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Melatonin produces a rapid onset and prolonged efficacy in reducing depression-like behaviors in adult rats exposed to chronic unpredictable mild stress

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Highlights

- Six-week chronic unpredictable mild stress is sufficient to caused the depressive-like behavior in Wistar rats.
- Melatonin produces a rapid antidepressant effect in Wistar rats.
- The sustained antidepressant effect of melatonin in rats is demonstrated by behavioral test.
- Low-dose daytime melatonin administration will ameliorate depressed behaviors and cognitive function in CUS models.

Abstract

The present study was aimed at evaluating the rapidity and duration of melatonin as an antidepressant in a rat model of depression. The rats were subjected to a six-week period of unpredictable mild stress followed by melatonin treatment. Three groups of rats were included in this study: Controls (CON – no stress exposure), Chronic Unpredictable Mild Stress (CUS) and CUS followed by melatonin (MT). Stressors consisted of exposure to rotation on a shaker, placement in a chamber maintained at 4°C, lights off for 3h, lights on overnight, exposure to an aversive odor, 45° tilted cages, food and water deprivation and crowding and isolated housing. Subsequently, the saline vehicle (CUS) or melatonin was administered at a dose of 10mg/kg for 14 days period. Body weight and behavioral tests were used to evaluate depression-like behavior and its recovery following melatonin treatment.

While body weight increases were significantly lower in rats exposed to CUS versus CON, body weights of the MT group increased significantly following melatonin treatment as compared with the CUS group. With regard to results obtained with behavioral assays indicative of depression, rapid and long-term functional recoveries in depression were observed in the MT as compared to the CUS group.

The results indicate that not only does melatonin induce an antidepressant-like action within this rat model of depression, but does so with a rapid onset and prolonged efficacy. As most current treatments for depression require an extended period of administration, our current results suggest that melatonin may prove to be a particularly effect agent to promote a rapid onset and prolonged behavioral benefits in the treatment of depression.

Key words

Melatonin; Depression; Behavior Test; Rats

1. Introduction

Depression affects millions of people and is one of the leading causes of disability worldwide. It has been reported that depression is strongly associated with major disruptions in circadian rhythms [1], although the underlied mechanisms remain unclear. Moreover, stress plays a key role in depression [2,3]. As a result, a depression model can be established in rats subjected to chronic unpredictable mild stress (CUS) [4].

In recent years, there has been a dramatic increase in the prescription of antidepressants that act by interacting with the serotonin and norepinepherine neurotransmitter systems. Unfortunately, such treatments are limited in their efficacy because their therapeutic benefits take several weeks before setting in and they are only effective in approximately one third of depressed patients [5]. Beyond that, antidepressant drugs are often associated with extrapyramidal side effects, such as increase the body weight, electrocardiac changes, hyperglycemia, hyperlipidemia and other adverse reactions [6]. Thus, there exists an urgent need for antidepressants with better efficacy and reduced side effects.

Melatonin is the main biologically active substance secreted by the pineal gland, an organ long considered as a vestigial structure isolated in 1958. Subsequent investigations have revealed that melatonin is the mediator of photoperiodic information to the central nervous system in vertebrates and allows a central circadian regulation of numerous physiological homeostatic processes, including circadian rhythms, sleep, and mood [7,8].

Although the exact role of melatonin in pathogenesis of depression is uncertain, it has been reported that decreased nocturnal melatonin levels are found both in animal models of depression as well as in depressed patients [9,10]. A deficiency in melatonin is associated with a predisposition for melancholic depression characterized by psychopathological and neurobiological disturbances, including anhedonia, agitation, sleeping disturbances, circadian fluctuations in mood, weight loss, and increases in monoamine oxidase activity and plasma cortisol levels [11].

Findings from both clinical and epidemiological studies suggest a strong association between melatonin and depression, and melatonin-related drugs have been shown to be effective in the treatment of depression as indicated in several clinical studies [12]. Moreover, results from preclinical studies have indicated that the combination of antidepressants (imipramine or buspirone) with melatonin serving as a particularly effective strategy for the treatment of depression [13,14]. Based upon studies using preclinical antidepressant behavior-based assays, it has been revealed that the melatonin receptors, MT_1 and MT_2, are important targets for the development of novel antidepressants [15,16]. Further support for this belief was provided from research demonstrating that rapid MT_2 receptor desensitization kinetics could facilitate melatonin-mediated antidepressant-like effects following treatment with an MT_1/MT_2 receptor agonist [17].

Although these antidepressant effects of melatonin have been clearly demonstrated in animal models, as well as in humans with depression [18,19,20], the time course of these effects remain to be established. Specifically, the onset and duration of melatonin's effects upon depression remain unclear. These represent important issues with regard to the use of melatonin in the treatment of depression. Therefore, the present study was designed with two goals: (1) to determine the onset of melatonin's effects upon depression as assessed after a relatively short-term two-week administration period and (2) to determine the duration of melatonin's effects in a CUS model of depression.

2. Materials and methods

2.1 Animals

Thirty male Wistar rats (2 months, body weights = 200–250 g) were obtained from the animal center of Weifang Medical University, China and were group-caged under controlled conditions of lighting (lights on: 07:00–19:00h) and temperature (25 ± 2°C) with food and water available *ad libitum*. Rats were allowed to acclimate to these laboratory conditions for seven days prior to use in the experiment. The study was approved by the Institutional Animal Care Committee of Weifang Medical University. Rats were randomly divided into three groups (N = 10/group): Controls (CON), Chronic Unpredicted Stress (CUS), and CUS treated with melatonin (MT). Animals were subjected to CUS for a 6-week period followed by a 2-week period of melatonin administration and euthanized after completion of all behavioral tests.

2.2 Animal model of depression

With the exception of the CON group, rats in the CUS and MT groups were subjected to chronic unpredictable mild stress. A total of 10 different stressors was used (rotation on a shaker, placement in an ambient chamber maintained at 4°C, lights off for 3h, lights on overnight, exposure to an aversive odor, 45° tilted cages, food and water deprivation, crowding and isolation housing). On each day, rats were exposed to two types of stressors that were randomly administered and were of a three-hour duration. There was a minimum four-hour period between the two stressors administered.

2.3 Drugs and treatments

The melatonin was manufacturer by the Kang Long Group Corp. (POMONA CA91766

USA). Melatonin(10mg/kg, i.g.) was freshly prepared each day and dissolved in a minimum volume of sterilized saline solution (0.9% NaCl). The melatonin was wrapped to prevent light-induced degradation [16]. The final volume of ethanol in vehicles was less than 1%.The basic design (time course) of this experiment as follows, Figure 1.

(Insert Figure 1 here)

2.4 Methods of behavioral assessments

2.4.1 Body weight

For evaluation of long-term effects of melatonin administration on weight gain, the body weight at 0 month was taken as the baseline of 100%. Body weights were recorded at the initiation of the experiment and then weekly throughout the duration of the experiment.

2.4.2 Sucrose Preference Test

Rats were individually housed for 3 days prior to the onset of the test. They were permitted a 3-day training period to familiarize them with consuming water from two bottles. During this 3-day training period, the two bottles containing water were replaced for 1 h a day with two bottles filled with a 2% (w/v) sucrose solution. Forty-eight hours later the rats were tested for their ability to discriminate and select between the two bottles, one containing water and the other the sucrose solution. To avoid conditioned place preference learning, the position of the bottles was altered at the middle of the light and dark phases. Bottles were weighed at the onset of the light and dark phases in order to measure the amount of sucrose water consumed**.**

2.4.3 Elevated plus maze Test (EPM)

The EPM apparatus consisted of a plus-shaped maze elevated 60 cm above the floor. Two opposite arms (42×14.5 cm for each arm) were enclosed with walls (22.5 cm), while the other two arms were open. Rats were placed in a central square and allowed to freely explore the maze for 5 minutes. Behaviors recorded included mobility distance and the time spent and entries in the open arms (non-anxiety state) of the maze. Data were analyzed using the SMART video tracking system**.**

2.4.4 Open Field Test (OFT)

Rats were placed at the corner of a white-painted open field arena (100 × 100 × 40 cm). The arena was divided by transverse lines into 25 equal squares. Each rat was placed in the same corner of the arena facing in the same direction and was allowed to freely explore the arena for 5 minutes. When both hind limbs entered a square, a crossing was recorded. Locomotor and exploratory activity were evaluated by counting the number of line crossings and rearing events. The sessions were recorded with a digital video camera. Mobility distance, the number of boundaries traversed and time spent in the center were analyzed using the SMART video tracking system.

2.4.5 Forced Swim Test (FST)

Thirty-six hours after the last injection, rats were habituated to the FST testing room for 30 min prior to the onset of the test. Rats were subjected to a pre-swim before the final test session by placing them into cylinders containing a depth of 30 cm of clean tap water at 22°C for 15 min to induce a state of behavioral despair. The testing area was dimly illuminated to reduce stress or anxiety. The sessions were videotaped for subsequent analysis. Rats were forced swimming for total 6 min (the same conditions as pre-swim), the cumulative immobility time was recorded during the last 4 min of the test by a trained observer who was unaware of the rat's treatment condition [16]. After testing, each rat was gently dried, placed in a preheated holding cage with normal bedding, and covered by an absorbent paper towel for 30 min. Subsequently, rats were returned to their home cages.

2.4.6 Water Maze Test (WMT)

The water maze (Morris) consists of a pool of water diameter: 1.55 m, 50-cm high rim. For first task a 12×12-cm escape platform is hidden at one of four quadrants (E, S, W, and N) in the pool with the top surface 2-3 cm below the water level. In this way the platform is not visible to the performing rat. Rats were trained to locate the platform at different locations for 5 consecutive days. On day 6, the original platform was removed and the quadrant that had contained the platform was defined as the target quadrant. The actual path of the rat and the time of escape latency is recorded using the SMART video tracking system.

2.5 Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM), and performed using the statistical software package SPSS18.0. Specifically, student-*t* (two-tailed) test was used for comparison of body weight and FST results between groups, while one-way analysis of variance (ANOVA) was used for comparison of SPT, EPMT, OFT and WMT results. The difference between groups was considered statistically significant when P-value was less than 0.05.

3. Results

3.1 Weight changes

Reductions in body weight increases represent a major symptom of depression in the CUS model. Both the CUS and MT groups showed significantly decreased growth over the initial six-week stress exposure period (Mean ± SEM in gm: CON=375±24.9, MT=253±17.8, CUS=265±18.6, P<0.05). However, by the sixth week of melatonin treatment, body weights of the MT group started to increase and were now significantly greater than that of the CUS rats (MT=342±12.5, CUS=263±15.2, P<0.05). When expressed as the percent of weight gained, similar gains in body weight were observed between the MT and CUS animals.

(Insert Figure 2 here)

3.2 Behavioral test results

3.2.1 Sucrose Preference Test (SPT)

Anhedonia, a major symptom of depression, was evaluated using the sucrose preference test. Exposure of rats to the six-week stress regime was successful in eliciting an anhedonia-like condition as demonstrated by significantly lower Percent Sucrose Uptake scores within the MT and CUS rats as compared to CON ($F_{1, 28}$=6.37, P=0.018). On day 64 of melatonin treatment, the Percent Sucrose Uptake scores of the MT group were significantly increased as compared to CUS group ($F_{1, 18}$=6.07，P=0.021).

(Insert Figure 3 here)

3.2.2 Elevated Plus Maze (EPM) Test

In the elevated plus maze test, the number of entries into, and durations of time spent within, the open arms of the maze are negatively correlated with anxiety in rats. When tested after six-weeks of the stress regime, both the MT and CUS groups showed significantly lower ratios of entry into the open arm as compared with CON ($F_{1, 28}$ = 6.52, P= 0.012). These results indicate that a higher level of anxiety was present within these two groups of stressed rats. On day 113 (8 weeks after melatonin treatment), rats in the MT group showed significantly more entries, and longer durations within the open arms as compared with their scores at six weeks ($F_{1, 9}$ = 5.52, P= 0.027). In contrast, the CUS group failed to show any significant changes between these two time periods (P = 0.062). Such reductions in anxiety scores with melatonin suggest an antidepressant effect of this treatment.

(Insert Figure 4 here)

3.2.3 Open Field Test (OFT)

Spontaneous motor activity in the open field test also provides an index of anxiety/depression that can be present in rats subjected to stress. With this test, reductions in carding and crossing numbers indicate higher levels of anxiety/depression. Carding numbers at six weeks were significantly decreased in the MT and CUS groups as compared with CON ($F_{1, 28}$ = 5.826, P= 0.024; Fig. 5a). Crossing numbers of the MT group at day 113 (8 weeks after melatonin treatment)were significantly higher than those obtained at six weeks ($F_{1, 9}$ = 10.526, P= 0.024; Fig. 5b). No statistically significant differences among the three groups were obtained for the number of upright postures (Fig. 5c).

(Insert Figure 5 here)

3.2.4 Forced Swim Test (FST)

The forced swim test was also employed to assess the anti-depressant effects of melatonin. In this test, shorter latencies of immobility indicate higher levels of depression. Following the six-week period of chronic stress MT and CUS rats showed shorter latencies of immobility (110.50±5.77 s) as compared with CON (150.50±3.75 s). However, on day 64 (days after melatonin treatment), post-melatonin immobility latencies of the MT group (170.43±7.56 s) were significantly increased ($P < 0.01$) as compared with their scores at six weeks, as well as to that of the CUS rats on day 64 (125±4.82 s). Immobility latency times of the CUS group failed to increase significantly between six weeks of stress exposure and day 64, post-saline treatment.

(Insert Figure 6 here)

3.2.5 Water Maze Test (WMT)

As memory can also be affected with depression, we assessed memory function in these rats with the Water Maze Test. When tested in the water maze test, reductions in escape latencies and distance traveled to the platform provide an index of memory function. Within the CON group escape latency and distance traveled decreased over the training period, indicating that an effective memory process was present. Escape latencies of the MT and CUS groups were significantly increased in comparison to CON（$F_{1, 28}$ = 4.326, P= 0.021). After treatment with melatonin, escape latencies of the MT decreased significantly compared to CUS group($F_{1, 9}$ = 5.526, P= 0.032)(Table 1).

With regard to distance traveled, the number of entries and the time spent in the target quadrants by the CON was significantly greater than that of the MT and CUS groups（$F_{1, 28}$ =5.36, P= 0.026). After melatonin treatment, the MT group demonstrated significantly more entries into the target quadrants than that of the CUS group（$F_{1, 28}$ = 6.37, P= 0.021).

(Insert Table 1 here)

4 Discussion

4.1 The depression model in rats

Depression is related to many psychiatric conditions, especially stress. Chronic unpredictable mild stress is widely used in animal studies to create models for investigating the neurobiological characterizations of psychiatric disorders, like depression, and the efficacy of drugs for the treatment of these disorders [21]. Our study demonstrates that a six-week chronic stress regime was sufficient to establish a depression model in rats as revealed by the results of these behavioral tests,such as stress exposed rats exhibited reduced weight gain (Figure 2) and a diminished preference for saccharin (Figure 3) and so on. which are in accordance with previous findings [22]. While a previous study had shown that a four-week exposure to stress did not affect spatial learning in the WMT [23], we found that a six-week stress regime was effective in impairing memory in this test (Figure 7).

4.2 Rapidity of antidepressant effects of melatonin

It had been reported that a duration of melatonin administered in low doses was effective in diminishing behavioral and cognitive changes observed in depressed rats and no significant side effects [24,25,26]. In the present study we show that a two-week administration of melatonin can produce a relatively rapid antidepressant effect as indicated by its capacity to alleviate anhedonia (Figure 3). Melatonin act similar to agomelatine (melatonin analog) also showed analogous result which induced comparatively rapid antidepressant effect both in neurogenesis level and behaviour tests

[27]. Such effects were observed by responses within a number of tests, including time spent in the open arms of the elevated plus maze (Figure 4), increases in the number of entrances within the center of the open field (Figure 5) and decreases in immobility times in the forced swim test (Figure 6).

4.3 Duration of antidepressant effects of melatonin

Our current findings are supported by numerous studies which have shown that melatonin treatment results in long-term behavioral and cognitive changes associated with antidepression in rats as demonstrated in a variety of paradigms such as sucrose preference, forced swimming, elevated plus maze, open field and the water maze test as well as in different models of depression, including learned-helplessness, environmental stress, chronic mild stress, prenatal restraint stress and transgenic mouse models of depression [28]. Results obtained from studies utilizing anxiety tests indicated that high doses of melatonin produced beneficial effects upon long-term exposures to chronic mild stress [29]. However, in our study involved with testing the effects of lower doses of melatonin, clear antidepressant effects, which persisted over an eight-week period of drug administration, were observed in rats as demonstrated in several behavioural test paradigms. The results testify that melatonin not only can be as an antdepressant, but can do so over an extended period of time.

4.4 Limitations and future outlooks

Our present results demonstrate that melatonin treatment not only induced a rapid onset, but also a persistent long-term antidepressant effect in rats, and provide important referenced value for the clinical treatments in long-term curing or improving depression-like behaviors by melotonin and melatonin-related drugs. Meanwhile, our current results show that daytime melatonin administration was sufficient to improvement depression, although melatonin levels peak during the night phase in rodents as well as humans [30]. Therefore, future studies will be required to elucidate the mechanisms underlying this effect, as well as any potential metabolic side effects that may result from these treatments. And the possibility exists that a nocturnal strategy of melatonin administration might prove even more beneficial than that achieved during the daytime. Besides, numerous researches indicate that melatonin, neurogenesis, stress, hippocampal function are relevant strongly, thus future studies would be of great value in assessing this relationship among them[31,32,33].

4.5 Conclusion

The present work revealed that melatonin exerts rapid and prolonged antidepressant and anxiolytic effects within a CUS rat model of depression. The sustained antidepression effects were demonstrated in a number of test paradigms including sucrose preference, FST, EPMT, OFT and WMT. Accordingly, the findings presented in this study provide new insights into the time-course efficacy of the antidepressant effects of melatonin.

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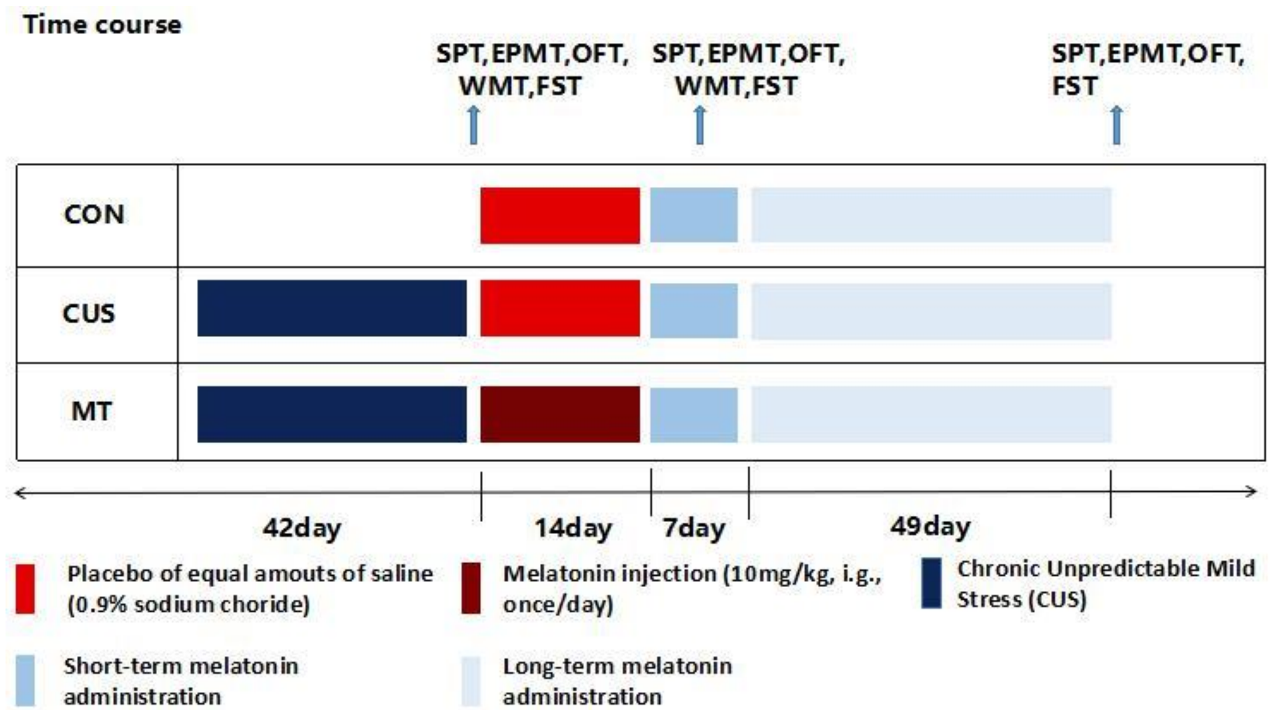

Fig. 1. Time course**.** CON: Control, no stress exposure; CUS: Chronic Unpredictable Mild Stress; MT: CUS followed by melatonin.

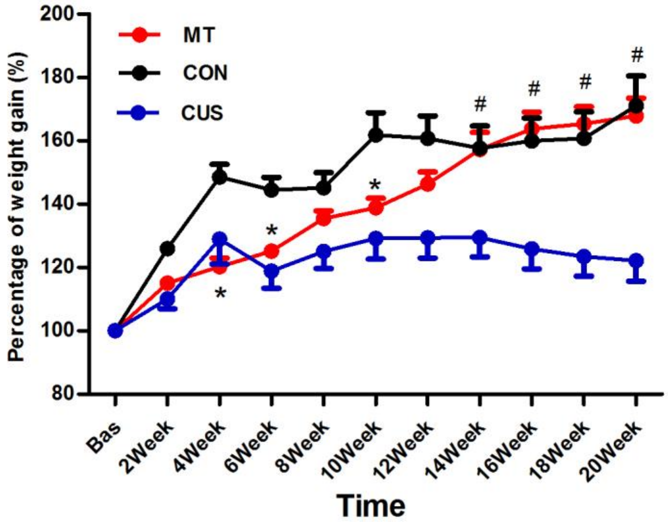

Fig. 2. Body weight.The final body weights were expressed as the percentage of the body weights at 0-month (taken as 100%) when the experiment started. The line charts reveal the effect of chronic melatonin administration on percent of body weight gain in rats. Data represent as mean±SEM. Differences among groups were analyzed using one-way ANOVA followed by Newman–Keuls comparison test. The gains in body weights of the CUS and MT groups were significantly lower than that of the CON group during the initial 6-weeks of stress exposure (P<0.05). A significant increase in body weight was observed in the MT (P<0.05). * and # indicate statistically significant differences between and within groups, respectively.

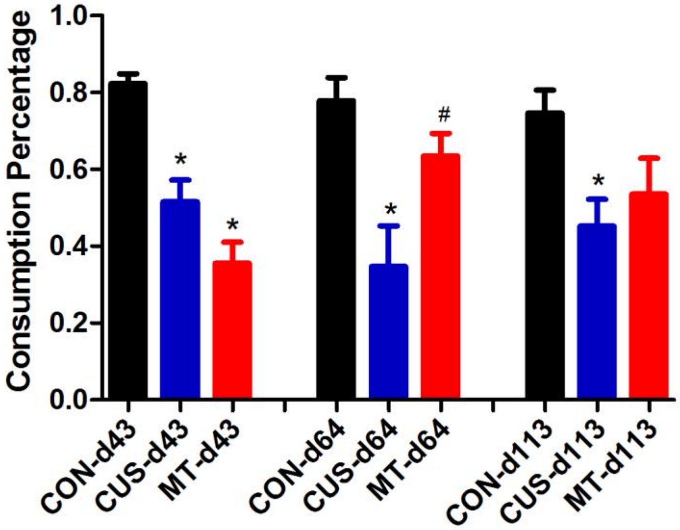

Fig. 3. Sucrose preference test (SPT). Histograms show that the percent of sucrose consumed by the MT and CUS groups was significantly lower than that obtained with CON ($P<0.05$). A significant increase percent of sucrose consumed by the MT group was observed after the eight week of melatonin administration ($P<0.05$).* and # indicate statistically significant differences between and within groups, respectively.

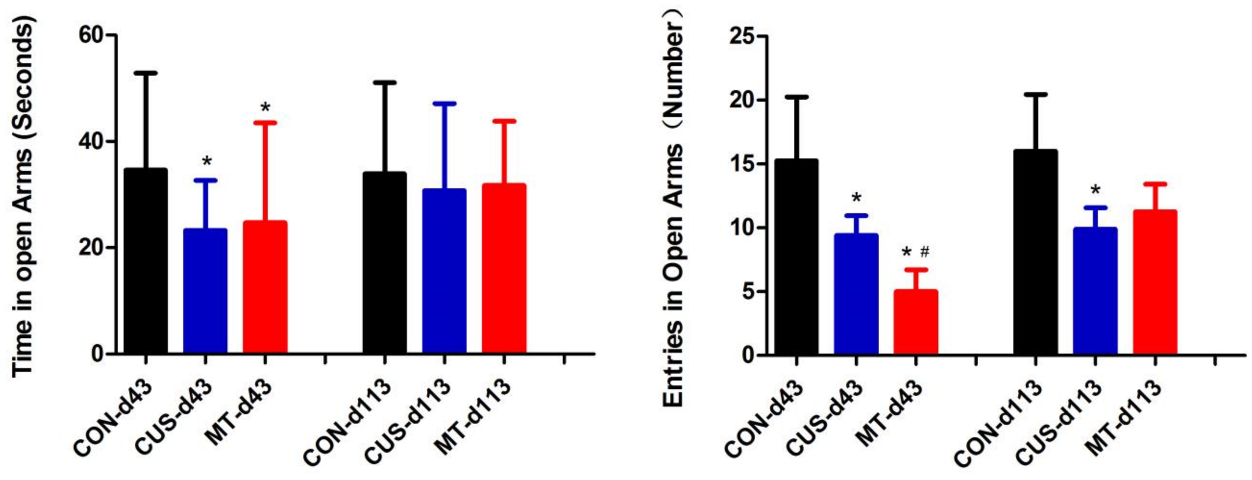

Fig. 4. Elevated Plus Maze (EPM) test. The histograms illustrate that the entry times and entry numbers of the MT group into the open arm of the maze were significantly increased after melatonin administration (P<0.05). No such significant effects were obtained with the CUS group. Values were expressed as mean ± SEM. * indicates statistically significant differences between groups.

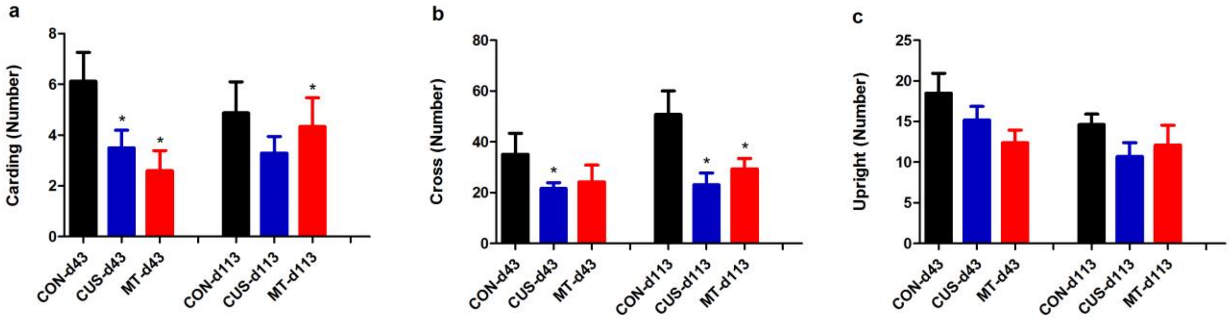

Fig. 5. Open field test (OFT). a. The histograms illustrate that a statistically significant increase in carding number was obtained in the MT group after melatonin administration (P<0.05). b. The histograms illustrate that a statistically significant increase in cross numbers was obtained in the MT group after melatonin administration (P<0.05). c. No statistically significant differences were obtained among the three groups for the display of upright posture (P>0.05). Data are presented as mean ± SEM.

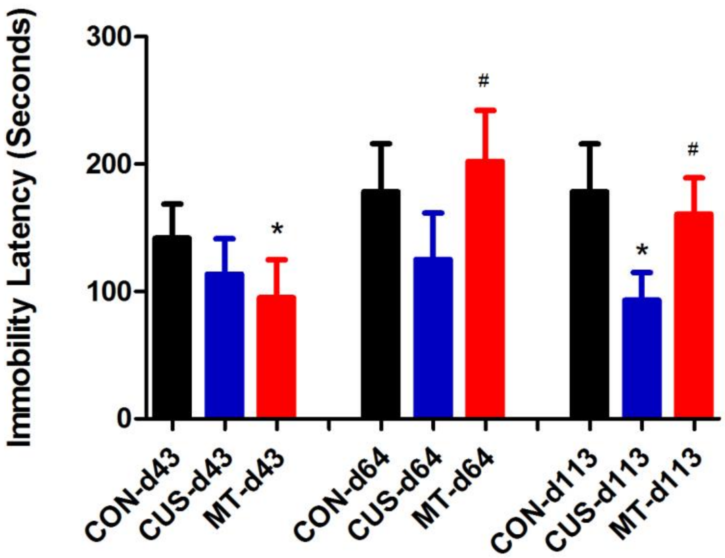

Fig. 6. Forced Swim Test (FST). Effect of chronic melatonin administration on the latency to first immobility, in the forced swim test paradigm, of rats after 6 weeks of stress exposure and inject melatonin on the 8 weeks. Data expressed as mean ± SEM. The histograms illustrate that significantly higher immobility latency times were obtained in the MT group after melatonin administration as compared with CUS group (P<0.05). * and # indicate statistically significant differences between and within groups, respectively.

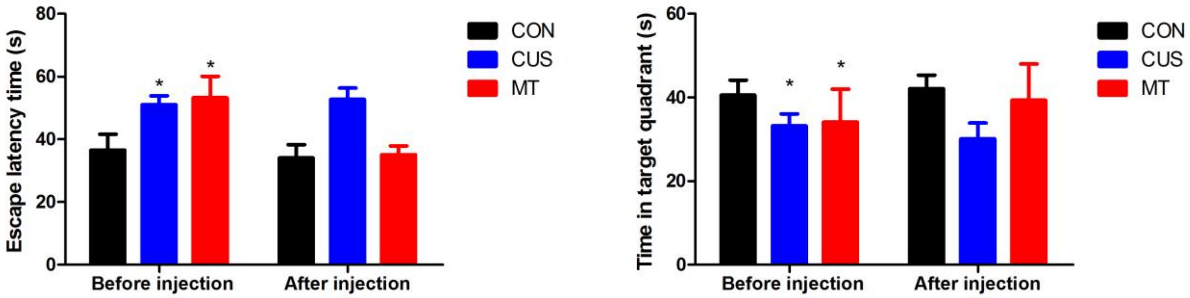

Fig. 7. Water Maze Test (WMT). Values are expressed as mean ± SEM. * indicates statistically significant differences among groups; while # indicates statistically significant differences within a group $p < 0.05$.