JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY

ACCEPTED ARTICLE

Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats

Amri Naziha[1]*, Habbachi Sarra[1], Samai Ibtissem[2], Bairi Abdelmadjid[1], Tahraoui Abdelkrim[1]

1. Applied Neuroendocrinology Laboratory, Department of Biology, BadjiMokhtar University 23000 Annaba POBox 12 El Hadjar Annaba / Algeria.

2. Laboratory research of soil and sustainable Development, department of biology, faculty of sciences, Badji Mokhtar University23000 Annaba POBox 12 El Hadjar Annaba / Algeria.

Corresponding author: amrinaziha23@gmail.com

Telephone number +213 559 333 115

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

Abstract

In the present work, we sought to determine the effects of an individual application of prenatal stress and postnatal stress and their combination on the anxious and locomotor behavior of first-time mother rats. Indeed, three batches of rats were exposed to three types of daily repeated stress: one batch exposed to prenatal restraint stress (SC): 01h a day from day 11 to day 19 of gestation, one batch exposed to postnatal mother prenatal stress (SS): 03h a day from the third to the fourteenth postnatal day (JPN), and one batch exposed to combined restraint stress: 01h from day 11 to day 19 of gestation followed by a postnatal mother separation of 03h a day from the third to fourteenth JPN (CSS); a fourth control batch (T) of female rats that did not undergo any type of stress was also part of our study. At postnatal days 15 and 20, the behavior of the female rats in the four batches was assessed using the Open field Test along with the Plus Maze, and at JPN 30, the female rats were decapitated, the blood of which was collected for plasma adrenocorticotropic hormone (ACTH) level evaluation. Our findings show that all studied behaviors were very highly affected relative to the results of the control batch after individually applying prenatal restraint stress and mother separation. We noticed an intensified anxious behavior in CSS with a significant increase in plasma levels of ACTH.

Keywords: Early life stress, anxiety, ACTH

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

1. Introduction

Two symbolic situations are depicted by the prenatal period and the postnatal period in all mammals (Missonnier 2003). Exposure to a stressful environment during the prenatal period, i.e. gestation, leads to a weakening and favors the appearance of cognitive and behavioral disorders in the mother as well as a major influence on the neuro-development at the embryonic stage (Aaron et al. 1998). The postnatal period is a significant introduction to mothering in self-confidence of mothers; however, many physical and internal/external stressors can be present, which will lead to negative consequences on the mother and will have an impact on its relationship with their growing baby and disturb its environment to which it is exposed in its early life (Bales 2015). The stressful events activate the hypothalamic-pituitary-adrenal axis (HHS) and enhance the corticotropin-releasing factor hormone from the paraventricular nucleus of the hypothalamus, which causes adrenocorticotropin (ACTH) secretion from the anterior pituitary. Studies were conducted on pregnant women. These revealed more consistently that the psycho-social factors (such as stress or the social support) are significantly correlated with the incidence of diseases (pathological) at birth (premature) (Barbosa et al. 2000; Da Costa et al. 2000; Graignic-Philippe et al. 2005).

High levels of prenatal maternal anxiety were also found to be related to the duration of labor (Pagel et al. 1990; Graignic-Philippe et al. 2005). Critical periods may be spotted during the growth of an individual. Exposure to stressors during the perinatal period may give rise to the advent of disorders in the long term. The brain growth period stands mainly in the last quarter of pregnancy in humans and between the fifth and fifteenth days in a rat's life. (Bayer et al. 1993). The combination of these stress types, namely, the prenatal and postnatal stress stimulate in our experimental model a myriad of stressors occurring in a daily environment.

2. Material and methods

2.1. Biological material

The rats used in this work were white male and female rats Ratusratus of the Wistar strain, a class of nocturnal mammals of the rodent order brought from Pasteur Institute in Algiers. The rats were coupled and their offspring (the male rat pups) were the subjects of this study. The

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

animals were raised in easy-to-clean polyethylene cages. The experimentation took place after an acclimation period during which the rats were subjected to a temperature of 25 °C, a hygrometry of 50%, and the natural photoperiod. The distributed food was in the form of pellets provided by the ONAB AL-KASER BEJAIA-ALGERIE National Livestock Feeding Board. The drinking water was supplied in ad libitum bottles to meet the animals' needs. All experimental procedures described in this work are following the guidelines of Care and Use of Laboratory Animals of the University of Badji Mokhtar Algeria.

2.1.2. Gestation

To induce and synchronize the estrous cycle in females (Whitten 1958), some cages containing two males were positioned next to those of females. After 07 days of exposure to male pheromones, females were placed in pairs with one male for mating. Day 0 of gestation matched the day of the presence of spermatozoids, which was verified by using a vaginal smear. Pregnant females were split into four experimental batches of six rats each.

2.1.3. Distribution of pregnant females

- (T) female rats did not undergo any stress (n = 6)
- (SC) female rats were exposed to restraint stress 1 h/day from day 11 to 19 of pregnancy (n = 6)
- (SS) female rats were exposed to maternal separation stress 3 h/day from postnatal day 3 to 14 (n = 6)
- (SSC) female rats were exposed to combined stress (restraint stress followed by mother separation) (n = 6)

2.2. Methods

2.2.1. Applying the restraint stress

The stress model we use in our laboratory is that of Bardin et al. (2009). The female rats were placed in a plastic cylindrical bottle, the bottom of which was cut off to let the animal in. The other end was fitted with an opening, which acted as an air vent with a 01-cm diameter.

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

From day 11 of gestation to day 19, (SC) and (SCC) batches were exposed every day from 10:00 am to 11:00 am in the same room to this stress. All female rats were taken back to their breeding cages when the protocol was over.

2.2.2. The separation process

The pregnant females were housed individually. The day of birth was named D 0. On day 3, groups of 6 rats were carried out. Only males were randomly retained so that every female rat had the same number of rats in her cage. The maternal separation protocol was done from D 3 to D 14 for batches (SS) and (SSC). Each rat was separated from the litter for 3 h (Meaney et al. 2008) from 09:00 am to 12:00 pm daily in the same room. The latter was moved to another cage and returned to its original cage with its litter.

2.2.3. The Combined stress

Here, pregnant rats from the (SCC) batch were exposed to restraint stress and then separated from their pups after parturition in exact accordance with the same aforementioned durations.

2.3. The Open field test

Described by Hall in 1934, it was developed to measure rodent anxiety, locomotor activity, and behavior. The open field test was performed for 5 min. The animal was placed in the center of the device and its movement was monitored by measuring the number of crossed squares and the time spent in each zone, which indicated locomotor activity and anxious behavior, respectively. The latter was particularly noted when the rat spent more time in the peripheral zone than in the central zone. Its exploration represents a sign of less anxiety. At the end of this test, the following parameters were measured:

• Time spent in the periphery in seconds.

• Rectification time in seconds (animal positioned on its two posterior, right legs, equilibrium in the vacuum or against a wall).

• Number of crossed spaces, which corresponds to the traveled distance.

2.4. The Elevated plus maze test

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

The Plus Maze test, commonly known as the Raised Cross Maze, is a behavioral test that was developed by Montgomery in 1955. It is used to assess the anxiety level in the tested animal. True to its name, this device is made up of 04 perpendicular wooden arms 10 cm wide and 50 cm long. Two arms are open while 02 are surrounded by a 40-cm-high plexiglass border. The maze was raised 50 cm off the ground, and the following parameters were measured: the up-righting time and time spent inside the open arms.

2.5. Blood sampling

The blood sample was taken after slaughtering the female rats from the four batches: control batch (T), restraint stress batch (SC), separation batch (SS), and the combined batch (SSC). The blood samples were collected in heparin pipes for the ACTH assay.

2.6. ACTH assay

The ACTH assay was performed by using the "IMMULITE ACTH" kit. The kit contains a solid phase for the chemiluminescent sequential immunometric assay (Menarini, 2010). This test was carried out in two steps for the quantitative determination of serum levels of ACTH. The solid phase was a bead-coated with murine anti-ACTH monoclonal antibody. The liquid phase was alkaline phosphatase (an enzyme that enhances chemiluminescence for antigen detection) conjugated to an anti-ACTH rabbit polyclonal antibody in the ACTH reagent.

2.7. Statistical survey

The results were obtained using the XLSTAT 2014 software in the form of histograms and were treated by using the parametric variance comparison test, which allowed us to compare the results of the experimental batches (SC, SS, and SSC) with those of the Control batch (T).

3. Results

3.1. Variation of the Open field test parameters

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

The female rats were subjected to an open field test session on the 15th day of testing for the four batches (control and stressed).

Figure 1A, The results obtained in this study show a highly significant decrease between the number of cases crossed by stressed female rats (SC) and the control female rats (Fobs=5.15 ;p:0.008). The stressed female rats (SC), (SS) and (SSC) cross on average (45.66.±3.44), (32 ±2.28) and (29.50±2.88) cases in comparison with control female rats that use (88 ±9.24) cases.

Figure 1B, The results obtained in this study show a major drop of the uprighting (Fobs=4.007; p:0.022). The average uprighting time in stressed female rats (SC),(SS) and (SSC) is (25.83s ±11.23) (15.50 s ±7.79) et (13.33 s ± 6.37) seconds while it reached (40.83s ±14.90) in control female rats.

Figure 1C, The results obtained in this study show a substantial increase of time spent in the peripheral part of the Open Field Test (F_{obs}=3.20 ; P = 0.045). The stressed female rats (SC), (SS) and (SSC) spend respectively (181.83s ± 7.65), (202.33 ±21.80s) and (223.3±8.72 s) in the periphery whereas the control female rats spent on average, 74.5 ± 14.61 s.

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

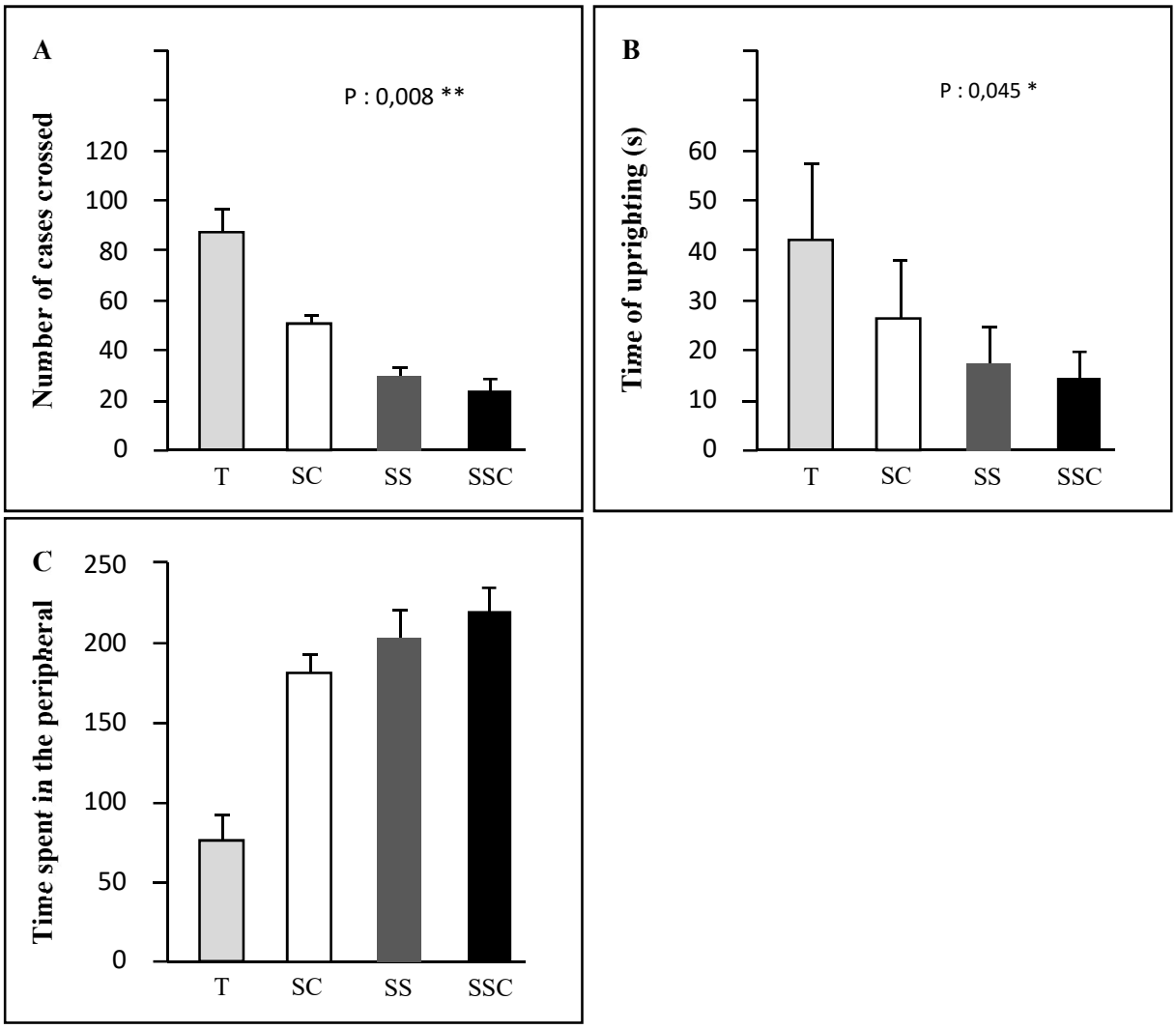

Figure 1 Variation of the Open field test parameters. The results are expressed on average ± SEM, (n = 6).
Ns. Insignificant difference $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3.2. Parameters Variation in the Elevated plus maze test

Rats were exposed to an EPM test session on the 20th day of experimentation on four batches (control and stressed).

Figure 2A, The time spent inside the open arms of the device by the stressed female rats (SC), (SS) and (SSC) (69.16±35 s), (60±15.65 s) and (46.6 ±9.48 s) is lower than that of the control female rats (204.167s ±43.14) in a highly significant way ($F_{obs} = 5.16$; $P = 0.008$).

Figure 2B, The results of our study indicate a very high time of the uprighting (Fobs 11.66 ; p:0.000) in the stressed female rats (SC), (SS) and (SSC) (13.83 ±0.75s), (15.33 ±9.28s) and (16.33 ±12.45s) relative to control female rats (45.16 ±15.52s)

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

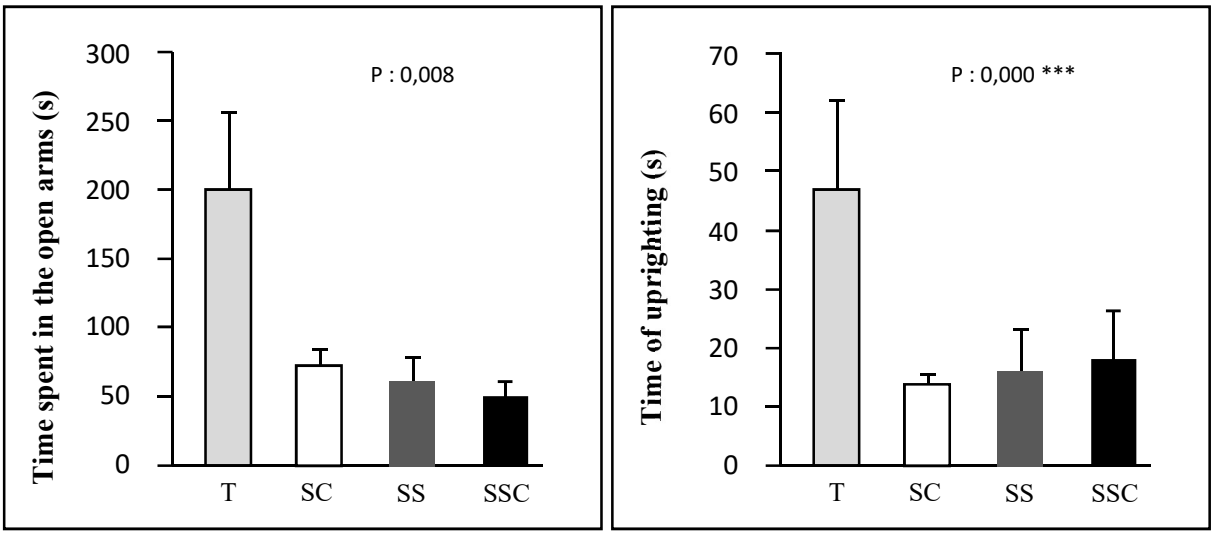

Figure 2 Parameters variation in the Elevated plus maze test. Results are outlined on average ± SEM, (n=6).
Ns. Minor difference $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

3.3.Variation in ACTH rate

The attained findings of our study show that the ACTH averages of stressed female rats (SC), (SS) and (SSC) (391.66±14.72 ng/ml), (426.66±5.16 ng/ml) and (440±5.47 ng/ml) are higher than that of the control female rats (271.5 ± 3.45 ng/ml). This uptick is statistically highly considerable (Fobs=5.41 ; $P = 0.007$)

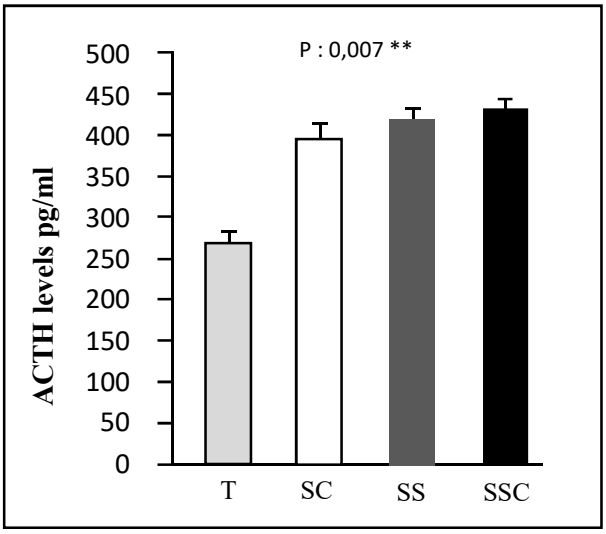

Figure 03: Variation of the ACTH rate in pg /ml. The results are expressed on average ± SEM, (n = 6).
Ns. (insignificant difference $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

4. Discussion

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

The maternal behavior alters the expression of several hundreds of genes as well as a significant part of the offspring's future behaviors, including possible anxio-depressive behavior. Abandoning a newborn as an early emotional deprivation may leave behind some marks in the growing baby's brain through epigenetic mechanisms resulting most often in hypermethylation of genes promoters, leaving them inactive. These brain prints will show up in the form of anxious and depressive moods that can persist in adulthood and be passed on from one generation to another (hereditary). However, these epigenetic effects may be definitive or reversible (Weaver 2006). Our findings display that the stressed female rats (SS), (SC), and particularly (SSC) spend more time in the peripheral zone than the control female rats; besides, there is a reduction is the up-righting time, which is an evidence of the increase in the anxious behavior, and this finding is consistent with those of the following studies: stress can also have an impact on aggressiveness (Barreto-Medeiros et al. 2007; Veenema et Neumann 2007) and particularly causes a rise in the anxiety level in the open field test.

The study results show a decline in the number of boxes passed through by the stressed female rats (SS), (SC), and (SSC) relative to the control female rats. The diminution of the distance covered in the device for stressed female rats indicates a decline in the exploratory activity, which is an evidence of a higher anxiety level in the female rats (Prut et Belzung 2003).

Every newborn child is cared for by his mother and stays in direct contact with her over a certain period. This early time of life is of great importance in the individual's emotional and cognitive development while providing a shape to its responses to stressors in adulthood (Kafman et al. 2007).

In rodents, the maternal interaction is highly influential on the development and future function of the HHS. An extended maternal separation (SM) is enough to increase the ACTH and corticosterone levels, especially when the studied rat is aged 07 days (Aguilera 1994). The results found in our experimentation show very high ACTH levels (very highly significant) in stressed female rats and prove hyperactivity of the hypothalamic-pituitary-adrenal axis compared to the control rats. In response to a stressor, the paraventricular nucleus (NPV) of the hypothalamus synthesizes and secretes mainly the corticotropin releasing factor (CRP). Arginine vasopressin is also secreted by the paraventricular nucleus, which has the effect of bolstering the stimulatory effect of the CRP. The latter is then moved to the anterior pituitary lobe to bind with its receptor. The attachment of CRP on CRHR1 enables the synthesis of

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

proopiomelanocorticotropin (POMC), the forerunner of adrenocorticotropin (ACTH). The POMC is subsequently cleaved by the prohormone convertases PC1 and PC2, allowing ACTH release and melanotropin (MSH). The ACTH then binds with the receptors of type 2 melanocortins (MC2-R) in the adrenal cortex. Activation of MC2-R enables glucocorticoid synthesis: cortisol in humans, and corticosterone in rodents (Rodrigues 2009).

Some glucocorticoid receptors (RG) are expressed in the hippocampus, hypothalamus, and pituitary gland. At a very high concentration, the glucocorticoids (GC) exert negative feedback over the production of CRP and ACTH via the glucocorticoid receptors (Toledano 2014).

5. Conclusions

Our findings reveal that all studied behaviors were significantly affected in comparison with the results of the control batch after the individual implementation of the prenatal restraint stress and the mother separation stress in the (SC) and (SS) batches. We also noted the onset of an intensified anxious behavior, as well as a very diminished locomotor behavior in female rats from the (CSS) batch. Moreover, the ACTH plasma levels were also affected by the three stress types with a major upsurge in the (SS) and (CSS) batches**.**

Conflict of Interest

The authors declare no conflict of interest.

References

Aaron J N, Field T, Fox NA, Davalos M, Lundy B, Hart S (1998) Newborns of mothers with depressive symptoms are physiologically less developed - Infant Behavior andDevelopment 21:537–41.
Aguilera G, Lightman SL (1994) Transcriptional and post- Transcriptional regulation ofcorticotropin relasing hormone and vasopressin expression by stress and gluco corticoides. Stress: Neural, Endocrine and Molecular Studies 1:96-276.
Bales M (2015) Perinatal psychological difficulties: risk factors and development of amultifactorial model in the general population. Results of the French Longitudinal Study since Childhood (ELFE).
Barbosa GA (2000) The association of life events to gestational age at delivery among low- income, urban,African-american women. J Perinatol 20:438–42.
Bardin L, Malfetes N, Newman-Tancredi A, Depoortère R (2009) Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance tohuman stress-associated painful pathologies.Behavioural Brain Research 205: 360–366.
Barreto-Medeiros J, Queiros-Santos A, Cabral-Filho JE, Ferreira E Silva WT, Leandro CG,Deiró TC, Manhaes-de-Castro R (2007) Machado Barbosa de-Castro CM.: Stress/aggressivenessinduced immune changes are altered in adult rats submitted to neonatal malnutrition. Neuroimmunomodulation 14:229-334.
Bayer SA, Altman J, Russo RJ, Zhang X (1993) Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14:83-144.
Da Costa D, Larouche J, Dritsa M, Brender W (2000). Psychosocial correlates of prepartum and postpartum depressed mood. J Affect Disord 59:31–40.

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005

Graignic-Philippe R, Tordjman S, Granier-Deferre C, Ribeiro A, Jacquet AY, Cohen-Salmon C, Fortes S, Gérardin P (2005) Le stress prénatal: état de la question et perspectives. Neuropsychiatrie de l'enfance et de l'adolescence 53: 54–61.
Hall C S (1934) Emotional behavior in the rat: Defection and urination as measures of individual differences in emotionality. Journal of Comparative psychology 18:385-403.
Kaffman A, Meaney M (2007) Neurodevelopmental sequelae of postnatal maternal care inrodents: clinical and research implications of molecular insights. J Child Psychol Psychiatry 48:224–244.
Meaney MJ, Brake W, Gratton A (2008) Environmental regulation of the development ofmesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse. Psychoneuroendocrinology 27:127-138.
Missonnier S (2003) Perinatal therapeutic consultation. Toulouse, Erès.
Montgomery KC (1955) The relation on skip between fear induced by novel stimulation exploratorybehaviour.J.comp.physio 48: 254-260.
Pagel MD, Smilkstein G, Regen H (1990) Psychosocial influences on new born outcomes: a controlled prospective study. Soc Sci Med 30:597-604.
Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxietylike. Behaviors: a review. Eur J Pharmacol 463: 3-33.
Rodrigues SM, LeDoux JE, Sapolsky RM (2009) The influence of stress hormones on fear circuitry. Annu Rev Neurosci 32: 289–313.
Aguilera G (1994) Regulation of pituitary ACTH secretion during chronic stress. Front Neuroendocrinol 15: 321–50.
Toledano D (2014) Base physiologique commune entre état de stress post-traumatique et dépendance aux drogues: caractérisation comportementale et tentative thérapeutique chez le rat 4:27-70.
Veenema, A. H , Blume A , Niederle D , Buwalda B, Neumann I. D (2006) Effects of early life stress on adult male aggression and hypothalamic vasopressin and serotonin. The European Journal of Neuroscience 24:1711-1720.
Weaver CG, Meane MJ (2006) Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. Proceedings of the National Academy of Sciences 802:687-698.
Whitten WK (1958) Inactivation of human chorionic gonadotropin extracted from chorionic tissue and serum by neuraminidas Journal Endocrinol 14:160-163.

Cite this article as: Naziha A, Sarra H, Ibtissem S, Abdelmadjid B, Abdelkrim T (2021) Amplified neurobehavioral and hormonal alterations following the combination of prenatal and postnatal stress in first-time rats. *Journal of Animal Behaviour and Biometeorology*, doi: https://doi.org/10.31893/jabb.21005