Effect of prenatal and postnatal environmental enrichment on laboratory rats' welfare

Abstract

The welfare of laboratory animals is one of the most distrusted issues concerning animals under human control. Environmental and biologic factors can influence experimental results by exerting subtle influences on an animal's physiologic characteristics, behavior, or both. Therefore great attention must be paid to the wellbeing of every laboratory species to ensure the high quality of both science and ethical practice. A total number of 30 female rats were randomly divided into two groups, control group (raised under standard housing conditions) and enriched group (raised under enriched housing conditions from zero day of pregnancy). Offspring from enriched group were divided into two groups; one raised under standard and the other raised under enriched condition from day 23 to day 35 postnatal meanwhile the neonates 'behaviour was observed, on day 36postnatal, rats subjected to elevated plus maze and open field tests, and on day 42 postnatal, rats were blood sampled and humanely sacrificed to obtain brain samples for histopathological examination. The results showed significant ($p<0.05$) increase in sleeping behaviour in prenatally enriched group and significant ($p<0.01$) increased in E+EC group. Play and fighting behaviour were significantly ($p<0.05$) increased in duration in enriched group. E+EC group showed high exploratory behaviour but not statistically significant. The behavioural tests results revealed significant ($p<0.05$) increased time spent in open arm, open arm entries and time in center in E+EC group and USAP were significantly ($p<0.01$) increased in E+EC group. Freezing time in open field test were significantly ($p<0.01$) decreased in enriched group, while grooming

frequency were significantly increased ($p<0.05$) in E+EC group. Corticosterone level were significantly ($p<0.05$) decreased in E+EC group and the mean of tertiary processes of cytoplasmic processes in cross section of hippocampal region were significantly increased ($p<0.05$) in E+EC group.

Introduction

Animal welfare was described as the avoidance of abuse and exploitation of animals by maintaining appropriate standards of accommodation, feeding and general care, the prevention and treatment of disease, and the assurance of freedom from harassment, and unnecessary discomfort and pain **(Bousfield and Brown, 2010)**.

A lot of research focuses on the welfare issues concerning the maintenance and use of laboratory animals, searching for better alternatives to husbandry routines, experimental techniques, as well as alternatives to animal in research. This has led to several principles, guidelines and recommendations which ensure the welfare of animals' and the reliability of research **(Kaliste, 2007** and **Patterson-Kane and Golab, 2014)**.

Assessing welfare is a complex problem, and a number of approaches have been taken to try and resolve it. There are general indicators that can be directly and objectively measured, providing clear indicators that an animal's welfare may be compromised such as body weight, physical state, physiological state (such as levels of stress hormones), Psychological state (Changes in behaviour such as increased aggression to cage mates) ,monitoring of mortality, food and water consumption and measuring pleasure **(Baumans, 2005;Leacch and Main, 2008**; **Hawkins et al, 2011)**. Most animal care professionals would agree that an enriched captive environment enhances the psychological, physiological well-being and behaviour of animals since the animals from an enriched environment may be

able to cope with variations in different housing conditions which resulted from differences between breeders as well as various experimental procedures **(Russell and Burch, 1959)**; therefore, researchers assuming that enrichment enhances animal welfare **(Mellen and SevenichMacPhee, 2001)**. Also environmental enrichment found to affect the central nervous system both during the critical period and during adulthood inducing functional and neuroanatomical changes (neuronal soma size, length of synapses and glial cell counts, dendritic branching, dendritic length, spine density) in brain regions **(Mohammed et al., 2002; Leggio et al, 2005 and Baroncelli et al., 2010).** Environmental enrichment may be in the form of social, physical, Nutritional, Cognitive or Sensory enrichment **(Van de Weerd and Baumans 1995; Young, 2003** and **Karen Worley, 2011)**.

This research was designed to investigate the effects of environmental enrichment during gestation on behaviour, physiology and brain histology of enriched and non enriched offspring rats.

Material and Methods

This study was carried out in the laboratory animal house of animal and poultry behavior and management, Department of Hygiene, Management and Zoonoses at Faculty of Veterinary Medicine, Beni-suef University on a total number of 30 pregnant female wistar rats which were purchased from a commercial breeder (Giza governorate) and their neonates which were the subject of this study to investigate effects of environmental enrichment on the improvement of the laboratory animal welfare via measuring changes occur in behaviour, physiology and brain histology of enriched offspring rats.

1) Animals management:-

Animals were housed in well-ventilated room and allowed to accommodate in the new environment for two weeks after arrival before starting the experiment.

Rats were housed in plastic shoe box-type cages with available space 350cm² for adult rats and 95cm² for young rat and include either standard condition where no modifications in house or enriched condition where all cages enriched with enrichment tools/ equipment such as wheels, tunnels, wooden house, chewing blocks, glass jars and nyl bone which were changed twice weekly.

Feed was available adlibitum twice a day using commercial balanced diet, continuous adequate supply of clean fresh water fortified with mineral and vitamin premix was available all the day according to **NRC 1995** with provision of safety margin.

The temperature, relative humidity were recorded daily using digital hygrothermometer during the whole period of study. Lighting system was maintained depending on the natural and artificial lighting using a reversed 12hr light dark cycle.

Polygamous breeding system was used for breeding rats and detection of pregnancy was done by gross observation of yellowish protein coagulates (remnants of the copulatory plug) on vaginal smears of mated females made on clean glass slides according to **Ochiogu et al, (2006)**.

2) Experimental design:-

The study was designed to investigate the effects of environmental enrichment during prenatal and postnatal life of the rats on the welfare of offspring. A total number of 30 pregnant female weighting 190-220gm at

the age of 3to4 months were used in this experiment. The rats were randomly divided into two groups:

* Control group: pregnant rats were raised under standard housing conditions.

* Enriched group: pregnant rats were raised under enriched housing conditions from zero day of pregnancy.

All offspring were weaned on day 22 postnatal then offspring for enriched group divided into two groups; one raised under standard and the other raised under enriched condition from day 23 postnatal to day 35 postnatal. On day 36postnatal, rats subjected to elevated plus maze and open field tests, and on day 42 postnatal, rats were humanely sacrificed using diethyl ether anaesthesia and brain samples were preserved in a special fixative for histopathological examination.

3) Behavioural measurements:

3.1) Behavioural observation: behavioural recording occurs by personal observation using video camera from a distance don't disturb animal then videos were analyzed manually throughout 12 consecutive days from day 23 postnatal to day 35 postnatal (15minutes\cage, twice daily at the daily hours of 9:00-12:00hrs in the morning and 15:00to18:00hrs in the evening, Focal observation was done for rats identified by dyes **(Brown, 2005** and **Abou-Ismail etal; 2010)**.

Observation Ethogram as following:

General activities	1. Intake maintenance (ingestive behaviour).	-Feeding and drinking.
	2. Non intake maintenance (Body care).	-Self-grooming.
	3. Movement activities.	-Movement and/or climbing the cage lid.
	4. Exploratory behaviour.	-Sniffing cage wall, cage top and sniffing air outside the cage. -Digging and pushing or pulling bedding material. -Burrowing: The animal tries to bury its body inside the bedding material. -Rearing: Standing on the hind paws, stretching the body and taking the Fore paws off the ground, while sniffing the air inside the cage.
Comfort behaviour	1. Rest.	-Sitting or lying flat, with eyes normally opened or nearly closed. - Awake non-active: alert (eyes opened) but with no directed attention while standing or leaning against a cage side.
	2. Sleep.	- Sleep: Lying un alert with both eyes closed, apparently asleep. - Self-generated sleep Interruptions: Sleep grooming, position change during sleep.
Other behaviour:	1. Social/Play Behavior.	-Social play in the form of playing Fighting between neonates.
Enrichment directed behaviours:	1. Enrichment directed Behaviours.	Sniffing, chewing, climbing, hide in and manipulating the enrichment objects.

3.2) Behavioural tests:-

3.2.1) Elevated plus maze test:

Elevated plus Maze is used to measure rat anxiety **(Kalinichev et al., 2002).** Rat was placed in the central area and allowed to explore the apparatus for 5 minutes.

Scoring the elevated Plus Maze:

Duration of entries open arm	Time spent in it (all 4 paws crossing)
Number of entries open arm	Frequency of crossing the line into the open arm with all 4 paws.
Duration of entries closed arm	Time spent in it (all 4 paws crossing)
Number of entries closed arm	Frequency of crossing the line into the closed arm with all 4 paws.
Time in the center	Time in the center recorded.
Protected stretch attend postures (SAP)	Where 2-paw enters the arm; where one portion of the body (generally up to half) is in the center or closed arm.
Unprotected stretches attend postures (USAP)	Occur while one portion of the body (generally up to half) is in the open arm.
Grooming	Recorded.
Number of fecal boli	Recorded.

3.2.2) Open field test:

The Open Field Test provides simultaneous measures of locomotion, exploration and anxiety according to **Walsh and Cummins, (1976)**. Rat was placed into one of the four corners of the open field and allowed to explore the apparatus for 5 minutes.

Scoring the open field:

Freezing	Duration with which the rat was completely stationary.
Peripheral squares crossing	Number of Peripheral squares crossed with all four paws.
Center Square Entries	Frequency with which the rat enters Center Square with all four paws.
Center Square Duration	Duration of time the rat spent in the central square.
Rearing	Frequency with which the rat stand against wall of the maze.
Stretch Attend Postures	Frequency with which the animal demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
Grooming	Duration of time the animal spent licking itself while stationary.
Urination	Number of streaks of urine.
Defecation	Number of fecal boli produced.

4) Measurements: the following parameters were measured.

4.1) Physical parameters:

4.1.1) Body weight:

Animal's birth weight were recorded and then weighted once every week; body weight gain was calculated as BWG= w3-w1.

4.2) Physiological parameters:

4.2.1) Blood sampling:

Blood samples were collected from retro-orbital venous plexus by using hematocrit micro-capillary tubes in a clean centrifuge tubes which were lifted to stand in a room temperature for at least 30 minutes for clot formation then centrifuged at 3000 rpm for 10 minutes **(Coles, 1986)** to obtain serum which stored at -20°c for further analysis.

4.2.2) Determination of serum cortisol level:

By using cortisol ELISA kits according to **Burtis and Ashweed, (1994)**.

4.3) Histopathology:

Rat neonates were humanely sacrificed on postnatal day 42 using diethyl ether anesthesia and brain samples were preserved in special fixatives according to Golgi copsh staining technique **(Tömböl, 1966)**.

5) Statistical analysis:

All data were analyzed using one way analysis of variance (One way ANOVA) and independent T-test using SPSS version 20 statistical software.

Results and discussion

Changes occur in the prenatal environmental factors may induce a critical influences and long-term effects on the fetus, on the young and on the mature animals during its development and these processes are recognized as "early programming" , environmental enrichment

has many profound effects on young and mature rodents such as increases social behaviour , enhances learning and memory and decreases anxiety-like behaviour in rats. Environmental enrichment also reduce incidence of abnormal behaviour **(Van Dellen et al.,(2000);Francis et al., (2002); Marashi et al., (2003); Sáenz et al., (2006)**and **Rosenfeld and Weller, 2012)**.

The obtained results as shown in **Table (1)** are showed that feeding behaviour was significantly ($P<0.01$) decreased in duration and frequency in prenatally enriched neonates (enriched) (1.05min, 0.62) in comparison with rat neonates which were reared in standard housing condition (control) (2.46 min, 1.32) respectively and in duration only with prenatally and postnatal enriched neonates(E+EC) (1.27min). Such findings coincides with that obtained by **Van de Weerd et al., (1994);Tomchesson, (2004)**and **Abbott et al., (2006)**who found that rats and mice from the enriched environments (exposed to acute change in environment) consumed significantly less food than the standard housed animals, in contrast, **Abou-Ismail et al., (2014)** found that feed intake increased in physically enriched rats in comparison with socially enriched or standard housed rats, while **Beale et al., (2011)**found that environmental enrichment had no significant difference between groups.

Regarding body weight there was no significant difference between groups while it was noticed that rat neonates which were reared in standard housing condition (control) (94.59gm) show increased weight gain in comparison with prenatally enriched neonates (enriched) (89.28gm) and prenatally and postnatal enriched neonates (E+EC)(86.03gm),similar results were obtained by **Van de Weerd et al., (1994)**; **Olsson and Sherwin., (2006)** and **Beale et al., (2011)**who suggested that body weight not significantly affected by housing

condition and in some degree is consistent with **Tomchesson, (2004); Peńa et al., (2009) and Harati et al., (2013)**who reported that animals housed in enriched housing have reduced body weight in comparison with standard housed animals.

On the contrary **Abou-Ismail et al., (2014)** indicated that physically enriched animals weighing heavier and gaining more weights than socially enriched and standard housed animals and **Van de Weerd et al., (2002)** mentioned that enriched mice weighed more than mice from standard housing conditions.

The decreased feed intake and body weight in enriched animals may be due to increased sleep duration in enriched animals while in E+EC animals as a result of consuming more time using enrichment tools either playing in wheel or hide in tunnel or wooden house or due to reduced heat loss where enriched cages had enrichment tools in which animal can sleep and thus providing them with a good insulation **(Van de Weerd et al., 1994)** or due to enduring changes in metabolism (**Peńa et al., 2009)**.

From **Table (2)** it was clear that sleep behaviour was significantly ($P<0.05$) increased in duration and frequency in enriched group (12.30min, 0.98) respectively followed by control (standard) group (10.22min, 0.94) respectively and was significantly ($P<0.01$) decreased in duration and frequency inE+EC group (6.19min, 0.49) respectively.

These results are in consistent with the previous observation of **Sawin and Scerbo, (1995)** and**Kass et al.,(2001)**who acknowledged that sleep may also increase due to the exposure to boring (un stimulated) environments.

A notable finding is that mice in furnished cages spent less time resting, conversely spent more time in exploration and locomotion behaviour than mice in standard condition **(Olsson and Sherwin, 2006)**. On the contrary, **Orok-Edem and Key, (1994); Abou-Ismail et al.,(2010, 2014)** reported that enriched rats showed increased level of sleep relative to control group due to the ability of them to accommodate to their environment by avoiding disruptive effect of white light or increased activity directed to the enrichment object.

The alleviated level of sleep behaviour in the previously mentioned results could be attributed to timespent by rats interacting with various enrichment tools (4.76min) which is absent in other groups in addition E+EC rats showed significant preference toward tunnel and wooden house in which they can hide and performcertain behaviour which couldn't be seen(may spend time in sleeping), soit might be revealed increased sleep behaviour in the group relative to control group.

Regarding grooming behaviour it was noticeable that grooming duration and frequency were significantly ($P<0.05$) increased in control (standard) group (0.10min, 0.36) in comparison with enriched groups (0.05min, 0.14) respectively and only in frequency with E+EC group (0.21).

These results are, to some extent, in agreement with that of **D'Aquila et al., (2000)** and **Moyaho and Valencia, (2002)** who stated that increased grooming is seen when rats are stressed, as a part of their coping mechanism, while **Baumans, (2004)**and **Abou-Ismail., (2014)** mentioned that high levels of grooming may reflect a positive state and thus used to indicate good welfare and can also reflect higher amount of sleep in animals.

Regarding enrichment-directed behaviour rats in E+EC group spent large part of time interacting with various enrichment tools either wheels, tunnels, wooden house, jars or restrainer cover, they showed great preference for wooden house (2.29min) and tunnels (1.95min) and these findings are more similar to that detected by **Chmiel and Noonan, (1996)** who found that rats spent majority of time in the shelter (dark part of the cage) thus providing choices to animals may improve their welfare.

Table (3) demonstrated that play and fighting behaviour were significantly ($P<0.05$) increased in duration in enriched group (0.18min) in comparison with other groups (control, 0.06min and E+EC, 0.04min).

This result may be explained in the light of obtained result in **Table (6)** that enriched group has low corticosterone level and from this data it was clear that play behaviour decreased as a result of low level of neonatal steroid hormones which organize the juvenile play behaviour **(Auger and Olesen., 2009)** and this explanation may be reinforced by results of **Meaney et al., (1982)** who found that decreased level of play and fighting behaviour was observed in neonates administrated glucocorticoids.

The same table showed high exploratory behaviour in E+EC group but not statistically significant in comparison with control(standard) and enriched groups such as exploration against wall (1.01, 0.89, 0.56) and exploration outside cage (0.42, 0.33, 0.25) respectively and exploration by walking with enriched group(1.35, 0.77)and these results are, to some extent, in agreement with the data obtained by **Orok-Edem and Key, (1994)**and **Townsend's, (1997)** who observed increased exploration in enriched cages and **Olsson and Sherwin., (2006)**who noticed that mice

housed in furnished cages spent more time in exploration and locomotion behaviour than mice in standard condition.

The elevated plus maze results were demonstrated in **Table (4)** which revealed significant (P<0.01) increased time spent in open arm and open arm entries in E+EC (34.18sec , 2.21) in comparison with enriched group (14.54sec, 1.28) respectively. It is also appeared that unprotected stretch attend posture frequency (USAP) were significantly(P<0.01) increased in E+EC group (3.81) in comparison with enriched group(1.95) and time in center were significantly (P<0.05) increased in E+EC group(41sec) in comparison with control(standard) group(18.25sec).

Regarding time spent in the middle platform of the elevated maze may be indicative of a decision-making period(process) to enter any of the maze arms or the time spent at the center of the maze as indices of risk-assessment behaviours **(Rodgers, et al., 1992; Trullas and Skolnick, 1993; Cruz et al., 1994**and **Rico et al, 2009)**.

These results are, to some extent, in agreement with**Tomchesson, (2004) Penaet al. (2006).Galaniet al. (2007); Leal-Galicia et al. (2008)**who reported that environmental enrichment decrease anxiety like behaviour in rats.

Regarding the open field test results as shown in **Table (5)** freezing time were significantly (P<0.01) decreased in enriched group(0.21sec) in comparison with control (standard) group (0.99sec) and were significantly(P<0.05) decreased in E+EC group (0.29sec) in comparison with control group, in addition grooming frequency were significantly increased (P<0.05) in E+EC group (1.58) in comparison with control(standard) (0.96) and enriched group (0.92).

Similar results were obtained by **Archer, (1973)and Walshand Cummins,(1976)** who suggested that freezing (the absence of activity) is a parameter usually taken as indicative of a high-stress state. In addition **Amart et al, (2008) and Ali et al, (2009)** attributed the increased locomotor activity to decreased freezing in response to medial prefrontal cortex activation. Also **Gould et al., (2009)** declared that time spent in grooming in the open field considered as a sign of comfort within the environment, however **Hines and Minton, (2012)** suggested that increased grooming time associated with inhibition of fear response by environmental enrichment.

From **Table (6) and Figure (1)**it was clear that corticosterone level were significantly ($P<0.05$) decreased in E+EC group (2.69µg\dl)in comparison with control (standard) group (4.42µg\dl**)**.

These data are parallel to that achieved by**Brown and Grunberg, (1995);Welberg et al., (2006)** and **Urakawa et al ., (2014)** Although **Bakoset al., (2004)** reported increased corticosterone levels in males reared in enriched environment.

In **Table (7)** it was found that the mean of tertiary processes in each secondary process of cytoplasmic processes in cross section of hippocampal region (spine density) were significantly increased ($P<0.05$) in E+EC group (7.20) in comparison with control (standard) group (5.63).

These findings are supported by **Fiala et al., (1978)** who stated that hippocampus region of brain was influenced by environmental enrichment which induce increase in granular cells, dendritic branching and overall size of the dendritic field and these changes were observed in enriched juvenile but not in adult rats, in addition **Moser et al., (1994)**

mentioned that exposure to complex environment induces increased spine densities in the hippocampal pyramidal cells.

Conclusion

Providing experimental laboratory rats with physical enrichment tools in prenatal and post natal life, improve their behaviour, reduce anxiety and fear responses, reduce basal cortisol level and increased spine densities in the hippocampal pyramidal cells; all of which were reflected on their welfare.

References

Table (1): Effect of prenatal and postnatal enrichment on ingestive behaviour and body weight of rat neonates:

Group	Feeding		Drinking		Birth weight (gm)	Weaning weight (gm)	Weight gain (gm)
	Duration (min)	Frequency	Duration (min)	Frequency			
control	2.46±0.14[a]	1.32±0.21[a]	0.11±0.03	0.35±0.11	16.48±8.28	39.85±3.52	94.59±7.31
Enriched	1.05± 0.11[b]	0.62±0.10[b]	0.18±0.07	0.40±0.13	15.30± 2.08	38.47±4.84	89.28±2.24
E+EC	1.27±0.29[b]	1.00±0.15	0.15±0.05	0.45±0.15	13.50±2.08	43.50±7.66	86.03±4.55

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.

Table (2): Effect of prenatal and postnatal enrichment on comfort, body care (grooming)and enrichment-directed behaviour of rat neonates:

Group	Rest		Sleep		Grooming		Enrichment-directed behaviour	
	Duration (min)	frequency	Duration (min)	frequency	Duration (min)	frequency	Duration (min)	frequency
control	0.41± 0.15	0.25± 0.08	10.22± 0.49[b]	0.94±0.06[ab]	0.10±0.01[a]	0.36±0.03 [a]	-	-
Enriched	0.30±0.10	0.16±0.04	12.30±0.44[a]	0.98±0.04[a]	0.05±0.02[b]	0.14±0.04[c]	-	-
E+EC	0.43±0.12	0.22±0.07	6.19±0.28[c]	0.49±0.02[ac]	0.06±0.01	0.21±0.05[b]	4.76±0.38	1.45±0.10

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.

Table (3): Effect of prenatal and postnatal enrichment on exploratory and playing behaviour of rat neonates:

Group	Exploration against cage wall	Exploration outside cage	Exploration by walking	Exploration of bedding		Exploration by chewing bedding		Exploration by Burrowing		Play and fight		Hanged on lid cage	
				Duration (min)	Frequency	Duration (min)	Frequency	Duration (min)	Frequency	Duration (min)	Frequency	Duration (min)	Frequency
control	0.89 ± 0.19	0.33 ± 0.07	1.37 ± 0.25	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.06 ± 0.03[ab]	0.13 ± 0.06	0.02 ± 0.01	0.05 ± 0.02
enriched	0.56 ± 0.10	0.25 ± 0.07	0.78 ± 0.18	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.18 ± 0.05[a]	0.15 ± 0.04	0.04 ± 0.02	0.10 ± 0.05
E+EC	1.01 ± 0.17	0.42 ± 0.11	1.35 ± 0.34	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.03	0.04 ± 0.0[ac]	0.10 ± 0.03	0.01 ± 0.00	0.03 ± 0.01

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.

Table (4): Effect of prenatal and postnatal enrichment on behaviour of rat neonates in elevated plus maze:

Behavior scoring in elevated plus maze											
Groups	**T. spent in open arm (Sec)**	**No. of entries open arm**	**T. spent in closed arm (Sec)**	**No. of entries closed arm**	**SAP**		**USAP**		**grooming**	**No. of fecal boli**	**Time in center (Sec)**
					Time (Sec)	**Frequency**	**Time (Sec)**	**Frequency**			
control	24.96 ± 5.56	1.50 ± 0.09[b]	125.58 ± 9.09	6.71 ± 0.82	16.14 ± 2.54	6.33 ± 0.82	12.33 ± 1.71	2.92 ± 0.26	1.58 ± 0.21	0.96 ± 0.34	18.25[b] ± 3.17
enriched	14.54 ± 2.52[b]	1.28 ± 0.12[c]	119.78 ± 16.87	6.38 ± 0.84	16.23 ± 1.63	5.83 ± 0.39	16.45 ± 4.29	1.95 ± 0.18[b]	1.55 ± 0.42	0.52 ± 0.19	35.69 ± 6.16
E+EC	34.18 ± 7.09[a]	2.21 ± 0.32[a]	86.10 ± 13.09	5.33 ± 0.52	19.49 ± 1.61	5.44 ± 0.38	16.23 ± 6.53	3.81 ± 0.59[a]	2.22 ± 0.46	0.42 ± 0.14	41.00 ± 9.01[a]

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.

SAP: protected stretch attend posture. USAP: unprotected stretch attend posture.

Table (5): Effect of prenatal and postnatal enrichment on behaviour of rat neonates in open field test:

	Behavioural Scoring in open field									
Group	**Freezing (Sec)**	**No. of Peripheral. Sq. cross**	**No. of Central Sq. cross**	**Central sq. Duration (sec)**	**Grooming**		**Rearing**	**Defecation**	**Urination**	**Stretch attend posture**
					Duration (sec)	**Frequency**				
control	0.99 ± 0.24[a]	66.22 ± 7.43	3.63 ± 0.31	6.17 ± 0.78	3.27 ± 0.66	0.96 ± 0.08[b]	9.57 ± 0.74	1.46 ± 0.32	0.06 ± 0.06	3.01 ± 0.73
enriched	0.21 ± 0.14[b]	75.13 ± 6.32	2.30 ± 0.88	3.46 ± 1.16	4.11 ± 0.79	0.92 ± 0.19[b]	9.58 ± 1.76	2.92 ± 0.66	0.08 ± 0.05	2.96 ± 0.73
E+EC	0.29 ± 0.12[b]	60.20 ± 2.17	3.38 ± 1.04	6.17 ± 2.02	4.89 ± 0.71	1.58 ± 0.28[a]	9.75 ± 1.13	1.92 ± 0.62	0.13 ± 0.09	3.38 ± 0.97

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.

Table (6): Effect of prenatal and postnatal enrichment on corticosterone level (µg\dl) of rat neonates:

Group	Corticosterone (µg\dl)
control	4.42±0.75[a]
enriched	2.97±0.44
E+EC	2.69±0.35[b]

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.

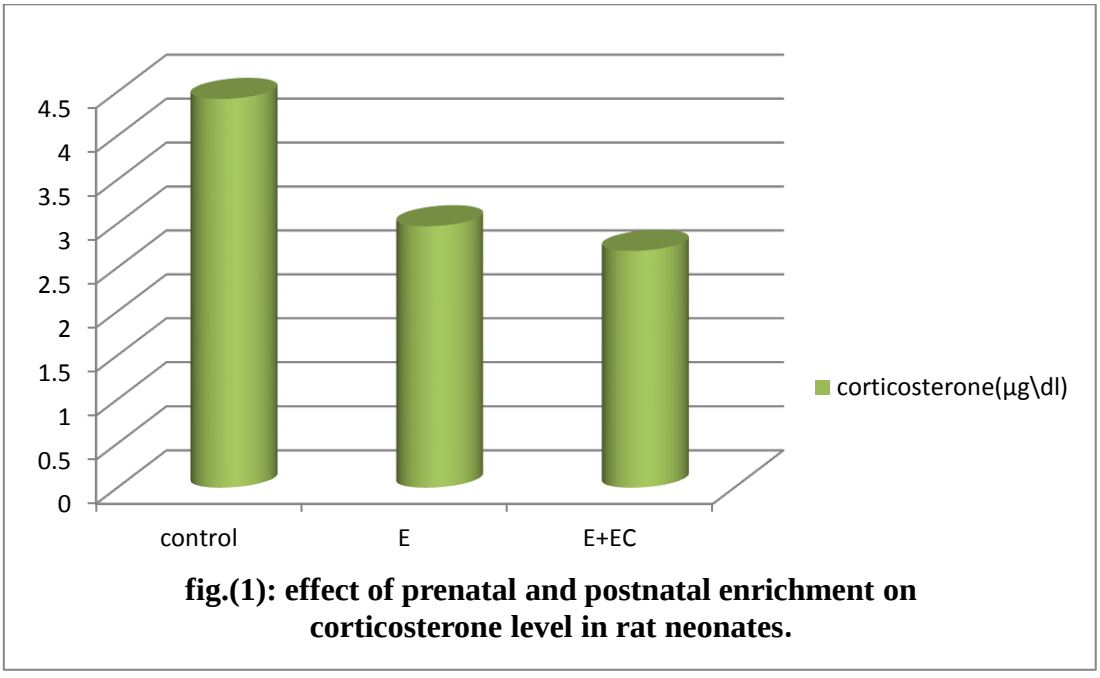

fig.(1): effect of prenatal and postnatal enrichment on corticosterone level in rat neonates.

Table (7): Effect of prenatal and postnatal enrichment on the spine density of hippocampal neurons of rat neonates:

Group	Number of cytoplasmic processes in cross section
control	5.63 ± 0.53^{a}
enriched	5.80 ± 0.37
E+EC	7.20 ± 0.37^{b}

Results are expressed as means ± standard error.

a,b,c superscripts within rows indicate significant difference at P<0.05.

Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.

E+EC: dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 day postnatal.