Physical and Psychological Stressor Exposure during Pregnancy Impacts the Expression of Synapsin and Neuronal Cells Number of MUS Musculus Offspring

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Abstract

Introduction: Stress during gestation can lead to inappropriate fetal brain development, especially psychological stress. Psychological stress play pivotal role in offsprings' brain development.

Objective: The aim of this study was to investigate the effects of maternal exposure to physical and psychological stress during pregnancy on the cerebrum in mice offspring.

Method: This study was an analytical experiment study with the subject were 24 female mice *Mus musculus*. The sample were divided into treatment and control group. Treatment groups divided into three conditions, 1 group was given physical stress, 1 group with psychological stress, and 1 group was given combination of physical and psychological stress intervention. The data were analyzed with ANOVA test then followed by LSD to find the differences between all groups.

Results: The ANNOVA test results showed significant differences of the expression of synapsin with p = 0.0000. The number of neuronal cells also represented significant differences with p = 0.000 on the cerebrum.

Conclusion: Stress exposure during pregnancy can induce bad impacts in brain development, especially the expression of synapsin and the number of neuronal cells on the cerebrum in mice offspring.

Keywords: Cerebrum, neurons, pregnancy, stress, synapsin

Introduction

The stress that occurs during pregnancy results in an increased risk of preterm birth, a higher neonatal abnormality, and delays in motor and cognitive development. The prevalence of pregnancy stress is quite high. Psychosocial stress research during pregnancy conducted on Asian, African and white races states that 6% of pregnant women experience mild stress, 78% experience severe stress and 16% experience no stress at all.(1) In Canada, it shows that pregnant women experience low levels of psychosocial stress and 6% of high levels. Pregnant women in Spain have 30% lower chance of experiencing stress, while in Indonesia there are 64.4% of pregnant women who experience severe stress.(2)

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Previous study used exposure to light stressors proved that the number of brain neurons in the intervention group was significantly smaller than the non-intervention group.[3] The noise as stressor has been shown to reduce the number of neurons in the CNS in which is the cerebral cortex.[4] Synapsis plays a role in communication between neuron cells, so that synaptic structures play an important role in memory and learning. The greater the number of neuron cells, the more synapses. The multiple synapses cause a reduced apoptotic process. As a result, the brain processes information faster.[5] Stress during gestation can lead to inappropriate fetal brain development, especially psychological stress. Maternal who had experienced psychological stress during pregnancy have higher risks for cognitive, behavioral, and emotional problems. Exposed to psychological stress can damage structural brain development in which facilitated by programming of the hyperactivity of the Hypothalamus-Pituitary-Adrenal cortex (HPA) axis.[6]

The impact of stress on synapsin caused a decrease in synapsin-I mRNA in the amygdala by microaray analysis, the stressor showed an increase in high CRH expression and an increase in glucocorticoid receptors.[7] Also found that a disturbance at the beginning of fetal growth it will cause inhibited synapses formation which can inhibit cognitive enhancement.[8] The reduction in Syn-I will result in impaired axon differentiation, neurite growth, inhibit the formation and mechanism of synapses and allow neurological disorders in the brain.[9]

Objectives

To compare the expression of synapsin and neuronal cells number of *Mus musculus* offspring exposed to physical stress, psychological stress, the combination of psychological and physical stress, and without stress exposure.

Materials and Methods

This study used experimental laboratory research method with posted-only control group design. Animal subject was obtained from Faculty of Veterinary, Universitas Airlangga in which 24 pregnant female mice Mus musculus. Sample size was calculated based on replication formula of Frederer. Samples for each group has 6 pregnant mice. Pregnant Mus musculus were divided into 4 groups, 1 control group in which was not treated and 3 treatment groups. Treatment group 1 (G1) were given exposure to physical stress by making the pregnant mice swimming every morning for 5 minutes. Treatment group 2 (G2) were given exposure to psychological stress by giving noisy sound every morning with an intensity of 90 dB in a soundproof box for one hour. Treatment group 3 (G3) were given exposure to psychological stress then followed by physical stress every morning. There were no pregnant mice experiences preterm birth, abortion, or death. The interventions start from 6th day until 15th day of pregnancy. On the 16th day pregnant mice were sacrificed and the fetuses were taken by section caesarea. Then three newborn were selected based on the heaviest, moderate, and light weight. Selected newborn mice were sacrificed by decapitation. The brain of the selected offspring were taken and immunohistochemistry preparation. The expression of synapsin-I in the cerebrum were examined through immunohistochemistry with Syn-I antibody and calculated with Immuno Reactive Score (IRS) which is viewed under microscope with 5x visual field with 400 magnification. The number of neuronal cells in the cerebrum were calculated after Hematoxylin Eosin staining under the microscope with 5x visual field with 400x magnification. The data were analyzed with ANOVA test then followed by LSD to find the differences between all groups.

Results

The research sample consisted of 24 female mice based on inclusion criteria and randomized into four groups. Mean and standard deviation of the expression of synapsin in the cerebrum can be seen in Table 1.

Table 1. Data of the expression of synapsin in the cerebrum.

	Mean ± SD			
	G1	G2	G3	Control
Synapsin	7,80 ± 1,56	6,20 ± 0,82	4,03 ± 0,46	9,70 ± 1,71

Table 1 showed that treatment group 3 has the lowest synapsin expression in the cerebrum of *Mus musculus* offspring. Treatment group (G2) which had been exposed to psychological stress has lower expression of synapsin compare to physical stress exposure group (G1). The differences of synapsin expression between groups were analyzed by one-way ANOVA test then followed with Least Significant Difference (LSD) test. ANNOVA test resulted p= 0.000 which there are significant differences in the expression of synapsin in the *Mus musculus*' offspring cerebrum.

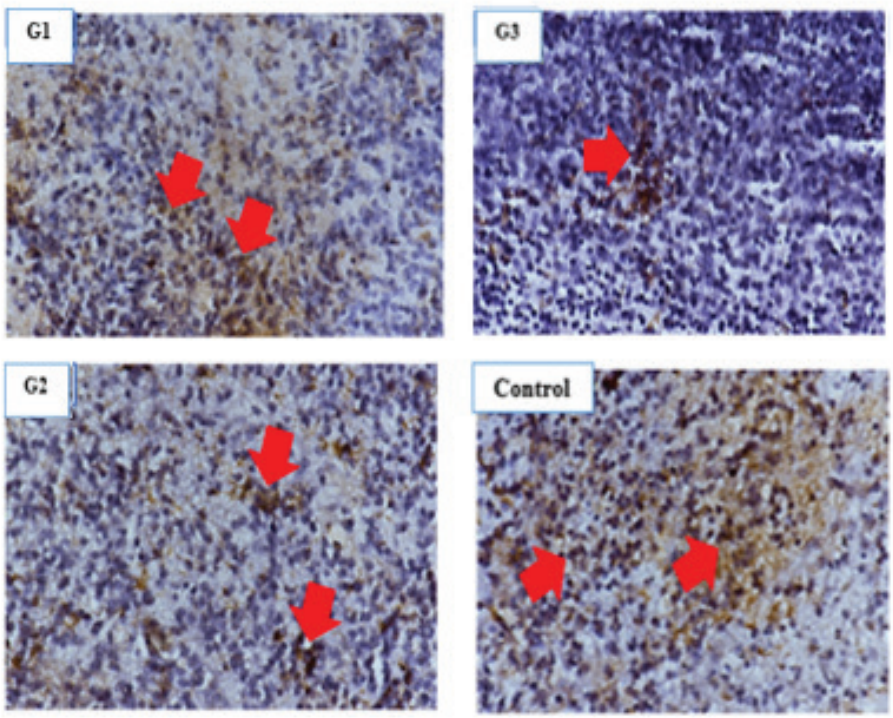

Figure 1. Comparison of synapsin expression in the cerebrum of Mus musculus offspring (G1, G2, G3, Control). The red arrow indicates the presence of synapsin expression in the cerebrum which is indicated by the presence of a chromogenic brown color. Viewed after immunohistochemistry with 400x magnification.

The number of neuronal cells in the offspring's cerebrum were calculated after Hematoxylin Eosin staining procedure. Mean and standard deviation of the number of neuronal cells in the cerebrum can be seen in Table 2.

Table 2. Mean of neuronal cells number in the cerebrum.

	Mean ± SD			
	G1	**G2**	**G3**	**Control**
Neuronal cells number	9,67 ± 1,19	7,87 ± 1,89	6,00 ± 1,46	11,63 ± 0,87

The results showed the mean difference between G1, G2, G3, and Control group, as shown in Table 2. The decrease in neuronal cells number occurred in the stress exposure groups. Statistical analysis used the One Way Anova test with p-value = 0.000, which means significant differences between groups. Post Hoc LSD test (Least Significant Difference) shows significant differences between G1, G2, G3, and Control group.

Discussion

Stress during pregnancy affects the well-being of the mother and the fetus. Stress caused disadvantages such as low birth weight, premature birth, and delayed child development after birth. It can cause brain mass atrophy and reduce its weight.[10]

This research found that on the cerebrum of newborn *Mus musculus* showed the mean of synapsin expression in the physical stress exposure group (G1) proved to be lower than the control group. Physical activity for 1 hour can significantly increase oxidative stress.[11] Stress and mental conditions have a critical impact on physical health because the mind and body are one unit and psychological stress can adversely affect the health of the body.[12]

Stress changes brain function by modifying the structure and function of neurons and astrocytes. Astrocytes play an important role in synaptic transmission by expanding the subtle processes around the synapses. These astrocytes express GluA1, a subunit of glutamate receptors that is known to prolong astrocyte processing. Astrocytic structural changes are associated with decreased levels of the GluA1 protein.[13]

This study also showed that in the cerebrum of the newborn *Mus musculus*, the mean synapsin expression in the psychological stress exposure group (G2) proved to be lower than the control group. The effects of stress not only result in changes in the number of synapses, but stress can also result in significant remodeling of the ultra-structural morphology of the individual excitatory synapses. In particular, exposure to stress reduces the length of the synaptic active zone in the dentate gyrus and reduces the thickness of the postsynaptic density in the CA1 area.[14]

The stress-induced morphological changes of the GABAergic tissue are complemented by in vitro-electrophysiological findings, documenting malfunctioning GABAergic neurotransmission in the pressurized hippocampus.[15] In addition, vivo-electrophysiological studies focused on tissue function documented long-term potentiation-induced impairment and decreased basal synaptic transmission at the hippocampal CA3-CA1 synapses, and this was accompanied by decreased dendritic spine density in the CA1 and CA3 pyramidal neurons.[16]

Stress patterns can be divided into two main categories, namely physical stress and psychological stress.[17] Various studies have shown that these two stress patterns can lead to behavioral disorders, brain atrophy and cognitive dysfunction, abnormal neurotransmitters and cytokines, irregular hormone levels, and increased inflammatory factors in experimental animals.[18] Synapse loss has been reported in PFC depression patients. Synaptic dysfunction has been suggested as a key factor contributing to emotional distress. The mechanistic theory prevailing in perinatal psychiatry to explain mood-related effects on offspring is through changes in the mother's Hypothalamic-Pituitary-Adrenal (HPA) axis during pregnancy. Research result found that basal levels of plasmatic corticosterone were higher in animals under prenatal stress compared to controls. Corticosterone is a fat-soluble glucocorticoid that penetrates the placenta and interacts strongly with various fetal cells and tissues.[19]

This research found that mean of neuronal cells in the cerebrum was shown to be lower than in the G1 than control group. Structural changes include disorders of atrophy and neurogenesis. In addition, chronic stress can increase plasma cortisol and cause a decrease in the number of dendritic branches and the number of neuron cells, as well as structural changes in synaptic terminals and decreased neurogenesis in the hippocampal tissue.[20] Research on exposure to psychological stress in the form of noise causes the release of stress hormones, including glucocorticoids.[21] When suffering from stress, the central nervous system (CNS) is affected, the individual can experience anxiety and depression, which can have detrimental effects. Brain-Derived Neurotropic Factor (BDNF), a well-known neurotropic factor, is widely expressed among various brain areas, playing an important role in neuron maintenance and survival and neurogenesis.[22] Chronically elevated GC impairs neurotrophic factor expression.[23] This causes changes in neuron structure such as decreased synaptic activity, changes in morphology and neuron proliferation capacity.[24] The effects of stress were more pronounced in the psychological group than in the physical group at the end of the treatment week even though exposure to psychological stress was responded more slowly than exposure to physical stress.[25]

Physical and psychological health during pregnancy affects fetal nerve development.[26] The combined exposure group of physical stress and psychological stress (G3) showed a lower mean number of neuron cells than the group given one exposure (G1, G2). Stress involves two-way communication between the brain and other systems through nervous and endocrine mechanisms. Stress exposure and stress hormones can produce maladaptive effects in this brain region throughout the course of life. Psychological stress give worse impacts than physical stress with the result that the mean number of neuron cells is lower in the psychological stress exposure group than in the physical stress exposure group.

Conclusion

The expression of synapsin and neuronal cells number in Mus musculus offspring cerebrum exposed to physical and psychological stress proved to be lower than pregnant mice with no stressor exposure.

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