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Evaluating the Impact of a Modified UCMS Protocol with a Psychological Stress Device on Neurobiological and Behavioral Responses in Rats

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ABSTRACT

The antidepressant effect of drugs can be tested using various methods, including unpredictable chronic mild stress (UCMS). Although the initial UCMS protocol involved administering stressors for 21 days, many modifications have been made, one of which is shortening the duration of the stressors to 10-15 days. This study aims to modify the UCMS model to increase the stress response in male Wistar rats. This study replaced predator odor (2,5-dihydro-2,4,5-trimethylthiazoline) as in the previous protocol with a psychological stress device (PSD). Forty male Wistar rats (150-200 grams) were given UCMS treatment for 10 and 15 days. Sucrose consumption, coat score, body weight, and serum corticosterone levels were measured. Immunohistochemical examination of 5-HT$_{1A}$ receptors, TNF-α, NOX2, and NF-κB was performed in the hippocampus part of the brain. All data were analyzed using the Mann-Whitney and Spearman's correlation test. UCMS treatment for 10 and 15 days reduced sucrose consumption and body weight and increased the coat score. UCMS treatment elevated corticosterone levels in plasma, reduced 5-HT$_{1A}$ receptor expression in the hippocampus, and increased TNF-α, NOX2, and NF-κB expression. The primary behavioral response during PSD was head dip as a preparatory behavior for jumping. Modifying the UCMS model using a PSD can increase stress response.

Keywords: animal model, depression, unpredictable chronic mild stress, psychological stress device

INTRODUCTION

Major depressive disorder typically carries a high mortality risk, significant morbidity, substantial costs, and an elevated risk of suicide (Angst et al., 2002; Ferrari et al., 2013; Gustavsson et al., 2011; Lopez et al., 2006; Murray et al., 2012; Wittchen et al., 2011). The spectrum that includes various responses to stress is explained by variables related to stress exposure, such as duration, intensity, and predictability; even though the neuroendocrine, neurochemical, and genetic factors determine the threshold at which the stress response changes from a physiological to a harmful state (Franklin et al., 2012). Moreover, these factors explain variations in perception and stress response, which justify modifying chronic stress protocols to adjust for the animal's species and, consequently, stressors when utilizing animal models.

The most commonly used chronic stress animal model is unpredictable chronic mild stress (UCMS), based on stress effects on sucrose consumption (Markov, 2022). UCMS is a depressive animal model created in the 1980s and has acquired popularity (Katz, 1982, 1984; Katz et al., 1981). UCMS involves continually placing animals in stressful conditions for at least four weeks, sometimes longer. Intermittent exposure to stressors, such as food or water deprivation, simulate stress in daily life (Monteiro et al., 2015). Within the last decades, the UCMS protocol has emerged as one of the most translationally relevant models for studying the pathophysiology of depression in rodents (O'Leary & Cryan, 2013).

UCMS is based on the fundamental concept that chronic exposure to stressors disrupts stress response systems and ultimately leads to the development of depressive disorders (Frisbee et al., 2015). This concept is supported by the fact that it was established based on etiological relevance since chronic stress, which is variable, unpredictable, and uncontrollable, causes pathology in animals and is known to be a risk factor for depression (Lesch & Mössner, 2006). Moreover, the UCMS model has been linked to the onset of anhedonia, or a decrease in reward sensitivity, identified as the leading indicator of an animal's depressive-like state (Willner et al., 1987, 1992). Because the UCMS has a strong face and conceptual validity, it models stress-induced behavioral changes like some essential features of MDD.

The initial UCMS procedures involve the administration of unpredictable shock, food deprivation, 4^{o}C cold swim, 40^{o}C heat stress, shaker stress, light/dark cycle reversal, switch cage mates, or increasing housing density (Katz et al., 1981). Searching on PubMed using the exact keywords Willner (2017) used found more than 9000 UCMS studies until November 2023. The UCMS model may be time-consuming and laborious, even though it is effective. Therefore, there was an innovation to shorten the UCMS duration to five days but increase the stressor administration frequency (Remus et al., 2015). The study shows that the modification involved administering predator odor (2,5-dihydro-2,4,5-trimethylthiazoline/TMT).

TMT is a chemical compound in fox feces that can cause anxious behavior in mice. Its use still causes controversy since the results obtained are inconsistent. The fear caused by predator odor can depend on an animal's genetics, environment, and surroundings (Morrow et al., 2002; Sotnikov et al., 2014). Previous studies also stated that the predator odor stressors did not result in corticosterone levels as high as the psychological stress device (PSD) (36,139 vs. 4,972; $p<0.05$) (Daeng et al., 2015). In this research, TMT was replaced with PSD, and the neurobiological and behavioral responses of rats given UCMS for 10 and 15 days were measured.

MATERIAL AND METHODS

Animals

The number of rats used in this study was calculated using G*Power software ver. 3.1.9.7 (Erdfelder et al., 2009) with 95% power and 5% error based on rat sucrose consumption from a preliminary study. The number of rats was 40 male albino Wistar strain rats (*Rattus norvegicus*) (n=10 per group). The rats were three months old and 150-200 grams (procured from a local veterinarian breeder in Surabaya, Indonesia). Rats were transported to the animal laboratory and housed at room temperature (25-27^{o}C) under a 12-hour light/dark cycle. The rats were acclimatized for seven days. The rats were given food in pellets (Ratbio, PT. Ina Citrafeed Mill, Indonesia) and mineral water *ad libitum*. Weight and general behavior were recorded daily. If the rats do not exhibit signs of illness and do not lose >10% of their weight, they are considered healthy and employed in this study. The rats were randomly grouped into control (20) and UCMS (20). This study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital (KE/FK/0806/EC/2023).

Unpredictable Chronic Mild Stress (UCMS)

The UCMS stress procedure was divided into two stages: the first was carried out for ten days, while the second was carried out for 15 days. The stress protocol was a cycle of five days (Fig. 1) adapted from the previous UCMS protocol by Remus (2015). In the first stage, the duration of the stressor was 10 days according to the previous protocol; but in the second stage, the duration of the stressor was increased by one cycle, so the total became 15 days. The TMT stressor in the previous protocol was replaced using PSD tools. Stressors were administered for four consecutive days (twice daily in the morning and afternoon). The sucrose preference test and coat score assessment were performed on day five. On the first day, the UCMS rats were placed inside restrainers for 60 minutes and then fasted from food and water overnight (18 h). The following day, the rats were placed into foot shock chambers for 30 min and given two shocks (13.8 V, 2A; 2s each) delivered at 15 and 17 min. The selection of current and voltage strength on our modified footshock device is based on the animal's response to the footshock device as mentioned by Adriaan Sitsen & De Jong (1983), which can be a mild response such as slightly moving the legs or head or a more intense response such as jerking, running, making noises, or jumping with evidence of no burns on the rat. The lights in the animal lab were left on overnight starting at 5 p.m. On the third morning, the rats were placed on a PSD and rotated for 5 min. At 2 p.m., 500 mL of water was added to the rats' cage to wet the wood pellets (damp bedding) for 18 h. On the morning of the fourth day, the rats were treated with electrical footshock (as on the second day); and in the evening, the rats were deprived of food and water for 12 hours. The next morning (Day 5), a sucrose preferences test and coat score evaluation were performed. The administration of stressors at 10 and 15 days of UCMS was carried out by repeating the five-day protocol described above.

Psychological Stress Device (PSD)

PSD is made from a transparent acrylic platform and a pole, as described before by Daeng *et al.* (2015) (Fig. 2A). The PSD was modified to rotate in this research. This modification was made to improve platform instability and increase stress intensity. That behavior resembled the state of anxiety that some individuals experienced. The pole height was 100 cm from the ground. Meanwhile, the size of the acrylic platform was 30 cm in length and 20 cm in width.

The rats were placed on the PSD platform for five minutes (based on the preliminary study data), and their responses were observed: head dip, head raise, grooming, hunch, urination, defecation, or jumping. Head dip and head raise are visual scanning behaviors that indicate the mice's preparation for jumping from the PSD (King, 1998). Those behaviors are the efforts of the rats to escape the aversive conditions (escaping behavior). Hunched posture, urination, and defecation are rat-specific stress behaviors (Deal et al., 2018).

To ensure that the PSD specifically induced psychological stress without causing significant physical harm, the PSD was rotated slowly at two rotations per minute. The rats were monitored continuously during stress exposure for any signs of physical distress or injury.

Sucrose Preference Test (SPT)

In the morning of day 5, two bottles containing 150 mL with 2% sucrose solution and 150 mL mineral water were placed in each rat's cage. The two bottles were left in the cage for one hour to give the rats time to drink. The position of the two bottles was continuously changed in every SPT. The sucrose and water bottles were weighed before and after the sucrose test, and the difference in weight was recorded. Sucrose preference percentage (% sucrose preference) was determined by calculating the amount of sucrose consumed by rats (grams) divided by the total sucrose and water consumption (grams) multiplied by 100.

Coat Score

Coat score assessment (Fig. 4) was performed on the same day after the sucrose test. Scoring was carried out on the fur of the rats' head, neck, back, stomach, front and hind legs, tail, and genitals. The scoring system considers the condition of the fur. It assigns a score of 0 to areas that are smooth and shiny, 0.5 to areas that arre piloerected, and 1 to dirty and piloerected areas. The maximum total coat score is 8.

Plasma Corticosterone (CORT) level

Blood sampling was performed via cardiac puncture, while the mice were anesthetized with a ketamine-xylazine cocktail. Plasma was collected using Na2EDTA as an anticoagulant. The sample was diluted five times and centrifuged for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. The supernatant was collected to carry out the assay. The CORT level in plasma was measured using an ELISA kit (Cat. no: E-OSEL-R0002, Elabscience Biotechnology Inc., Wuhan, China) per the manufacturer's instructions. The absorbance of the samples was read at 450 nm using a spectrophotometer (Thermo Fisher Scientific, Multiskan Go). The results were calculated using OriginPro Version 2023b (OriginLab Corporation, Northampton, MA, USA).

Immunohistochemistry assay

Immunohistochemistry (IHC) observation was performed to determine the expression of 5-HT_{1A} receptor (Cat. No. bs-1124R), TNF-α (Cat. No. bs-2081), NOX2 (Cat. No. bs-3889R), NF-κβ (Cat. No. bs-0465R). All antibodies were purchased from Bioss Inc., Woburn, MA, USA. Histological preparation was prepared from rat hippocampus tissue through a sagittal section from the paraffin block. The hippocampus tissue was observed using a microscope (Olympus CX-31; Olympus, Tokyo, Japan) with a digital camera (Olympus DP21) with 100x magnification, and the intensity of brown tissue could display the expression of each variant. The image was saved in a .jpg format. Pigment discoloration at each tissue incision was measured using a semi-quantitative method which was based on protocols developed by Crowe and Yue (2019) using Fiji-ImageJ software with an additional plugin (https://imagej.net/Fiji/Downloads). Color deconvolution using an HDAB vector was used to separate the image staining. The maximum threshold on the DAB image was adjusted and compared to the original image to ensure that the positively stained cells were identified. The threshold values were set based on the average threshold of five images. The staining intensity was measured as the standard deviation of gray values and presented as a percentage of control group values.

Statistical Analysis

The non-parametric Mann-Whitney test confirmed the statistical significance while comparing the two groups, namely the control and UCMS groups, since the Shapiro-Wilk test shows that the data distribution was not normal p<0,05). A box plot was created to identify outliers. The Mann-Whitney test was performed based on the exact (two-tailed) p-value. The results of biomarker measurements were associated with behavioral test data, namely sucrose consumption, coat score, and body weight, using the Spearman correlation test. Statistical analyses were performed with GraphPad Prism (Version 8.0.2; Graph Pad Software Inc., Boston, Massachusetts USA, www.graphpad.com).

RESULTS AND DISCUSSION

Psychological Stress Device (PSD)

A life-threatening event triggers a variety of primitive responses; some of which include an increase in the heart rate and freezing. This research uses a PSD tool (Fig. 2A), which evokes an escape response in threatening situations, which is more aversive than predatory threats in laboratory animals. As in the previous study, the situation to be imitated is an unstable, elevated, and exposed environment (King, 1998). In the PSD test, the rats showed various responses, including head dip, head raise, grooming, hunch, urination, defecation, and jumping (Fig. 2B). The frequency of each behavior varied on the 1st, 2nd or 3rd PSD test, but the head dip response always occurred. The head dip is a position where it looks as if the rat is about to jump. Unlike fall, which was usually triggered by scrabbling and foot slippage, jumping was preceded by a preparatory step. This response attempts to escape from the stressful situation because, in general, if a patient experiences stress, they will run away or divert activities, and rats will escape by throwing themselves down (King, 1998). Rats also exhibited urination and defecation in response to applied stress. Urinating and defecating are acceptable measures of animal emotional behavior and valid indicators of the individual emotionality of the subjects (Hall, 1934). The intensity of a rat's emotionality can be assessed based on the frequency of urination or defecation in a field environment (Hall, 1934).

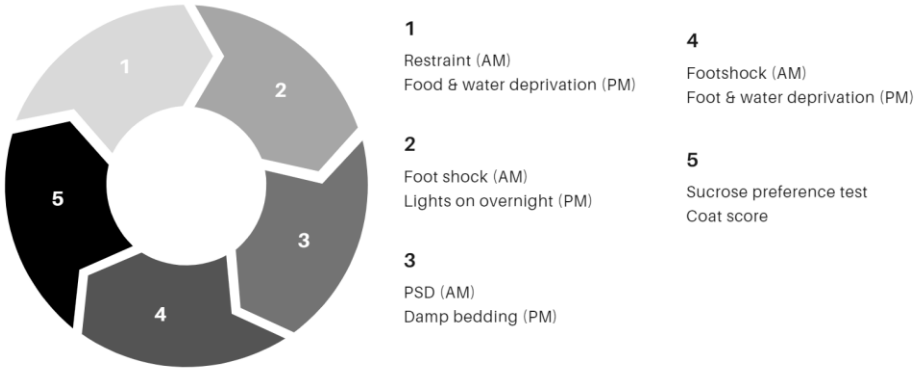

Figure 1. The five-day cycle of the UCMS protocol. This cycle was repeated twice for the 10-day UCMS protocol and three times for the 15-day protocol. PSD: Psychological Stress Device; UCMS: unpredictable chronic mild stress.

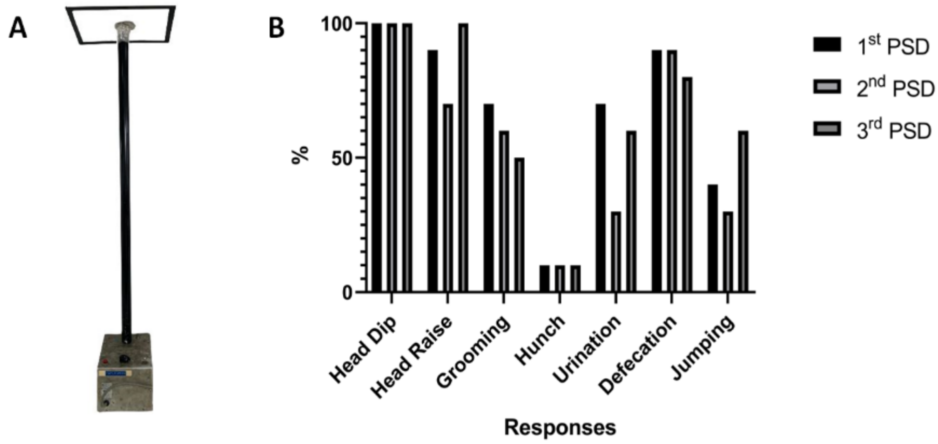

Figure 2. Modified PSD used in this study (A) and rat responses to PSD (B). Responses are expressed in units of % of rats showing a positive response. PSD: psychological stress device.

Sucrose Preference Test (SPT)

Chronic stress causes a variety of physiological changes unrelated to behavior, such as depression and anhedonia (Strekalova et al., 2011). The UCMS model has been widely used since it was firstly developed by Katz et al. (1981). In the UCMS, rats are exposed to mild stress, which causes anhedonia, a parameter used to test the antidepressant activity of drugs (Willner et al., 1987, 1992). Anhedonia in rats can be measured by sucrose consumption and coat score (an indicator of lack of grooming behavior).

The previous UCMS method by Remus (2015) showed a decrease in % sucrose preference after ten days of UCMS. This study showed a significant decrease in sucrose consumption after administering stressors for 10 and 15 days (p<0.01; Fig. 3A,3D). For ten days, stressed rats showed lower levels of sucrose consumption than control rats (65.99% vs. 91.57%; $p_{(\text{UCMS } vs. \text{ control})}$ <0.0001). Rats in the 15-day UCMS group also showed a decrease in the % sucrose preference (37.50% vs. 85.40%%; $p_{(\text{UCMS } vs. \text{ control})}$=0.0040). Therefore, the decrease in the sucrose preference was more pronounced in the 15-day UCMS group.

The results of a survey conducted by Willner (2017) showed that 70% of respondents needed more than two weeks to get the effect of UCMS. A previous study by Gouirand & Matuszewich (2005) showed no difference in the % preference for sucrose in the control group compared to the UCMS group (79.9 ± 5.6 vs. 84.5 ± 2.5) after ten days of UCMS treatment in male Sprague-Dawley (SD) rats. However, Shilpa et al. (2017) mentioned a decrease in % sucrose consumption in male Wistar rats that were immobilized two hours daily for ten days. Similar studies in male SD rats also showed a decrease in

% sucrose consumption (Ampuero et al., 2015; Luo et al., 2015). The duration of stress administration also affects sucrose intake. Research by Ma et al. (2011) in male C57BL/6J mice showed a significant decrease in sucrose consumption compared to the control group since the 10th day of stressor administration. The decline became significant on the 17th and 24th days. Meanwhile, on day 31, sucrose consumption increased.

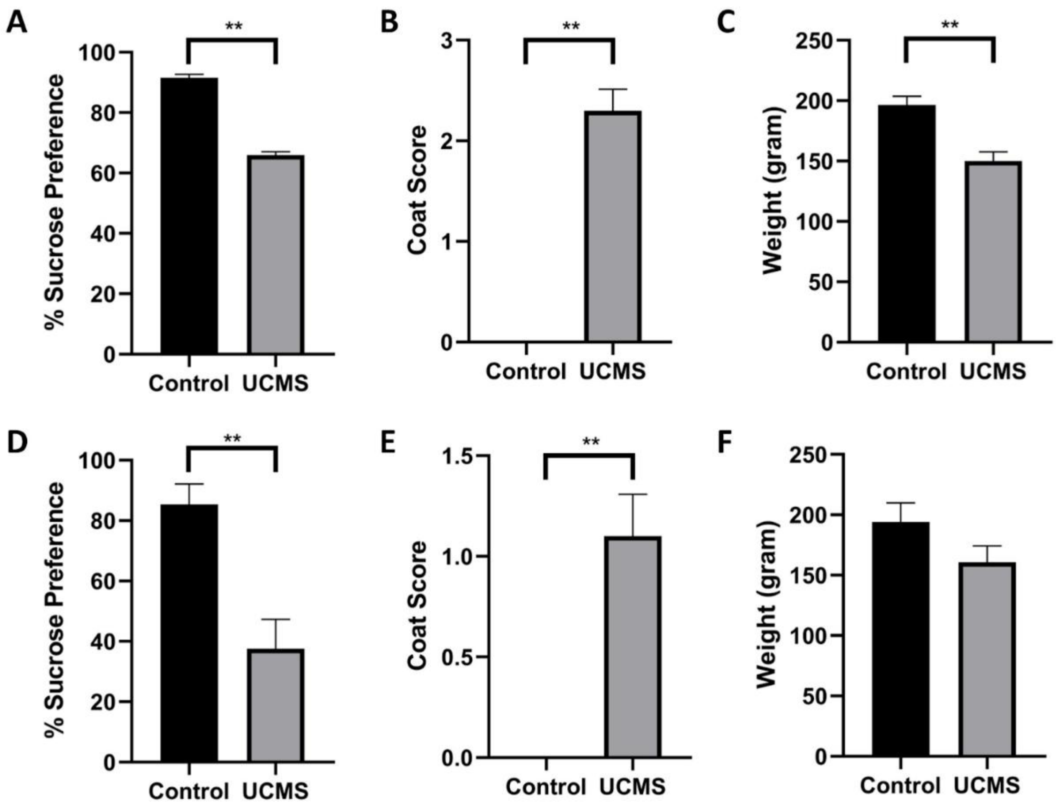

Figure 3. The effects of stress on the behaviors of 10-day (A, B, C) and 15-day (D, E, F) UCMS-treated rats. The depressive behaviors were evaluated by % sucrose preference (A, D), coat score (B, E), and the changes in body weight (C, F). Data are shown as mean ± SEM. **, $p< 0.01$ between groups. n = 10 *per* group. UCMS, unpredictable chronic mild stress.

Coat Score

Grooming is a measure of the animal's motivation toward self-centered activities, and it is done routinely (Nollet, 2021). The coat score value shows the stress level in rats, representing the nature of the self-centered activity (Frisbee et al., 2015). Chronically stressed animals and human depression are similar in that they exhibit decreased grooming behavior and the inability to perform even the most minor tasks as coping behaviors (Griebel et al., 2002; Alonso, 2004; Surget and Belzung, 2009). In rodents, grooming plays an essential role in their behavior, and it is affected by stress (Berridge and Whishaw, 1992; Berridge et al., 1987; Van Erp et al., 1995; Kalueff and Tuohimaa, 2004a; Kametani, 1988; Sachs, 1988; Spruijt et al., 1992; Kalueff and Tuohimaa, 2004b). All UCMS animals in this study increased their coat scores due to decreased grooming behavior (p<0.01; Fig. 3B,3E). Alonso (2004) has confirmed that the UCMS severely affected the coat score of BALB/c mice even after seven weeks of UCMS. However, the coat score in the UCMS group did not show an increase, nor did the duration of the stressor treatment was extended. In addition to the 10-day and 15-day UCMS procedures performed at different times with different mice, previous studies by Frisbee et al. (2015) showed the possibility that the coat score could improve during the UCMS procedure. The coat score can be influenced by several factors, namely grooming behavior, stress intensity, adaptation, and genetic heritage (Nollet, 2021).

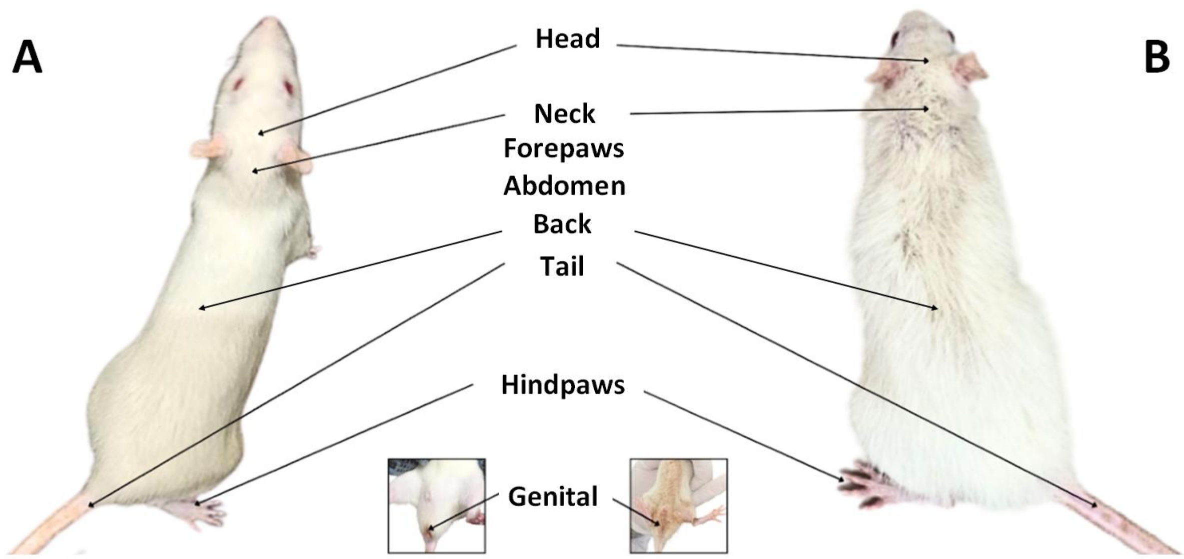

Figure 4. Assessment of the coat state. (A) Low coat score rat; (B) High coat score rat. The coat state score results from a qualitative scoring of different body parts, including the head, the neck, the forepaws, the abdomen, the back, the tail, the hindpaws, and the genital. Each zone scored 0 if it is smooth and shiny, 0.5 if it shows piloerection, and one if the fur is dirty or piloerection. In the figure, (A) rat would be scored 0 for each zone, (B) rat would be scored one on the head, one on the neck, one on the back, one on the abdomen, and one on the genital. Thus, it would have a global score of 5.

Body Weight

UCMS treatment causes anhedonia and shows other characteristics of depression, such as weight loss. In 10-day UCMS rats, the body weight between control and stress rats was significantly different (150 vs. 196.5; $p_{(\text{UCMS } vs. \text{ control})}$=0.0007; Fig. 3C), while in 15-day UCMS, the body weight did not differ between groups (160.7 vs. 194.0; $p_{(\text{UCMS } vs. \text{ control})}$=0.1903; Fig. 3F). However, body weight loss cannot explain the stress-induced reduction in sucrose intake, and UCMS does not require a weight reduction to decrease its sucrose intake (Willner et al., 1996). **Serum Corticosterone (CORT) Level**

Giving stress can increase CORT levels (Estévez-Cabrera et al., 2023). This study measured the effect of giving UCMS for ten days on CORT. Although CORT levels in the control group increased, they were insignificant (132.6 vs. 117.7; $p_{(\text{UCMS } vs. \text{ control})}$=0.8413; Fig. 5). Spearman correlation test was conducted on behavioral data (% sucrose preference, coat score, and body weight) and CORT level and found no correlation ($P > 0.05$).

A previous study using a similar 10-day UCMS (TMT instead of PSD stressor) showed an insignificant increase in CORT (Remus et al., 2015). It is known that the duration of the stressor influences serum CORT levels. Giving acute stress for six hours can significantly increase serum CORT levels, while giving chronic stress causes a decrease in CORT levels (Piskunov et al., 2016). Furthermore, the study showed an increase in CORT in the hippocampus, indicating that local delivery of corticosterone was more significant compared to circulating levels (Piskunov et al., 2016).

The hypothalamic-pituitary-adrenal axis can adapt to chronic stress conditions, and exposure to UCMS for ten days can cause adaptation to stressors (Stepanichev et al., 2016). Apart from that, CORT levels fluctuate depending on when the blood sample is taken (Beerling et al., 2011; Stepanichev et al., 2016). In the 10-day UCMS model, the blood sample was taken on the 10th day after the final stressor (food and water deprivation). This type of stressor may have less influence on increasing CORT. Previous research showed that food deprivation for 24 days reduced CORT levels, and it took more than five days to increase basal CORT (De Boer et al., 1989).

Therefore, the administration of the UCMS stressor was extended for 15 days in a new group of rats, and stress-related parameters were measured directly in the hippocampus using the IHC method.

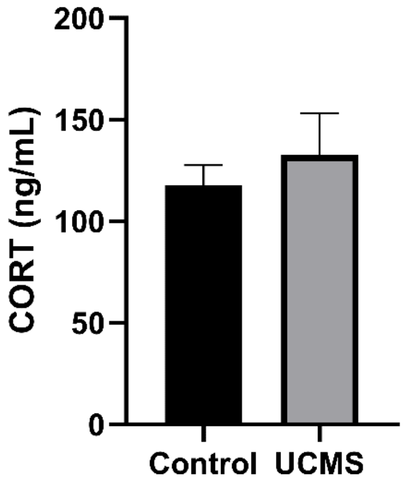

Figure 5. The effects of stress on the corticosterone level in 10-day UCMS-treated rats. Data are shown as mean±SEM. n = 5 *per* group. UCMS: unpredictable chronic mild stress; CORT: corticosterone.

Immunohistochemistry (IHC) of 5-HT$_{1A}$ receptor, TNF-α, NOX2, and NF-κB in hippocampus tissue

Previous study results showed that UCMS for 60 days can cause oxidative stress and low-grade chronic inflammation (López-López et al., 2016), and the results of this study are in the same line. The IHC results showed that 15-day UCMS influenced the expression of both 5-HT$_{1A}$ receptor, TNF-α, NOX2, and NF-κB (Fig. 5). The increase in expression from hippocampal cells for each of these parameters was not significant (p>0.05) probably because the duration of stressor administration was only 15 days.

Serotonin is related to the regulation of synaptic plasticity, which is related to neurogenesis, dendritic maturation, neuroprotection, and astroglial interactions, which is based on the activity of serotonin receptors, especially the postsynaptic 5-HT$_{1A}$ receptor (Kraus et al., 2017). In this study, IHC staining using 5-HT$_{1A}$ antibody revealed a 5-HT$_{1A}$ receptor expression decrease in the UCMS group (Fig. 5A, E) (p=0.1 > 0.005). Previous research also showed decreased 5-HT$_{1A}$ expression in hippocampus tissue (L. Chen et al., 2020). The results of a meta-analysis of the UCMS effect on rodents' serotonin receptor expression showed no change in 5-HT$_{1A}$ expression in the CA1, CA2, and CA3 areas of the hippocampus (Lages et al., 2021). Significant changes occurred only in the dentate gyrus or when analysis was performed on the hippocampus.

The 5-HT$_{1A}$ receptor is widely expressed in the postsynaptic area, especially in the hippocampus, which acts as the primary inhibitory receptor for serotonin in the brain (Carhart-Harris & Nutt, 2017). In anxiety and stress conditions, hyperactivity occurs in the hippocampus, and 5-HT overcomes this hyperactivity by inhibiting postsynaptic 5-HT$_{1A}$ (Engel et al., 2009; Tada et al., 2004). It has been proven that 5-HT$_{1A}$ knockout rodents experience more severe symptoms of anxiety and depression, possibly due to a lack of postsynaptic 5-HT$_{1A}$ signaling (Heisler et al., 1998). The results of post-mortem studies on depressed patients who committed suicide also showed decreased 5-HT$_{1A}$ receptor binding in the cortex and hippocampus (Savitz et al., 2009). Therefore, it can be concluded that a decrease in 5-HT$_{1A}$ receptor expression in the hippocampus is related to the emergence of stress, impulsiveness, aggression, and anxiety.

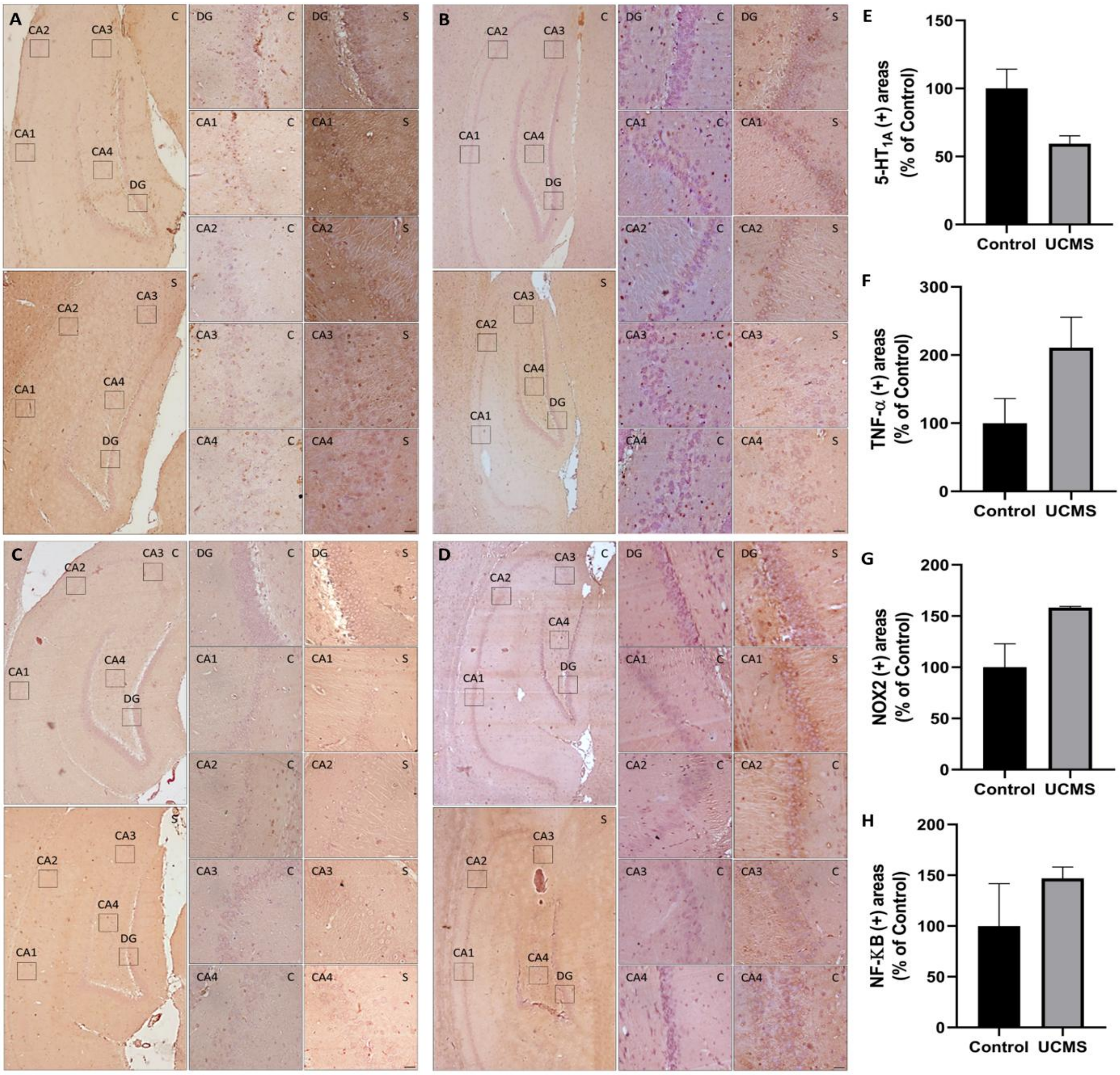

Figure 6. Five-HT_{1A} (A, E), TNF-α (B, F), NOX2 (C, G), and NF-κB (D, H)-positive cells in the hippocampus (DG, CA1, CA2, CA3, CA4) in control (C) and UCMS (S) rats (Scale bar, 500µm). The positive areas are expressed as mean ±SEM. n=3 *per* group. CA, Cornu ammonis, DG, dentate gyrus.

The hippocampus's elevated levels of various immune system chemicals, such as IL-1, IL-1β, IFN-γ, and TNF-α, are linked to CMS-induced depression in rodents. This study shows an increase in TNF-α expression in the hippocampus of UCMS rats (Fig. 5B, F) ($p = 0.2 > 0.005$). These results are in line with the results of previous research, which showed an increase in protein levels and mRNA expression of IL-1β, IL-6, and TNF-α in the hippocampus after 3- or 5-week UCMS (W. Wang et al., 2022; Xu et al., 2022). Examination of these three inflammatory markers in plasma using the ELISA method showed a significant increase after 4-5 weeks of UCMS (Y. Chen et al., 2022; Li et al., 2022). Another study reported increased IL-1, IL-1β, IFN-γ, and TNF-α after giving UCMS for four months (Lu et al., 2017).

Oxidative damage arises due to an imbalance between the production of reactive oxygen species (ROS) and the body's ability to inactivate it due to pathological conditions such as depression or anxiety (Giustarini et al., 2009). Oxidative damage, expressed as an increase in related markers such as lipid oxidative markers and DNA damage, plays a role in the progression of depressive illness (Black et al., 2017; Che et al., 2010; Lindqvist et al., 2017; Matchkov et al., 2015; Rossetti et al., 2020). Oxidative damage will damage brain cells and disrupt neurotransmission and neuron function (Cobley et al., 2018).

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) are essential for endogenous ROS production by catalyzing electron transfer from NADPH to oxygen (Bedard & Krause, 2007; Kim et al., 2015). The brain is susceptible to oxidative stress because it consumes 20% of the body's oxygen contains iron and copper to catalyze the formation of ROS has high amounts of polyunsaturated fatty acids in brain cell membranes, and has a low GSH level as an endogenous antioxidant (X. Wang & Michaelis, 2010).

One of the NOX2 subunits is expressed in various parts of the rodent brain, including the hippocampus, and is a significant contributor to ROS (Bedard & Krause, 2007; Serrano et al., 2003). Research on NOX2 knockout mice showed that giving UCMS for three weeks increased NOX2 expression in the hippocampus (Lv et al., 2019). Meanwhile, in this study, giving UCMS for 15 days increased NOX2 expression in the hippocampus of rats ($p=0.1 > 0.05$). UCMS treatment has previously been shown to increase NOX2 protein levels in the prefrontal cortex of mice for seven weeks (Paladini et al., 2021). Furthermore, social isolation treatment in mice increased NOX2 gene expression in the amygdala, hippocampus, nucleus accumbens, prefrontal cortex, and striatum (Schiavone et al., 2009).

Nuclear factor (NF)-κB is a family of transcription factors which are essential for cells' survival and proliferation during stress, immune, and inflammatory responses (J. Chen & Chen, 2013). NF-κB regulates various physiological processes in the body (Koo et al., 2010; Piva et al., 2006). Stressors such as acute immobilization will activate the brain's NF-κB signals, which induces behavioral changes (J. Madrigal et al., 2002; J. L. M. Madrigal et al., 2001). In this study, 15-day UCMS increased NF-κB expression ($p=0.4 > 0.05$). Previously, it took a longer time; for example, three weeks (Koo et al., 2010), 35 days (Pesarico et al., 2016), six weeks (X. Wang et al., 2020), or nine weeks to significantly increase NF-κB expression in the brain (Avolio et al., 2017). The activation of NF-κB mediates responses to stress, such as inhibition of neurogenesis and anhedonia (Koo et al., 2010). This activation also contributes to the regulation of inflammatory processes.

Correlation between behavioral and neurobiological changes

The results of behavioral tests showed symptoms of anhedonia as indicated by a decrease in % sucrose preference, an increase in coat score, and a decrease in body weight after administration of UCMS. In addition, the results of neurobiological tests showed an increase in corticosterone levels, a decrease in 5-HT$_{1A}$ expression, and an increase in TNF-α and NF-κB expression. However, the results of the Spearman correlation test showed no correlation between behavior and neurobiological test results ($p>0.05$).

It should be noted that the limitations of this study are related to the number of samples, measurements taken at random, and the gender of the rats used (only male). Further research with more samples and periodic data collection during the experiment is needed to get a complete picture of the relationship between behavioral and neurobiological changes.

CONCLUSION

Using PSD as a stressor, the modified 10- and 15-day UCMS model induces behavioral and neurobiological alterations in rats. Behavioral changes include decreased sucrose preference, increased coat score, and decreased weight. Neurobiological alterations involve decreased expression of the 5-HT$_{1A}$ receptor and increased expression of TNF-α, NOX2, and NF-κB in the hippocampus. Future studies should explore additional modifications to enhance the stressor intensity of the short-term UCMS model.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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