

THE DURATION OF UNPREDICTABLE CHRONIC MILD STRESS (UCMS) ADMINISTRATION FOR CAUSING DEPRESSIVE BEHAVIOR IN RATS

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ABSTRACT

The unpredictable chronic mild stress (UCMS) model has been widely accepted as an appropriate and valid model for testing animal depression. Various studies have examined UCMS in rats for durations of 2–8 weeks with various types of stressors. The aims of the study is to determine the duration of UCMS administration that causes depressive behavior in rats. The methods used an experimental laboratory study with a true experimental design (randomized control group, pre-test, and post-test design) was conducted with *Rattus norvegicus* at the Institute of Bio-Science of Universitas Brawijaya. Five rats were given UCMS treatment for eight weeks. The depressive behavior of the rats was assessed through a sucrose preference test (SPT), a tail suspension test (TST), and an open-field test (OFT) before treatment (pre-test) and after four weeks (post-test I), six weeks (post-test II), and eight weeks (post-test III) of treatment. The data analysis used a paired t-test and SPSS statistical tests with $\alpha = 0.05$. This study found decreases in the sucrose consumption (the SPT test) four weeks (post-test I) and six weeks (post-test II) after the pre-test measurements. We also found increases in the duration of immobility (the TPT test) six weeks (post-test II) after the pre-test measurements. In contrast, no statistically significant changes were observed for the area units covered by the rats (the OFT test) in any of the post-tests. UCMS administration causes depressive behavior in rats after six weeks.

Keywords: UCMS, rats, depression, SPT, TST, OFT

1. INTRODUCTION

The model of laboratory rats with depressive behavior was first introduced successfully by Willner and co-workers in 1957 using unpredictable chronic mild stress (UCMS). This model simulates exogenous factors for depression. The main symptom observed was decreased consumption of sweet liquid food (Li et al., 2015). The depressive conditions of rats appear gradually over time in response to more natural stress (Yan et al., 2016). The exposure to a series of chronic stresses that cannot be predicted and resembles the situations in human lives with mild and chronic stressors (Li et al., 2014).

The UCMS paradigm has been widely accepted as a suitable and valid model for depression and antidepressant therapy research in animals (Zhao et al., 2018; Kushwah et al., 2016; Yan et al., 2016; Nguyen et al.,

2014; Refojo and Deussing, 2012). This model emphasizes the dominant role of stress as the etiology of depression. The symptoms of depression in animals have similarities with depression in humans (Zhao et al., 2018; Yan et al., 2016; Refojo and Deussing, 2012). The UCMS model has high validity, both predictive validity (antidepressant drugs can correct behavior changes), face validity (chronic stress induces behavioral changes according to depressive features), and construct validity (chronic mild stress decreases sensitivity in the brain's reward system) (Kushwah et al., 2016).

In the UCMS model, a series of mild stressors are alternately and randomly given every day for a week. Next, the stressors are randomized again for the next 2–8 weeks. The stressors include cramped isolation or cages, food or beverage deprivation, light and darkness disturb-

ances, tiled cages, 45° sloped cages, wet cages, horizontal shock, group feeding (combining two cages), friends-switching, being paired with other stressed rats, shock waves in their legs, and heat stimulation (Farhan and Haleem, 2016; Kushwah et al., 2016; Wu et al., 2016; Yan et al., 2016; Yang et al., 2016; Li et al., 2014; Nguyen et al., 2014; Refojo and Deussing, 2012). The duration of each stressor in a 24 hour period is listed in Table 1.

One drawback of UCMS is the lack of inter-laboratory reliability (Nguyen et al., 2014; Refojo and Deussing, 2012). Using the UCMS method to cause depression in rats also raises bioethical considerations (Nguyen et al., 2014). The types of UCMS stressors in this study were chosen for their minimal discomfort to the rats. Therefore, a study is needed to determine the duration of UCMS administration that creates a depressive behavior in *Rattus norvegicus*.

2. MATERIALS AND METHODS

For two weeks, five male Wistar rats (*Rattus norvegicus*) aged 2–3 months, weighing 107–187 g, were given an adaptation time at the Institute of BioScience, Universitas Brawijaya. Chronic mild stressors that could not be predicted consisted of fasting (no food and drink) for 24 hours, being in 45°-sloped cages for 24 hours, being in wet cages for 24 hours, light and darkness disturbances, being held in a pipe for one hour, being in cages with no sawdust, and being in tiled cages for 24 hours. The treatment was given alternately and randomly every day for a week and then randomized again for the following eight weeks. The time at which the treatment was initiated was also carried out randomly during the working hours of the laboratory staff.

The assessment of depressive behavior was done before (pre-test) and after the administration of UCMS at four weeks (post-test I), six weeks (post-test II), and eight weeks (post-test III). Three tests were conducted to assess

depressive behavior – a sucrose preference test (SPT), a tail suspension test (TST), and an open-field test (OFT). For the SPT, an adaptation of 1% of sucrose solution (w/v) was carried out for seven days, and then the rats fasted for 24 hours. Next, the rats were placed for 2 hours in individual cages equipped with bottles containing 200 ml of sucrose solution and 200 ml of water. The amount of sucrose consumed was calculated as (sucrose solution consumption / water + sucrose solution consumption) × 100%. The TST procedure involved isolating the rats acoustically and visually in an isolation room. Next, they were hung for 6 minutes on a horizontal bar 30–50 cm from the floor with a tape placed about 5 cm from the tip of the tail. The rats were considered immobilized when they became passive and did not move. At this stage, the duration of their immobility was recorded. The OFT test provided an area of 50 × 50 cm with a 30 cm wall. The base of the area was divided into 25 fields. The rats were placed in the middle, and then for 5 minutes, the number of fields crossed by all four of their legs was counted. The rats were considered depressed if at least two of these tests showed a significant difference between the post-test and the pre-test.

3. RESULTS

The data from the SPT, TST, and OFT measurements are shown in Table 2 and Figure 1. The SPT pre-test had an average sucrose solution consumption of 67.32%, the consumption in post-tests I–III was 29.29%, 35.73%, and 34.21%, respectively.

For the TST pre-test, the average duration of immobility was 179 s, while for post-tests I–III, the durations were 231.6 s, 250 s, and 189.33 s, respectively.

For the OFT pre-test, the average number of units covered in five minutes by the rats was 45.8 units, while the average results for post-tests I–III were 56 units, 19.25 units, and 30 units, respectively. Rat 5 died at the end of

the sixth week and Rat 1 dropped out at the end of the eighth week.

The results of a statistical analysis of the SPT, TST, and OFT measurements are shown in Table 3. We found that for post-test I the average sucrose consumption was significantly smaller than in the pre-test, but the differences in TST and OFT tests were not statistically significant. In post-test II, there was a significant decrease for the SPT test and a significant increase for the TST test, compared to the pre-test measurements, but the decrease for the OFT test was not statistically significant. In post-test III, the decreases observed for the SPT and OFT tests, and the increase observed for the TST test were not statistically significant compared to the pre-test.

4. DISCUSSIONS

This study found decreases in the sucrose consumption (the SPT test) four weeks (post-test I) and six weeks (post-test II) after the pre-test measurements. We also found increases in the duration of immobility (the TPT test) six weeks (post-test II) after the pre-test measurements. In contrast, no statistically significant changes were observed for the area units covered by the rats (the OFT test) in any of the post-tests. From these results, we concluded that the rats were depressed after being given a UCMS in the sixth week.

Our findings suggested that responsive behavior due to chronic mild stress began to appear in the fourth week and later became more pronounced. An adaptation had begun to occur by the eighth week. It has been proposed that adaptation occurs in response to chronic stress. Habituation is an adaptive response to repetitive stressors, which results in a decreased response. This decrease also occurs with chronic stressors that are varied or unpredictable. The areas of the brain involved in this habituation are the limbic area, the ventral prefrontal cortex, the basolateral amygdala, the orbitofrontal cortex, and the paraventricular thalamus (Herman, 2013).

The General Adaptation Syndrome theory developed by Hans Selye proposes that an individual's reaction when experiencing stress consists of three phases i) the warning phase (alarm), ii) the resistance phase (adaptation), and iii) the fatigue phase (adaptation fails). Individual reactions to stress that threaten a person's life aim to reduce the impact of stressors and restore homeostasis. This resilience and adaptation are likely to involve endogenous glucocorticoids, cytokines, and neurotrophins (Sadock et al., 2015).

The habituation of chronic mild stress takes place through the HPA axis, meaning to look at the level of glucocorticoid, whether it is decreased or stable at homeostatic circadian levels and to observe the level of negative feedback from the hippocampus. This habituation leads to changes in response to acute stress, which increases susceptibility to future acute stressors (Rao and Androulakis, 2019; Herman, 2013).

The depressive behavior found in this study represents the variations between samples. The rats used were heterogeneous because they were not genetically identical, even though they came from the same breeder. Genetic diversity can increase behavioral variation among experimental animals after exposure to stress (Refojo and Deussing, 2012). The lack of inter-laboratory reliability is also an obstacle in UCMS research (Nguyen et al., 2014; Refojo and Deussing, 2012).

Conflict of interests

The authors declare no conflict of interest.

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