

**Identification for the Specific Depression-like Behavior of 60-Minute Maternal Deprivation Rats in Early Life**Mu-Hsin Chen<sup>1</sup>, Jen-Haur Lin<sup>1</sup>, Chih-Hung Lin<sup>1,\*</sup><sup>1</sup>Department of Optometry, Chung-Hwa University of Medical Technology, Tainan City, Taiwan.[Earlylife555@yahoo.com.tw](mailto:Earlylife555@yahoo.com.tw)

**Abstract:** In clinic, it is well known that maternal deprivation can lead to certain forms of depression. However, the evidence from basic researches on the relationship between maternal deprivation and depression is still inadequate, a major impediment in this research field is lack of validated animal models. In previous studies, most evidence showed that animals experienced maternal deprivation in early life would behave more anxiously, it means that maternal deprivation animals will form anxiety animal models. However, Just little evidence showed that maternal deprivation animals would express depression-like behavior. Therefore, it is so urgent to develop a steady and optimal maternal deprivation animal model for the research of depression. In this study, rats after parturition will submit to maternal deprivation process for 60 minutes a day from postnatal day 2 to 14. When the isolated rats wean, they will be separated from mother and raised independently. In addition to fundamental physiological condition measurements, 5-week-old maternal deprivation rats will proceed to forced swim test, spontaneous motor activity test, antidepressant treatments and active avoidance test. However, the results show that maternal deprivation rats experiencing all the tests will express depression-like behavior, but not anxiety-like behavior. Therefore, we find a novel depression animal model due to rats experiencing maternal deprivation for 60 minutes daily in early life. It is expected to offer more beneficial contributions to basic medical studies on the relations between maternal deprivation and depression by this animal model.

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**1. Introduction**

It is not until the middle part of the 19<sup>th</sup> century that the brain became the focus of efforts to understand the pathophysiology of depression [1]. However, a major impediment in depression research is the lack of validated animal models. It is so urgent to develop validated depression animal models for the depression research. It is well known that animal models of depression are used both as screening tests to discover and develop novel antidepressant drug therapies and as simulations for investigating various aspects of the neurobiology of depressive illness including the neuropharmacological mechanisms mediating the effects of antidepressant treatments [2, 3, 4]. Although there are some pitfalls for depression animal models in depression research, nevertheless, depression animal models are still very required for the field to formulate several hypotheses by which depression may occur and antidepressant treatments may work.

Depression is often described as a stress-related disorder, and there is good evidence that episodes of depression often occur in the context of some form of stress [1].

Animal models of depression are typically generated by exposure of animals to stressors of various kinds, resulting in behavioural changes reminiscent of aspects of depression [5, 6, 7, 8]. Some early life experiences are also known to increase the

risk for depression, particularly parental deprivation. Clinically, maternal deprivation induced early life stress is a crucial source of stress-related depression [9]. However, little evidence is revealed in basic research of maternal deprivation induced predisposition to depression. Therefore, the development of optimal maternal deprivation induced depression animal model is a pivot in studying the relationship between maternal deprivation and depression.

In previous studies, maternal deprivation induced animal models have been applied commonly [10]. However, there is a discrepancy existed in the final expression of behavior in rats experiencing isolation of early life. Most evidence shows that rats experiencing maternal deprivation produce anxiety-like behavior [11, 12, 13, 14]. And little evidence shows depression-like behavior in maternal deprivation rats of early life [15]. The difference in animal behavior may be due to various conditions of isolation protocols, it means different isolation duration and intensity will lead to different behavioral expression. Therefore, proper isolation conditions will be tested for obtaining optimal maternal deprivation induced depression models.

Tests for the validations of new built animal models are necessary. All available animal models of depression rely on two principles: actions of known antidepressant or responses to stress [16, 17, 18,

19]. Therefore, when rats experiencing 60-min maternal deprivation protocol in early life are grown up to 5 weeks old, they will be tested in forced swim test, spontaneous motor activity test, antidepressant treatments and active avoidance test. These validation criteria are conducted to identify this new depression animal model, and the results reveal that rats experiencing maternal deprivation for 60 minutes once a day from postnatal day 2 to postnatal day 14 will express behavior of predisposition to depression.

## 2. Material and Methods

### Animals

This study is conducted in conformity with the policies and procedures detailed in the "Guide for Animal Care and Use of Laboratory Animals". The animal experimental protocols are confirmed by the Institutional Animal Care and Use Committee (IACUC) of Chung-Hwa College of Medical Technology and IACUC. Young adult male Sprague-Dawley rats weighing 130-150g at the time of testing are housed at a constant room temperature ( $22\pm 1^{\circ}\text{C}$ ) and humidity ( $50\pm 10\%$  RH) with a 12-h light: dark cycle. Food and water are available ad lib. The source of feeding diet comes from Lab Diet<sup>R</sup> company in U.S.A. and trade code is "rodent diet 5001".

### Maternal deprivation

The experimental protocols are modified from Husum et al. [20]. Timed -pregnant Sprague-Dawley rats arrived at the animal facility on gestational day 12. The day of delivery is designated as postnatal day (PND) 0. On PND 2, the pups were sexed and culled into litters of 8-10 male pups and randomly assigned to maternal deprivation for 60 min per day (MDP60) or untreated control group. The separation procedures took place at 8:00~9:00 P.M. daily from PND 2 to PND 14. During the separation, the pups are placed in a neonatal incubator to prevent metabolic changes caused by change of body temperature. Non-handled pups are left undisturbed until PND 23 when all pups were weans. From this day onwards, the rats are maintained four per cage at a constant room temperature of  $22\pm 1^{\circ}\text{C}$  in a 12-h light/dark cycle (light on at 6:00 A.M.) with free access to chow and water.

### Basal physiological conditions monitor

In this study, we measure two items of basal physiological conditions including body weight and temperature of rats. Five-week-old rats are under measurements. We put rats which are limited in a small transparent cage (the weight of small cage has been zeroed first) on the electronic weight scale and record the digital number of body weight. After weight measurement, animal-specific rectal thermometer is applied to measure the body temperature of rat.

Vaseline treated thermometer is put into 2-3 cm rectal lumen of rat and kept for 5 min, then take the thermometer out and record the body temperature.

### Forced swimming test

Rats are immersed in plexiglass cylinders (diameter 18 cm, height 38 cm) filled to a depth of 25 cm with water at  $25^{\circ}\text{C}$ . On the first experimental day, rats are gently placed in the water for a 15 min period of habituation. On removal from the water, they are placed in a plexiglass box under a 60W bulb for 30 min to dry. The next day, they are replaced in the cylinders and observed for 5min. During this period, the total time that spent immobile (i.e., making only the movements necessary to remain afloat) is measured. After 5-min test, the rats are removed from the water.

### Spontaneous motor activity test

In view of the physiological nature of rats in spontaneous running. We assign 4 rats at a time on the treadmill (Columbus Instruments) which partitioned into 4 channels by plastic boards (90 cm x 8 cm x 27 cm for each channel). The test protocol needs two days. On the first day, rats were placed at the center of treadmill for 10 min for familiarization with the environment. The next day, the training load of 9 m/min for 10 minutes is applied to the animals and test its spontaneous running time. The treadmill machine was modified. The start point of every run path is equipped with a sensor, which is responsible for the recording of retention time. After 10 min test, the spontaneous running time will be available by abstracting the retention time from total test time. A rat of low spontaneous running time is considered as impairment of moving activity due to physiological reasons. Based on the vivacious instinct of rats, the measurement of the spontaneous running time by treadmill can be used to assess the spontaneous motor activity of rats.

### Antidepressants treatment protocol

Rats are treated with desipramine HCl and fluoxetine HCl (from Sigma Chemical Company) intraperitoneally. The treatment doses of desipramine and fluoxetine are 10mg/kg and 5mg/kg respectively. The treatment duration for desipramine is 21 days and that for fluoxetine is 3 days. The timing for the treatments of antidepressants is earlier than the time that rats grow up to 5 weeks old.

### Active avoidance test

The anxiety level of rats is evaluated by active avoidance test. The apparatus is consisted of two compartments: one light area (27 L x 27 W x 27 H cm) illuminated by 100-W desk lamp is transparent, and the other dark area (18 L x 27 W x 27 H cm) is painted

black. The dark box is enclosed completely and the light box is open on the ceiling cover. The experiments are performed between 09:00 and 14:00. The retention time of rats in light area will be recorded by a timer. According to the recording time, we will quantify the anxiety level of rats. The more retention happens in the dark area, the more anxious level produces in rats.

### Statistics

Results are expressed as mean  $\pm$  SEM. Sample sizes are indicated by n. Comparisons between groups are carried out with a one- or two-way analysis of variance (ANOVA). Differences between two groups are compared by using unpaired or paired Student's t-test with  $p < 0.05$  considered statistically significant.

## 3. Results

### The basal physiological conditions measured in normal and MDP60 rats

Five-week-old normal and MDP60 rats are submitted to the measurement of body weight and temperature. In body weight, figure 1A shows that there is no obvious difference in these two groups (normal rats: MDP60 rats,  $141.2 \pm 1.1$  :  $140.0 \pm 2.2$ ,  $n=8$ , respectively,  $F(1,14)=0.44$ ,  $p > 0.5$ ) and the average weight of rats is about 140g. Figure 1B also shows that there is no significant difference between these two groups in body temperature (normal rats : MDP60 rats,  $37.9 \pm 0.1$  :  $38 \pm 0.03$ ,  $n=8$ ,  $F(1,14)=1.81$ ,  $p > 0.5$ ) and the average body temperature of rats is about  $38.0^\circ\text{C}$ .

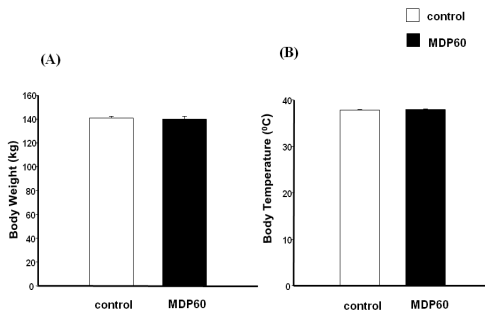


Figure 1

Figure 1. Measurement of body weight and temperature in normal and MDP60 rats 5-week-old normal and MDP60 rats are submitted to the measurement of body weight and temperature. (A) The data show that there is no difference between normal and MDP60 rats in body weight ( $n=8$  in each group,  $p > 0.5$ ). (B) The data also show that there is no difference between normal and MDP60 rats in body temperature ( $n=8$  in each group,  $p > 0.5$ ). It represents that rats experiencing maternal deprivation have no alteration in basal physiological conditions (vs. normal rats).

### The immobility time measured in normal and MDP60 rats in FST

After the measurement of basal physiological conditions, rats are subjected to forced swim test. Figure 2 shows that rats experiencing maternal deprivation for 60 minutes once a day from PND2 to PND14 expressed more immobile than normal rats did in forced swim test (normal rats: MDP60 rats,  $45.5 \pm 2.5\%$  :  $75 \pm 2.6\%$ ,  $n=8$ , respectively,  $F(1,14)=76.5$ ,  $***p < 0.001$ ). It means behavioral alterations happen in rats experiencing maternal deprivation in early life and the basal level of immobility in MDP60 rats is significantly higher than that in normal rats.

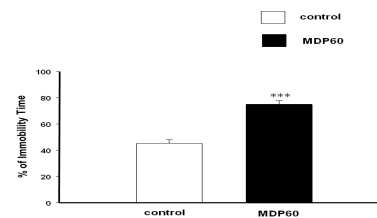


Figure 2

Figure 2. Measurement of immobility time in normal and MDP60 rats during FST. Forced swim test is conducted to normal and MDP60 rats. The data show that rats experiencing maternal deprivation in early life express significantly higher immobility time than normal rats do (normal rats: MDP60 rats,  $45.5 \pm 2.5\%$  :  $75 \pm 2.6\%$ ,  $n=8$ ,  $***p < 0.001$ ). It demonstrates that behavioral alteration happens in MDP60 rats (vs. normal rats).

### Spontaneous motor activity test in normal and MDP60 rats

Spontaneous motor activity test is conducted to evaluate the spontaneous motor activity of rats. Rats after two-day test protocol, figure 3 shows that rats experiencing maternal deprivation still express strong motor activity as well as normal rats (normal rats: MDP60 rats,  $95.0 \pm 1.2\%$  :  $97 \pm 1.8\%$ ,  $n=8$ , respectively,  $F(1,14)=4.54$ ,  $p > 0.5$ ). And the percent of motor activity in MDP60 rats is almost 100%, it suggests that maternal deprivation seem not to influence the physiological motor function of rats.

### The effects of antidepressants on forced swim MDP60 rats

MDP60 rats are treated with two antidepressants: one is desipramine, the other is fluoxetine. After the treatments of antidepressants, MDP60 rats are submitted to forced swim test and the effects of antidepressants on MDP60 rats are evaluated. Figure 3A shows that rats after 21-day desipramine treatment produce a significant decrease in immobility

time (MDP60 control : desipramine treated MDP60 ,  $75.7\pm 4.2\%$ :  $39.4\pm 3.4\%$ ,  $n=6$ , respectively,  $F(1,10)=62.0$ ,  $***p<0.001$ ). In figure 3B, the data also show the inhibitory effect of 3-day fluoxetine treatment on the immobility behavior of MDP60 rats (MDP60 control: fluoxetine treated MDP60,  $72.0\pm 4.5\%$ :  $39.0\pm 7.0\%$ ,  $n=6$ , respectively,  $F(1,10)=27.5$ ,  $***p<0.001$ ). There is no change on immobility of MDP60 rats by treatment of saline solution ( $69.5\pm 3.7\%$  in fig.3A,  $69.0\pm 4.5\%$  in fig.3B,  $n=6$  in each group,  $p>0.5$ ).

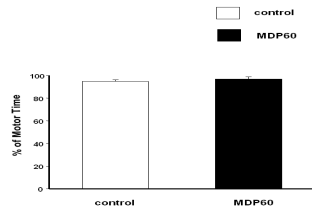


Figure 3

Figure 3. Measurement of spontaneous motor activity in normal and MDP60 rats. The monitoring on the spontaneous motor activity of rats is carried out. After two-day test protocol, the data show that no matter normal or MDP60 rats, there is no retardation took place in both rats (normal rats: MDP60 rats,  $95.0\pm 1.2\%$ :  $97\pm 1.8\%$ ,  $n=8$  in each group). It means rats experiencing 60-min maternal deprivation protocol has no alterations in motor activity.

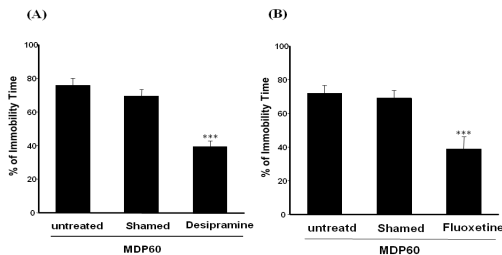


Figure 4

Figure 4. The inhibitory effects of antidepressants on the immobility time of MDP60 rats in FST. MDP60 rats are treated with two antidepressants: desipramine (10mg/kg, i.p.) and fluoxetine (5mg/kg, i.p.). (A) After 21-day desipramine treatment, MDP60 rats produce a significant reduction in the immobility time in FST ( $n=6$  in each group,  $***p<0.001$ ). (B) After 3-day fluoxetine treatment, MDP60 rats also show a obvious reduction in the immobility time in FST ( $n=6$  in each group,  $***p<0.001$ ). There is no effect on both rats with 0.9% normal saline treatment. It demonstrates that antidepressants can work on MDP60 rats very well.

### The immobility time measured in normal and MDP60 rats in FST

After the measurement of basal physiological conditions, rats are subjected to forced swim test. Figure 2 shows that rats experiencing maternal deprivation for 60 minutes once a day from PND2 to PND14 expressed more immobile than normal rats did in forced swim test (normal rats: MDP60 rats,  $45.5\pm 2.5\%$  :  $75\pm 2.6\%$ ,  $n=8$ , respectively,  $F(1,14)=76.5$ ,  $***p<0.001$ ). It means behavioral alterations happen in rats experiencing maternal deprivation in early life and the basal level of immobility in MDP60 rats is significantly higher than that in normal rats.

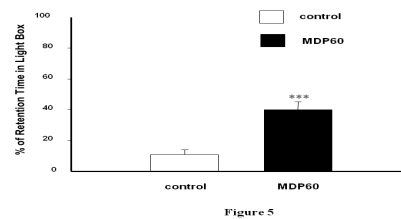


Figure 5

Figure 5. Measurement of anxious level in normal and MDP60 rats. Active avoidance test is carried out to estimate the anxious levels of normal and MDP60 rats. The data show that rats experiencing 60-min maternal deprivation protocol behave less anxious manner than normal rats (retention time in light box: normal rats: MDP60 rats,  $11.0\pm 3.0\%$  :  $40.0\pm 5.05$ ,  $n=8$  in each group,  $***p<0.001$ ). It can demonstrate that MDP60 rats could not be an appropriate anxiety animal model.

### 4. Discussion

Early life stress involved in the etiology of depression has been revealed. However, the detailed mechanisms for the involvement of early life stress in depression are still inadequate, therefore, it is necessary to build a well early life stress animal models for the further research of depression. Here we find out MDP60 rats, a stable and solid maternal deprivation animal model. Rats experiencing 60-min maternal deprivation protocol in early life will express depression-like behavior, but not anxiety-like behavior. In previous studies, various maternal deprivation protocols show different animal behavior. 15-min isolation protocol makes no alterations in animal behavior, however, 180-min isolation protocol makes animals behave anxiously [20,21]. Here, we conduct 60-min isolation protocol and it makes the animals express a steady depression-like behavior. It may suggest that different isolation intensity or duration would make different final animal behavior, and 60-min isolation is an optimal protocol for the

formation of depression animal models.

In figure 1, MDP60 rats have no difference from normal rats in basal body weight and body temperature. The evidence elucidate that the basal physiological conditions or even the basal metabolism rate (BMR) in rats experiencing 60-min maternal deprivation protocol in early life have no alterations (versus normal rats). In brain regions, hypothalamus is the regulatory center of appetite and body temperature in mammals [22]. Since there is no changes in body weight and body temperature of MDP60 rats, it seems to suggest that rats submitted to this isolation protocol show no harm on the hypothalamus of rats. In other words, it seems to suggest that mild early life stress would not impact the normal function of hypothalamus, at least in the control of body weight and temperature. However, HPA axis dysfunction involved in depression has been well stated [1, 23]. Thus, it is worthy to investigate further whether 60-min isolation early life stress would elicit the dysfunction of HPA axis and contribute to the depression-like behavior formation in rats.

In figure2, MDP60 rats express an obvious increment in immobility time in FST (vs. normal rats). In view of the statement of Porsolt et al, the immobility time in forced swim test represents “despair” state [24, 25, 26]. Therefore, the interpretation for the behavior of MDP60 rats is depression-like behavior. On the other hand, the immobility time in normal rats will be interpreted as basal despair level. In human beings, it is accepted that men has certain basal level of emotional despair, and the persons that express more disappointed emotion will be understood as more depressive. However, there is still a pitfall for identifying the depression syndrome in animals. Therefore, other remedies will be applied to identify the depression state of animals. In this studies, we conduct spontaneous motor activity test 、antidepressant treatments and active avoidance test to identify the behavior of MDP60 rats.

It is very common to question that whether the alteration on immobility time of rats in forced swim test is just a change of motor activity of rats, but not a mental change. For responding to the point, spontaneous motor activity test is used in the study. In figure 3, the data show that MDP60 rats behave a strong spontaneous motor activity as well as normal rats (normal rats : MDP60 rats,  $95.0 \pm 1.2\%$  :  $97 \pm 1.8\%$  ). From this result, it suggests that rats experiencing 60-min isolation protocol have no damage or hurt on the motor activity of rats. Therefore, it can be deduced that the alterations on immobility time of MDP60 rats is due to mental changes but not physiological issues.

Through the tests of forced swim test and spontaneous motor activity test, it seems more convincing to identify the depression-like behavior of

MDP60 rats. However, antidepressant treatment is also a useful screening platform for depression-like behavior in animals. Here we treat MDP60 rats with desipramine and fluoxetine and observe how they are going on. In figure 4, desipramine, a tricyclic antidepressant, and fluoxetine, selective serotonin reuptake inhibitor, are treated in MDP60 rats. The data show that MDP60 rats treated with desipramine and fluoxetine show a significant reduction on immobility time in FST. It represents that antidepressants can also improve the immobility behavior of MDP60 rats as well as they work in clinic patients. Taking the data from figure2, figure3, figure 4 together, it get more convincing to the identification for the depression-like behavior of MDP60 rats.

Although MDP60 rats can express depression-like behavior, however, as we discussed in first paragraph of discussion section, various isolation protocols for rats may produce anxiety behavior. Therefore, active avoidance test is conducted to dissect the anxious level of MDP60 rats in the study. In figure 5, MDP60 rats spend more time in retaining in light area, it means that MDP60 rats behave less anxious than normal rats do (retention time in light box: normal rats : MDP60 rats,  $11.0 \pm 3.0\%$  :  $40.0 \pm 5.0\%$  ). And the difference between normal rats and MDP60 rats in anxious level is significant. Based on this result, rats experiencing 60-min maternal deprivation protocol in early life show less anxiety behavior, reversely, we can state that the behavior of MDP60 rats is more specifically depression-like.

## 5. Conclusion

The establishment for depression animal models is a necessity in the research field of depression. We first demonstrate that rats experiencing 60-min maternal deprivation from PND2 to PND14 in early life will produce a stable and specific depression-like behavior, it means, an optimal depression animal model has been addressed, especially, a maternal deprivation induced depression animal model. Thus, it is expected that more fruitful and beneficial evidence will be elucidated by means of this animal model. At final in the research of maternal deprivation induced depression. In addition to the search of depression animal models, searching the screening platforms for identifying the depression syndromes or behavior in animals is still another crucial issue.

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