Chronic prenatal restraint stress induced memory impairment in passive avoidance task in post weaned male and female Wistar rats

Saju Binu Cherian1, K L Bairy2 & Muddanna S Rao3
1Department of Anatomy, Melaka Manipal Medical College, Manipal Campus, ICHS, Manipal, Karnataka, 576 104, India
2Department of Pharmacology, and 3Department of Anatomy, Kasturba Medical College, Manipal, 576 104, India

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With a view to examine the effect of chronic maternal stress on cognitive function in the offspring during young age, pregnant Wistar rats were subjected to restraint stress from embryonic day 11 till delivery. Male and female pups born to these stressed rats were subjected to passive avoidance test on postnatal day 30 and 31. Results were compared with rats of the same age and sex born to control mothers, which were not stressed. The results showed that prenatal maternal restraint stress impairs the memory retention during young age in both sexes. The memory retention deficit induced by maternal restraint stress was evident in the decreased latency to enter the dark compartment of passive avoidance apparatus by the rats born to stressed mothers. The observed behavioral deficit may be due to the insult of stress on the developing hippocampus, a structure of the brain concerned with learning and memory. The results suggest that prolonged prenatal stress leads to long lasting malfunction in the behavioral development during young age in both male and female young rats. However when compared to their respective stress naïve controls, it seems evident that prenatal restraint stress has a less effect on females which could be due to their oestrogenic effects. These data reinforce the view that prenatal stress affects cognitive development in a sex-specific manner.

Keywords: Cognition, Hippocampus, Passive avoidance test, Prenatal stress.

Life exists by maintaining a complex dynamic equilibrium that is constantly challenged by a variety of intrinsic and extrinsic adverse stressors. During the perinatal period, the development of an organism is subjected to complex environmental influences. A vast number of factors such as genetic makeup1,2 epigenetic factors like nutrition3, and environmental toxins4,5 and stressful condition6,8 play a major role on normal growth in the intrauterine development. In most of such instances, affect of such insults is carried to perinatal and young age and even to whole lifespan of individual9,10. Early adverse experiences such as prenatal stress significantly affect the development of brain and the organization of cognition11-16. Early environmental experiences have long-lasting effects on adult cognition in humans17,18 and animals19.

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 gain20. This particular type of stress has been shown to decrease the locomotor activity21 and immobility in the constrained swim test20. Prenatal stress is reported to increase the anxiety like behavior in elevated and plus maze or in open field22-25 and decrease the spatial learning and memory in T-maze21,26-29, diminution of time spent in target quadrant in the water maze30,31, spontaneous alternation test in Y-maze21,31, and passive avoidance learning20,21,26,27,32.

In male rats prenatal stress is reported to decrease the learning ability in water maze33,35 and increase the tight rope test score36, increase the emotionality in open field37, and depression like behavior in forced swim test37. In female rats prenatal stress results in increased learning in water maze24,31,33, decreased learning ability and memory38 and elevated anxiety like behavior35. Both male and female prenatally stressed rats showed decreased performance in spontaneous alternation and delayed alternation in Y-maze21,26, delayed memory deficit, spatial and non spatial memory and short and long term memories. Thus there exists sexual dimorphism in the effects of prenatal stress on the postnatal cognitive behavioral development.

*Correspondent author
Telephone: 91- 9448819568
E-mail: sajubinu@rediffmail.com
Literature on passive avoidance learning in the prenataly stressed rats is also sexually dimorphic. Both male and female prenataly stressed rats will show decreased performance in passive avoidance19. Male rat’s learning performance will be decreased when they are prenataly stressed20. Step through latency and percentage of animals reaching the criterion are decreased in the stressed female rats21. Thus there exists a controversy regarding the performance of prenataly stressed male and female rats particularly in passive avoidance learning and memory retention as well. Hence present study has been undertaken to assess the passive avoidance behavior in the prenataly stressed male and female rats.

Materials and Methods

Animals and housing conditions — In-house bred adult female rats of Wistar strain were housed in presence of a male rat. The animals had free access to food and water under a constant light-dark cycle (12:12 h) with controlled temperature (22±3°C) and approximately 50±10% RH in an air conditioned animal house. Institutional Animal Ethical Committee (I.A.E.C) approval (IAEC/KMC/06/2005-2006) was obtained before the conduct of the experiment and care was taken to handle the rats in humane manner.

Timed pregnancy in rats — For breeding, virgin female rats were placed with adult males overnight during whole estrus cycle. A vaginal smear39 was examined on the next day morning. The presence of sperms in the smear will confirm the mating and that day is taken as day zero of pregnancy for further counting the days. Each pregnant female was separated and kept in an individual cage and fed with standard feed. Pregnant females were assigned randomly into control mothers (n=6) and prenatal stressed mothers (n=6) groups.

Prenatal stress protocol — Pregnant rats in the stressed group were stressed daily from embryonic day 11 till delivery. They were exposed to a regimen of restraint stress by placing in a wire mesh restrainer for 6 hours per day40. The wire mesh restrainer has a wooden base and stainless steel wire mesh restrainer hinged to the base. The restrainer is of 11cm (L) × 6cm (B) × 6cm (H) dimensions (modified from Sunanda et al.41.). This type of restrainer restricts the animal’s movement only without any pain or suffocation. All dams delivered at term (21-22 days of gestation). Control mothers were left undisturbed in the home cage for the duration of their pregnancies. The offspring of both groups were raised by their biological mothers until weaning (21 days after birth).

Experimental design — After weaning, two male pups, and two female pups were selected from each of the control mother and designated as normal control (NC, n=12) group. Similarly, two male pups and two female pups were selected from each of the stressed mother and designated as stressed (ST, n=12) group. Rats in both NC, and ST group were subjected to passive avoidance test on 30th and 31st postnatal day as described below.

Passive avoidance test — To test the memory retention, 30 days old rat pups in normal control and stressed (n=12) groups were subjected to a passive avoidance test42. The test determines the ability of a rat to remember a foot shock delivered 24 h prior to the memory retention test. The apparatus used for this purpose was the passive avoidance apparatus which consisted of two compartments: (i) bright, larger compartment and (ii) dark smaller compartment. The smaller compartment was equipped with a grid floor which is attached to a foot shock source. Experiment begins by placing a rat in the illuminated larger compartment for exploration. The door between the two compartments remains open at this time. The rat is allowed to explore both the compartments for 5 min. This is followed by three test trials of 5 min each. At the end of 3rd test trial, as soon as the animal stepped into the dark compartment, the door between the two compartment was closed and a single foot shock was delivered through the grid floor (1.5 mA, 1 sec). The rat was held in the dark compartment for an additional 10 sec, to allow the animal to form an association between the properties of the chamber and the foot shock. It was then returned to its home cage. The memory retention test was done 24 h after foot shock. The rat was placed in the bright compartment and the time taken (the step-through latency) for it to enter the dark compartment for the first time was recorded using a stop-watch. A maximum of 180 sec were given for the rat to explore. Fraction of time spent in the dark and bright compartment for each rat was noted.

Statistical analysis — Data were presented as mean ±SE. Unpaired, two tailed Student’s t-test was used to compare the statistical significance of the data using Graph pad in stat software. Probability (P) value < 0.05 was considered statistically significant.
Results

The rats born to the control mothers and stressed mothers were healthy during the study period. There was no visible abnormal behavioral development in the rats born to stress mothers.

I. Passive avoidance performance in male rats:
During passive avoidance exploration test, behavior of the rats born to control mothers (normal control group, NC) and rats born to stressed mothers (stressed group, ST), was not distinguishable. Both the groups of rats spent similar time in the dark and bright compartments of the passive avoidance apparatus (data not shown).

(a) Latency to enter the dark compartment in male rats — During passive avoidance retention test, the rats born to the stressed mothers took shorter time to enter the dark compartment than the rats born to the control mothers. Normal rats remember the foot shock given in the dark compartment of the passive avoidance apparatus at the end of exploration, and avoid entering the dark compartment during retention test for a long period of time or will not enter the dark compartment, there by avoid their normal behavior of exploring the dark chamber. A rat having cognitive impairment fails to suppress their natural behavior of exploration and enter the dark compartment very early, and stays there during rest of the retention test period. In the present study, rats in stressed group (ST), entered the dark compartment as early as 6 sec (6.33±2.69, n=12) from the commencement of the retention test, whereas normal control group (NC) of rats delayed their entry to the dark compartment as long as 41 sec (41.20±2.43; NC vs ST, P<0.001, Fig. 1).

(b) Time spent in the bright compartment in male rats — On assessing the total time spent in the bright compartment of the passive avoidance apparatus during the retention test, it was found that normal control rats spent 70% of the total retention test duration (180 sec) in the bright compartment, whereas stressed rats spent only 6% of total time in the bright compartment (126.67±9.97 sec in NC, vs 10.33±3.98 sec in ST, P<0.0001, Fig. 2).

(c) Time spent in the dark compartment in male rats — On assessing the total time spent in the dark compartment of the passive avoidance apparatus during the retention test, it was found that normal control rats spent 30% of the total retention test duration (180 sec) in the dark compartment, whereas stressed rats spent 94% of total time in the dark compartment (53.33±14.49 sec in NC, vs 169.7±14.49 sec in ST, P<0.001, Fig. 3).

Fig. 1 — Time taken (latency) to enter the dark compartment of passive avoidance apparatus by normal control (NC) and rats born to stressed mothers (ST) group of rats (male and female) during retention test. (Note the significantly reduced latency in the stressed group. NC vs S, P values: ***<0.001; **** <0.0001; Unpaired, two-tailed Student’s t-test).

Fig. 2 — Time spent in the bright compartment of passive avoidance apparatus by normal control (NC) and rats born to stressed mothers (ST) group of rats (male and female) during retention test. (Note the significantly reduced time spent in the bright compartment by the stressed group. NC vs ST, P values: ***< 0.001; ****< 0.0001, Unpaired, two tailed Student’s t-test).

Fig. 3 — Time spent in the dark compartment of passive avoidance apparatus by normal control (NC) and rats born to stressed mothers (ST) group of rats (male and female) during retention test. (Note the significantly increased time spent in the dark compartment by the stressed group. NC vs S, P values: ***< 0.001; **** < 0.0001; Unpaired, two tailed Student’s t-test).
II. Passive avoidance performance in female rats:
(a) Latency to enter the dark compartment in female rats — During passive avoidance retention test, the female rats born to the stressed mothers took shorter time to enter the dark compartment than the female rats born to the control mothers. In the present study, rats in stressed group (ST), entered the dark compartment as early as 4.00 sec (4.16±0.65) from the commencement of the retention test, whereas normal control group (NC) of rats delayed their entry to the dark compartment as long as 33 sec (33.5±2.67; NC vs ST, \(P<0.0001\), Fig. 1).

(b) Time spent in the bright compartment in female rats — Normal control female rats spent 30% of the total retention test duration (180 sec) in the bright compartment during retention, whereas stressed rats spent only 4% of total time in the bright compartment (55.66±12.54 sec in NC, vs 6.83±2.33 seconds in ST, \(P<0.001\), Fig. 2)

(c) Time spent in the dark compartment in female rats — Normal female control rats spent 68% of the total retention test duration (180 sec) in the dark compartment, whereas stressed female rats spent only 95% of total time in the dark compartment (122.75±15.77 sec in NC, vs 172.6±3.23 sec in ST, \(P<0.001\), Fig. 3).

III. Comparison of passive avoidance performance by normal control males and females:
On comparing the passive avoidance performance by normal control males and females it was found that normal control male’s memory retention is poorer than the male rats as they (females) had shorter latency (poor memory) to enter the dark compartment during memory retention test (33.5±2.67 sec vs 41.20±7.43 sec in males, \(P<0.05\), Table 1).

IV. Comparison of passive avoidance performance by stressed males and females:
On comparing the passive avoidance performance by stressed males and females it was found that there was no significant difference between the groups (Table 1).

### Table 1 — Summary of passive avoidance test performance in males (normal and stressed) and females (normal and stressed)

<table>
<thead>
<tr>
<th>Passive avoidance test parameters</th>
<th>Normal Male</th>
<th>Stress Male</th>
<th>Normal Female</th>
<th>Stress Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to enter the dark compartment (sec.)</td>
<td>41.20 ± 7.43</td>
<td>6.33 ± 2.69</td>
<td>33.5 ± 3.67</td>
<td>4.16 ± 0.65</td>
</tr>
<tr>
<td>Time spent in bright compartment (%)</td>
<td>70</td>
<td>6</td>
<td>30**</td>
<td>4</td>
</tr>
<tr>
<td>Time spent in dark compartment (%)</td>
<td>30</td>
<td>94</td>
<td>68**</td>
<td>95</td>
</tr>
</tbody>
</table>

Note the latency to enter the dark compartment in different groups is not significant (NS) during passive avoidance learning, suggesting that rats in all groups learnt the task equally. However performance of normal females are poor during memory retention test as the latency to enter the dark compartment, percentage of time spent in the bright and dark compartments are significantly different in normal males and females. Normal male vs normal female, \(P\) values: *< 0.05; **< 0.001, Unpaired, two tailed Student’s \(t\)-test.

### Discussion
The results of the present study revealed that memory retention ability of male and female young adult rats are affected by prenatal stress, and there is significantly poor memory retention ability in normal female rats compared to normal male rats. Both male and female rats born to stressed mothers learnt the passive avoidance; however they showed impairment in passive avoidance memory retention. During the retention test, they entered the dark compartment of the passive avoidance apparatus, very quickly, without remembering the foot shock given on the previous day. These rats spent very less time in the bright compartment of passive avoidance apparatus, unlike rats born to normal mothers, which spent more time in the bright compartment during memory retention test. These observations are novel and potentially important since only a few animal or human studies have utilized females as subjects.

1. **Restraint stress method:**
The method of stress used in the present study is one of the well known methods of stress. Different methods of stress procedures have been used such as forced immersion in cold water, social stress by exposing the rats to cat, and electric foot shock. Stress by restrainer method used in the present study is convenient and animals will not suffocate, but at the same time stress them. Stress by this method is known to increase the adrenal gland weight and glucocorticoid hormone level.
ii. Effect of prenatal stress on passive avoidance learning and retention in male and female rats:

Results of the present study are consistent with earlier reports. Both male and female prenatally stressed rats showed decreased performance in passive avoidance test. Male rats, learning performance was decreased when they are prenatally stressed. Step through latency and percentage of animals reaching the criterion are decreased in the stressed female rats. The chronic maternal stress induced memory retention impairment, may be due to the influence of stress hormone on the developing brain. Exposures to excessive glucocorticoids during critical windows of neuroendocrine development have been reported to lead into cognitive deficits. The hippocampus is one of the targets for glucocorticoid hormone and is highly vulnerable regions of the brain. Chronic repeated psychosocial, restraint stress, chronic treatment with corticosterone or adrenal steroids cause apical dendritic atrophy in CA3 pyramidal neurons of the hippocampus and cause specific cognitive deficits in spatial learning and memory. Damage limited to the hippocampus is sufficient to impair memory. Decreased dendritic arborization and dendritic spine in the hippocampal CA3 region and dentate gyrus in the male prenatally stressed rats has been reported, which was correlated with decreased performance in spatial learning in a water maze. Prenatal stress can enhance or suppress the development of hippocampal neurons depending on the stress intensity. Further, mineralocorticoid and glucocorticoid receptors contribute to stress-induced morphological changes. Prenatal stress can even alter the hippocampal gene expression and changes in cognition. Prenatal stress alters synaptic plasticity and enhances the effects of acute stress on synaptic plasticity in the hippocampus, which may be the mechanism for the impaired spatial learning and memory in young rat offspring.

iii. Passive avoidance learning and retention in normal male and female rats:

The data on the poor memory retention in normal female rats compared with normal male rats is consistent with earlier reports. These findings suggest that females are more susceptible to memory retention impairments. This memory impairment showed by the normal female rats could be the influence of increased oestradiol on the brain. Sexual dimorphic effect of prenatal stress has been discussed in detail in several extensive reviews.

Conclusions

In conclusion, the present results obviously show the major influence of maternal stress on the cognitive development of the offspring in a sex-specific response. The observed behavioral deficit may be due to degeneration of the neurons in specific parts of the brain, particularly hippocampus which in turn may be due to altered glucocorticoid levels circulating during critical periods of fetal and neonatal development. The enhanced performance shown in females could be due to the organizational and activational oestrogenic effects. These findings underscore the point that many effects of prenatal stress obtained in males cannot be generalized in females and highlight the need to investigate the stress response in both sexes.

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