) is the result of infant's intrauterine learning. The development of the infant's brain will be better if there is adequate stimulation and nutrition. In the womb, brain cells undergo the process of proliferation/differentiation, mi-gration, organization and myelinization. The process of migration to different areas of the brain, such as cerebrum, cerebellum, hippocampus and other structures, is almost entirely completed at the time of term pregnancy, although neurogenesis can still continue after the baby is born up to adulthood. On the other hand, subcortical structures at birth are relatively similar to that of adulthood. Therefore, adequate stimulation and nutrition given during pregnancy play an important role in the function of the fetal brain. Disruption of cellular and molecular formation during this critical period can cause permanent damage and long-term disturbance to adulthood. The pathophysiological mechanism of such occurrence is known as "perinatal programming of adult diseases," or "developmental origin of health and adult diseases (DOHAD)".[1,3,4,13-15]

Nutritional disorders in pregnant women are associated with developmental obstacles and fetal brain maturation that is influenced by various growth factors, one of which is neurotrophin. Research in rats found that neurotrophin that has the highest concentration in the brain is the brain derived neurotrophic factor (BDNF), which is present especially in the hippocampus and hypothalamus. In early life, BDNF affects almost all aspects of brain development. Research in rats with 50% food restriction (FR 50) to describe nutritional deficiencies during pregnancy shows a decrease in BDNF expression and impairment of Tyrosine Kinase B (TrkB) receptor that inhibits brain development.[12,16] In addition to the influence of nutrients, fetal brain development is also influenced by the presence of stimulation from outside environment since intrauterine life. One of such influence is the provision of classical music as a stimulation. Based on research on rats, exposure to Mozart composition during pregnancy can actually increase BDNF expression in *Rattus norvegicus* offsprings' cerebrum.[5-11] This study examined the influence of Mozart composition exposure on BDNF expression in cerebellum and cerebellum of *Rattus norvegicus* from FR50 model.

MATERIALS AND METHODS

This study was an experimental analytic study with single blind randomized post test only control group design. This study used experimental animal of *Rattus norvegicus* FR50 model that received stimulus of Mozart composition. Subjects were divided into two groups at random. The treatment group received exposure to Mozart composition approximately 1 hour during the night since pregnancy day 10, while the control group did not get exposure to Mozart's composition. The study was conducted at Experimental Animal Cage, Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya, from May to July 2016. The inclusion criteria were pregnant *Rattus norvegicus* who were malnourished with food restriction 50% on day 1 pregnancy, has never given birth, and the newly born *Rattus norvegicus* offsprings. The exclusion criteria were *Rattus norvegicus* mothers with anatomic abnormalities and illness before treatment.

Sample size estimation resulted in sample size of 11 in each group. From each *Rattus norvegicus* mothers, two offsprings with the highest weight were taken. To achieve the sample size of 11 *Rattus norvegicus* offsprings in each group, 22 pregnant *Rattus norvegicus* were involved, randomly divided into 2 groups, and marked. Once born, the *Rattus norvegicus*' offsprings were sacrificed by using chloroform, weighed, the brains were taken and immunohistochemical preparations were made. BDNF expression in each sample was calculated. The groups were compared using statistical tests. Ethical eligibility was obtained from Research Ethics Committee, Faculty of Veterinary Medicine, Airlangga University.

RESULTS AND DISCUSSION

Subjects characteristics

Pregnant *Rattus norvegicus* in this study had a weight range of 137 to 152 grams, which we randomized in each group of 11 samples. No deaths were found in those pregnant *Rattus norvegicus*. All of the *Rattus norvegicus*' offsprings were weighed, then two *Rattus norvegicus*' offsprings were sacrificed and their brains were sliced. Two brains of *Rattus norvegicus*'s offspring

were made into one preparation and are stained. Shapiro-Wilk test showed normal distribution of data on the bodyweight of *Rattus norvegicus*' mothers and offsprings in the group with exposure to Mozart composition and control (p> 0.05).

Table 1. Characteristics of *Rattus norvegicus* based on pregnancy age

Gestation age	Control		Treatment	
	N	%	N	%
19 days	4	36.36	1	9.10
20 days	7	63.64	9	81.80
21 days	-	-	1	9.10
Total	11	100	11	100

Table 2. Mean birth weight of the mothers and offsprings of *Rattus norvegicus*.

Groups	Mothers' BW (gram)			Offsprings' BW (gram)		
	Mean	S/D	p	Mean	S/D	p
Control	147.82	±3.09	0.112	4.60	±0.52	0.713
Treatment	145.09	±4.48		4.52	±0.49	

To measure the expression of BDNF, data of each sample were assessed semiquantitatively according to modified Remmele method. Remmele scale index (Immuno Reactive Score/IRS) is the result of multiplication between percentage score of immuno-reactive cells with color intensity score in immunoreactive cells. Data of each sample were presented as the average IRS value observed at 10 (ten) different visual fields (VF) at 400x magnification.

Table 3. Characteristics of *Rattus norvegicus* offsprings based on BDNF expression in the brain.

Groups	Cerebrum	Cerebellum
Control	68.4	63
Treatment	75.8	81.5

Analysis of the results in the cerebrum

Result of normality test of apoptosis index of in cerebrum using Shapiro-Wilk showed normal data distribution (p> 0,05), so we used independent T-test. Table 4 shows the results of the T-test on cerebrum BDNF expression with p=0.403 (p> 0.05), indicating no significant difference in BDNF expression between exposure to Mozart composition and control.

Table 4. Independent T-test results on cerebrum BDNF.

Groups	Mean ± S/D	p value
Control	6.22 ± 1.99	0.403
Treatment	6.89 ± 2.52	

Analysis of the results in the cerebellum

The result of normality test of BDNF expression in the cerebellum showed abnormal data distribution in control group with p=0.025 (p<0.05), so we used Mann-Whitney test. Table 5 shows the results of Mann-Whitney test on the expression of BDNF in the cerebellum, obtaining p=0.146 (p>0.05), indicating that there was no significant difference in BDNF expression between exposure of Mozart composition and control.

Table 5. Mann-Whitney test results on BDNF in the cerebellum.

Groups	Mean ± S/D	p Value
Control	5.73 ± 2.45	0.146
Treatment	7.41 ± 2.67	

This study was conducted since the 10th day of *Rattus norvegicus* pregnancy since the perfection of ear formation in *Rattus norvegicus* was gestation age of at 9-10 day.[17] Ernawati (2008) calculated the apoptotic index to assess the effect of Mozart composition at the beginning of pregnancy compared to that on 10-day gestation, and significant differences were not found. This is possible because when the ear is fully formed the stimulation of sound waves is received through the ear, then converted into electrical pulses and forwarded to the auditory cortex of the brain through the auditory nerve. Therefore, the stimulation of the composition will begin to influence after the formation and func-tioning of the ear and after the formation of synapses, ie at 20 to 24 weeks of gestation or equivalent to 10-day pregnancy in mice.[18] Several other studies on avian animal models also show that sound stimulation can stimulate calcium-dependent signaling pathways that trigger connectivity of the auditory pathway of the brain. This activates neurons in the hippocampus area which is thought to play an important role in shaping the hippocampal function as a center of memory and learning. The calcium-signaling pathway activates neu-rons through cAMP response element-binding (CREB), which leads to an increase in BDNF, which maintains the survival of neurons, dendritic growth and synapse plasticity in the brain.[19-21]

A study on the stimulation of Mozart's composition in humans proves that the provision of Mozart composition affects fetal biophysical profile where there is an

increase in the rate of acceleration, the length of acceleration, increased fetal heart rate during acceleration, and the amount of fetal motion due to exposure to Mozart composition during the day.[22] In addition, there is increase in newborn BDNF serum levels and BDNF serum blood levels of newborn cord blood.[6,7] There was no significant difference in BDNF expression in group receiving exposure to Mozart composition compared to that without exposure in newborn *Rattus norvegicus* FR50 model (see Figure 1). Although there was an increase in BDNF expression in the treatment group versus control, the difference was not statistically significant.

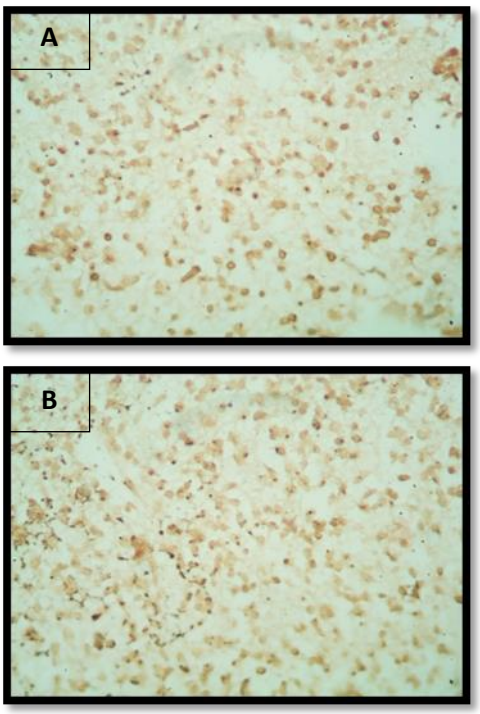

Figure 1. BDNF expression in *Rattus norvegicus*' offsprings cerebrum. A. Without exposure to Mozart's compositions; B. With exposure to Mozart's composition.

A study by Hermanto (2002) suggests that the Mozart composition during pregnancy increases the number of rats'brain cells higher than the gamelan, dangdut and control (no exposure) composition group.[2] Other studies in rats have shown that, similar to humans, rats receiving exposure to Mozart compositions have better learning and memory skills with increased BDNF, CREB and synapsein I (SYN 1) gene expression in the hippocampus, compared with those exposed to noisy or no exposure.[8-10] Furthermore, studies in rats suggest that exposure to Mozart composition during pregnancy may increase BDNF expression in cerebrum in *Rattus norvegicus*' offspring.[11] Similarly, in the cerebellum, responses to auditory stimulation are also seen through Positron Emission Tomography (PET) and Functional Magnetic Resonance Imaging (fMRI) examinations. BDNF has also been shown to increase in the cerebellum in terms of neuron cell plasticity.[23,24]

Biomolecularly, exposure to the Mozart composition has been shown to increase Calcium Binding Protein (CaBP) that stimulates BDNF and TrkB receptors in rats' offsprings, especially in the hippocampus, thus improving learning performance.[20] There is a significant difference in the number of BDNF and protein TrkB in the cortex which is the center of memory, especially in the auditory area. Thus, Mozart composition is known to affect the BDNF/TrkB pathway through the intracellular pathway of phophoinositidide 3-kinase (PI3-K)/Akt. PI3-K/Akt is mediated by Pyruvate dehydrogenase kinase-1 (PDK1) which activates Akt. Mozart composition has been shown to increase PDK1 protein levels in the cortex and hippocampus.[25] However, in the above studies, the rats were not treated with malnourishment. A study by Coupé et al. (2009) showed that BDNF level in rat fetus was highly sensitive to maternal nutritional status. BDNF is known to play an important role in brain development, with a 50% reduction in maternal nutrition in the embryonic (E)14, postnatal (P)7, P14 and P21 periods affects BDNF levels in rat fetuses in the hippocampus and hypothalamus. Maternal perinatal undernutrition affects BDNF expression in the hippocampus and hypothalamus. However, the difference between them is based on the period of brain development in the embryonic and postnatal periods. The observations also showed a difference in the expression of the BDNF receptor gene TrkB.T2 caused obstacles to neurotrophic BDNF/TrkB pathway during postnatal period.[16] Other studies have also shown that protein restrictions as much as 70% from day 8 of pregnancy to week 4 postnatal causes a decrease in both body weight and brain weight, as well as BDNF concentration in the hippocampus in P28.[26]

This study also proved that there was no significant increase in BDNF in mice treated with malnutrition deficiency with FR50 that started since pregnancy despite the exposure to Mozart composition. It can therefore be assumed that the activation of neurons by BDNF from calcium signal pathway that stimulates the brain through auditory area cannot work optimally in malnutrition despite the provision of compositional stimulation due to the inhibition of TrkB receptor, where the availability of BNDF for TrkB receptors decreased, thus making it not sensitive. In addition, BDNF expression also reduced. As a result, the intracellular signaling pathway PI3K/Akt, mediated by PDK1 in the stimulation of Mozart composition, did not run well.[16,25] Other studies have also shown that mater-

nal malnutrition conditions can lead to increased oxidative stress, resulting in reactive oxygen species (ROS). As a result, if the condition persists, the defense mechanism by superoxide dismutase (SOD) in brain neuron cells cannot compensate the occuring oxidative stress and the decrease in SOD results in reduced cellular energy with the final result of the decrease in the number of neuron cells. In addition, increased ROS also causes epigenetic changes that stimulate cell apoptosis so that the number of neurons decreases. Such epigenetic changes also cause abnormalities of gene expression, including the BDNF, TrkB and CREB genes that are found to have been damaged, which is lasting into adulthood.[27-29]

CONCLUSION

The exposure of Mozart composition during pregnancy has no effect on changes in BDNF expression in cerebrum and cerebellum of *Rattus norvegicus* offsprings born from FR50 model.

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