International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 4 | Issue 2 | April - June - 2016
www.ijamscr.com

Research article *Medical research*

Effects of Chronic Prenatal Restraint Stress on Motor Coordination in Post weaned Male and Female Wistar Rats

Saju B Cherian*, Bairy KL[1], Muddanna S Rao[2]

**Department of Anatomy, Apollo Institute of Medical Sciences and Research, Jubilee Hills, Hyderabad- 500096, India.*

[1]*Department of Pharmacology, Kasturba Medical College, Manipal- 576104, Karnataka, India.*

[2] *Faculty of Medicine, Kuwait University, Safat- 13110*

***Corresponding author: Saju B Cherian**

Email id: sajubinu@gmail.com

ABSTRACT

Background

Maternal stress has a variety of deleterious effects on developing fetus that manifests into adulthood.

Aims

The present study was designed with the view to examine the gender- specific effects of chronic maternal stress on sensorimotor coordination following prenatal stress in rats.

Materials and Methods

Pregnant Wistar rats were subjected to restraint stress from embryonic day 11 till delivery. Male and female rat pups on the 33rd day of postnatal life were subjected to rotarod test for motor coordination and balance.

Results

It was observed that prenatal stress caused significant effects in stressed males when compared to that of control and did not show any effect in stressed females.

Conclusion

These data reinforce the view that prenatal stress affects motor coordination in a sex-specific manner and the diminished effect seen in females could be due to the estrogen- mediated neuroprotection on hippocampal function.

Keywords: Prenatal Stress, Hippocampus, Rotarod Test, Motor Coordination.

INTRODUCTION

Development is shaped by a highly complex process involving the interplay of complex biological and environmental factors. Prenatal or intrauterine development plays a critical role in normal physical, mental and behavioral development of an individual. Any insults to the environment of the fetus can have an effect upon development beginning at conception and not at birth. The environment does indeed begin to influence the individual as soon as he or she is conceived.

Maternal nutrition[1], exposure to environmental toxicants[2], and stressful disturbances[3] of the pregnant female are among the many variables that

can affect *in utero* conditions and impair the maturational trajectory of the fetus. All sorts of early environmental influences can leave indelible imprints and influence the development of an offspring. In most of the cases, affects of such insults will be carried to the young age or even to the whole life span of the individual[4]**.** Though any system of the body is the target of flawed development, nervous system becomes the main target of faulty development.

Prenatal stress evokes a cascade of neurohumoral events which triggers HPA axis hyperactivity in response to stress throughout life[5]. It can affect foetal neurodevelopment and result in increased risk of depression in adulthood. It promotes increased maternal hypothalamo-pituitary-adrenal gland (HPA) secretion of glucocorticoid (GC), leading to increased foetal and maternal GC receptor activity[6].

Prenatal stresses of different nature and duration applied during various gestational periods have shown delay in developmental reflexes such as cliff aversion, startle, righting, forelimb placing, grasping, bar holding, hair growth, appearance of ear, eye opening and decreased body weight gain[7]. This particular type of stress has been shown to decrease the locomotor activity and immobility in the constrained swim test[7]**.** Gestational stress is reported to increase the anxiety like behavior in elevated plus maze or in open field[8] and decrease the spatial learning and memory in T-maze [9]**,** diminution of time spent in the target quadrant in the water maze, spontaneous alternation test in Y-maze[10] and passive avoidance learning [11]. Thus, there are many instances in which neural function and cognition are either facilitated by prenatal stress[12] or even not affected[13].

Literature suggests that prenatal stress does not exert the same neuroendocrine effects in males and females. However, these previous studies have used a range of prenatal stress paradigms and have not consistently compared males and females in sexually differentiated behavioral tasks.

Hence an attempt has been made here to find out the effect of the chronic prenatal stress paradigm on the sensorimotor coordination in post weaned rats and to compare the gender- specific effects if any.

MATERIALS AND METHODS

Experimental animals and housing conditions

In-house bred male and female Wistar strains of rats were used in the study. Animals were bred in the Central Animal Research Facility of Manipal University, Manipal. Adult rats (3 months old) were housed in air conditioned animal rooms with constant light-dark cycle (12:12 h), controlled temperature (22±3ºC) and humidity (50±5%). The animals had free access to food (Gold Mohur; Lipton India Ltd.) and water *ad libitum*.

Breeding and maintenance of animals were done according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethical Committee (I.A.E.C) approval was obtained before the conduct of the study (IAEC/KMC/06/2005-2006) and care was taken to handle the rats in humane manner.

Timed pregnancy in rats

To get the pregnant rats of known gestational days, all female rats were subjected to vaginal smear test[14].The rats in the estrus cycle were mated with adult male rats overnight. Vaginal smear was examined within 12 hours after mating. The presence of sperms in the smear confirms the mating, and that day was taken as day zero of pregnancy for further counting the days. The pregnant female rat was separated from other rats and housed individually with proper label indicating the day of conception. Pregnant females were assigned randomly into 'No stress' and 'stress groups' (n=20 in each group). The rats in 'No stress group' remained without any further procedures and allowed to deliver the pups. The rats in the 'Stress group' were subjected to restraint stress.

Prenatal stress protocol

Pregnant rats in the 'stressed group' were subjected to daily restraint stress from 11^{th}gestational day, till they deliver the pups. The pregnant rats were restrained stressed by placing them individually in a wire mesh restrainer, 6 hours per day[15] (**Figure-1**). The wire mesh restrainer has a wooden base and stainless steel wire mesh restrainer hinged to the base. A padlock and latch will help to secure the rat in the restrainer. This type of restrainer is claimed to restrict the animal's

movement without any pain, discomfort or suffocation.

Experimental animal groups

The pups born to 'No stress' and 'Stress' group of pregnant rats remained with their respective mothers until 21 days after birth. Number of pups for each dam were culled to six on 8th post-delivery day by removing the extra pups if any in both groups of dams. The male and female pups from 'No stress' dams were designated as normal control-male (NCM) and Normal control-female (NCF) groups. The male and female pups from 'Stressed' dams were designated as normal Stressed-male (STM) and Stressed-female (STF) groups.

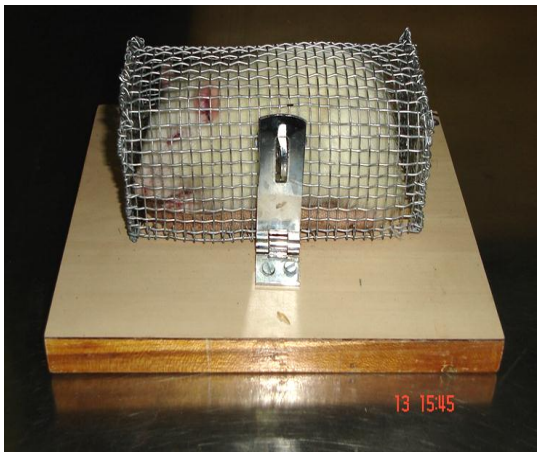

Figure: 1 A rat in a wire mesh restrainer

Rotarod test

The purpose of the present experiment was to assess the sensorimotor coordination following prenatal stress in rats. The rotarod apparatus consists of a rotating rod, which was placed in the center (20cm from the periphery) of an aluminium drum (**Figure -2**). The drum was divided into six compartments. Rats were trained to hold on the rotating rod (5 RPM rotation speed) of the apparatus in 2 trials of one minute each. After training, animals were placed on the rotating rod at an initial speed of 5 RPM and an acceleration of 7 rpm$^{2.}$ The duration for which a rat can hold the rotating rod is recorded. For each rat four trials were given.

Figure -2: A-Rotarod apparatus (Closed)
B-Rotarod apparatus (Open)

Result

Rotating rod holding time in the rotarod instrument was significantly decreased in the male rats born to the stressed mothers compared to normal controls (P<0.001, P<0.001, P<0.001 on 1st, 2nd and 3rd trials respectively). However there was no difference between the control female and stressed females. (Fig.3).

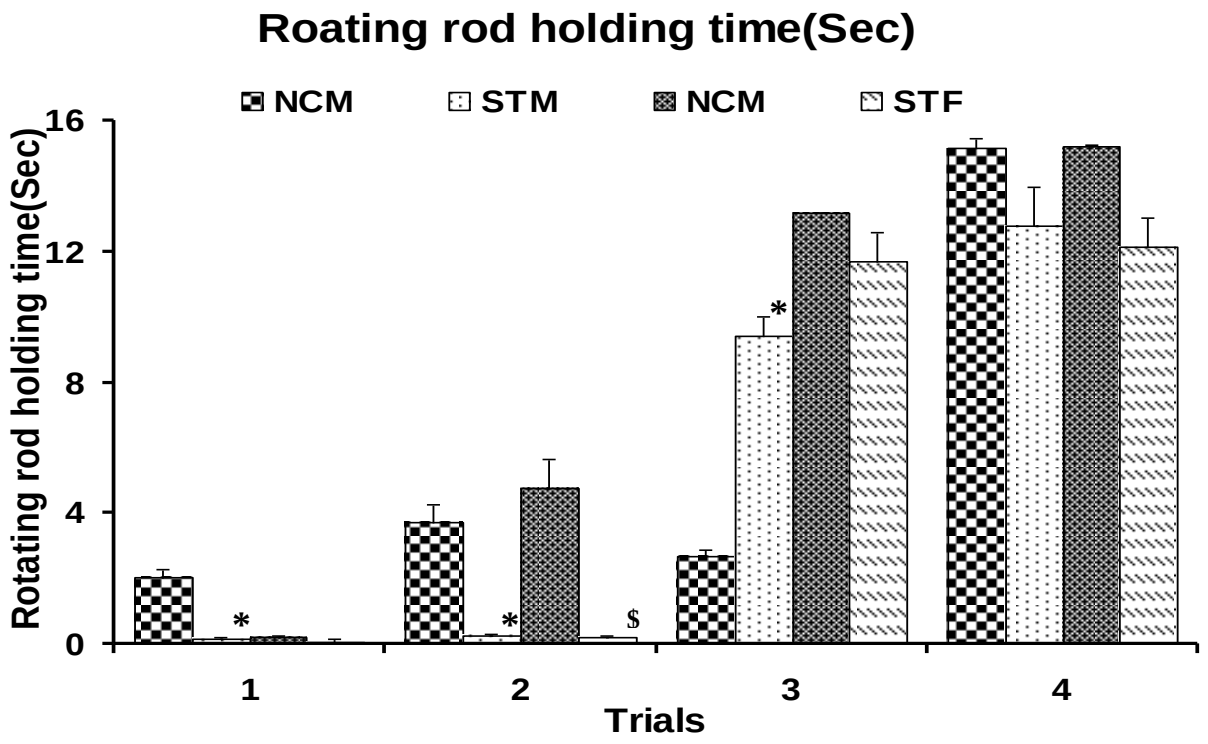

Figure 3: Rotating rod holding time for rats in different groups in rotarod test. NCM-normal control male (n=12), NCF- normal control female (n=12), STM -stressed male (n=12), STF-stressed female (n=12). Note (i) stressed males were holding the rotating rod in the rotarod apparatus for significantly less time in the first two trials compared to control males and hold significantly more time in 3rd trial. NCM vs STM: ***P<0.001; NCF vs STF: $$$ P<0.001; STM vs STF and NCM vs NCF: not significant. (One way ANOVA, Bonferroni's test. Each data represents mean± SEM).

DISCUSSION

The use of rotarods to measure balance in rodents (evaluating the duration that they can maintain balance at increased rotation speed) has been described since the 1950s[16]. The rotarod is used to assess the ability of an animal to balance on a rotating rod. As the speed of rotation is increased, it becomes difficult for the animal to keep its balance. In the present study, we observed that prenatal stress affected the motor co-ordination in male rats when compared to controls and not in female rats, which shows that effect of prenatal stress on motor coordination is gender specific.

The cerebellum is a region of the brain that plays an important role in motor control. The cerebellum does not initiate movement, but it contributes to coordination, precision, and accurate timing. It receives input from sensory systems and from other parts of the brain and spinal cord, and integrates these inputs to fine tune motor activity[17].Changes in cerebellar structure claimed to be responsible for impaired motor function[18].It has

been shown that maternal stress caused a decrease in granular layer of cerebellum[19]. Intrauterine stress alters the morphology of granule cells and causes a profound and long-lasting deficit in their inter-neuronal connectivity which could be the reason for our finding in the experiment. The results of this study suggest that prenatal exposure to chronic stress affects cerebellar development and the response to prenatal stress appears to be different in male and female rats. Prenatal stress did not affect the motor co-ordination in stressed female offspring which is due to the estrogen – mediated neuro protection on hippocampal function.

CONCLUSION

These data show that excess stress-induced CORT (corticosterone) can alter the programming of the foetal brain and predispose it to alterations in motor coordination that are gender specific revealing the decisive importance of nine months of pregnancy for the rest of the child's life and that of the adult it will become.

REFERENCES

[1]. Firth EC, Rogers CW, Vickers M, Kenyon PR, Jenkinson CM, Blair HT. The bone-muscle ratio of fetal lambs is affected more by maternal nutrition during pregnancy than by maternal size. Am J Physiol Regul Integr Comp Physiol 294(6), 2008, 1890-94

[2]. Gilbert ME, Kelly ME, Samsam TE, Goodman J. H. Chronic developmental lead exposure reduces neurogenesis in adult rat hippocampus but does not impair spatial learning. Toxicol Sci. 86(2), 2005, 211-13.

[3]. Igosheva N, Klimova O, Anischchenko T, Glover V. Prenatal stress alters cardiovascular responses in adult rats. J. Physiol. 557(1), 2004, 273- 85

[4]. Roman E, Nylander I. The impact of emotional stress early in life on adult voluntary ethanol intake – results of maternal separation in rats. Stress 8(3), 2005, 157-74

[5]. Vall'ee M, Maccari S,Dellu F, Simon H, Le Moal M ,Mayo W. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. Eur J of Nerosci 11, 1999, 2906-16

[6]. Hauser J, Feldon J, Pryce CR. Direct and dam-mediated effects of prenatal dexamethasone on emotionality, cognition and HPA axis in adult Wistar rats. Horm Behav. 56(4), 2009, 364-7

[7]. Drago F, Di Leo F , Giardina L. Prenatal stress induces body weight deficit and behavioural alterations in rats: the effect of diazepam. Eur Neuropsychopharmacol. 9, 1999, 239- 45

[8]. Kohman RA, Tarr AJ, Day CE, Mc Linden KA ,Boehm GW. Influence of prenatal stress on behavioral, endocrine, and cytokine responses to adulthood bacterial endotoxin exposure. Behav Brain Res. 193(2), 2008, 257- 68

[9]. Son GH, Geum D, Chung S, Kim EJ, Jo JH, Kim CM et al. Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity. 26(12), 2006, 3309-18

[10]. Darnaudéry M, Perez-Martin M, Bélizaire G, Maccari S ,Garcia-Segura LM. Insulin-like growth factor 1 reduces age-related disorders induced by prenatal stress in female rats. Neurobiol Aging. 27(1), 2006, 119- 27

[11]. Wu J, Song TB, Li YJ, He KS, Ge L,Wang LR. Prenatal restraint stress impairs learning and memory and hippocampal PKCbeta1 expression and translocation in offspring rats. Brain Res. 1141, 2007, 205-13

[12]. Yang J, Han H, Cao J, Li L , Xu L.Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring. Hippocampus 16(5), 2006, 431-6

[13]. Schwalbe L, Schächinger H, Kloet ER, Oitzl MS. Stress impairs spatial but not early stimulus-response learning .Behav Brain Res. 2010.

[14]. Lesage J, Del- Favero F, Leonhardt M. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behavior disturbances in the aged rat. J Endocrinol 181, .2004, 291-96

[15]. Igosheva N, Klimova O, Anishchenko T and Glover T. Prenatal stress alters cardiovascular responses in adult rats. J Physiol 557(1), 2004, 273-285

[16]. Dunham N.W and Miya T.S. A note on a simple apparatus for detecting neurological deficits in rats and mice. J.Am. Pharnac. Assoc. Sci Ed. 46, 1957, 208-209

[17]. Fine EJ, Ionita CC, Lohr L. "The history of the development of the cerebellar examination". Semin Neurol. 22 (4), 2002, 375–84.

[18]. Nguon K, Ladd B, Baxter MG, Sajdel-Sulkowska . Development of motor coordination and cerebellar structure in male and female rat neonates exposed to hyper gravity. Advances in Space Research 38(6), 2006, 1089-1099

[19]. Ulupinar E, Yucel F. Prenatal stress reduces inter neuronal connectivity in the rat cerebellar granular layer. Neurotoxicol Teratol. 27(3), 2005, 475-84