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1-CONSEQUENCES OF UNPREDICTABLE CHRONIC MILD STRESS (UCMS) AS A MODEL OF DEPRESSION ON THE PHYSICAL AND BEHAVIOURAL PARAMETERS OF MALE SWISS MOUSE

By

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ABSTRACT

The current study was carried out to investigate the role of unpredictable chronic mild stress as a model of depression on the physical and behavioral 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. Body weight and body coat were assessed. Sucrose consumption test, reward maze test, elevated plus maze and forced swim test were conducted. UCMS mice displayed a reduction in body weight, and decreased sucrose consumption, while exhibited an increase in the latency to pass 1st gate of the reward maze. UCMS mice also showed a tendency of being in the closed arm of the elevated plus maze for a longer duration compared to mice in the control group, a tendency of being immobile in forced swim test. In conclusion, physical changes in conjunction with the significant behavioral changes strongly support the efficacy of UCMS as a model of depression in the human being.

Key words:

Depression; Swiss mice; Unpredictable chronic mild stress; Anhedonia; Sucrose preference; Forced swim test.

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).** Anhedonia is considered one of the most prominent symptoms of a major depressive episode **(GrØnli, 2006).** It is defined according to the DSM-IV **(American Psychiatric Association, 1994)** as loss of the ability to experience pleasure of any sort. It can be induced easily in rodents by decreasing responsiveness to rewards reflected by reduced consumption of sweetened solutions **(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. There are many literatures on the influence of CMS on mouse behaviour agreed on the great difference of the mouse strain in their vulnerability and response to the CMS procedure **(Mineur *et al.,* 2006. Pothion *et al.,* 2004),** examined three mouse strains and measured physical as well as behavioural parameters including consummatory behaviour of sucrose solution. Surprisingly, only one strain showed a decrease in sucrose consumption along with changes in other behavioural and physiological parameters. This experiment was performed to address the physical and behavioural outcomes of the UCMS as an indicator of depressive like behaviour in Swiss male mice.

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 performed with 25 male Swiss Albino mice weighing 25 -30 gm at arrival. Mice were housed in plastic shoebox-type cages with stainless steel wire lids. Cages were supplied with saw dust as a bedding material. 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:

Physical Parameters:

Body weight: throughout the eight-week experimental period mice were weighed weekly in grams using an electric sensitive balance.

Coat condition: The coat was assessed on the following seven body areas: head, neck, back, abdomen, tail, fore paws and hind paws and was scored for each area as follows: 1 (good) for smooth and shiny fur, with no tousled, spiky patches; 0.5 (moderate) for slightly cottony fur with some spiky areas; 0 (bad) for cottony fur on with slight staining. The scores for all seven body parts were summed to obtain an overall score, with a maximum possible score of seven **(Nollet *et al.*, 2013).**

Behavioural parameters:

At the sixth week of the unpredictable chronic mild stress paradigm, all mice in the two treatments were exposed to behavioural tests starting at 9.00 a.m. and ending at 12.00 p.m. the behavioural tests were conducted in the following order:

Elevated plus maze (EPM) test: all mice were tested individually in the light phase of the light/dark cycle on the same day between 09:00 and 12:00 h. Each mouse was gently placed at the junction area of the elevated plus maze, facing one of the open arms for 5 min.

the arms of the plus-maze were carefully cleaned with ethyl alcohol after each individual mouse was tested. The following measures were taken: the total numbers and durations of entries into closed and open arms **(Walf and Frye, 2007).**

Reward maze test: Reward maze apparatus was used in this test. It consists of three aligned wooden chambers (20-cm length × 20-cm width × 20-cm height) connecting by two doors (controlled by the experimenter). Only the colors of the walls and the floor are different between the chambers: white for the first one where the mouse is placed, gray for the second, and black for the third chamber where the cookie is available.

A piece of cookie (~2 cm × 2 cm) was placed in the center of the black box after habituation of the animals for the cookie, 2 weeks before the test, by placing one cookie in each cage and removing it before conducting the test by one week.

All mice were tested individually in the light phase of the light/dark cycle on the same day between 09:00 and 01:00 h. Each mouse was gently placed at the other end of the device in the white chamber (head facing opposite to the opening) for 5 min. The chambers of the reward maze were carefully cleaned with ethyl alcohol (Pharma One, Cairo, Egypt) after each individual mouse was tested.

The following measures were recorded: the latency for the animal to pass through the first gate (when the four legs have crossed the door) (seconds), frequency of passing second gate and latency to chew cookie in third gate (seconds) **(Nollet *et al*. ,2013).**

Forced swim test: each individual mouse was placed in a plastic rectangular water tank (60 ×60 cm) containing clean tap water at 25 ºC (13.5 cm deep) for 6 min. After the test, the mice were taken and allowed to dry before being returned to their home cages.

The test was performed between 09:00 - 1:00 h in the light phase of a light/dark cycle, the 1st 2 minutes was discarded from the measurement. Mobile movement indicated by active swimming movement or diving and /or active movement with forepaws against walls, while immobile movements indicated by floating without struggling and doing movements to keep the head above the water were recorded. **(Martinez-Mota *et al*., 2008).**

Consummatorybehaviour(Sucroseconsumption test): After 1 week of sucrose consumption training phase, mice were given, for a period of 24h, a free choice between two bottles, and one with 1% sucrose solution and another with water. This was conducted twice at the beginning of the experiment to determine their baseline then, after the end of stress period (8 weeks). **(Strekalova *et al.*, 2006).** Switching the position of the bottles halfway through the

procedure was necessary to prevent side preference in drinking. Regular sugar, stored in paper bags, well protected from sources of flavor, was used for the test.

The consumption of water, sucrose solution and total intake of liquids were measured by weighing the bottles, with balances which resolution was 0,1g. The preference for sucrose was calculated as a percentage of the consumed sucrose solution (Vs) over the total intake of liquids (Total (T) = V water + Vs), using the following formula:

Sucrose preference = (Vs / T) x 100%

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). For time series analysis (Body weight) repeated measures ANOVA with Greenhouse-Geisser correction for non- sphericity was used. 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. For forced swim test, Data were analyses using non-parametric test (Mann-Whitney test) and expressed as mean rank. The significance was set at 0.05.

RESULTS

Physical performance:

Body weight.

The output of student -*t* test revealed a significant effect to the unpredictable chronic mild stress on body weight in mice. Mice of UCMS group had a reduced body weight (t_{67}= 9.02, p = 0.0001; Fig. (1 B), as compared to those of the control group who showed a steady increase in body weight over 8 weeks Fig. (1 A).

Coat assessment:

The qualitative scoring of different body parts of mice revealed that coat deterioration generally began on the neck and on the back and then proceeded to the head, abdomen and the hind paws. Mice experienced chronic mild stress procedures has a more deteriorated body coat than mice in the control group as demonstrated by a decreased coat state score (t_{33}= 4.709, p=0.001; Fig. (2).

Behavioural performance:

Consummatory behaviour (Sucrose consumption test).

Student *t*-test yielded a statistical significance in sucrose preference between two groups (t_{15} =2.523, p=0.023). Mice experienced UCMS had decreased sucrose consumption as compared to mice in the control group Fig. (3).

Reward maze test (Cookie test).

Statistical analyses revealed a significant difference in the latency to pass 1st gate of the reward maze apparatus between the two groups (t_{35} = 1.637, p =0.01), whereas there was no a significant statistical difference between the two groups in the frequency of passing 2nd gate (t_{35} = 0.271, p=0.788).

Concerning the latency to chew cookie, a significant decrease was observed in mice of UCMS group as compared to those of the control group (t_{35} = 3.209, p=0.003) (Table 2).

Elevated plus maze:

Unpredictable chronic mild stress had a significant effect on the number of closed arm entry (t_{28} =-2.089, p=0.04), time spent in closed arm (t_{28} = 2.70, p=0.01) and time spent in open arm (t_{28} =2.700, p=0.01). Whereas there was no significant effect to the UCMS on the number of open arm entry (t_{28} = 0.507, NS). Means of measures of anxiety in two groups are shown in (Table 3) Forced swim test Data in forced swim test did not meet assumption of parametric test so, Mann - Whitney U test was applied and different parameters were demonstrated as mean rank as shown in (Table 4).

DISCUSSION

The findings of this experiment demonstrate clear differences in physical and behavioural, 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 in UCMS group displayed a reduction in their body weight, a significant deterioration in their body coat state, and decreased sucrose consumption at the end of the 8 weeks housing period. They also exhibited an increase in the latency to pass 1st gate of the reward maze apparatus and in the latency to chew cookie but displayed no significant effect on the latency to pass 2nd gate compared to mice in the control group. Mice in UCMS group also showed a tendency of being in the closed arm of the elevated plus maze for longer duration compared to mice in control group, whereas there was no significant effect on the number of entry to open arms. In addition, they showed tendency of being immobile in forced swim test in comparison with

control group. Chronic mild stress has been shown to correlate with decreased body weight **(Cooper *et al.,* 2009)**. The reduction in body weight displayed by the mice in UCMS group could be a result of presence of multiple stressors in their environment in a chronic and unpredictable way. Similar findings of decreased body weight in mice exposed to UCMS has been reported by **Nollet *et al.* (2013), Strekalova, (2008), Ducottet and Belzung, (2005), Strekalova *et al.* (2004), and Schweizer (2009).** Deterioration of body coat displayed by mice in UCMS group can be related to a decrease of grooming which results in a disturbance in self-directed behaviour of mice. This could be easily observed in many depressed patients demonstrated by poor personal hygiene, so it still an important measure of depression due to sensitivity of auto grooming behaviour to stress in rodents **(Kalueff and Tuohimaa, 2004).** A similar finding of body coat deterioration displayed by mice in UCMS group has been reported by **Nollet *et al.* (2013).** Anhedonia in rats and mice is considered as the hallmark of depression **(GrØnli *et al.,* 2005; Craft and Devries, 2006).** It can be measured by many ways as sucrose preference test **(Grippo *et al.,* 2006).** The low percentage of sucrose consumption displayed by mice in UCMS could be a reflection of the decrease in their body weight. However, it could also be due to a decrease in the attraction of mice in UCMS toward palatable solutions because of stress **(Corriea, 2010)** therefore, a reduction in their sucrose consumption. A similar finding of decreased sucrose preference displayed by mice in UCMS has been reported by **(Willner, 2005 and Strekalova *et al.,* 2004, 2008).** Another measure of anhedonia is reward maze test which measure the appetence of the mice to the pleasurable food (cookie consumption) **(Nollet *et al.,* 2013).** The reduction of the latency to pass 1st gate and latency to chew cookie displayed by mice in UCMS could indicate that stressors induce an anhedonic state. Some parameters, such as frequency of passing 2nd gate was not affected by UCMS. The finding of reward maze test is in accordance with that of **Nollet (2013), Isingrini *et al.* (2010) and Surget *et al*. (2011).** The results of the elevated plus maze (EPM) showed that UCMS mice displayed an increase in number of closed arm entry and increased in time spent in it compared to control group, which can be interpreted as a sign of anxiety **(Strekalova *et al.,* 2005).** Interestingly, anxiety-like behaviors often precede the onset of depressive-like behaviors **(Dzirasaa and Covington, 2012).** The finding of EPM is in accord with those of **Correia, (2010).** The forced swim test (FST) is commonly used for evaluating depressive like behaviour in psychiatric animal models **(Overstreet *et al.,* 2005).** Mice in

UCMS displayed a higher mean rank in immobility and a lower mean rank in mobility during the forced swim test compared to controls which could be due to behavioural despair, another hallmark feature of depression **(McArthur and Borsini, 2006).**

Therefore, based on the findings of the current experiment, it appears that mice exposed to UCMS displayed higher levels of various physical and behavioural changes as reduced body weight, coat deterioration, reduced sucrose preference, increased latency to pass 1st gate and to chew cookie, being in closed arm for longer duration, stayed immobile in forced swim test, compared to mice in control group. Therefore, UCMS dramatically affect the physical and behavioural profile of the mice rendering it an effective tool for modeling major depressive disorders in human.

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(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 behaviour of male *Swiss* albino mice in reward maze test.

Group	Reward maze test (Cookie test)		
	Latency to pass 1st gate (s)	Frequency of passing 2nd gate	Latency to chew cookie (s)
Control	**5.89 ± 1.328[a]**	**17.56±1.59**	**75..44±16.68[a]**
UCMS	**15.11±3.13[b]**	**18.18±1.193**	**199.29±21.05[b]**

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

(Table 3): Effect of UCMS on behaviour of male *Swiss* albino mice in Elevated plus maze.

Group	Elevated plus maze			
	No of entries to closed arm	Time spent in closed arm (second)	No of entries to open arm	Time spent in open arm (second)
Control	**4.67± 0.645[a]**	**67.33±14.1[a]**	**6.33±1.61**	**223.67±14.1[a]**
UCMS	**7.57±0.861[b]**	**197.19±14.4[b]**	**5.05±0.623**	**102.8±14.4[b]**

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

(Table 4): Effect of UCMS on behaviour of male *Swiss* albino mice in Forced swim test.

Group	Forced Swim test (mean rank)		
	Mobility	Immobility	Probability
Control	25.79[a]	10.21[a]	.024
UCMS	16.05[b]	19.95[b]	.024

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.

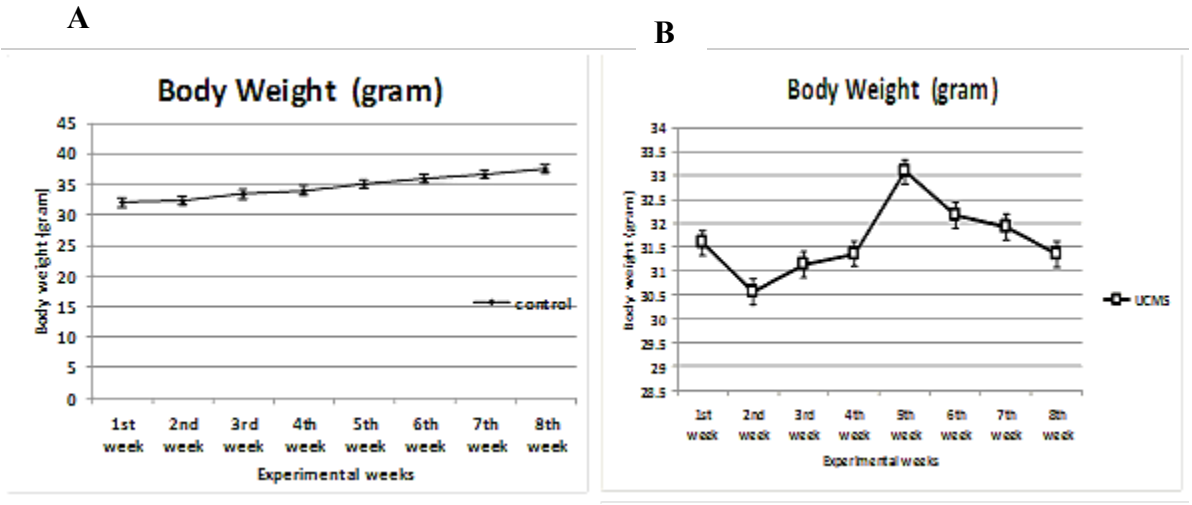

Fig .(1): Effect of UCMS on average body weight (g) of mice in the two treatments within 8 Weeks, A. Average body weight in control group, B. Average body weight in UCMS group. Data on the graph are expressed as estimated marginal means± SE.

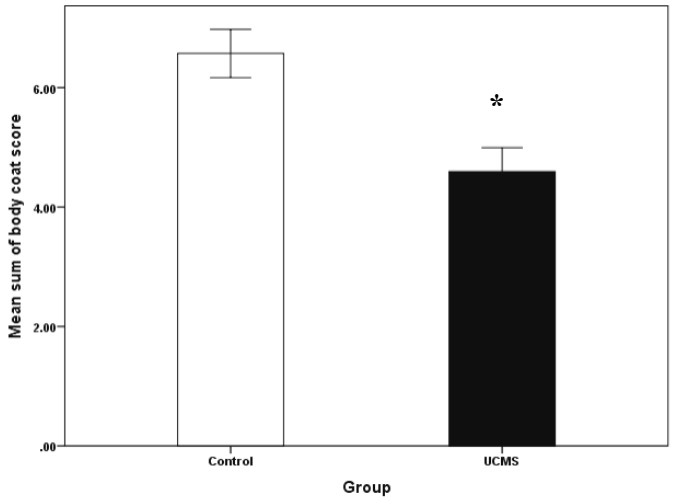

Fig. (2): Effect of UCMS on body coat state of the mice in the two treatments. Data on the graph are expressed as estimated marginal means± SE.*p<0.05.

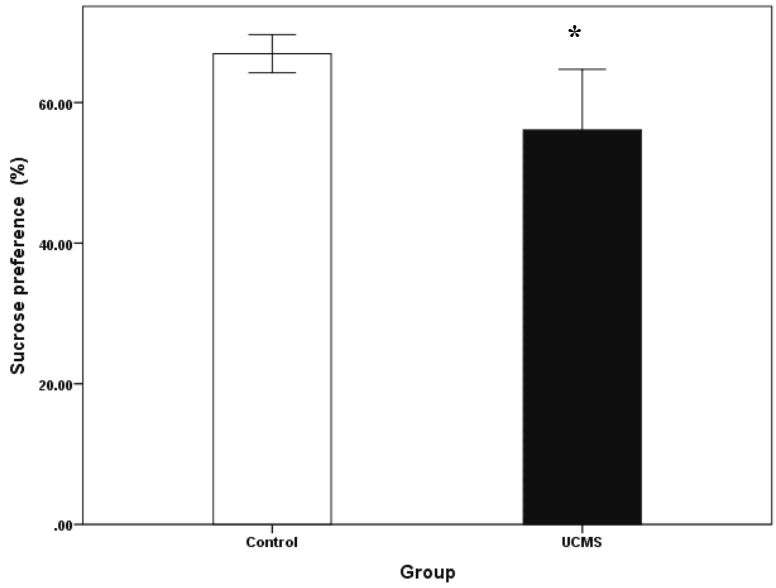

Fig. (3): Effect of UCMS on sucrose consumption of the mice in the two treatments. Data on the graph are expressed as estimated marginal means± SE.*p<0.05.