

# The Pyramid Counteracts Chronic Prenatal Restraint-stress Effects on the Milestones, Anthropometry, and Body, Brain and Adrenal Gland Weights of Pups in Rats

Mitchel Constance George<sup>1</sup>, Krishna Dilip Murthy<sup>1\*</sup>, Zainal Arifin Mustapha<sup>1</sup>

<sup>1</sup>Department of Biomedical Sciences and Therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

\*Corresponding author's email: dkmurthy@ums.edu.my

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## ABSTRACT

Prenatal exposure to chronic stress during critical periods of foetal development produces depression, attention and learning deficits, hormonal imbalances and affects the brain. The effect of prenatal restraint-stress on the postnatal developmental milestones, anthropometric measurements, and the body, brain and adrenal gland weights of the pups were examined and compared with the unrestrained control and the restrained group under the pyramid at postnatal day 10 and 21. Pregnant rats were restrained (9h/day) from gestation day 7 until parturition. Results showed significant delay in the milestones by one day in the restraint control (RC) compared to the unrestrained normal control (NC), while pups of the restrained pyramid (RP) group did not show the delay. Significant decreases in the anthropometric measurements, body and brain weights in RC group were observed at both postnatal days, while the RP group results matched with the NC group. Significant increase in the adrenal weights was found in the RC group compared to NC group and not the RP group. Results suggest prenatal restraint-stress definitely hampers the developmental milestones, anthropometric measurements, and body and brain weights of the young offspring. Results suggest, pyramid environment counteracts and protects the deleterious effects of chronic prenatal stress.

**Keywords:** anthropometry, milestones, prenatal stress, pyramid

## INTRODUCTION

Any disruption of the maternal environment during gestation leads to behavioural changes in the offspring. Studies have shown that stress during gestation can induce early and long-lasting effects on neurobehavioural development of the pups. Zaneta et al. reported

high maternal cortisol levels to impact the foetal growth particularly in the male offspring.<sup>1</sup> Earlier reports<sup>2-11</sup> show effects of prenatal stress on cognitive, behavioural and psychosocial aspects to be mostly mediated by the effects of maternal stress on the structure and function of the foetal brain. Earlier research<sup>10, 11</sup> has shown the significant effect of prenatal stress on the CA3 hippocampal pyramidal neurons as well as the beneficial effects of the pyramid environment. However, there is very little information on the effect of gestational stress on the overall postnatal developmental milestones, anthropometric measurements, and body and brain weights in the rats.<sup>12</sup> This research focused on the effect of prenatal stress outside and under a wooden pyramid on the postnatal development of the offspring at postnatal day 10 and 21.

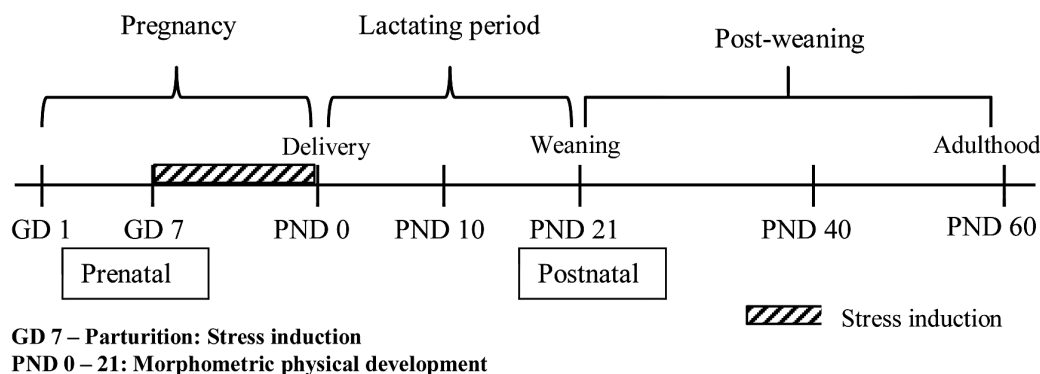
## MATERIALS AND METHODS

Sprague Dawley rats weighing 180 – 250 g were used in the study. Pregnant rats were housed in polypropylene cages (25 × 47.5 × 20 cm) individually. The environmental conditions were controlled at 23 ± 2°C, 50 ± 5% RH on a 12:12 h light/dark cycle. They were allowed to food and water *ad libitum*. All procedures were performed in accordance with the guidelines of National Institute of Health Guide for Care and Use of Laboratory Animals,<sup>13</sup> and the study was approved by the Animal Experiments Ethics Committee of the Institution. All efforts were made to minimize the suffering and number of animals. Food pellets were purchased from Cargill Farm Animal Food Product which consisted of 16% protein, 2.5% crude fat, 18% crude fibre, 13% moisture, 0.75% calcium and 0.45% phosphorus.

### Prenatal Stress (PS)

The pregnant rats were randomly divided into normal control (NC), restrained (stressed) control (RC) and restrained (stressed) under the pyramid (RP) groups. The RC and the RP groups were exposed to restraint stress in a wire-mesh restrainer ( $L = 15$  cm;  $W = 7$  cm;  $H = 7$  cm) for nine hours per day from gestation day 7 until parturition.<sup>10-11</sup> The NC group of pregnant

rats was left unstressed in their home cages. Pups ( $n = 10$ /group) born to these respective mothers were the subjects of the study. At birth pups were housed with their respective mothers until postnatal day 10 and 21. However, they were observed every day for the development of the milestones. The animals of all the groups were weighed on postnatal days (PND) 10 and 21, euthanized and the brain and adrenal glands were dissected out and weighed (Figure 1).



**Figure 1** Periods of prenatal and postnatal development in the rat

### Pyramid Model

This consisted of a wooden pyramid-shaped model, locally fabricated having the dimensions of height 30”, base 45” and the sides 41.5”. Holes were drilled on all sides for ventilation and a glass window on one side for observation. The four sides had an angle of 51° to the base and met at the apex of the pyramid as reported earlier.<sup>8, 10-11, 14-15</sup>

### Pyramid Housing

The pyramid was aligned to face the four cardinal north, south, east and west directions. It was placed to face in the true magnetic north-south axis to provide the maximum beneficial effects as earlier reported.<sup>8, 10-11, 15</sup> Pregnant rats were restrained in the wire-mesh restrainers and placed on an elevation at one-third the height (10 inches) from the base of the pyramid to attain the maximum effect of the pyramid environment.<sup>8, 14</sup>

### Statistical Analysis

Data obtained were analysed using one-way ANOVA and Bonferroni test with SPSS version 17 software.  $p < 0.005$  was considered significant. Results are expressed as the mean  $\pm$  SE.

### RESULTS

The results are expressed as mean  $\pm$  SEM. There was a delay in the developmental milestones in the restrained control group compared to the unrestrained group; while the group restrained under the pyramid did not show the delay. The body weights and brain weights of pups prenatally exposed to restraint significantly decreased compared to the unrestrained controls at postnatal day 10 and 21 respectively. However, no such change was observed in the pups born to mothers restrained under the pyramid but the results compared well with the unrestrained control group at both the postnatal days. Adrenal glands on the other hand showed a significant

increase in weight in the restrained group of pups while the weights of the adrenals of the pups restrained under the pyramid were closer to the unrestrained control group.

### Developmental Milestones

The appearance of fur, opening of the eye and ear as well as detachment of the pinna were

observed and compared. There was a delay by one day in all the four milestones observed in the restrained group compared to the unrestrained control group which was significant ( $p < 0.003$ ), while there was no difference in the pyramid restrained group but compared well with the unrestrained control group (Table 1).

**Table 1** Effect of chronic restraint-stress on the day of appearance of developmental milestones (in days)

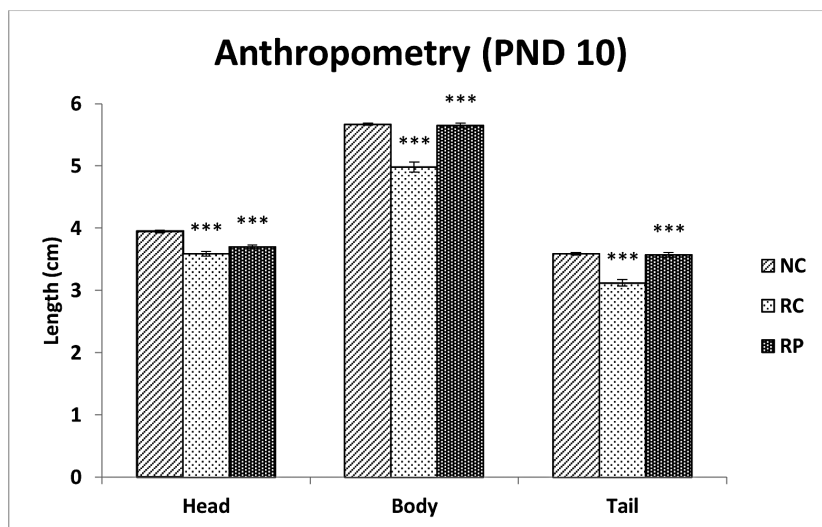
Milestone (day)	NC (n = 10)	RC (n = 10)	RP (n = 10)	ANOVA	
				F	p
Appearance of fur	5.10 ± 0.18	6.00 ± 0.21	5.00 ± 0.21	<b>7.51</b>	<b>0.003***</b>
Detachment of pinna	5.30 ± 0.21	6.20 ± 0.20	5.10 ± 0.18	<b>8.75</b>	<b>0.001***</b>
Opening of the ear	15.20 ± 0.20	16.20 ± 0.20	15.10 ± 0.23	<b>8.26</b>	<b>0.002***</b>
Opening of the eyes	16.00 ± 0.21	17.00 ± 0.21	15.90 ± 0.18	<b>0.17</b>	<b>0.001***</b>

Notes:  
Values are mean ± SE from 10 rats/group  
\*\*\*ANOVA  $p < 0.003$ ]

### Anthropometry

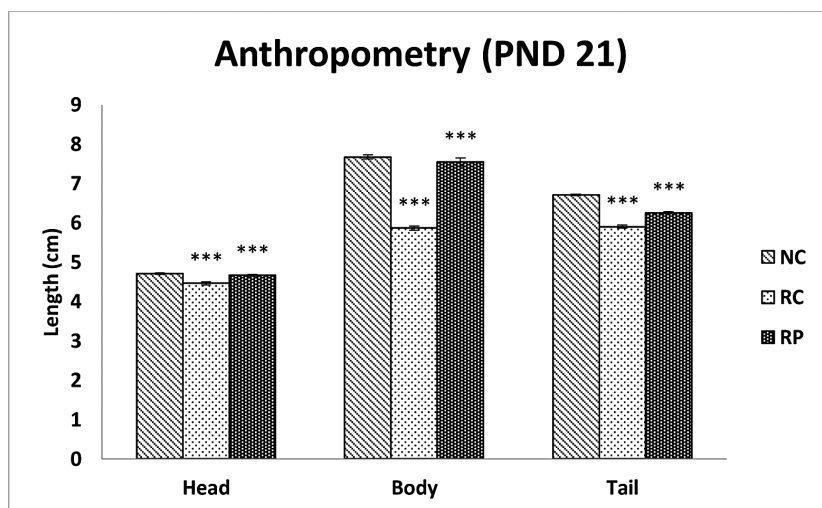
Anthropometric measurements of the lengths of the head, body and tail were also compared at postnatal day 10 and 21 among the groups. Once again there were significant ( $p < 0.003$ )

decreases in the head, body and tail lengths in the RC groups compared to the unrestrained NC group, while the results of the group restrained under the pyramid (RP) compared well with the unrestrained NC controls but was significantly increased compared to the RC group (Figures 2 and 3).



[NC = Normal unrestrained control; RC = Restrained control outside; RP = Restrained under the pyramid. Values are mean  $\pm$  SE from 10 rats/group; \*\*\*ANOVA,  $p < 0.001$ ]

**Figure 2** Effect of chronic restraint-stress on anthropometric measurements at postnatal day (PND) 10



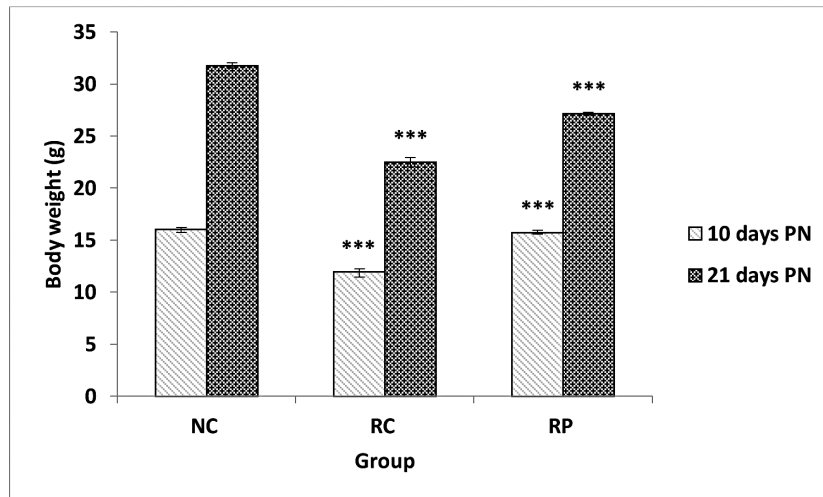
[NC = Normal unrestrained control; RC = Restrained control outside; RP = Restrained under the pyramid. Values are mean  $\pm$  SE from 10 rats/group; \*\*\*ANOVA,  $p < 0.001$ ]

**Figure 3** Effect of chronic restraint-stress on anthropometric measurements at postnatal day (PND) 21

### Body and Brain Weights

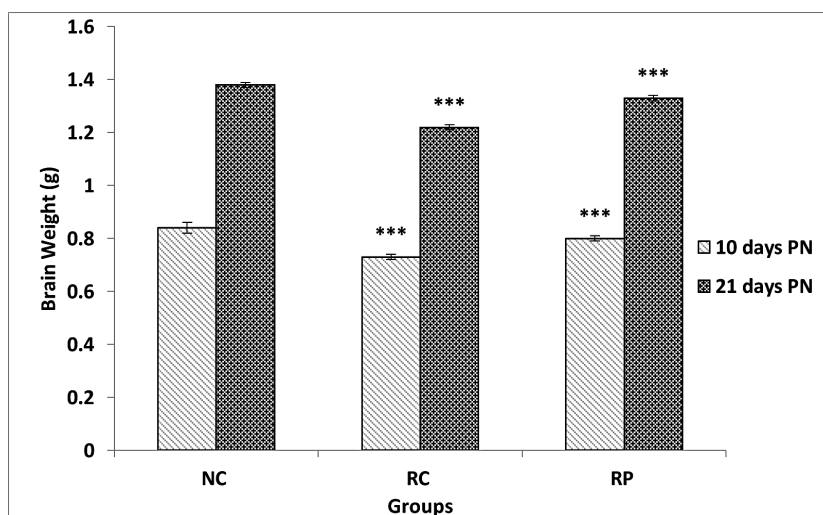
The RC group of animals showed a significant reduction in their body weights at PND 10 ( $11.94 \pm 0.29$ ;  $p < 0.001$ ) and PND 21 ( $22.47 \pm 0.47$ ) compared to NC group ( $16.01 \pm 0.22$

and  $31.77 \pm 0.26$ ;  $p < 0.001$ ) respectively. On the other hand the RP group of animals' body weights were significantly higher compared to the RC group ( $15.72 \pm 0.23$  and  $27.16 \pm 0.13$ ) but similar to those of the unrestrained NC group at both PND 10 and 21 respectively (Figures 4 and 5).



[NC = Normal unrestrained control; RC = Restrained control outside; RP = Restrained under the pyramid. Values are mean  $\pm$  SE from 10 rats/group; \*\*\*ANOVA,  $p < 0.001$ ]

**Figure 4** Effect of chronic restraint-stress on the body weight on postnatal day (PND) 10



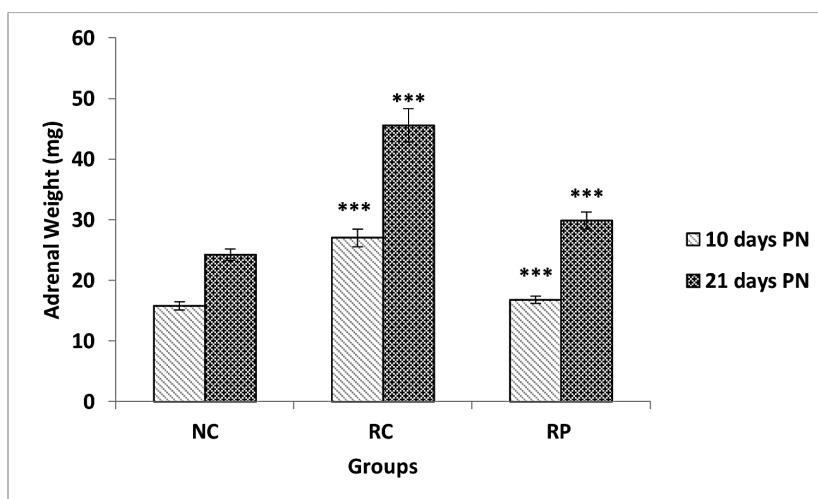
[NC = Normal unrestrained control; RC = Restrained control outside; RP = Restrained under the pyramid. Values are mean  $\pm$  SE from 10 rats/group; \*\*\*ANOVA,  $p < 0.001$ ]

**Figure 5** Effect of chronic restraint-stress on the brain weight on postnatal day (PND) 21

### Adrenal Gland Weights

Weights of the adrenal glands were used as an indicator of the stress. The restrained RC group adrenal glands weighed significantly ( $p < 0.001$ ) more ( $27.01 \pm 1.42$ ) compared to the unrestrained NC group ( $15.81 \pm 0.70$ ), while the

restrained-pyramid (RP) group adrenals ( $16.80 \pm 0.56$ ) were closer to those of the unrestrained NC group at PND 10. Similar trend was also seen at PND 21 (Figure 6). When compared with the RC group the adrenal weights of the RP group were significantly reduced at both the ages.



[NC = Normal unrestrained control; RC = Restrained control outside; RP = Restrained under the pyramid. Values are mean  $\pm$  SE from 10 rats/group; \*\*\*ANOVA,  $p < 0.001$ ]

**Figure 6** Effect of chronic restraint-stress on the adrenal gland weight on postnatal day (PND) 10 and postnatal day (PND) 21

## DISCUSSION

Though stress is essential and triggers homeostatic mechanisms to combat, chronic stress is found to be deleterious to the well-being. Gestation period is vulnerable to various external stimuli, such as stress. It affects the postnatal development of the central nervous system inducing neurological deficits as reported earlier,<sup>16–20</sup> report prenatal continuous light exposure, a form of stress to have adverse behavioural effects leading to increased chronic oxidative stress and altered gene expression. On the other hand, other researchers<sup>21–23</sup> have shown that environmental enrichment during gestation effectively prevents behavioural deficits and abnormal structure of synapses in prenatal stressed offspring.

Results of the offspring were compared between the unrestrained control, restrained control and those restrained under a wooden pyramid. The offspring of the stressed group of rats showed a significant delay in the opening of the eyes and ears growth of fur, and detachment of the pinna compared to the control group. However, these changes of delay were not seen in the stressed groups under the wooden pyramid. In fact the results were similar to the

unstressed control group. In other words, the animals did not show evidence of stress when kept under the pyramid. These results are in agreement with the results on the corticosterone levels and the dendritic branching of the CA3 hippocampal neurons under similar conditions reported earlier.<sup>10–11</sup>

Dancause et al. have reported prenatal stress results in shorter long bones in adulthood, independently of effects on overall body size.<sup>24</sup> The present study adds to a growing body of evidence suggesting prenatal stress is a risk factor for not only poor linear growth of bones as earlier reported,<sup>23, 25</sup> but also the overall postnatal physical development of the pups. Maternal stress retards foetal development in rats with delay in the postnatal milestones. Results of the present study also suggest that the geometric shape of the pyramid and the energy within reduces or ameliorates the effects of restraint stress. Results of the present study compare well with previously reported results on the plasma levels of corticosterone and dendritic arborisation of CA3 neurons in the hippocampus<sup>10, 11</sup> and the effect seen on the oxidative stress parameters.<sup>26</sup> Seckl et al. state that the excess maternal cortisol (corticosterone in rodents), is typically changed by the foetal-

placenta into the inactive form (cortisone), which reaches the foetus in high concentrations and is responsible for the alterations in the foetal development and growth.<sup>27</sup>

## CONCLUSION

Stress and the environment in which it is experienced have deleterious effects on the overall physical development, the foetus being most vulnerable during gestation. Effects are seen on the development of the offspring born to the mothers stressed during gestation. The current results also suggest that the pyramid's geometric shape helps reduce stress and its deleterious effects. However, it will be interesting to explore whether the pyramid environment can reverse the effects of chronic stress. Further studies on analysis of the quality and quantity of energy developed within the pyramid structure should help us better understand how such environment acts as an anti-stressor.

## CONFLICT OF INTEREST

The authors have no conflict of interest to declare regarding the study described in this article and in the preparation of the article.

## ACKNOWLEDGEMENTS

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