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A comparison between chronic exercise training and desipramine as treatments for the depression-like behavior of early-life maternal deprivation rats

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ABSTRACT

We have established a depression-like behavioral animal model through maternal deprivation in rats during early life. By using this model, the curative effects of exercise training and desipramine, a tricyclic antidepressant, on the improvement of depression-like behavior in rats were compared. Four-week-old male maternal deprivation (MDP) or normal rats were divided into control and exercise groups. The exercise group animals were subjected to a protocol of running on a treadmill at a moderate intensity for 60 min/day, 5 days/week, for 5 weeks. Forced swimming and light/dark box tests were conducted here to identify the behavioral characters. In addition, the short treadmill test was also used to clarify their movement motivation. Our data showed that the immobility during the forced swimming test and retention time spent in the light box of MDP rats were significantly longer than those for the control group. However, there was no difference between MDP and normal rats in the short treadmill test. MDP rats subjected to the 3-week exercise training course could produce less immobility in the forced swimming test and less retention in the light box. However, with the long-term desipramine HCl treatment (21 days, 10 mg/kg, i.p.), MDP rats could express less immobility in the forced swimming test, but more immotility in the short treadmill test. These results suggested that exercise training and desipramine may mediate different mechanisms to ameliorate depression-like behavior in MDP rats.

Recently there has been a rise in the diagnosis of depression and an increase in the number of related prescriptions [13]. However, side effects including sexual dysfunction, weight gain, sedation, respiratory depression and cardiovascular toxicity are common in the pharmacological treatment of depression [8], and thus there is obvious interest in developing better therapeutic treatments.

Over the past few decades, many studies have found evidence of the positive effects of exercise training for the treatment of many diseases and conditions, including mood disorders [1,3,10,12,16]. To date, a majority of the studies on the effects of exercise on mood disorders are based on human subjects. However, at least two obvious limitations exist in the current evidence derived from such studies. Firstly, clinical data from people who suffer from depression is limited, since not every sufferer is easily motivated to exercise, and this has made it difficult to demonstrate the successful treatment of mood disorders with exercise training. Secondly, when using human subjects little can be done to explore the underlying curative mechanisms of the effects of exercise. Therefore, further research using nonhuman models to reveal the mechanisms of beneficial effects of exercise on mood disorder still needs to be undertaken.

Maternal deprivation during early life in rodents is a common technique in setting up mood-perturbation animals [5]. Evident depression-like behavior rats were achieved in our lab by applying proper stress intensity protocols during early life [6]. The maternal deprivation animal model with our lab protocol was conducted throughout the whole experiment to objectively evaluate the effects of diverse interventions on the focal mood disorder.

The changes in behavior of the rats were evaluated with the forced swimming test, a light/dark box, and a short time treadmill test [2,3,14,15]. We tried to confirm the curative effect of chronic exercise training on the focal mood disorder with the use of the animal depression model. Furthermore, we compared the curative and adverse effects of chronic exercise training and desipramine, a tricyclic antidepressant which is popular in the clinical treatment of depression [11,17], with regard to the treatments of animals with depression-like behavior, and the results are expected to reveal an optimal intervention for the treatment of depression in human subjects.

This study was conducted in conformity with the "Guide for Animal Care and Use of Laboratory Animals" [7], and the animal experiment protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung-Hwa Uni-

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versity of Medical Technology. Adult male Sprague–Dawley rats weighing 150–170 g at the time of testing were housed at a constant room temperature (22 ± 1 °C) and humidity (50 ± 10% RH) with a 12 h light/dark cycle (light on at 6:00 A.M. and off at 6:00 P.M.). Groups of six rats were raised in a cage with the size of 47.0 cm × 26.0 cm × 20.0 cm ($L \times W \times H$). Food and water were available ad lib.

Maternal deprivation in early life was utilized to produce depressed rats, as described previously [6]. Briefly, timed-pregnant Sprague–Dawley rats arrived at the animal facility on gestational day 12. The pups were sexed and divided into litters of 8–10 male pups and randomly assigned to maternal deprivation for 60 min/day (MDP) for 13 days or the untreated control group. Non-handled pups were left undisturbed. When all the pups were weaned, the rats were maintained four per cage at a constant room temperature of 22 ± 1 °C in a 12 h light/dark cycle (light on at 6:00 A.M.) with free access to food and water.

The exercise protocols were modified from Chen and Chiang [3]. Briefly, the rats ran on a treadmill (Columbus Instruments) partitioned into four channels with plastic boards (90 cm × 8 cm × 27 cm for each channel) at an intensity of ~70% of maximal oxygen consumption for 5 days/week, for 6 weeks. The exercise training protocols began on the 28th PND. In the 1st week, the rats were placed in the treadmill for training with speed of 9 m/min, 10 min/day for 5 days. On the subsequent 5 weeks of training, the running time was eventually extended to 60 min/day at the end of the entire training protocol. The training loads were readjusted according to the animal's newly determined peak oxygen consumption every 2 weeks. The training loads at 1, 3, 6 weeks were 9, 12, 15 m/min, respectively. The sedentary control groups were placed in the treadmill without running for 10 min/day. No electric shock device was installed on the floor of treadmill. A photo sensor timer was mounted on the bottom wall of the treadmill to monitor the total running time during the training. When the rats were put into a channel during the training, the trainer tapped the bottom of treadmill to encourage them to begin running.

Rats with or without maternal deprivation were immersed in plexiglass cylinders (diameter 18 cm, height 38 cm) filled to a depth of 25 cm with water at 25 °C. This test was carried out on two consecutive days. On the 1st day, rats were gently placed in the water for a 15 min period of habituation. On removal from the water, they were placed in a plexiglass box under a 60 W bulb for 30 min to dry. The next day, they were replaced in the cylinders and observed for 5 min. During this period, the total time that they spent immobile (i.e. making only the movements necessary to remain afloat) was measured and the immobility time was calculated by immobility time/total testing time × 100%. After a 5-min test, the rats were removed from the water and were again placed in a plexiglass box under a 60 W bulb for 30 min to dry and rest.

The anxiety level of the rats was evaluated by the light/dark box test. The more time spent in the illuminated part of the cage, the less anxiety the rats have. The apparatus for the light/dark transition test consisted of two compartments: one light area (27L × 27W × 27H cm, 400 lx) illuminated by 100-W desk lamp was painted white, and the other, dark area (18L × 27W × 27H cm, 4 lx) was painted black. The floor of the light area was divided into nine equal squares (9 cm × 9 cm) by black lines, and the dark area was divided into six equal squares (9 cm × 9 cm) by white lines. The experiments were performed between 09:00 and 14:00. An animal was placed in the center of the light area with its back to the opening. The total time spent in the illuminated part of the cage during 5 min was recorded, and the retention time was calculated as the retention time/total testing time × 100%. The apparatus was cleaned thoroughly between trials. All behavioral recordings were carried out with the observer unaware of the treatment of the rats.

Based on their vivacious nature, most rats will spontaneously run on a treadmill when they are familiar with the environment. Therefore, it is useful to measure the spontaneous running time on the treadmill to assess the activity or motor ability of rats. To familiarize them with the environment, the rats were placed in the center of the treadmill for 10 min. A training load of 9 m/min was applied to the animal in order to test its spontaneous running time for 10 min, and the motility time was calculated as the motility time/total testing time × 100%. A rat with low spontaneous running time was considered as having impaired motor activity due to the apparent loss of movement motivation.

The results are expressed as mean ± SEM. Sample sizes are indicated by n. Repeated testing groups were carried out with repeated measures ANOVA. Differences between two groups were compared by using unpaired Student's t-test or Student–Newman–Keul's test. The p value < 0.05 was considered statistically significant.

We carried out a forced swimming test, light/dark box test, and short time treadmill test to characterize the behavior profiles of MDP and control rats. The immobility time during the forced swimming test of MDP rats was significantly higher than that of control rats (Fig. 1A, control, 45.5% ± 2.3; MDP, 76.0% ± 3.0; $p < 0.01$ compared to control, $n = 7$ in each group). To determine whether the anxiety level of rats may be influenced by maternal deprivation, we subjected control and MDP rats to the light/dark box test. In MDP rats, time spent in the light compartment was significantly higher than for the control rats (Fig. 1B, control, 11.2% ± 3.3; MDP, 39.5% ± 6.8; $p < 0.001$ compared to control, $n = 7$ in each group). In the short time treadmill test, both MDP and control rats expressed the same motility ability (Fig. 1C, control, 4.7% ± 1.1; MDP, 3.8% ± 1.8; $p > 0.1$ compared to control, $n = 7$ in each group).

Before testing the effects of the chronic exercise training on depression, we traced the chronic phenotype of depressive characterization in MDP and control rats using the Porsolt forced swimming test for 5 weeks since the 28th PND. In control rats, there was no alteration in the immobility time during continuous forced swimming tests (Fig. 2A, week 5, 43.4% ± 4.1; week 6, 44.7% ± 2.8; week 7, 43.4% ± 3.6; week 8, 44.2% ± 2.2; week 9, 42.7% ± 3.9, $n = 7$ in each group). However, the immobility time of MDP rats reached a peak (vs. control rats) at the age of 5 weeks and maintained at a plateau from the 42nd to the 63rd PND (Fig. 2B, week 5, 72.0% ± 3.7; week 6, 66.4% ± 4.1; week 7, 66.0% ± 2.0; week 8, 64.3% ± 2.2; week 9, 64.0% ± 4.9, $n = 7$ in each group).

Data from the photo timer revealed that both MDP and control rats at 28 days old ran spontaneously when the treadmill was on. Most of the rats spent more than 90% of the time on spontaneous running during each trial. In the control rats, there was no effect of the chronic exercise on the immobility time of the forced swimming test (Fig. 3A, week 5, 45.0% ± 5.7; week 6, 41.0% ± 4.0; week 7, 45.0% ± 7.8; week 8, 39.0% ± 6.7; week 9, 44.0% ± 4.9, $n = 6$ in each group). Fig. 3B shows that chronic exercise training could significantly reduce the immobility time of MDP rats in the forced swimming test (week 5, 65.0% ± 7.4; week 6, 62.0% ± 6.6; week 7, 30.0% ± 7.5; week 8, 41.0% ± 6.3; week 9, 39.0% ± 7.1 s, $n = 6$ in each group).

The data shows that the MDP rats had lower anxiety levels (Fig. 1B), and thus we tried to investigate whether the chronic exercise training would also alter the dark/light box behavior of these rats. In addition, we also tried to discover the influence of such training on the rats' performance of the short time treadmill test. Table 1 shows that after the training the MDP rats spent as much time in the light box as the control rats did (control: 11.0% ± 3.0; MDP after chronic exercise: 11.0% ± 4.0, $n = 8$ in each group). In addition, the dark/light behavior of the control rats was not altered after the chronic exercise training (control: 11.0% ± 3.0; control after chronic exercise: 12.0% ± 5.0, $n = 8$ in each group). In the short