Journal of Medicine and Biomedical Research. 23 (2) 38-45
https://dx.doi.org/10.4314/jmbr.v23i2.6

ORIGINAL ARTICLE

Effect of Lauric Acid on Neurodevelopmental Parameters and Hippocampal BDNF Levels in Offspring of Wistar Rats Exposed to Prenatal Stress

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ABSTRACT

Background: Unfavorable environmental experiences can affect fetal neurodevelopment negatively by lowering brain-derived neutrotrphic factor (BDNF) in offspring of rodents. This study examined the effect of Lauric acid (LA) on some neurodevelopmental parameters and hippocampal BDNF levels in male offspring of pregnant Wistar rats exposed to sleep deprivation.

Methods: Thirty-six male offspring from 24 pregnant female Wistar rats were used for this study. The pregnant rats were sleep-deprived for 20 hours daily using the multiple water platform method from day 9-19. Group 1 received distilled water, group 2 served as stress control while 3,4 and 5 were treated with LA at 125mg/kg, 250mg/kg and 500mg/kg. Group 6 received vitamin C at 300mg/kg.

Results: LA significantly improved pinna detachment, incisor eruption, eye opening, air righting reflex ($p<0.05$) compared to the sleep deprived untreated group.

Conclusion: Lauric acid reversed the delay in some neurodevelopmental parameters by increasing the hippocampal BDNF level in male offspring of Wistar rats. This was reversed upon oral treatment with Lauric acid.

Keywords: Lauric acid; BDNF; sleep deprivation; neurodevelopment; Pregnancy

INTRODUCTION

Pregnancy, though a natural phenomenon is one of the most important periods in a woman's life comes with major physiological, psychological and social alterations. [1,2] Adverse environmental experiences during this period can negatively alter fetal neurobiological development [3] by increasing serum corticesterone [4] and lowering serum and hippocampal levels of brain-derived neutrotrophic factor (BDNF) in offspring of rodents.[5,6] Fetal communication with the mother is critical for the developing fetus and as such, the placenta is key since there is no other direct neural connection between them.[7] Stress during critical periods of pregnancy has been shown to greatly impact neonates which serves as a risk factor for postnatal neurodevelopmental deficits. It has also been reported to reprogram the hypothalamopituitary-adrenal axis (HPA), decrease placental barrier which can affect neurodevelopment. [8]

Disruption in sleep have been reported to be on the increase in the general population. Prenatal sleep restriction is a critical serious concern since it has been reported to negatively affect the mother's wellbeing and that of the developing fetal brain.[9,10] It has been reported to increase the risk of attention-deficit/ hyperactivity disorder (ADHD), [11] delays in behavioral development,[12] depression and anxiety,[13] altered

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antioxidant enzymes expression,[14] altered adult neurogenesis,[15] lower sexual behavior,[16] risk taking behavior [17] in offspring later in life.

Studies have shown that BDNF promotes the growth and maturation of stem cells [18] and decreased level of it can alter plasticity.[19] Lauric acid, a medium chain saturated fatty acid, is found in human breast milk, coconut oil, coconut milk and palm kernel oil. [20,21] It has been reported to possess antioxidant and anti-inflammatory activities. [22,23] This study aims to assess the impact of prenatal sleep restriction on early neurodevelopmental responses and the effect of lauric acid in the male offspring of Wistar rats.

METHODOLOGY

Chemicals

Lauric acid (LA), 98% (AK Scientific, Union City, CA, USA. CAS#: [143-07-7] Lot#: AG37411). The compound was dissolved in Tween 80 (Sigma-Aldrich) at a ratio of 1:2 and diluted to desired concentrations in distilled water. [24] Vitamin C (Ascorbic acid, Central Drug House, New Delhi, India, CAS No.99.99-0; 029395), Tween 80 (Sigma-Aldrich), Rat CORT ELISA Kit (ER0859, Wuhan Fine Biotech Co., Ltd. Wuhan, China).

Animals and grouping

Twenty-four (24) female Wistar rats weighing 180-210 g and fifty (50) male Wistar rats weighing 40-60 g were used in the research. The adult rats were bought, mated, and housed in normal cages in the animal house unit of the department of Human Physiology, Ahmadu Bello University Zaria. Commercial foods (Vital feeds) and tap water were provided, and their cages were routinely cleaned. Vaginal smear tests were used every day to check for pregnancy. Gestational day zero (GD 0) was the day the smear revealed sperm or the seminal (copulation) plug was first noticed.[25]

Ethical approval was sought and obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) (ABUCAUC /2021/107). One day following parturition, litters were reduced to 6 pups per mother (picked randomly) to avoid differences during lactation. Litters were housed together with their mothers until weaning at postnatal day 21 after which they were housed with same sex littermates in groups. A total of 6 male pups per group (at least one per cage in from each group) were selected randomly and used for the study.

Animal groups and treatments

Male Wistar rats from postnatal day 0-21 were used for early neurodevelopmental assessments while those from postnatal day 28-35 from each of the groups were sacrificed for assessment of hippocampal BDNF level

Induction of stress

Sleep deprivation was induced in the pregnant Wistar rats for 20 hours from GD9 to GD 19, [26] with 4 hours (7:00am-11:00am) rest each day, using the Modified Platform Method (MMP) as described by Medeiros *et al.*[27] and modified by Oh *et al.* [28] The modified multiple platform method, involves placing the rats in an acrylic water tank (123 x 44 x 44 cm) containing 14 circular platforms, 6.5 cm in diameter, with water up to 1 cm of their upper surface. This way, the rats can freely navigate inside the tank by moving from one surface of the platform to another. As soon as they fall asleep and reach the paradoxical stage of sleep, they lose muscle tone, and they will fall into the water and wake up. Food and water was provided *ad libitum* by placing chow pellets and water bottles on a grid located on top of the tank. The water in the tank was changed daily throughout the SD period.

Neurodevelopmental Assessments (Developmental reflex testing)

Maturation of neural signs and reflexes were carefully examined in all the male pups daily until weaning.[29]

Table 1: Animal grouping and treatment

Groups	Treatment	Number of Dams	Number of Pups (offspring)
Normal Control (Distilled water)	1ml/kg	4	6
Sleep deprivation (SD)	Untreated	4	6
SD+LA	125mg/kg	4	6
SD+LA	250mg/kg	4	6
SD+LA	500mg/kg	4	6
SD+ vitamin C	300mg/kg	4	6

SD (sleep deprivation), LA (lauric acid)

Physical Development Assessments

Pinna Detachment (unfolding of external ear).
This was observed from PND 2 to PND 4. [30]

Scoring: (record the day of unfolding of both ears)
0 when no visible pinna unfolding
1 when one pinna unfolds
2 when two pinna unfolds.[31]

Incisor Eruption

This was examined from PND 9 to PND 12 to determine the day of first appearance of the upper incisors.

Scoring
0 when no tooth erupts
1 when one tooth erupts
2 when 2 teeth erupt.[31]

Eye opening

This was conducted from PND 12 to 16. The day both eyelids opened was recorded

Scoring
0 when no eye opens
1 when one eye opened (left or right)
2 when two eyes opened.[31]

Developmental Reflex Assessments

Righting reflex

This test was conducted from PND 6 to PND 9. It involved placing the pup in a supine position, with all four paws upright and allowed to right itself. Each pup was allocated 15 seconds to achieve this goal. Time to achieve normal position was recorded. The righting reflex can be used to assess the reflection of sensorimotor coordination.[30, 31]

Air righting reflex

This test was conducted from PND 13 to PND 15. It involved dropping the pup in a supine position onto a bed of foam landing from a height of 50cm. The first day the pup lands on its four paws (righting) was recorded.

Scoring
0 when landing on its back
1 when landing on its side (left or right)
2 when landing on its paws.[29, 31]

Gait

This test was conducted from PND 6 to PND10. It involved placing the pup in the center of a white circular paper of 15cm diameter, and the day it began to move off the circular paper with both forepaws in less than 30 seconds was recorded. Each pup was allowed 30 seconds to complete the task. [31, 32]

Scoring

The gait was scored as the time in seconds the pup takes to move off the circular paper. Record 30 seconds, if the pup was unable to complete the task within the given time.

Determination of brain derived neurotrophic factor (BDNF) level

Hippocampal BDNF level of the male offspring assay was carried out using Rat BDNF ELISA Kit: ER0008 (Fine Test), Reactivity: rat, Sensitivity: 18.5 pg/ml, sourced from Wuhan Fine Biotech Co, China. The rat brain tissue was rinsed thoroughly with ice cold phosphate buffer solution (PBS) (0.01 M, pH= 7.4) to remove excess hemolyzed blood after which the hippocampus was gently dissected, separated and homogenized in the PBS (9 mL of PBS to 1g of tissue). The homogenate was then centrifuged at 5000×*g* for 5 minutes and the supernatant retrieved. Prior to commencement of the assay (30 minutes), all reagents and samples were brought to room temperature. The plate was washed twice with the wash solution before the addition of standard, sample and control wells. 50 µl of each solution was dispensed into the appropriate wells followed by addition of 50 µl of the biotin-detection antibody working solution to each well and covered with the plate sealer. The set up was gently tapped to ensure adequate mixing followed by 90 minutes incubation at 37°C. Afterwards, the solution was discarded and washed 2 times using the wash solution. This was carried out by filling each of the wells with wash buffer (350 µl) and allowed to soak for 1-2 minutes after which the residual wash-liquid was removed by aspiration. 0.1 ml of Histidine-rich peptide streptavidin conjugate (SABC) working solution was added into each well and the plate covered and incubated at 37°C for 30 minutes. The solution was then discarded and the plate washed 5 times. Afterward, 90 µl of TMB substrate was added into each of the wells and the plate covered and incubated again at 37°C for about 10-20 minutes avoiding the light. 50 µl of the stop solution was added into each well and the results read at 450 nm within 20 minutes.

RESULTS

Results from the study (Table 2), indicated that there was a statistically significant delay in the pinna detachment on PND 4 in the SD untreated group versus the normal control group :1-00 (1.00-2.00) vs 2.00(2.00-2.00). The LA 125 mg/kg :2.00(2.00-200), LA 250 mg/kg: 2.00(2.00-2.00), LA 500 mg/kg 2.00(2.00-2.00

and the Vitamin C 300 mg/kg 2.00(2.00-2.00) treated groups showed a statistically significant early onset of pinna detachment on PND 4 compared with the SD untreated group: 1-00 (1.00-2.00). The result obtained from the incisor eruption from this study (Table 2) showed that there was no significant difference in the SD untreated group versus the normal control group. However, there was a statistically significant difference in early tooth eruption on PND 10 in the LA 250 mg/kg: 1.00(1.00-1.00), LA 500 mg/kg 1.00(1.00-1.00) and Vitamin C 300 mg/kg 1.00(1.00-1.00) treated groups compared with the SD untreated group 0.00(0.00-0.00).

The result from the study on eye opening (Table 2) showed a statistically significant delay in eye opening on PND 16 in the SD untreated group: 1.00(1.00-1.00) compared with the normal control group: 2.00(2.00-2.00). The LA 250 mg/kg: 2.00(2.00-2.00), LA 500 mg/kg: 2.00(2.00-2.00) and the Vitamin C 300 mg/kg: 2.00(1.50-2.00) treated groups compared with the SD untreated group: 1.00(0.00-1.00), showed a statistically significant difference in early onset of both eyes opening on PND 16.

The result obtained from the study on righting reflex (Table 3) indicated there was no statistically significant difference in the time it took the pups to right or flip over on it paws in the SD untreated group compared with the normal control and all the LA untreated group. However, the result also showed a statistically significant delay in the time it took pups in the SD untreated group to right versus the Vitamin C 300 mg/kg group: 3.00(2.50-3.00) vs 2.00(1.00-2.00).

The result obtained from the study on air righting reflex (Table 3) on PND 14 showed a statistically significant difference in the ability of pups to achieve the goal in the normal control group versus the SD untreated group and all the LA treated groups, 2.00(1.50-2.00) Vs 1.00(1.00-1.00). Though they all achieved the goal on PND 15.

The result on gait (Table 3) on PND 10 showed there was no statistically significant difference in achieving the goal in the normal control group compared with the SD untreated group. However, there was an indication of a statistically significant difference in reaching the goal in the LA 250 mg/kg: 10.00(9.00-10.00) and Vitamin C 300 mg/kg: 11.00(8.00-12.50), compared with the SD untreated group: 18.00(17.00 -25.00).

Table 2: Effect Lauric acid on Maternal Chronic Sleep Deprivation-Induced-Stress on some early physical developmental parameters in Male Offspring

Activities/Groups	Control	SD untreated	LA 125 mg/kg+SD	LA 250 mg/kg+SD	LA 500 mg/kg+SD	Vit C 300mg/kg+SD
Pinna detachment (PND 4)	2.00 (2.00-2.00)	1.00(1.00-2.00)[a]	2.00(2.00-2.00)[b]	2.00(2.00-2.00)[b]	2.00(2.00-2.00)[b]	2.00(2.00-2.00)[b]
Incisor eruption (PND 10)	1.00(0.50-1.00)	0.00(0.00-0.00)	1.00(0.00-1.00)[b]	1.00(1.00-1.00)[b]	1.00(1.00-1.00)[b]	1.00(1.00-100)[b]
Eye Opening (PND 16)	2.00(2.00-2.00)	1.00(0.00-1.00)[a]	2.00(1.00-2.00)	2.00(2.00-2.00)[b]	2.00(2.00-2.00)[b]	2.00(1.50-2.00)[b]

PND: postnatal day, SD: sleep deprivation, LA: Lauric acid, Vit C: Vitamin C

Table 3: Effect Lauric acid on Maternal Chronic Sleep Deprivation-Induced-Stress on some early reflex developmental parameters in Male Offspring

Activities/Groups	Control	SD untreated	LA 125 mg/kg+SD	LA 250 mg/kg+SD	LA 500 mg/kg+SD	Vit C 300mg/kg+SD
Righting Reflex (secs) (PND 9)	2.00(1.50-2.00)	3.00(2.50-3.00)	2.00(2.00-2.00)	2.00(2.00-3.00)	2.00(2.00-2.00)	2.00(1.00-2.00)[b]
Air righting reflex (PND 14)	2.00(1.50-2.00)	3.00(2.50-3.00)[a]	1.00(1.00-1.00)[b]	1.00(1.00-1.00)[b]	1.00(1.00-1.00)[b]	1.00(1.00-2.00)
Gait (secs) (PND 10)	15.00(13.00-16.50)	18.00(17.00-25.00)	11.00(9.50-14.00)	10.00(9.00-10.00)	14.00(12.00-17.50)	11.00(8.00-12.50)[b]

PND: postnatal day, SD: sleep deprivation, LA: Lauric acid, Vit C: Vitamin C

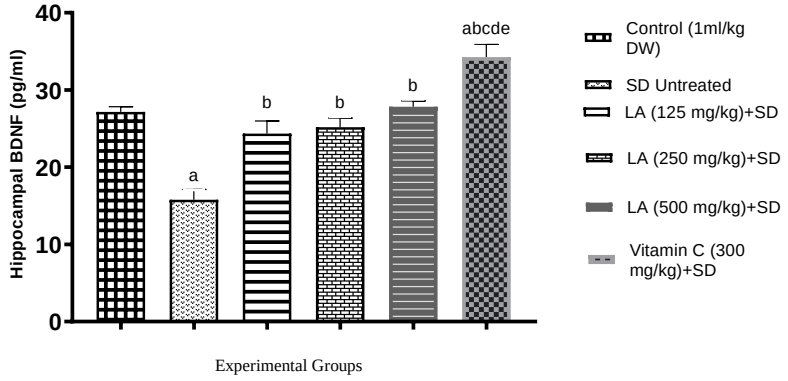

Figure 1: Hippocampal brain-derived neurotrophic factor (BDNF) level (pg/ml) in male offspring of pregnant rats exposed to sleep deprivation

Superscripts a,b,c,d,e, indicate statistically significant difference (p≤ 0.05) compared to control,, SD untreated, LA 125 mg/kg, LA 250 mg/kg and LA 500 mg/kg respectively. DW- Distilled water, SD- sleep deprivation-induced-stress, LA- Lauric Acid

The hippocampal BDNF concentration (Figure 1) was statistically significantly decreased in the SD untreated group when compared with the normal control: 15.78±1.33pg/ml vs 27.16 ± 0.66pg/ml; [F (6, 36) =22.59; p=0.0001]. The result also showed that there was a statistically significant increase in the Hippocampal BDNF level in the male offspring treated with the graded doses of LA (12mg/kg, 250 mg/kg and 500 mg/kg) and that the increase was in a dose dependent manner when compared with the SD untreated group: 24.36 ± 1.62pg/ml vs 15.78 ± 1.33pg/ml; 25.18 ± 1.18 vs 15.78 ± 1.33pg/ml; 27.82 ± 0.76pg/ml vs 15.78 ± 1.33pg/ml respectively [F (6, 36)=.22.59; p=0.0001]. The Vitamin C treated group also indicated a statistically significant increase in the hippocampal BDNF level compared with the control and the SD untreated group and with all the LA treatment groups; (34.26 ± 1.66pg/ml vs 27.16 ± 0.66; 34.26 ± 1.66pg/ml vs 15.78 ± 1.33pg/ml; 34.26 ± 1.66pg/ml vs 24.36 ± 1.62pg/ml; 34.26 ± 1.66pg/ml vs 25.18 ± 1.18pg/ml; 34.26 ± 1.66pg/ml vs 27.82 ± 0.76pg/ml respectively; [F (6, 36) = 22.59; p=0.0001].

DISCUSSION

Early development assessments provide sensitive indications of the changes in the offspring nervous system caused by prenatal stress.[25,30,33] Early neurodevelopmental landmarks such as pinnae detachment, incisor eruption, eye opening, righting reflex, air righting reflex and gait were slightly delayed in the male offspring as seen from our study. The LA and Vitamin C treated group showed early apparition of these parameters, suggesting a preventive role of LA and Vitamin C against the prenatal stress. The delay in the SD untreated group from our study could be due to prolonged fetal exposure to glucocorticoids, [34] altered BDNF level. [35, 36] Although there are relatively few data available on the effect of preventive treatment against prenatal stress, our study to the best of our knowledge is one of the earliest to report that treatment with Lauric acid enable early appearance of these early neurobehavioral developmental landmarks. Prenatal stress during different stages of pregnancy did not show any significant difference in these parameters. The difference in the result from our study could be due to the type of model used to induce the prenatal stress, duration of stress and the period of gestation when the stress was induced.[25]

Hippocampal serum levels of BDNF have been reported to be lowered in the male offspring as result of prenatal stress in rodents [5,6,37] as seen in our study. This could be due to elevated levels of corticosterone.[4] Higher levels of cortisol may be due to the over activation of the HPA axis leading to the atrophy of the hippocampal neurons, apoptosis and reduction in the granular cell regeneration of the dentate gyrus.[38] Decreased BDNF levels have been associated with Parkinsonism,[39] Huntington's disease[40] and multiple sclerosis.[41] Rodents with impairments in hippocampal-dependent learning and memory have been reported to express low hippocampal BDNF level.[42] LA from our study increased the level of hippocampal BDNF level in the male offspring of Wistar rats exposed to prenatal stress. It may be acting by decreasing the level of corticosterone, since BDNF level have been reported to be inversely associated with cortisol.[43,44] From this study, graded doses of LA which was shown to reverse the impact of maternal sleep restriction-induced stress male offspring may be acting via decreasing the levels of corticosterone and upregulation of hippocampal BDNF level, since corticosterone level is inversely associated with the level of BDNF.[43] Decrease in BDNF level has been reported to be responsible for the pronounced cognitive deficit and motor coordination disorder in some studies.[45]

Conclusion: Lauric acid reversed the delay in some neurodevelopmental parameters by increasing the hippocampal BDNF level in male offspring of Wistar rats. This was reversed upon oral treatment with Lauric acid.

Competing Interest: Authors declare that this manuscript is free of any form of competing interest whether financial or otherwise.

Source of funding: TETFUND

Authors' contributions: MIJ and AS-conceptualization of the research problem, literature review and research design**;** UPI, OS and MIJ-acquisition of data, statistical analysis; JAC, OE and ADA- manuscript editing and review for submission.

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