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Behavioral Changes and Immune Responses in Response to Stress Separation in Wistar Rats

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Abstract: This study is part of a research program on the effect of separation of mother and offspring applied to male rats for 5 minutes; 30 minutes and one hour, studying the consequences of stress on the behavior of Wistar rats revealed by tests and open Field maze. And immune system. The observed time lag argue in favor of an anxiogenic effect induced separation. The exploration of the open arms was significantly lower than the control and confirms a probable depression. Also valued through rates lymphocytes immunosuppression indicates an immune alteration compared to the control.

Key words: Rat · Separation · Immunity · Plus · Maze · Open Field

INTRODUCTION

According to the World Health Organization, mental disorders are expected to increase 50% in 2020, becoming internationally one of the five leading causes of morbidity in children. This increase is referred to as the crisis of the twenty-first century [1].

Such periods as prenatal development, infancy, childhood or adolescence are periods of high vulnerability to stress. Exposure to stressors during these periods can lead to hypersensitivity to stressors in adulthood to a more rapid onset and severe disease correlated [2].

It is affective disorders and anxiety that are according to the report most of this percentage. Overall estimated one in four children suffer from emotional problems and personality disorders for various reasons (war, separation, parental death and divorce ... etc.) [3].

The consequences of hype ractivation of stress systems are physical and behavioral and physiological and behavioral manifestations neuropsychiatric.

Mental health is an essential component of human health. A significant proportion of children and adolescents (22% worldwide) suffer from mental illness, that is to say some disorders of their mental activities, which interfere with their development, hindering their academic learning and jeopardize their future by an impact on the quality of daily life [4].

Similarly following genetic and environmental factors, hyper activation systems stress can lead to anorexia or obesity [5, 6].

Maternal separation / child is referred for a decade as the main cause identified adolescent disorders.

Understanding the mechanisms induced by this stress has led many researchers to assess the situation in animals experimentally introducing a stress says stress of separation. This was made possible by the introduction of new essentially neurobiological and behavioral approaches [7, 8].

Thus, critical periods could be identified during development and the intervention of stressors during these periods, as the separation of mother and isolation, would be involved in the development of visible long-term problems [9].

The period of brain growth that fits especially in the last trimester of gestation in humans would possess equivalent between the fifth and fifteenth day of embryonic development in rats "[10].

It has been demonstrated, in adult male rats become separated, compared to the unseparated adult rats:

- A behavioral response to various stresses exacerbated face: acoustic stress [11,12], drafts [3], confinement in a new environment [13], soft handling stress [12].

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- Increased fear and face different new environments anxiety: elevated plus maze [11].
- A dysfonctionnment HPA axis hypothalamic-pituitary-adrenal: the increased ACTH and corticosterone hormone Adenocorticotropin following stress responses [3, 11, 12, 14, 15] to be related to a decrease in the negative feedback normally exerted by glucocorticoids on HPA axis and an increase in hippocampus mRNA levels of CRH or CRF basal induced stress [14, 16].

It seems interesting to note that certain classes of depressed humans have a disturbance in the regulation of stress hormones going in the same direction [16,17].

- The development of a preference for alcohol [3]

This separation model could therefore be used for research on alcoholism in humans.

Our work focuses on behavioral and adaptive changes in the rat is an attempt to identify the behavioral parameters evaluated from the open feild and plus maz and immune lymphocyte levels through rates.

MATERIALS AND METHODS

Biological Material: The animals used in this study were adult male rats Rattus rattus of the Wistar strain, class nocturnal mammals of the order of rodents from Pasteur Institute of Algiers. Gestation lasts 20 -23 days and litters are 8 -14 youth. These pèsent5-7g, they are hairless and blind and open their eyes between le12emeet le16eme day; the coat grows to completely 10emejours. The young people can be separated from the mother from about 20 days.

Rats reach puberty between 50 and 60 days after birth in both sexes, testicular descent occurs before puberty, usually around the age of weaning. A rat can live on average between 2 to 3 years depending on the strain, sex and environmental conditions.

Terms of Breeding: The animals are acclimatized to the conditions previously in our animal facility for the duration of the experiment; being reared in cages polyethylene.la litter is made of wood chips and changed every two days. Because of the extreme susceptibility of different system of these animals any kind of aggression; which attached great importance to the environment and conditions of daily handling.

The rats are maintained in batches prepared under the conditions of temperature 22°C, a humidity of 50% and natural photoperiod. Water and food are ad libitum present in cages suitable for bottles. These animals were divided into four (04) experimental groups (group T, group 5, group 30 and group 1).

Methods

Protocol Separation: In an attempt to replicate as closely as possible to promote the known diseases in humans predicted circumstances have developed a number of experimental animal models. And it is of fundamental importance, in all species, the relationship between the mother and the newborn in the harmonious development of the latter underlying relevance model mother / newborn separation.

These were used to try to describe the consequences of early stress on the development of children. The mother serves as the primary link between the environment and the animal development; maternal separation could therefore have visible long-term adverse consequences, cause alterations in neuronal function, manifested. Maternal deprivation alters both behavior and neurotransmitter systems in many species.

There are several models of mother / newborn rat's separation. They differ mainly in the separation time (five minutes, thirty minutes, one hour). Induced changes are different according to the protocol used for separation.

Four pregnant females were individually high. The day (D) of the birth of the young was named D0. Maternal separation protocol began in D3. A D3, were conducted from May to December batches pups and divided into four experimental groups

Group T: no treatment.
Group 5: separation of 5 minutes.
Group 30: separation of 30 minutes.
Group 1: Separation of one hour.

Behavioral Analysis: The innate anxiety behavior is fundamental component of the overall behavior of rodents is manifested by the elevation of the animal to fear when placed under prior experience in an aversive environment. This behavior can be assessed using validated experimental devices, the most used are: the open field (open field), elevated cross maze (plus maze).

Evaluation of Exploratory Behavior in a Stressful Context of the Open Field Test (The Open Field) [20]: The open field is a device consisting of a large open rectangular box (90 cm x 70 cm x 60 cm x) in white and brightly lit from above (500 lux). Black lines demarcating ground tiles (10 x 10 cm) [20]. This is the most common. It involves placing the animal in a strange new environment, low stimulation, having no possibility of escape or shelter.

The animal is then in a situation of social isolation, which is already a stressful situation, or aversive situations. The first to have used is Hall in 1934.

Each rat was initially placed in one corner of the open field, facing the corner head. His behavior was observed for 5 minutes. Five parameters were measured by the experimenter:

Time spent in the center in seconds.
Time spent in the preferred in seconds.
The total number of adjustments (animal positioned on both legs
Posterior right balance in vacuum or against a wall).
The total number of grooming.
The total number of bowel movements.

Thus, this test assesses the ability of exploratory rat in a stressful environment. The number of adjustments reflects its exploratory activity and emotional state. The other parameters are rather indications of emotional state.

Test of the Maze (Plus Maze) [21,22]: In the test, each rat was initially positioned at the center of the maze located at the intersection of the four arms, the head facing one of the open arms (OA); had free access to all four arms for a period of 5 min. The maze was placed in a soundproof room. The experimenter visualized the behavior of rats outside of the room with a video camera; he raised the number of visits and time spent respectively in (OA) and the closed arms (CA); visit was recorded when the rat had all four paws in an arm.

The results were expressed as:
Number of entries into the open arms and closed arms.
Number for entry into the proximal portions of the open arms and closed arms.
Number of entries into the distal portions of the open arms and closed arms.
Number of recovery.
Past time (s) in the center.
Past time (s) in the open arms (OA) and closed arms (CA).
Recovery time (s).
Number of bowel movements.
Number of grooming (urine).

Collection of Bloods: After decapitation, blood is taken in EDTA tubes for analysis of NSF. The comparison will be for all measured parameters versus controls.

Statistical Analysis: Results are expressed as mean and standard deviation and illustrated by charts and histograms.

Using the ANOVA test we compared the averages for each variable depending on the treatment in use.these calculations were performed with the help of MINITAB software analysis and statistical data processing.

RESULTS AND DISCUSSION

AI early age, the brain has a high plasticity which makes it very sensitive to prenatal or postnatal environmental programming. This programming can alter brain homeostasis and thus influence the reactivity of the organism to stressful stimuli, their behavioral responses to new situations, but also during aging [23].

The effects of perinatal events can be very nature divergent as more or less invasive stimulation. Neonatal stimulation of the animal handling (handling) or by maternal care leads to adulthood, a decrease in the HPA response, a reduction in the fear of the new and protected by a neuroprotective effect body learning deficits associated with age [24, 25]. On the contrary, prolonged maternal separation may have adverse effects in adulthood, including increased HPA response and increased memory deficits [26, 27]. The main function of the HPA system is to ensure the body's response to stressors, but it also plays an important role in the regulation of many physiological functions, including food intake, anxiety and learning. Since the HPA axis is particularly sensitive to perinatal programming, regulation of these physiological functions may be altered by neonatal environmental factors [23].

Maternal separation has persistent effects on different neural systems HPA axis and behavior, which were studied in adult rats. The body weight of these animals is generally decreased regardless of age separation [28- 31] Stress can cause behavior aggressive reactions of fear, passivity and also inhibit reproductive behavior (libido) and appetite [32].

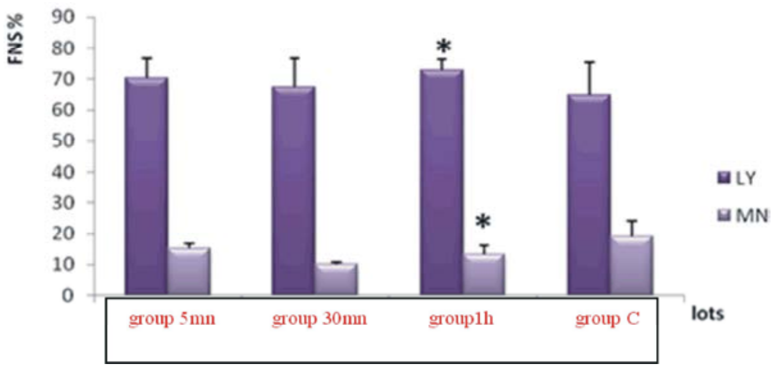

Fig. 1: Changes in rates of lymphocytes and monocytes of treatment and control groups (n = 4) (LY: lymphocytes; MN: monocytes)

P: level of significance (P LY: 0.330; P MN: 0011).

*: Significant difference separated vs control ($p \leq 0.05$).

ns: non-significant difference vs control separate ($p \leq 0.05$).

Table 1: Behavior settings and control groups recorded more maze treaties

	Rat's				
Variables	Group 5 mn (n=9)	Group 30 mn (n=5)	Group 1 h (n=4)	Group t (n=4)	P
Number of entries into the open arms	3.555 ± 1.943	2.8 ± 2.387	2.75 ± 2.5	2.25 ± 05	0.718 ns
Number of entries into the closed arms	3.777 ± 3.855	2.4 ± 2.073	2.5 ± 1.290	2.75 ± 0.957	0.430 ns
Number of entries into the proximal portions of with open arms	5.777 ± 3.032	4.00 ± 3.535	3.75 ± 3.304	3.75 ± 0.957	0.533 ns
Number of entries into the proximal portions of the arms closed	8.00 ± 4.795	6.2 ± 5.263	5.00 ± 3.651	5.5 ± 1.732	0.633 ns
Number of entries into the open arms of the distal	2.333 ± 1.5	1.4 ± 1.140	1.00 ± 0.81	1.75 ± 0.957	0.315 ns
Number of entries into the distal portions of arm closed	5.666 ± 3.082	3.8 ± 3.033	3.25 ± 2.217	3 ± 0.816	0.289 ns
Time spent in the center(s)	0.396 ± 0.272	0.074 ± 0.068	0.467 ± 0.327	0.517 ±0.509	0.153 ns
Time spent in closed arm(s)	0.682 ± 0.581	1.38 ± 2.060	0.52 ± 0.59	3.3 ± 1.241	0.557ns
Time spent in open arms (s)	3.973 ± 0.779	3.536 ± 2.021	3.997 ± 0.817	1.172±0.779	0.773 ns
Temps de redressement(s)	0.074 ± 0.051	0.144 ± 0.111	0.057 ± 0.045	0.092±0.022	0.221 ns
Number of recovery	0.666 ± 1.118	7.00 ± 5.612	3.5 ± 2.380	7.25±1.5	0.468 ns
Number of bowel movements	0.222 ± 0.440	2.80 ± 3.033	4.00 ± 2.160	0.5±0.577	0.22 ns
Number of urination (action of urine)	6.333 ± 3.535	0.2 ± 0.447	0.25 ± 0.5	1.25±0.957	0.035 *

P: level of significance.

*: Significant difference separated vs control ($p \leq 0.05$).

ns: non-significant difference vs control separate ($p \leq 0.05$).

These animals exhibited signs of lower anxiety, they spent less time in the central part (00 ± 00 S lot VS 0.07 ± 0.034 T lot) and more time in the peripheral part (lot S 5.00 VS 0 ± 4.92 ± 0.029 lot T) as the number of high defecation (4.00 ± 2.160 S lot lot VS 1.5 ± 2.380 T). This indicator behavior of an advanced state of stress, confirms the importance of the mother-offspring [33].

Glucocorticoids regulate the electrical activity of certain neurons in the hippocampus, which could explain the behavior change involving limbic structures (such as loco motor activity, memory or anxiety) [32].

This is the fundamental importance in all species, the relationship between the mother and the newborn in the harmonious development of that which underlies the relevance of animal models of separating mother / newborn that are being developed. This separation is an early stress, because it occurs during a period of intense neuronal development, is likely to cause abnormal brain function sustainably. These models are designed to try to describe the adverse behavioral and neurobiological consequences of early stress on development. In contrast, longer separations greater than or equal to 3 hours per day, amplify behavioral responses to stress [34].

he test results at the Open Field show a different behavior changes between the control groups and separate groups (stress) [35].

Table 2: Parameters of behavioral treatment and control groups recorded in open Field.

	Rats				
Variables	Lot 5 mn (n=9)	Lot 30 mn (n=5)	Lot 1 h (n=4)	Lot t (n=4)	P
Time in the periphery (s)	4.972 ± 0.332	4.956 ± 4.049	5.00 ± 0	4.92±0.029	0.016 *
Time in the center (s)	0.260 ± 0.329	0.038 ± 0.037	00 ± 00	0.07± 0.034	0.038 *
Recovery time (s)	0.0675 ± 0.069	0.144 ± 0.105	0.062± 0.105	1.182 ± 0.029	0.26 ns
Number of adjustments	4.375 ± 3.777	8.6 ± 4.159	4.25 ± 6.652	14.25 ± 2.753	0.09 *
Number of defecations	2.75 ± 2.121	3.8 ± 2.489	4.00 ± 2.160	1.5 ± 2.380	0.382 ns
Number of urination (action of urine)	0.25 ± 0.462	00 ± 00	0.25 ± 0.5	0.25 ± 0.5	0.721 ns

P: level of significance.

*: Significant difference separated vs control ($p \leq 0.05$).

ns: non-significant difference vs control separate ($p \leq 0.05$).

The test Open Field with the parameters to study seems to validate inappropriate anxiety in animals treated with the test against more Maze shows significant differences compared with controls regarding:

Time in the periphery, while in the center, a number of recovery.

Separation alters the exploratory nature that characterizes the ethogram rodents. This loss of activity is accompanied by an immune stimulation especially for group 1 hour compared to C.

Observations show that time passes in the central and peripheral indicate a time of high anxiety and immobility higher number of defecation down are the main defensive response in rats [36]. These parameters characterize any animal initially placed in a threatening situation or potentially threatening [37]. As against the time spent in the corners is a security zone [36, 35].

Immune appreciated through variations of lymphocyte clearly indicate a set of tracks that integrate the immune system in place of the control loop of homeostasis. Three major systems integration allow the body of vertebrates adapted to function in the outside environment and they also ensure the integration of signals and homeostasis within the body. These are the nervous systems (SN), endocrine (SE) and immune (SI) [38]. Other studies have shown immune changes in rats subjected to a restraint stress [39].

The Values obtained for the separate animals were significantly higher than the control (64,825 ± 10,712) and separated (72.92 ± 3.45, 67.37 ± 9.283). Immune activation prepares the animal to the confrontation of an aversive situation and lays the groundwork for a probable anticipation [40].

The sympathetic axis is deemed to take effect more quickly than the adrenal axis on the immune system. Two other routes are likely to occur: the endocrine system and the immune system itself. This immune impairment itself will produce an increased susceptibility to disease and changes in health status. These disorders may be increased by a direct effect of behavioral changes such as the reduction of muscle activity measured by the test of the open field. This string can be aggravated by brain disorders homeostasis cytokines that may they be generators of stress, both directly and through the environmental effects they induce [40].

Thus it appears that the stress of separation mother offspring, leads to a behavioral disturbance at the junction of anxiety and boosts the immune system. It should be in the light of these results to master the tests and the separation time where those 5 minutes and 30 minutes seem inconclusive.

Analysis of the variables obtained in the standard elevated plus maze shows that separate animals (stress) are longer in the central portion and the open arms. According to these high time the state of anxiety is decreed in rodents [36,37]. But in the absence of statistical significance due to test the device more Maze seems inappropriate to assess depression in separate groups.

CONCLUSION

At the end of the work on the effects of stress on behavioral and adaptive capacity in the rat we can move a certain number of tracks that remain to be explored. The stress of separation installs a fairly convincing anxiety and depression which, in turn interfere with all the cognitive and immune function.

The immune system in relation to the other two systems homeostatic regulation responds to the types of stress states and states of anxiety and fear conditioning.

In view of this preliminary study should be conducted an experiment with a time of larger (> 1 hour) separation. The assays to be performed concern the HPA axis (mainly cortisol), followed by a more comprehensive immune assessment.

REFERENCES

1. Lordi, B., V. Patin, P. Protais, D. Mellier and J. Caston, 2000. Psychophysiology, 37: 195-205.
2. Charmandari, E., T. Kino, E. Souvatzoglou and G.P. Chrousos, 2003. Pediatric stress: hormonal mediators and human development, Hormone Research, 59: 161-179.
3. Huot, R.L., K.V. Thrivikraman, M.J. Meaney and P.M. Plotsky, 2001. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment, Psychopharmacology, 158: 366-73.
4. Arnold, J.L. and S.M. Siviy, 2002. Effects of Neonatal Handling and Maternal Separation on Rough-and-Tumble Play in the Rat, Psychobiology, 41: 205-215.
5. Chrousos, G.P. and P.W. Gold, 1992.The concepts of stress and stress system disorders.Overview of physical and behavioral homeostasis, Journal of the American Medical Association, 26: 1244-1252.
6. Dallman, M.F., 2009. Stress-induced obesity and the emotional nervous system, trends endocrinology metabolism, Elsevier, 21(3): 159–165.
7. Conte-Devolx, B., V. Guillaume, M. Grino, F. Boudouresque, E. Magnan, M. Cataldi and C. Oliver, 1998. Stress. Neuroendocrine aspects, Encephale, 1: 143-146.
8. Kristensen, H.H., R.B. Jones, C.P. Schofield, R.P. White and C.M. Wathes, 2001. The use of olfactory and other cues for social recognition by juvenile pigs. Appl.Anim. Behav. Sci., 72: 321-333.
9. Champagne, F.A., D.D. Francis, A. Mar and M.J. Meaney, 2003. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiology & Behavior, 79: 359-371.
10. Tecoma, E.S. and L.Y. Huey, 1985. Psychic distress and immune response. Life Sciences, 36: 1700-1712.
11. Caldji, C., D. Francis, S. Sharma, P.M. Plotsk and M.J. Meaney, 2000. The Effects of Early Rearing Environnement on the Development of GABAA and Central Benzodiazepine Receptor Levels and Novelty-Induced Fearfulness in the Rat. Neuropsychopharmacology, 22: 219-229.
12. Kalinichev, M., K.W. Easterling, P.M. Plotsky and S.G. Holtzman, 2002. Long-lating changes in stress-induced corticosterone response and anxiety-like 73behaviors as a consequence of neonatal maternal separation in Long-Evans rats. Pharmacology Biochemistry Behaviour, 73: 131-140.
13. Meaney, M.J., W. Brake and A. Gratton, 2002. Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? Psychoneuroendocrinology, 27: 127-138.
14. Plotsky, P.M. and M.J. Meaney, 1993. Early postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence content and stress induced release in adult rats. Molecular Brain Research, 18: 195-200.
15. Liu, D., C. Caldji, S. Sharma, P.M. Plotsky and M.J. Meaney, 2000. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. Neuroendocrinology, 12: 5-12.
16. Seiden, A.M. and H.J. Duncan, 2001.The diagnosis of a conductive olfactory loss.Laryngoscope, pp: 111.
17. Nathan, K.I., D.L. Musselman, A.F. Schatzberg and C.B. Nemeroff, 1995. Biology of Mood Disorders. In, editors. The American Psychiatric Press Textbook of Psychopharmacology, Washington, American Psychiatric Press, pp: 439-477.
18. Willner, P., A. Towell, D. Sampson, R. Muscat and S. Sophokleous, 1987. Reduction of sucrose preference by chronic mild stress and its restoration by a tricyclic antidepressant. Psychopharmacology, 93: 358-364.
19. Lamouraux, S., N. Texier, C. Canestrelli, F. Beslot and V. Dauge, 1957. Dysfunctioning of opioidergic and cholecystokininergic systems after maternal separation in adult rats. Psychopharmacology, Soumis.
20. Dauge, V., P. Rossignol and B.P. Roques, 1989. Comparison of the behavioural effects induced by administration in rat nucleus accumbens or nucleus caudatus of selective mu and delta opioid peptides or kelatorphan, an inhibitor of enkephalinmetabolism. Psychopharmacology, 96: 343-352.
21. Pellow, S., P. Chopin, S.E. File and M. Briley, 1985. Validation of open/closed arm entries in an elevated plus maze as a mesure of anxiety in the rat. Neuroscience, 14: 149-167.
22. Calenco-Choukroun, G., V. Dauge, J. Gacel Feger and B.P. Roques, 1991. Opioid delta agonists and endogenous enkephalins induce different emotional 71reactivity than mu agonists after injection in the rat ventral tegmental area. Psychopharmacology, 103: 493-502.

23. Penke, Z., 2002. Stress néonatal et régulation à long terme de la prise alimentaire chez le rat:explorations neuroendocriniennes, morphofonctionnelles et comportementales, Thèse de Doctorat en Biologie et Santé Université Henri Poincaré – Nancy, Paris, pp: 170.
24. Caldji, C., B. Tannenbaum, S. Sharma D. Francis, P.M. Plotsky and M.J. Meaney, 1998. Maternai care during infancy regulates the development of neural systems mediatingthe expression of fearfulness in the rat. Proceedings of the National Academy of Sciences. U. S. A., 95(9): 5335-5340.
25. Mohammed, A.H., B.G. Henriksson, S. Soderstrom, T. Ebendal, T. Olsson and J.R. Seckl, 1993. Environmental influences on the central nervous system and their implications for the aging rat, Behavioural Brain Research, 57(2): 183-191.
26. Oitzl, M.S., J.O. Workel, M. Fluttert, F. Frosch and E.R. De Kloet, 2000. Maternai deprivation affects behaviour from youth to senescence: amplification of individual differences in spatial learning and memory in senescent Brown Norway rats.Neurosciences, 12(10): 3771-3780.
27. Van Oers, H.J., E.R. De Kloet and S. Levine, 1997. Persistent, but Paradoxical, Effects on HPA Regulation of Infants maternally Deprived at Different Ages. Stress, 1(4): 249-262.
28. Suchecki, D. and S. Tufik,199. Long-term effects of maternai deprivation on the corticosterone response to stress in rats. Physiology, 273(42): 1332-1338.
29. Rots, N.Y., J. De Jong J.O. Workel, S. Levine A.R. Cools and E.R. De Kloet, 1996. Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. Neuroendocrinology, 8(7): 501-506.
30. Lehmann, J., C.R. Pryce, D. Bettschen and J. Feldon, 1999. The maternai separation paradigm and adult emotionality and cognition in male and female Wistar rats. Pharmacology Biochemistry and Behavior, 64(4): 705-715.
31. Leshem, M., M. Maroun and S. Del Canho, 1996. Sodium depletion and maternai separation in the suckling rat increase ifs salt intake when adult. Physiology Behavior, 59(1): 199-204.
32. Diane, A., 2006. Stress, axe corticotrope et caractéristiques nutritionnelles et métaboliques, thèse de doctorat en nutrition humaine, Ecole Doctorale ABIES, Paris, pp: 175.
33. Mcewen, B.S., 1998. Stress, adaptation and disease. Allostasis and allostatic load., Annals of the New York Academy of Sciences, 940: 22-44.
34. Knuth, E.D. and A.M. Etgen, 2005. Corticosterone secretion induced by chronicisolation in neonatal rats is sexually dimorphic and accompanied by elevated ACTH. Hormones and Behavior, 47: 65-75.
35. Poucet, B. and S. Benhamou, 1997. The neuropsychology of spatial cognition in the rat. Critical Reviews in Neurobiology, 11: 101-120.
36. Pellow, S., P. Chopin, S.E. File and M. Briley, 1985. Validation of open/closed arm entries in an elevated plus maze as a measure of anxiety in the rat, the Journal of Neuroscience, 14: 149-167.
37. Hall, C.S., 1934. Emotional behavior in the rat. Defecation and urination as measures of individual difference in emotionality, Journal of Comparative Physiology, 18: 385-403. Sciences Pharmaceutiques et Biologiques, Paris, pp: 160.
38. Bifulco, A., T. Harris and G.W. Brown, 1992. Mourning or early inadequate care? Reexamining the relationship of maternal loss in childhood with adult depression and anxiety, Development and Psychopathology, 4: 433-49.
39. Sansri, S., A. Bairi, M. Haloui, C. Rettem and A. Tahraoui, 2014. Behavioral Studies and Immune to Acute Restraint Stress in the Wistar Rat,the Journal of World Applied Sciences Journal.
40. Husum, H., E. Termeer, A.A. Mathe, A.A. Bolwig and B.A. Ellenbroek, 2002. Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonin-gene related peptide in adult rats. Neuropharmacology, 42(6): 798-806.