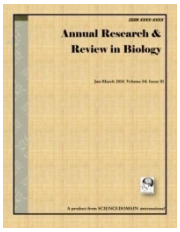

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Early Prenatal Stress Increases Body Weight and Reduces Nociception in Adult Male Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOA and IAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ODO managed the analysis of the study and literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Behavioral responses of 3-month-old male pups from female Wistar rats exposed to daily 1 hour or 3 hour restraint stress during the first 7 days of pregnancy were studied by tail flick and formalin test.
Methodology: Eighteen mature virgin female albino rats (140g-160g) were randomly allocated in a blinded fashion to 3 groups (n=6 each) and mated. Group 1 rats were the control and did not undergo restraint stress. Groups 2 and 3 rats were restrained for 1 hour and 3 hours respectively during the first 7 days of pregnancy. Six male rats only served purpose of copulation. At 3 month of age, 34 pups consisting of 10, 11 and 13 pups delivered by rats in groups 1, 2 and 3 respectively were randomly selected and studied for nociception.
Results: Body weights were higher in both 1 hour and 3 hour prenatally stressed pups compared to that of the control. The latency period during the tail immersion test in the pups prenatally stressed for 3 hour daily but not those stressed for 1 hour daily was significantly higher ($p<0.05$) compared to that of control. While there were no significant differences in the formalin score in pups prenatally stressed for 1 hour and 3 hour compared to the score of the control during the early phase, the formalin score of the pups

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prenatally stressed for 3 hour daily but not those stressed for 1 hour daily was significantly lower compared to that of the control during the late phase.
Conclusion: Our findings suggest that early prolonged prenatal stress modulates nociceptive sensitivity in 3-month-old rat and that different mechanisms are responsible for the effects of prenatal stress on acute and persistent pain in the formalin test.

Keywords: Body weight; pain; prenatal stress; Rat.

1. INTRODUCTION

It has been speculated in several prenatal studies that stressors presented during prenatal periods can have long-term effect on offspring. For instance, studies on prenatal restraint stress have proven that there are causal relationships between stress and certain factors such as disruption of reproductive events of early pregnancy [1,2], reduction in plasma levels of testosterone in male offspring [3] and an increase in behavioral attributes including emotionality, defensive behavior and anxiety [4].

Previous studies on the effects of prenatal stress on birth weight of offspring are controversial, possibly because of the difference in species and trimester studied, with some authors reporting increase [5], decrease [6-9] or no effect [10]. There is also some controversial evidence in rodent models that pre-natal stress may alter the pain experience of juvenile or adult animals. Daily prenatal stress during the last week of pregnancy caused reduction in pain perception in male rats [11] and mice [12], or increase in the severity of the spontaneous behavioral response to pain in rats [13,14].

While previous studies have investigated the role of prenatal stress during the mid period or last week of pregnancy (2^{nd} and 3^{rd} trimester respectively), this study sought to examine the effects of early prenatal restraint stress during the first 7 days of pregnancy (1^{st} trimester) on nociception in adult male rats.

2. MATERIALS AND METHOD

2.1 Experimental Animals

Sex differences in pain perception have been reported in numerous studies, with pain thresholds and pain tolerance being lower in females than in males. Previous studies on the estradiol modulation of nociception produced equivocal results, with some demonstrating longer latencies, [15,16] while another reported hyperalgesia [17]. Moreover, the estrous cycle in female rats has been shown to affect pain perception [18,19]. Therefore, we chose to investigate the effects of early prenatal stress on body weight and pain perception in adult male rats

Mature virgin female albino rats (140g-160g) were obtained from the animal house of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. They were housed at room temperature with free access to food and water *ad libitum* and were maintained on a 12-h light/dark cycle, with the lights on from 7:00 A.M. They were caged with mature males overnight during a whole estrous cycle. A vaginal smear was examined the next morning. Day 0 of pregnancy was marked by the appearance of a copulation plug. Females who failed to become pregnant after developing a copulation

plug were excluded from the study. Pregnant females were randomly allocated in a blinded fashion to 3 groups. Group 1 (n=6 pregnant rats) was the control and did not undergo restraint stress. Groups 2 (n=6 pregnant rats) and 3 (n=6 pregnant rats) were restrained for 1 hour and 3 hours respectively as described below. The male rats only served the purpose of copulation. A total of 52 (consisting of 21 males and 31 females), 50 (consisting of 22 males and 28 females), and 59 (consisting of 27 males and 32 females) pups were delivered by groups A, B, and C respectively. At 3 months of age, a total of 34 male pups consisting of about 50% each of the total pups delivered from groups 1 (n=10), 2 (n=11) and 3 (n=13) were randomly selected and studied. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by our institutional ethics committee.

2.2 Prenatal Stress Protocol

Restraint stress was performed from embryonic day 1 until day 7. The stress protocol involved placing the pregnant female in a restraint cage (19 cm×6 cm×9 cm) over which was poised two 100 W flood lights. Control dams were left undisturbed throughout gestation, while groups 2 and 3 dams respectively underwent 1 hour and 3 hour stress interventions three times on each day at 9.00 A.M, 1.00 P.M. and 4.00 P.M. This protocol has been employed in several previous studies and shown to significantly affect cardiovascular [20, 21], and neuroendocrine stress reactivity in adult offspring [22,23]. All offspring were weaned at 6 weeks of age. Food intake and body weight were monitored weekly.

2.4 Tail Immersion Test

At 3 months of age, rat pups were handled for 3 min and habituated to the testing room for 1 hour on two occasions before the day of testing and again on the day of testing. The rat was removed from its home cage and gently restrained in a towel, and its tail was immersed in 54°C water [24]. The latency to flick the tail was recorded three times; each time separated by 10 s, and the average of the three measures was calculated. All tail flick testing was performed between 9:00 A.M. and 1:00 P.M.

2.5 Formalin Test

At least 7 days later, the formalin test was administered. Tail flick testing 1 week before is not expected to affect formalin pain responses because others have reported no effect of repeated (formalin) testing at 1 week intervals [25-27]. The pups were habituated to the 30 X 30 X 30-cm transparent Plexiglas observation box for 30 min on two occasions before the day of testing and immediately before testing. The rat was removed from the observation box and restrained in a towel, and 50 µl of 1.5% formaldehyde was injected under the plantar surface of the left hind paw. The rats were placed in the observation box, and the pain behavior within the first 5 minutes of intraplantar formalin injection was recorded as early formalin score, while the pain behavior within 20^{th}- 40^{th} minute of formalin injection was recorded as the late phase. Below the floor of the box, a mirror at a 45° angle facilitated viewing of the injected paw. The behavior was scored as a 2 if the rat licked, bit, or shook the injected paw; as a 1 if the rat elevated the paw from the floor; or as a 0 if any part of the paw other than the tips of the digits was in contact with the box. The score was entered into a computer that recorded the last score entered once every half-second. A mean pain score (a weighted sum of the durations of each behavior) was calculated as the sum of the scores

divided by the number of scores in the time period. All formalin testing was performed between 9:00 A.M. and 2:00 P.M [28].

2.6 Statistical Analysis

Data were analyzed using SPSS version 16.0 for windows. All values given were the mean±S.E.M. of the variables measured. Significance was assessed by the analysis of variance (ANOVA) followed by a post-hoc Tukey multiple range test for multiple comparisons. P values of 0.05 or less were taken as statistically significant.

3. RESULTS.

The body weight was significantly higher in the pups exposed to daily 1 hour ($p<0.05$) and 3 hour ($p<0.01$) prenatal restraint stress compared to that of the control. However, there was no significant difference between the body weights of both groups of prenatally stress restraint pups (Table 1).

Table 1. Body weight and pain perception in 3 month old male rats exposed to repeated prenatal restraint stress. Values are expresses as Mean ± SEM. *P<0.05 and **P<0.01 vs control; $^{\#}$P<0.05 vs group 2.

Variables	Control pups (n=10)	Group 2 pups (n=11)	Group 3 pups (n=13)
Body weight (g)	146.43±8.50	171.43±6.52*	187.14±8.01**
Latency period (s)	4.07±0.22	5.86±1.33	7.90±0.91**
Formalin score			
Early phase (min); % inhibition	0.98±0.18	1.28±0.18; 0.31	0.85±0.09; 0.13$^{\#}$
Late phase (min); % inhibition	2.73±0.55	1.96±0.33; 0.28	1.47±0.18; 0.46*

The latency period of the control pups, though not significantly lower ($p>0.05$) compared to those exposed to daily 1 hour prenatal restraint stress, was significantly lower ($p<0.01$) compared to those exposed to daily 3 hour restraint stress during the first trimester. However, there was no significant difference ($p>0.05$) between the latency periods of both groups of prenatally stress restraint pups (Table 1).

The formalin scores during the early phase in both groups of prenatally stress restraint pups were not significantly different ($p>0.05$) from the control. However, it was significantly lower in those exposed to daily 3 hour prenatal restraint stress compared to those exposed to 1 hour restraint during the first trimester (Table 1).

The formalin score during the late phase in the control pups, though not significantly higher ($p>0.05$) compared to those exposed to daily 1 hour prenatal restraint stress, was significantly higher ($p<0.05$) compared to those exposed to daily 3 hour restraint stress during the first trimester. However, there was no significant difference ($p>0.05$) between the latency periods of both groups of prenatally stress restraint pups (Table 1).

4. DISCUSSION

The observed higher body weights than the control in pups prenatally stressed during the first trimester for 1 hour or 3 hour daily, as compared to the control group, is consistent with the previous study of Szuran et al. [5] but contrary to the previous studies that reported decrease [6-9] or no effect [10]. This increase might be a result of the previously reported decreased level of testosterone, which subsequently promotes visceral fat accumulation and obesity; or to alteration in sex steroids levels, thereby influencing fat metabolism and body composition of adult prenatally stressed males [20].

Previous non-prenatal studies have shown that chronic restraint stress caused sustained stress-induced analgesia in animals [29] and humans [30]. The increase in pain threshold in rats that underwent 3 hours prenatal restraint stress during the first trimester demonstrated that early prenatal stress could modulate pain perception in offspring during post-natal life. This observation is similar to the previous report in mice [12] but contrary to that in rats [5] on the effects of prenatal stress during the third trimester on pain threshold and may be a result of the previously reported alterations in the brain noradrenergic and serotonergic systems in adult rats exposed to early prenatal stress [31,32].

Prenatal exposure of piglets [33] and rats [13] to prenatal stress during 2^{nd} and 3^{rd} trimester respectively have been shown to increase the severity in spontaneous behavioral response to acute pain. However, the present study observed a reduced pain perception in prenatally stressed rat. The significant effect of prenatal restraint stress on the late but not early phase of formalin score in this study is consistent with previous studies [34,11]. The early (acute) phase had been reported in some studies to reflect direct effect of formalin on nociceptive C fibres, whereas the late (chronic) phase was found to be accompanied by well extended nociceptive response [35] and functional changes in nociceptive C fibres [36]. Experimental findings have indicated that substance P and bradykinins participate in the early phase while histamine, serotonin and prostaglandins are involved in the late phase [37]. Thus, when serotonin levels decrease, it is possible that certain mechanisms controlling the late phase response are altered. Studies on stress had shown that 2hours restraint stress caused decrease in brain serotonin levels [38]. Reduced nociceptive response to chronic pain may be associated with the previously reported alterations in brain noradrenergic and serotonergic systems in adult rats exposed to prenatal stress during early pregnancy [30, 31]. Further studies are needed to establish the reason for the variation in the two models of examining acute pain response observed in this study.

5. CONCLUSION

In conclusion, prolonged early prenatal restraint stress causes increased body weight and decreased nociception in adult male rats.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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