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Original Research Article

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

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ABSTRACT

This work was designed to investigate effects of environmental enrichment during gestation on behaviour, physiology and brain histology of enriched and non-enriched offspring rats. A total of 30 female wistar rats were randomly divided into two groups; control and enriched groups. Offspring from prenatally enriched group were divided after weaning into two groups; one raised under standard condition (enriched group) and the other raised under enriched condition after weaning (E+EC group) from the day 23 to the day 35 postnatal. Observing neonates' behaviour, on the day 36 postnatal, rats subjected to behavioural tests. On the day 42 postnatal, blood samples were collected and brain samples were obtained for histopathology. Behavioural tests revealed significant (P<0.05) increased time spent in open arm, open arm entries and time in center in E+EC group and unprotected stretch attend posture (USAP) were significantly (P<0.01) increased in E+EC group. Freezing time in open field test was significantly (P<0.01) decreased in the enriched group, while grooming frequency was significantly (P<0.05) increased in prenatally and post weaning enriched rats group (E+EC). The corticosterone level was significantly (P<0.05) decreased in prenatally and post weaning enriched rats group (E+EC). The mean of tertiary processes of cytoplasmic processes in cross section of hippocampal region were significantly (P<0.05) increased in prenatally and post weaning enriched rats group (E+EC) group. In conclusion, providing experimental laboratory rats with physical enrichment tools in prenatal and postnatal life can improve their behavioural and physiological status reflecting on their welfare.

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1. 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 led to several principles, guidelines and recommendations which ensure the welfare of animals' and the reliability of research (Kaliste, 2007; 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; Leach et al., 2008; Hawkins et al., 2011).

Most animal care professionals agreed that an enriched captive environment enhances the psychological, physiological well-being and behaviour of animals since animals from an enriched environment may able to cope with variations in different housing conditions 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 MacPhee, 2001). Moreover, the environmental enrichment affects 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). The environmental enrichment may be in the form of social, physical, Nutritional, Cognitive or Sensory enrichment (Van de Weerd and Baumans, 1995; Young, 2003; Karen Worley, 2011).

This study was designed to investigate effects of environmental enrichment during gestation on behaviour, physiology and brain histopathology of offspring rats.

2. Material and methods

A total of thirty pregnant female wistar rats were purchased from a commercial breeder (Giza, Egypt) and reared at 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.

2.1. Animal housing

Animals were housed in well-ventilated room and allowed to accommodate in the new environment for two weeks after arrival. They were housed in plastic shoe-box cages with available space 350cm² for adult rats and 95cm² for young rat.

Feed was provided *ad libitum* 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.

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

Polygamous breeding system used for breeding rats and the 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).

2.2. Experimental design

A total of 30 pregnant female weighting 190-220 gm and ages 3-4 months were used. Rats were randomly divided into two groups; a) Control group (*n*=15), when pregnant rats were raised under standard housing conditions. b) Enriched group (*n*=15), when pregnant rats were raised under enriched housing conditions from the zero day of pregnancy. All cages enriched with tools/equipment as wheels, tunnels, wooden house, chewing blocks, glass jars and nyl bone.

Offspring weaned on the day 22 postnatal. Offspring in the standard group raised in standard

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

2.3. Behavioural measurements

2.3.1. Behavioural observation

All behavioural patterns were recorded through video camera. Videos were manually analyzed through 12 consecutive days from the day 23 postnatal to the day 35 postnatal (15 minutes/cage, twice daily at the daily hours of 9:00-12:00 h in the morning and 15:00 to18:00 h in the evening. Focal observation was done for rats identified by dyes, and the behavioural ethogram was recorded based on Brown (2005) and Abou-Ismail et al. (2010). The following behavioural patterns were recorded; ingestive and drinking behaviour, body care, movement activities, exploratory behaviour, rest and sleep, playing behaviour as well as enrichment directed behaviour.

2.3.2. Elevated plus maze test

Elevated plus Maze was used for testing of anxiety and emotionality (Kalinichev et al., 2002). A rat was placed in the central area and allowed to explore the apparatus for 5 minutes. Duration and number of entries open arm, duration and number of entries closed arm, time in the center, protected stretch attend postures (SAP), unprotected stretches attend postures (USAP), grooming as well as Number of fecal boli were recorded.

2.3.3. Open field test

The Open Field Test provides simultaneous measures of locomotion, exploration and anxiety according to Walsh and Cummins (1976) and Gould et al. (2009). A rat was placed into a corner of the open field and allowed to explore the apparatus for 5 minutes. Freezing, peripheral squares crossing, center square entries and duration, rearing, stretch attend postures, grooming, and urination and defecation were measured.

2.4. Physical measurement

Animal's body weight was recorded once weekly to estimate the body weight gain.

2.5. Physiological assessment

To measure corticosterone level, thirty blood samples were collected from weaned rats on postnatal day 42 from retro-orbital venous plexus by using hematocrit micro-capillary tubes in a clean centrifuge tubes which lifted to stand in a room temperature for at least 30 minutes. Samples centrifuged at 3000 rpm for 10 minutes to obtain serum which stored at -20°C (Coles, 1986). ELISA kits were used to assess cortisol level according to Burtis and Ashweed (1994).

2.6. Histopathology

Weaned rats 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).

2.7. Statistical analysis

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

3. Results and discussion

Table (1) showed that feeding behaviour was significantly (P<0.01) decreased duration in prenatally and post weaning enriched rats group (E+EC) (1.27 min) and enriched group (1.05 min) compared to the control group (2.46 min). It had significantly (P<0.01) decreased frequency only in enriched group (0.62) compared with the control one (1.32).

Such findings coincide 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 standard housed animals. On the other hand, Abou-Ismail et al. (2014) found that feed intake increased in physically enriched rats compared to socially enriched or standard housed rats. Beale et al. (2011) revealed that the environmental enrichment had no significant difference among groups.

Table 1. Effect of prenatal and postnatal enrichment on ingestive behaviour of rat neonates.

Group	Feeding		Drinking	
	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
Enriched	1.05± 0.11[b]	0.62±0.10[b]	0.18±0.07	0.40±0.13
E+EC	1.27±0.29[b]	1.00±0.15[a]	0.15±0.05	0.45±0.15

- Results are expressed as means ± standard error.
- a and b superscripts within rows indicate significant difference at $P<0.01$.
- Enriched: dams were exposed to enrichment during gestation period till weaning of neonates.
- E+EC (prenatally and post weaning enriched rats group): dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 days postnatal.

Fig. 1 it revealed that there was no difference in the weight between groups till the 4th week, then at the 5th week. Less variations appeared when the control group was the first (65.05 gm), followed by enriched group (60.57 gm) then prenatally and post weaning enriched rats group (E+EC) (55.03 gm). At the 6th week, the enriched group was the first (125.47) group, followed by the control group (112.42 gm) with small difference in the weight then prenatally and post weaning enriched rats group (E+EC) (99.63gm) with moderate difference.

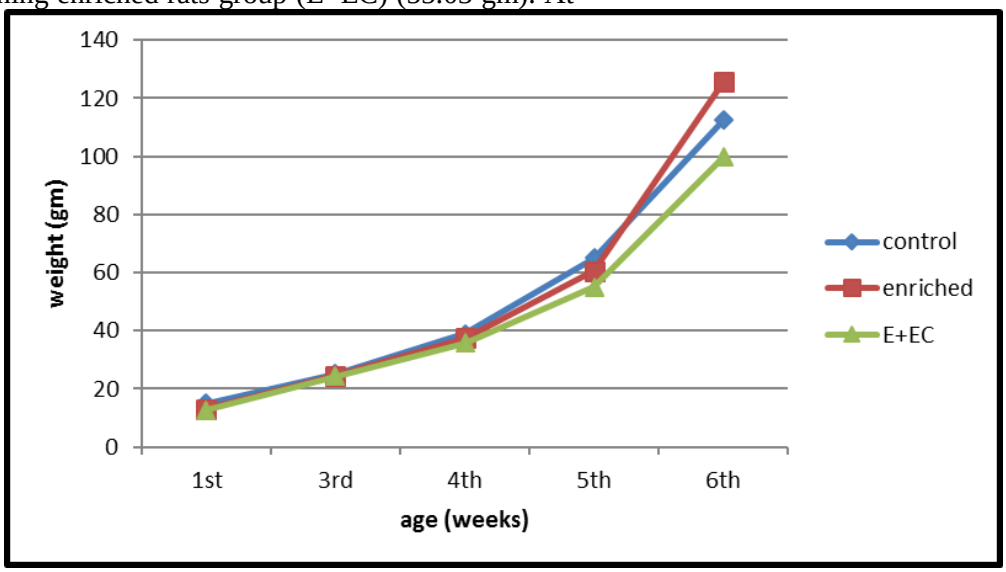

Fig. 1. The growth curve of rat neonates from first to the 6th week.

It was noticed that rat neonates reared in the enriched and standard groups showed increased weight gain compared to prenatally and post weaning enriched rats group (E+EC). Similar results were obtained by Tomchesson (2004), Peńa et al. (2009), and Harati et al. (2013) who reported that animals housed in enriched housing have reduced body weight compared to 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. Meanwhile, Van de Weerd et al. (2002) mentioned that enriched mice weighed more than those in standard housing conditions. Furthermore, Van de Weerd et al. (1994), Olsson and Sherwin (2006), and Beale et al. (2011) assumed that the body weight was not significantly affected by housing condition.

The decreased feed intake and body weight might be due to that prenatally and post weaning enriched rats group (E+EC) consuming more time in 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 providing a good insulation (Van de Weerd et al., 1994) or due to enduring changes in the metabolism (Peña et al., 2009).

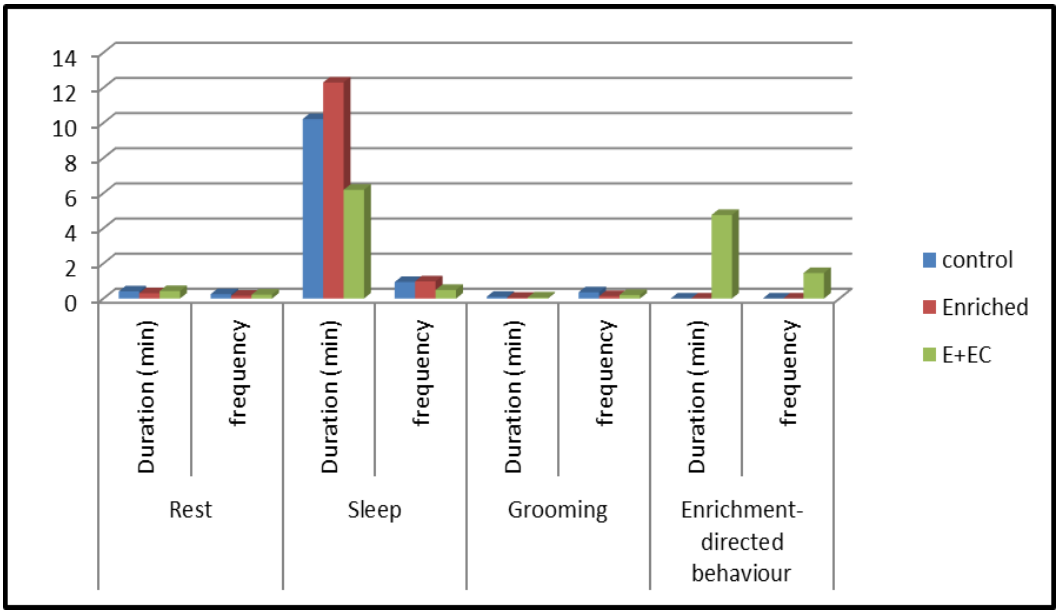

Fig. 2. Effect of prenatal and postnatal enrichment on comfort, body care (grooming) and enrichment-directed behaviour of rat neonates.

From Fig. 2, it was cleared that sleep behaviour was significantly (P<0.01) decreased in duration and frequency in prenatally and post weaning enriched rats group (E+EC) (6.19 min, 0.49), respectively, and was significantly (P<0.05) increased in duration and frequency in enriched group (12.30 min, 0.98), respectively followed by the control (standard) group (10.22 min, 0.94).

Such findings are in consistent with those of Sawin and Scerbo (1995) and Kass et al. (2001) who acknowledged that sleep may be increased due to the exposure to boring (un stimulated) environments.

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

Previous literature revealed that the alleviated level of sleep behaviour could be attributed to the time spent by rats interacting with various enrichment tools (4.76 min) which is absent in other groups. Meanwhile, prenatally and post weaning enriched rats group (E+EC) showed a significant preference toward tunnel and wooden house in which they can hide and perform certain behaviour couldn't be seen (may spend time in sleeping), so it might be revealed increased sleep behaviour in the group relative to the control group.

Regarding grooming behavior, it was noticeable that grooming were significantly (P<0.05) increased in duration and frequency in the control (standard) group (0.10 min, 0.36) compared to enriched ones (0.05min, 0.14), and increased only frequency in prenatally and post weaning enriched rats group (E+EC) (0.21). Such findings are, to some extent, in agreement with those obtained by 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 et al. (2014) mentioned that high levels of grooming may reflect a positive state and thus used to indicate a good welfare reflecting a higher amount of sleep.

Regarding enrichment-directed behavior, rats in prenatally and post weaning enriched group (E+EC) spent a large time interacting with various enrichment tools; wheels, tunnels, wooden house, jars or restrainer cover. They showed a great preference for wooden house (2.29 min) and tunnels (1.95 min). Such findings are more or less similar to those detected by Chmiel and Noonan (1996) who found that rats spent the majority of time in the shelter (dark part of the cage) providing choices for animals to improve their welfare.

Fig. 3. demonstrated that duration of playing and fighting behaviour was significantly (P<0.05) increased in the enriched group (0.18 min) compared to other groups (control, 0.06 min and E+EC, 0.04 min).

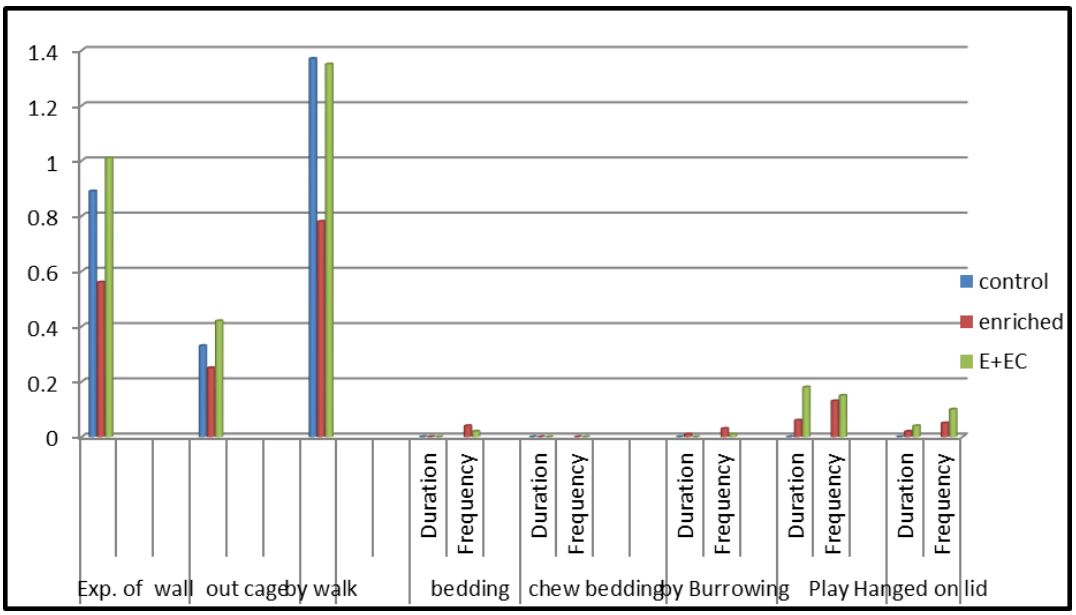

Fig. 3. Effect of prenatal and postnatal enrichment on exploratory and playing behaviour of rat neonates.

Such finding coincided with Auger and Olesen (2009) who demonstrated that playing behaviour decreased as a result of low level of neonatal steroid hormones which organize the juvenile playing behavior. Similarly, Meaney et al. (1982) found that a decreased level of playing and fighting behaviour was observed in neonates administrated glucocorticoids.

A high exploratory behaviour in prenatally and post weaning was seen in enriched rats group (E+EC) but not statistically significant compared to 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). Such findings are in parallel with data obtained by Orok-Edem and Key (1994) and Townsend (1997) who observed an increased exploration in enriched cages. Olsson and Sherwin (2006) noticed that mice housed in furnished cages spent more time in exploration and locomotion behaviour than those in standard conditions.

Elevated plus maze results were demonstrated in Fig. 4 revealing that time spent in an open arm was significantly (P<0.05) increased in prenatally and post weaning enriched rats group (E+EC) (34.18 sec) compared to enriched one (14.54 sec). In control (standard) group, variation was non-significant (24.96 sec). In addition, the open arm entries were significantly (P<0.05) increased in prenatally and post weaning enriched rats group (E+EC) (2.21) compared to the control (standard) group (1.50), and were significantly (P<0.01) increased in prenatally and post weaning enriched rats group (E+EC) (2.21) compared to enriched group (1.28). It has been found that unprotected stretch attend posture frequency (USAP) were significantly (P<0.01) increased in prenatally and post weaning enriched rats group (E+EC) (3.81) compared to enriched group (1.95). Time in center was significantly (P<0.05) increased in prenatally and post weaning enriched rats group (E+EC) (41sec) compared to the control (standard) group (18.25 sec).

Such results agreed with those obtained by Roy et al. (2001) and Peña et al. (2009) who demonstrated that post weaning enrichment increased the number of open arm entries, whereas the time spent into the open arms was not affected.

The time spent in the middle platform of the elevated maze might 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 behaviour (Rodgers et al., 1992; Trullas and Skolnick, 1993; Cruz et al., 1994; Rico et al., 2009).

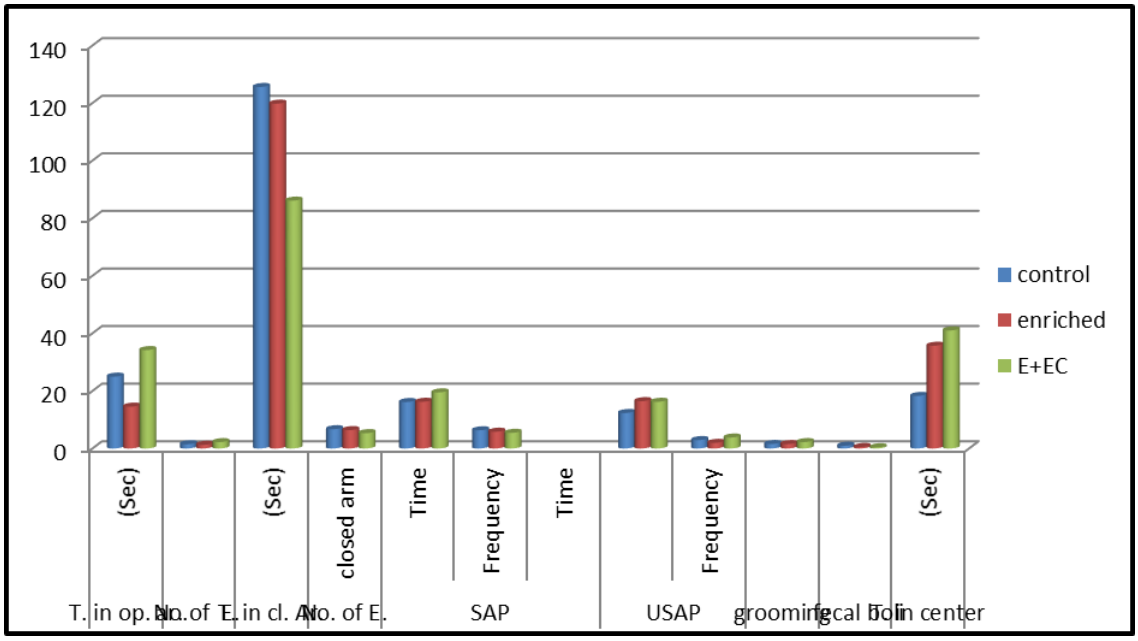

Fig. 4. Effect of prenatal and postnatal enrichment on behaviour of rat neonates in elevated plus maze.

Regarding the open field test, freezing time was significantly (P<0.01) decreased in the enriched group (0.21 sec) compared to the control (standard) one (0.99 sec), and it was significantly (P<0.05) decreased in prenatally and post weaning enriched rats group (E+EC) (0.29 sec) compared to the control one (Fig. 5). Moreover, the grooming frequency was significantly (P<0.05) increased in prenatally and post weaning enriched rats group (E+EC) (1.58) compared to the control (standard) (0.96) and enriched (0.92) groups.

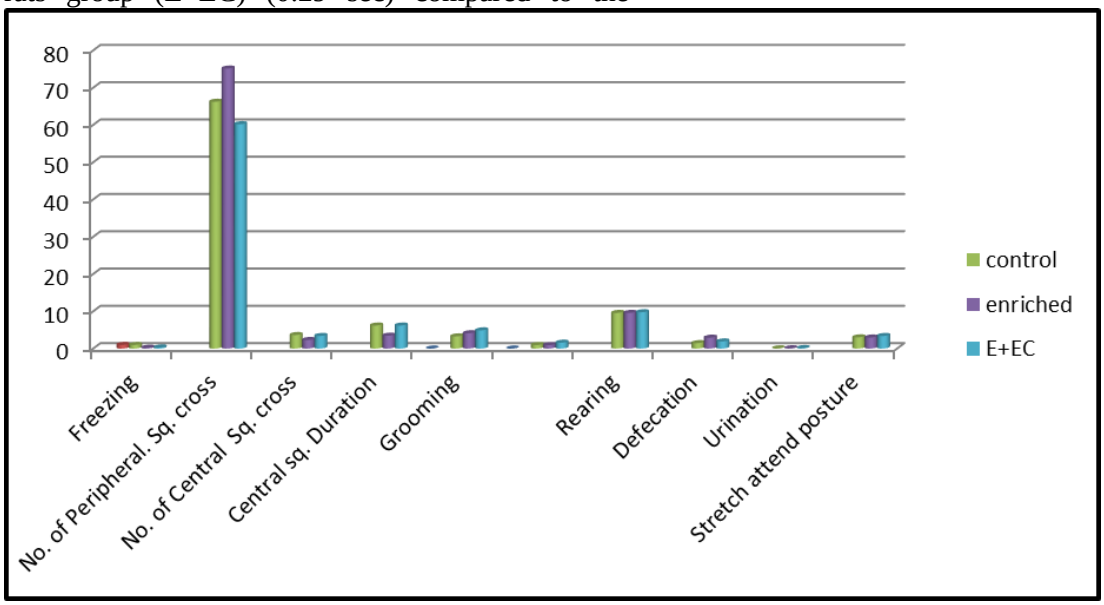

Fig. 5. Effect of prenatal and postnatal enrichment on behaviour of rat neonates in open field test.

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

It was cleared that corticosterone level was significantly (P<0.05) decreased in prenatally and post weaning enriched rats group (E+EC)

(2.69µg/dl) compared to the control (standard) one (4.42µg\dl) (Table 2).

Table 2. The 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[b]
E+EC	2.69±0.35[b]

- Results are expressed as means ± standard error.
- a and b 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 (prenatally and post weaning enriched rats group): dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 days postnatal.

The current data are parallel to those achieved by Brown and Grunberg (1995), Welberg et al., (2006), and Urakawa et al. (2014), however, Bakoset al. (2004) reported an increased corticosterone levels in males reared in the enriched environment (Table 3). The playing behaviour decreased as a result of a low level of neonatal steroid hormones organizing the juvenile playing behaviour in the enriched group (Auger and Olesen, 2009).

It was found that the mean of tertiary processes in each secondary process of cytoplasmic processes in a cross section of hippocampal region (spine density) was significantly ($P<0.05$) increased in prenatally and post weaning enriched rats group (E+EC) (7.20) compared to the control (standard) group (5.63) (Table 3).

Such findings are supported by Fiala et al. (1978) who stated that the hippocampus region of the brain was influenced by the environmental enrichment inducing an increase in granular cells, dendritic branching and overall size of the dendritic field. These changes were observed in enriched juvenile but not in adult rats. Meanwhile, Moser et al. (1994) mentioned that exposure to a complex environment induces an increased spine densities in hippocampal pyramidal cells playing a role in emotional, cognitive processes related to psychiatric disorders and modulation of anxiety. Recent studies showed that neurogenesis participates in the hippocampus-mediated learning and suppressed neurogenesis associated with increased in anxiety-related behaviours (Revest et al., 2009; Levone et al., 2015).

Table 3. The 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[b]
Enriched	5.80±0.37[b]
E+EC	7.20±0.37[a]

- Results are expressed as means ± standard error.
- a and b 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 (prenatally and post weaning enriched rats group): dams were exposed to enrichment during gestation period and neonates exposed to enrichment till 42 days postnatal.

4. 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 improved their status and reflected on their welfare.

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