Stimulation of Male Voice during Pregnancy Results in Higher Expression of Brain Derived Neurotrophic Factor in *Cerebellum* of Newborn *Rattus norvegicus*

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Abstract

Background: Recent studies in the field of Fertomaternal Medicine show that stimulation in the uterus can support brain growth and development. If in previous studies stimulation using several types of music, this study uses human voice which aims to analyze the effect of stimulation of male and female voices on differences in the expression of *Brain Derived Neutrotrophic Factor (BDNF)* in *cerebellum* of newborn *Rattus norvegicus*. **Methods:** This type of research is true experimental with post test only control group design. *Rattus norvegicus* was given stimulation of men voice and women voice on 6th day until 17th day of pregnancy. The expression of *BDNF* was examined using *immunohistochemistry*. **Results:** The mean ± standard deviation of the expression of *BDNF* in the *cerebellum* is 4.20 ± 1.75 (without stimulation), 6.04 ± 1.58 (male voice stimulation), and 5.60 ± 2.43 (female voice stimulation). The results of statistical tests show that there is a significant difference on *BDNF* expression between the control group and the group stimulated with male voice with a significance value <0.05 of 0.045 **Conclusion:** Giving stimulation using the male voice during pregnancy shows a significant increase in the expression of *BDNF* in *cerebellum* of newborn *Rattus norvegicus*.

Key words*: Stimulation, Pregnancy, Male and female voice, Rattus norvegicus, Cerebellum, BDNF*

Introduction

One way to measure the progress of a country, is not only dependent on indicators of economic growth. The Human Development Index (HDI) measures the progress of a country based on its human development dimensions; healthy and long-lived humans, knowledge, and a high standard of living. In 2019, Indonesia was ranked 111. Even though UNDP has classified Indonesia as a country with high HDI, this condition still deserves our attention, because we are still left behind with several developing countries such as the Philippines (106), Thailand (77), and Malaysia (61). Some of these countries outperformed Indonesia in their education indicators so that their human development index scores were better than Indonesia according to UNDP [(1)].

The high index of education is influenced by the quality of a country's generation. Several recent findings in the field of fetomaternal medicine state that to obtain a smarter generation, one of which is by enriching the environment during the gestation, especially the development of the fetal brain. Giving stimulation can support brain growth and development by stimulating the formation of synapses or connections between nerve cells and also inhibiting apoptosis or cell death. Apoptosis peaks at around 36-40 weeks of gestation, therefore it is highly recommended to provide optimal and routine stimulation during pregnancy [(2–4)].

Cerebellum is the second largest part of the brain after the *cerebellum*; several findings show the *cerebellum* has a more complex function. Most of the *cerebellum* is connected with brain association areas related to intelligence, social cognition, and emotional control [(5)]. *Brain Derived Neurotrophic Factor* (*BDNF*) is the main neuronal growth factor in the brain that regulates neurogenesis, neuron maturation, survival, and synaptic processes. Although neurogenesis in the hippocampus occurs until adulthood, most neurogenesis occurs in the prenatal and early postnatal periods. The main factor of Neurotrophin that plays a very important role in the learning process, memory, and behavior in the hippocampus is *BDNF* [(6)].

A frequency of about 8,000 Hz is very useful for filling (charging) cells in the brain. Music that is rich in high frequencies and has a high rhythm that is similar to the rhythm of the fetal heartbeat and can be linked to simulation and energizing functions [(7)]. In the sound production system in humans, the lungs produce air pressure which then passes through the windpipe (trachea). The pressure then vibrates the vocal cord that is located above the windpipe. The vibration of the vocal cord (opening and closing) produces sound which is then pronounced by mouth. The voice characteristics of each person are different because they are influenced by variations in the shape, length, and thickness of the vocal cord. Therefore, the sounds that sounded are significantly different in each person. The length of the vocal cord for a human is usually between 12 and 24 millimeters (mm), while the thickness is 3 to 5 mm [(8)].

In music theory, humans have different types of voices, both male and female. The types of voices in male are divided into tenor, baritone, and bass. Meanwhile, female voices are divided into soprano, mezzo-osopran, and alto [(9)]. The results of the study using the Backpropagation Neural Network as a system for identifying the types of human voices show that the algorithm used is very weak to detect the type of alto voice and tend to be good for detecting the type of tenor voice [(10)].

In recent years, there have been several issues regarding the brain development of infants that are given stimulation during pregnancy, such as a significant increase in the number of cerebral neuron cells of *Rattus norvegicus* after stimulation of Mozart music [(11)]; Similar studies on *BDNF* expression obtain a significant increase in *BDNF* expression with stimulation of Mozart's music [(12)]. The study uses two different types of human voice characters, namely male voice and female voice as stimulation during pregnancy in experimental animal, *Rattus norvegicus*, which aims to determine the effect of Ar-Rahman murottal stimulation on the expression of *BDNF* in *cerebellum* of newborn *Rattus norvegicus*.

Materials and Methods

This type of research is the experimental laboratory (true experimental) with a post-test only control group research design. Ethical eligibility was obtained from Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University. The research was conducted at the Animal Experiment Cage and Pathology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, from January to March 2021. The research subjects used were 30 pregnant *Rattus norvegicus* mothers who were divided randomly into 3 groups, namely the non-stimulated group (P1), the group stimulated with Surah Ar-Rahman by male voice (P2), and the group stimulated with Surah Ar-Rahman by female voice (P3). Stimulation was started from 6th until 17th days of pregnancy in a soundproof box with a sound intensity of 65 dB for 60 minutes at night. Inclusion criteria: *Rattus norvegicus* mothers who had never been used for previous studies, healthy, body weight of 120-160 g, and give birth 5-9 pups, newborn *Rattus norvegicus* born surgically in healthy condition. Meanwhile, the criteria for drop out were *Rattus norvegicus* which was stillborn and had anatomical abnormalities.

Two newborn *Rattus norvegicus* were selected from each mother with the heaviest and lightest weights. Then sacrificed, the head was weighed and preserved in 10% formalin solution which was then prepared for examination of *BDNF* expression by immunohistochemical methods. Antibodies used were primary antibody, anti-*BDNF* and DAB (3.3-diaminobenzidine tetrahydrochloride). *BDNF* expression of each subject was assessed according to the modified Remmele method [13], where the Remmele scale index (Immuno Reactive Score / IRS) is the result of the multiplication of the percentage score of immunoreactive cells and the color intensity score of the immunoreactive cells. Data of each subject represented the mean IRS value observed at 5x the field of view (LP) at 400x magnification. The data obtained were then statistically tested using the data normality test with the Shapiro-Wilk test. The data that normally distributed were analyzed using one way ANOVA followed by the Least Significant Difference (LSD) test among the three groups

Results and Discussions

The results of the normality test using the Shapiro-Wilk test on body weight after treatment are obtained a significance value (p-value) in the control group of 0.712, the group stimulated by male voice of 0.971, and the group stimulated by female voice of 0.958. These results indicate the distribution of parent body weight data in each group is normally distributed. The results of the homogeneity test of variety using the Levene test on data on body weight after treatment are obtained a significance value of 0.237. These results indicate that the variance among the three groups is homogeneous. The results of the normality test using the Shapiro-Wilk test on the treatment group are obtained a significance value (p-value) of fetal head weight in the control group is 0.083, the group stimulated by male voice is 0.243, and the group stimulated by female voice is 0.124. These results indicate that the distribution of data on fetal head weight in each group is normally distributed. The results of the homogeneity test of variance using the Levene test on fetal head weight are obtained a significance value of 0.691. These results indicate that the variance among the three groups is homogeneous.

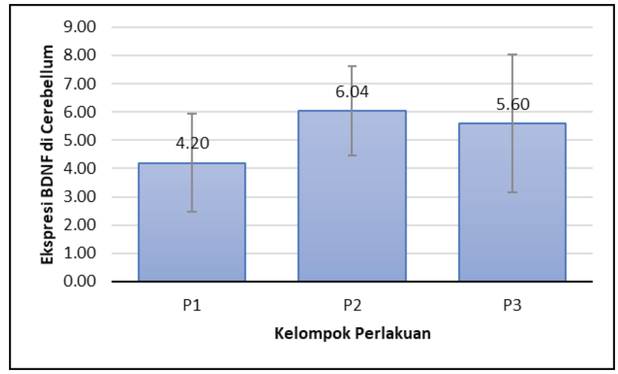

Figure 1. Graph of Mean and Standard Deviation of *BDNF* Expression in *Cerebellum* of newborn *Rattus norvegicus* in the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3).

Figure 1 shows the mean and standard deviation of *BDNF* expression in the *cerebellum* of newborn *Rattus norvegicus* in the control group of 4.20 ± 1.75, the group stimulated by male voice is 6.04 ± 1.58, and the group stimulated by female voice is 5.60 ± 2.43. The normality test used the Shapiro-Wilk test, *BDNF* expression data on the *cerebellum* of newborn *Rattus norvegicus* shows that the distribution of *BDNF* expression data in the *cerebellum* in each treatment group is normally distributed. It can be seen in Table 1.

Group	Significance value (p-value)	
	Male Voice	Female Voice
Control	0.045	0.121
Male Voice		0.619

Table 1. The Results of Shapiro-Wilk Normality Test and Levene Variety Homogeneity Test on *BDNF* Expression in *Cerebellum* of newborn *Rattus norvegicus*

Group	Statistic	p-value	Description
Control (P1)	0.861	0.079	Normal Distribution
Male Voice (P2)	0.904	0.242	Normal Distribution
Female Voive (P3)	0.897	0.201	Normal Distribution
Uji Levene	3.119	0.060	Homogeneous

The results of the homogeneity test of variance using the Levene test on *BDNF* expression data in the *cerebellum* are obtained a significance value of 0.060. These results indicate that the variance among the three groups is homogeneous. Based on the results of the normality test and the homogeneity test of variance, the data analysis to test whether there are differences in the treatment groups on *BDNF* expression in the *cerebellum* used the Analysis of Variance (ANOVA) test with the advanced Least Significant Difference (LSD) test.

Table 2. The Results of ANOVA Test on *BDNF* Expression in *Cerebellum* of Newborn *Rattus norvegicus*

Variable	Statistic	p-value	Description
BDNF Expression in Cerebellum	2.418	0.108	Not Significant

The results of ANOVA test on *BDNF* expression in *cerebellum* of newborn *Rattus norvegicus* are obtained a calculated F value of 2.418 with a significance value of 0.108 which indicates that there is no significant difference among treatment groups on *BDNF* expression in the *cerebellum* of newborn *Rattus norvegicus*. The p-value results of the LSD advanced test among the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3) can be seen in Table 3.

Table 3. The Results of LSD Test on *BDNF* Expression in *Cerebellum* of Newborn *Rattus norvegicus*

Group	Significance value (p-value)	
	Male Voice	Female Voice
Control	0.045	0.121
Male Voice		0.619

The results of LSD test show a significant difference in *BDNF* expression in the *cerebellum* of newborn *Rattus norvegicus* between the group stimulated by male voice and the non-stimulated group (Control). Meanwhile, the control group compared to the group stimulated by female voice shows a less significant difference, it means that there is less significant difference in *BDNF* expression in the *cerebellum* of newborn *Rattus norvegicus*. In the next group, the group stimulated by male voice compared to the group stimulated by female voice, the result is less significant. Although the result in the group stimulated by male voice is higher than the group stimulated by female voice, the difference is less significant or insignificant. Among the three groups, it is known that the group stimulated by male voice is obtained the highest expression results in *BDNF* expression in the *cerebellum* of newborn *Rattus norvegicus*

Gender affects the size of the vocal cords. The length of the female vocal cords is approximately 12.5 mm to 17.5 mm, whereas in male voice it is from 17.5 mm to 24 mm [(14)]. Because the vocal cords are longer, the pitch of the male voice is lower, therefore the male voice sounds heavier than the female voice. Heavy voices contain more sound intensity. Based on research on the voice identification system using Backpropagation Artificial Neural Network (ANN), the results of the voice identification system tend to be better at detecting the type of tenor voice compared to the type of alto voice, in which the tenor voice is one of male voice types, whereas alto voice is one of female voice types [(9)]. Research with similar results proposing a new feature for automatic gender detection using modified voice contour (MVC), found that the area under the MVC for male speakers is greater than for female speakers [(15)]. This is because male speakers have a higher intensity than female speakers.

Automatic gender detection applications have actually been widely used in the health world, for example in the health care monitoring system [(16,17)]; and the detection of abnormalities in the vocal cords [(18,19)]. Various researches on automatic gender detection (AGD) applications that have been developed have proven that male voices are easier to identify. Therefore, this study proves the theory that male voice is known to have a higher effect as a form of stimulation performed on experimental animal during pregnancy.

The red arrows in Figure 2 show the *BDNF* expression in the *cerebellum* which is indicated by the presence of a brown chromogen on the immunohistochemical examination which is observed at 5x the visual field (LP) at 400x magnification.

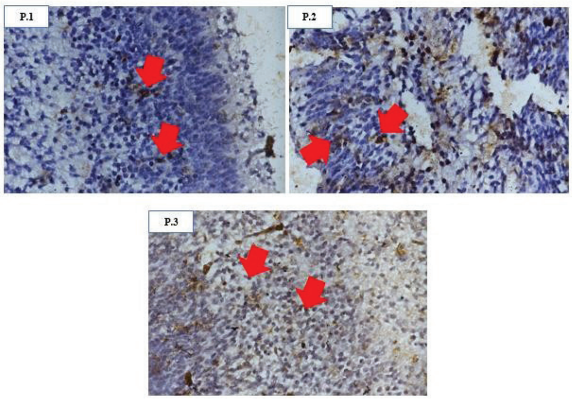

Figure 2 Comparison of *BDNF* expressions in the brain of the control group (P1), the group stimulated by male voice (P2), and the group stimulated by female voice (P3).

Conclusion

The stimulation of male voice during pregnancy has a significant effect on increasing the expression of *BDNF* in *cerebellum* of newborn *Rattus norvegicus* because the highest *BDNF* expression is shown in the group stimulated by male voice. Similar research in other parts of the brain needs to be more developed because this research is the first experiment of giving stimulation using the type of human voice in experimental animal.

Conflict of Interest : None

Source of Funding : Self

References

1. United Nation Development Programme. Human Development Report 2020 -The Next Frontier: Human Development and the Anthropocene, Briefing note for Uganda. 2020;1–7.
2. Chikahisa S, Sei H, Morishima M, Sano A, Kitaoka K, Nakaya Y, et al. Exposure to music in the perinatal period enhances learning performance and alters *BDNF*/TrkB signaling in mice as adults. Behav Brain Res. 2006;169(2):312–9.
3. Kim H, Lee MH, Chang HK, Lee TH, Lee HH, Shin MC, et al. Influence of prenatal noise and music on the spatial memory and neurogenesis in the hippocampus of developing rats. Brain Dev. 2006;28(2):109–14.
4. Sholichah AM, Joewono HT, Widjiati W. The Number of Cerebrum Neuron Cells in Mozart's Music Exposure Is Higher Than That of Indonesian Music. J Health Researchers" VOICE FORIKE. J Heal Res Forikes Voice. 2019;11(1):18–22.
5. Schmahmann JD. The *cerebellum* and cognition. Neurosci Lett. 2019;688(April):62–75.
6. Bienertova-Vasku J, Bienert P, Zlamal F, Splichal Z, Tomandl J, Tomandlova M, et al. Brain-derived neurotrophic factor and ciliary neurotrophic factor in maternal plasma and umbilical cord blood from pre-eclamptic and physiological pregnancies. J Obstet Gynaecol (Lahore). 2013;33(4):359–63.
7. de Voigt M, Vervoot J. LISTEN to LIVE-our Brain and Music: The Tomatis Listening training and therapy. MBL-Tomatis Network Listening Centre; 2018.
8. Hahn MS, Teply BA, Stevens MM, Zeitels SM, Langer R. Collagen composite hydrogels for vocal fold lamina propria restoration. Biomaterials. 2006;27(7):1104–9.
9. Wijayanto I, Dwifebrianti R. Types of Voice Range Types In Men and Women Using The Cepstral Coefficient Mel-Frequency Method and Neural Network Copy backpropagation. Natl Conf Syst Informatics. 2013;(October 2013):2–10.
10. Yudha IP, Tritoasmoro II, Atmaja RD. Human voice type identification system based on vocal range using artificial neural tissue backpropagation. 2012;621.382 2. Universitas Telkom Bandung.
11. Octariyandra SO, Joewono HT, Basuki M. Mozart Compilation During Pregnancy Gave Higher Number of Neurons of *Rattus norvegicus* Offsprings in Cerebrum Compared to Jazz, Blues, and Rock Compilation. Glob J Med Res. 2019;19(4):1–8.
12. Permatasari P, Joewono HT. Differences in The Influence of Mozart, Beethoven and Chopin's Exposure To Brain Derived Neurotrophic Factor Expression in The Nascent Crebellum *Rattus norvegicus*. Sci J Midwifery Gynaecol. 2019;11(3):46–50.
13. Nowak M, Madej JA, Dzięgiel P. Intensity of COX2 expression in cells of soft tissue fibrosacrcomas in dogs as related to grade of tumour malignancy. Bull Vet Inst Pulawy. 2007;51(2):275–9.

14. Titze IR, Martin DW. Principles of voice production Prentice Hall. NJ[Google Sch. 1994;
15. Alhussein M, Ali Z, Imran M, Abdul W. Automatic Gender Detection Based on Characteristics of Vocal Folds for Mobile Healthcare System. Mob Inf Syst. 2016;2016.
16. Hossain MS, Muhammad G. Cloud-assisted Industrial Internet of Things (IIoT) - Enabled framework for health monitoring. Comput Networks. 2016;101:192–202.
17. Hossain MS. Cloud-supported cyber-physical localization framework for patients monitoring. IEEE Syst J. 2017;11(1):118–27.
18. Muhammad G, Mesallam TA, Malki KH, Farahat M, Alsulaiman M, Bukhari M. Formant analysis in dysphonic patients and automatic Arabic digit speech recognition. Biomed Eng Online. 2011;10(1):1–12.
19. Muhammad G, Alsulaiman M, Mahmood A, Ali Z. Automatic voice disorder classification using vowel formants. In: 2011 IEEE international conference on multimedia and expo. IEEE; 2011. p. 1–6.