j.Egypt.net.med.Assoc 78, no 2. 301 – 308 (2018)

2-CONSEQUENCES OF UNPREDICTABLE CHRONIC MILD STRESS (UCMS) AS A MODEL OF DEPRESSION ON THE PHYSIOLOGICAL AND NEUROCHEMICAL PARAMETERS OF MALE SWISS MOUSE

By

Heba M. A. Khalil*; E. M. Abdel-Gawad[a]; M.Y. Matoock[a] and Marwa M. Abd-El-Rabo[b]

[a]Hygiene and Veterinary Management Department, Faculty of Veterinary Medicine, Cairo University, Egypt

[b]National Organization for Drug Control and Research (NODCAR), Giza, Egypt

*Corresponding author at: Hygiene and Veterinary Management Department, Faculty of Veterinary Medicine, Cairo University, P.O. Box 1221, Giza Governate, Cairo.

E-mail addresses: heba.ali315@gmail.com, heba.ali@cu.edu.eg (Heba Ali).

ABSTRACT

The current study was conducted to investigate the effect of unpredictable chronic mild stress as a model of depression on the physiological and neurochemical parameters of male swiss mice. Twenty- Five adult male *Swiss* albino mice were housed in either control group or unpredictable chronic mild stress (UCMS) group that was exposed to different physical stressors for 8 weeks. Plasma corticosterone and brain stem levels of dopamine and serotonin (5-OH) were measured. UCMS mice displayed an increase in the plasma corticosterone concentration and lower levels of both brain stem dopamine and 5- OHT compared to controls. In conclusion, the presence of physiological changes along with the significant neurobiological changes in the brain strongly support the efficacy of UCMS as a model of depression in the human being.

Key words:

Depression;Swiss mice;Unpredictable chronic mild stress;corticosterone; dopamine; serotonin

INTRODUCTION

Depression, formally termed major depressive disorder (MDD) is the most prevalent and prominent cause for disability worldwide. According to world health organization **(WHO, 2017),** over 300 million people are suffering from depression, equivalent to 4.4% of the world's population. Approximately half of these people live in the South-East Asia Region and Western Pacific Regions.

According to Diagnostic Manual of Mental Disorders (DSM-IV) **(American Psychiatric Association,1994),** diagnosis of depression depends on the presence of either depressed mood or anhedonia in combination with four additional symptoms related to weight changes, sleep disturbances, psychomotor retardation or agitation and, feelings of worthlessness for two weeks **(Berton and Nestler, 2006)**. Some of these symptoms can be reproduced and thus can be assessed in laboratory animals (e.g., weight changes, sleep disturbances, and, psychomotor retardation or agitation) **(Hasler *et al.,* 2004).** In contrast, other symptom cannot be reproduced in laboratory animals (e.g., depressed mood, and, feelings of worthlessness or suicidal thoughts) **(Deussing, 2006).**

Chronic stress is the leading cause of major depression because of disruption the overall homeostasis of the organism **(Surget and Belzung, 2009).** In addition, stressful life events and genetic background are known to increase susceptibility for depression**. (Zilkha *et al.,* 2014)** and lead to a transient hyperactivation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Consequently, an increase in the glucocorticoid secretion **(Cryan and Slattery, 2007),** suggesting that depressed patients must have an impairment of proper stress coping strategies **(De Kloet *et al.,* 2005).** Also, chronic stress can cause a down regulation of serotonin and dopamine receptors **(Zurawek *et al.,* 2013)**. Therefore, some models used to mimic depression in human are greatly based on exposure of the animals to stressful situation **(Schweizer *et al.,* 2009).**

Unpredictable chronic mild stress (UCMS) is the most commonly used paradigm to study depression **(Willner, 2017)**. The main advantages of this model are firstly, the mice cannot predict the stressors so they cannot habituate it. Secondly, the use of different physical stressors such as food deprivation or cage tilting. Thirdly, end phenotype of mice exposed to UCMS resembles aspects of depression in many features, including anhedonia **(Willner, 2017),** loss of appetite, loss of weight, a decrease in locomotor activity, and, increased immobility in the forced swim test.

This experiment was therefore performed to investigate the effect of UCMS on the physiological and neurochemical parameters of male Swiss mice, as well as increase the knowledge on the functioning of the HPA axis under chronic stress to understand the intimate link between stress response and the pathogenesis of depression.

1.Methods:

General animal housing and husbandry.

All aspects of experimental design were performed in compliance with the Guide for the Care and Use of Laboratory Animals **(National Research Council (U.S., 2011)** and approved by the Institutional Animal Care and Use Committee (IACUC) (2016) of Cairo University.

The current experiment was carried out with a total number of 25 male Swiss Albino mice obtained from (private animal house, Giza district, Cairo) weighing 25 -30 gm at arrival. Mice were housed in plastic shoebox-type cages (27×13×13 cm) with stainless steel wire lids. Cages were supplied with saw dust as a bedding material (1-2 cm thick).

Ordinary balanced diet (22.75% protein, 4.63% fats, and 5.35% fibers) and tap water were provided ad libitum unless otherwise stated. Mice were maintained on a 12:12 h light / dark cycle, at a constant temperature (20±2 ℃) and relative humidity (55 %).

Experimental procedures:

Mice were assigned to two test groups: (Group one (Control) (n=10) receiving the ordinary daily care and continued throughout the eight weeks of experiment and, Group two (Unpredictable chronic mild stress) (UCMS) (n=15) exposing to 2 or 3 different kind of stressors for 8 consecutive weeks in a chronic and unpredictable way, at any time of the day according to **(Nollet *et al.,* 2013)**. The stressors applied in this experiment are illustrated in (Table 1). Data collection Physiological parameters (serum corticosterone level) after the end of unpredictable chronic mild stress paradigm, the animals were sacrificed by decapitation 24 hours after the last behavioral test at 9.00 am. 10 trunk blood samples were collected (from each group in plastic plain vacutainer. All blood samples were centrifuged for 15 min at 3000 rpm. Serum was transferred to clean, labeled 1.5 ml micro centrifuge tubes. All serum samples were stored and frozen at - 20 ºC until radio immune assay. Measurement of serum corticosterone (Cort) using immune assay technique using corticosterone ELISA kit according to the manufacture instruction (Kono biotech Corporation, LTD).

Neurochemical parameters (High performance liquid chromatography (HPLC) Brain was removed, and placed on ice-cold surface for dissection. Brain stem region include pons, midbrain and medulla oblongata was removed, weighted and preserved in cryo tube at - 80 ºC until analyzed. Homogenization of the brain tissue was carried out on ice in 1/10 w/v of 75% aqueous HPLC grade methanol. The homogenate was spun at 4000 g for 10 min.

The resulting chromatogram identified each monoamine position and concentration of the sample as compared to that of the standard, and finally, the calculation of the content of each monoamine as mg per gram brain tissue was made according to **Pagel *et al.* (2000).** Statistical analyses. All statistical analyses were performed using the statistical package for social science (SPSS) 22.0 for windows **(IBM Corp., NY, Armonk, 2013).** Shapiro-Wilk's and Levene's tests were used to check the normality. A two-sided Student's *t*-test was applied for comparison of two experimental groups (control and UCMS group). When repeated measures ANOVA revealed a significant interaction effect of the factors week and group, data were further analysed with pairwise comparisons for each time point (Student's *t*-test). Data were expressed as mean ± S.E. The significance was set at 0.05.

RESULTS

1.1.Physiological parameters:

The output of student`s *t* test revealed that there was a significant effect of UCMS on serum corticosterone levels (t_5 = -2.949, p = 0.032; Fig. (1), with higher levels in mice of UCMS group than mice in the control group.

1.2.Neurobiological parameter:

Statistical analyses revealed that there was a significant effect of UCMS on brain stem levels of dopamine (t_{10}= 21.18 , p= 0.0001) and 5-OH-T (t_{10}= 12.07, p = 0.0001), with higher levels in mice of the control group than their counterparts in UCMS group as shown in (Table 2).

DISCUSSION

The findings of this experiment demonstrate clear differences in the physiological and neurochemical performance reported in the study between the mice exposed to different unpredictable chronic mild procedures in UCMS group and those of the control group.

Mice of UCMS exhibited higher levels of plasma corticosterone and lower brain stem levels of both dopamine and 5 - OHT compared to control mice.

The significant increase in serum corticosterone levels displayed by mice in UCMS compared to control group could be due to impaired feedback regulation in the hypothalamic-pituitary-adrenal (HPA) axis after exposure to UCMS **(Li, 2008)**. This result is in accordance with those of **(Krishnan and Nestler, 2008) and Anisman, 2009).**

According to the monoamine hypothesis of depression **(Maes, 1995)** and a dysfunctional serotonin system of depressed patients **(Gross *et al.,* 2002),** lower levels of brain stem

dopamine and 5- OHT were displayed by the mice in UCMS compared to controls. This could be due to that chronic mild stress induces neurochemical changes similar to those associated with depressed patients **(Anisman and Zacharko, 1990).**

Therefore, based on the findings of the current experiment, it appears that mice exposed to UCMS displayed higher levels of serum corticosterone and lower brain stem levels of dopamine and 5 - OHT compared to mice in control group. Therefore, UCMS may be considered an effective tool for modeling major depressive disorders in human.

REFERENCES

American Psychiatric Association (1994): Diagnostic and Statistical Manual of Mental Disorder (DSM-IV). Washington, DC.

Anisman H, Zacharko RM. (1990): Depression as a consequence of inadequate neurochemical adaptation in response to stressors. Br J Psychiatry 15:36 - 43.

Anisman H. (2009): Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. J Psychiatry neurosis 34 (1):4 -20.

Berton O, Nestler EJ. (2006): New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosis **7**:137-151.

Cryan JF, Slattery DA. (2007): Animal models of mood disorders: recent developments. Curr Opin Psychiatry 20:1-7.

De Kloet ER, Joëls M, Holsboer F. (2005): Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6:463 - 475.

DeussingJM.(2006): Animal models ofdepression. Drug DiscovToday,Disease Models 3 (4).

Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R. (2002): Serotonin1A receptor acts during development to establish normal anxiety like behaviour in the adult. Nature 416:396 - 400.

Hasler G, Drevets WC, Manji HK, Charney DS. (2004): Discovering end phenotypes for major depression. Neuropsychopharmacol 29:1765-1781.

IBM Corp (2013): IBM SPSS statistics for windows, Version 22.0, Armonk. NY.

Krishnan V, Nestler EJ. (2008): The molecular biology of depression. Nature 455.

Maes M (1995): The serotonin hypothesis of major depression. Psychopharmacology: The fourth generation of progress. K. D. Bllom FE. New York, Raven Press 933-944.

National Research Council (U.S.) (2011): Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (U.S.). Guide for the care and use of laboratory animals. National Academies Press.

Nollet M, Guisquet A, Belzung C. (2013): Models of depression: unpredictable chronic mild stress in mice. Current Protocols in Pharmacology.

Li S, Wang C, Wang W, Dong H, Hou P, Tang Y. (2008): Chronic mild stress impairs cognition in mice: from brain homeostasis to behavior. Life sciences 82:934 - 42.

Pagel P, Blome J, Wolf HU (2000): High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. J Chromatogr 746:297-304.

Schweizer MC, Henniger MS., Sillaber I. (2009): Chronic Mild Stress (CMS) in Mice: Of Anhedonia, 'Anomalous Anxiolysis' and Activity. PLoS ONE 4 (1): e4326.

Surget, A. and Belzung, C. (2009): Unpredictable chronic mild stress in mice. In Experimental An-imal Models in Neurobehavioral Research (A.V.Kalueff and J.L. La Porte, eds.) pp. 79 -112. Nova Science Publishers, New York.

Zilkha N, Feigin E, Barnea YG, Noam Zangen A. (2014): Induction of depressive-like effects by subchronic exposure to cocaine or heroin in laboratory rats. Journal of neurochemistry 130:575-582.

Zurawek, D., Faron-G, orecka, A., Ku Smider, M., Kolasa, M., Gruca, P., Papp, M., Dziedzicka-Wasylewska, M., (2013): Mesolimbic dopamine D2receptor plasticity contributes to stress resilience in rats subjected to chronic mild stress. Psy- chopharmacology 227, 583e593.

Willner P. (2017): Validity, reliability, and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134:319-329.

World Health Organization (WHO). [Online] (2017): Depression and other common mental disorders: global health estimates, Geneva. Available at http://apps.who.int/iris.

(Table 1): Types of stressors used in the unpredictable chronic mild stress model.

Stressors	Description	Duration
Empty Cage	**The sawdust was removed**	**1 to 6 hr**
Damped Cage	**About 125 ml of water was placed in each cage.**	**1 to 6 hr**
Bath	**The sawdust of each cage is removed and replaced by about 125 ml water at 20◦C (about 1 cm water)**	**15-30 min**
Soiled Cage	**About 60 ml of rat sawdust was deposited in each cage**	**1-2 hr**
Tilted Cage	**Cages were tilted backwards (45 degrees)**	**1-4 hours**
Water Deprivation	**Water bottles were removed for a certain time**	**12 hr**
Food Deprivation	**Food was removed for a certain time**	**12 hr**
Rat Exposure	**visual and olfactory contact were allowed with rat**	**15 hr**

(Table 2): Effect of UCMS on brain stem levels of dopamine and 5-OH-T in male *Swiss* albino mice of the two treatments.

Group	Brain stem levels (mg/gm wet tissue)	
	Dopamine (DA)	**5-OH-T levels**
Control	**2.16± 0.35^{a}**	**0.78±0.04^{a}**
UCMS	**0.91±0.07^{b}**	**0.10±0.01^{b}**

Table shows mean rank, Different small letters superscript within the same column denotes statistical significance at 0.05. UCMS refers to unpredictable chronic mild stress.

Fig. (1): Effect of UCMS on serum corticosterone concentration of the mice in the two treatments. Data on the graph are expressed as estimated marginal means± SE.*p<0.05.