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Melatonin ameliorates chronic mild stress induced behavioral dysfunctions in mice

Seenu Haridas, Mayank Kumar, Kailash Manda *

NeuroBehavior Laboratory, Division of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences, Delhi, India

HIGHLIGHTS

- Long term chronic mild stress-induced behavioral dysfunctions evaluated in mice
- Stress induced hedonic and cognitive deficits were not corroborated to anxiety.
- Night time melatonin administration ameliorated cognitive and affective disorders.

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ABSTRACT

Melatonin, a neurohormone, is known to regulate several physiological functions, especially the circadian homeostasis, mood and behavior. Chronic exposure to stress is involved in the etiology of human affective disorders, and depressed patients have been reported to show changes in the circadian rhythms and nocturnal melatonin concentration. The present study was conducted to evaluate a possible beneficial action of chronic night-time melatonin treatment against chronic mild stress (CMS) induced behavioral impairments. As expected in the present study, the stress exposed mice showed reduced weight gain, hedonic deficit, cognitive deficits and decreased mobility in behavioral despair test. Interestingly, CMS exposed mice showed less anxiety. Chronic night-time melatonin administration significantly ameliorated the stress-induced behavioral disturbances, especially the cognitive dysfunction and depressive phenotypes. In conclusion, the present findings suggest the mitigating role of melatonin against CMS-induced behavioral changes, including the cognitive dysfunctions and reaffirm its potential role as an antidepressant.

1. Introduction

Chronic stress has become an unfortunate, yet unavoidable part of our current lifestyle. Exposure to stress, whether chronic or acute, has been reported to cause depressive disorder [1,2]. The notable depressive symptoms in human include mood swing, irritability, low self-esteem, feeling of hopelessness, decreased ability to concentrate and rational thinking, changes in appetite and weight, sleep disturbances, fatigue, anhedonia and suicidal tendencies. These symptoms are, however, difficult to translate into a reliable animal model, as the symptoms of major depression like suicidal tendencies, low self-esteem and guilt cannot be scored in animals, especially rodents. Fortunately, Willner et al. developed the chronic mild stress (CMS) model for rodents to induce a depressive phenotype [3]. This model has been successfully employed for the development of the depression syndrome in rodents, with a predictive, face and constructive validity of the model, in many studies [4–8]. The symptoms that can be studied by this model include anhedonia, sleep disturbances, despair, circadian disturbances, anomalous sexual behavior, cognitive dysfunctions as well as changes in the social behavior, like increased aggression or dominance [9]. Since core symptoms of depression along with the associated behavioral dysfunctions can be induced by the CMS model, it has been proven to be a suitable model to study the effectiveness of anti-depressant drugs.

Currently, a wide variety of antidepressant agents are available. The commonly available classes of antidepressants include selective serotonin reuptake inhibitors, serotonin–noradrenergic reuptake inhibitors, tricyclic antidepressants and other atypical antidepressant drugs such as monoamine oxidase inhibitors [10]. Unfortunately, none of these antidepressants is devoid of side effects like sleep disturbance, cognitive impairment, apathy and sexual dysfunction [11]. Hence, there is a vital need for effective and better-tolerated antidepressants.

Melatonin, a hormone of darkness and the major secretory product of the pineal gland, has been reported to participate in a number of

Abbreviations: CMS, Chronic Mild Stress; CPSCEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; FST, Forced Swim Test; IAEC, Institute's Animal Ethical Committee; IST, Indian Standard Time; MT, Melatonin; NORT, Novel Object Recognition Test; OFT, Open Field Test; PSU, Percentage Sucrose Uptake; SAD, Seasonal Affective Disorder; SMA, Spontaneous Motor Activity.

* Corresponding author at: Institute of Nuclear Medicine and Allied Sciences, Brig. SK Mazumdar Road, Timarpur, Delhi-110054, India. Tel.: +91 11 23905371; fax: +91 11 23919509.
E-mail address: kailashmanda@gmail.com (K. Manda).

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physiological processes, such as mood and behavior, sleep, circadian rhythms and regulation of reproduction [12–15]. A decreased plasma nocturnal peaks and phase delay of melatonin rhythms have been reported in depressive patients [16–18]. Reports are also available to document the association of seasonal affective disorder (SAD) with an abnormal melatonin synthesis pattern [19,20]. Moreover, studies have shown that sleep disturbances, which is a common symptom of the depression syndrome, may be due to irregular circadian rhythms [21,22] and melatonin synthesis [23–25]. Since depression is frequently associated with desynchronization of circadian rhythms, the drugs which reset normal circadian rhythms may have antidepressant potential. Therefore, chronic melatonin treatment could be a promising strategy against long term mild stress-induced depression.

Our previous study [26] also reported the mitigating effects of chronic melatonin administration against radiation-induced behavior dysfunctions. Therefore, we hypothesized that melatonin treatment might help in mitigating the depressive phenotype induced by CMS model.

In the present study, we have examined the depressive syndrome with a robust and rigorous behavioral test battery, which included the affective behavioral changes observed in the open-field test (OFT), the behavioral despair by the forced swim test (FST) and the hippocampal dependent short term recognition memory using the novel object recognition test (NORT) test. Anhedonia and changes in the weight, food and water intake were also measured to assess the effects of the chronic night-time melatonin administration in drinking water as an anti-depressive strategy.

2. Materials and methods

2.1. Animals

Male C57Bl/6 mice of 6–8 week age group were obtained from an inbred colony. Mice were group housed (5 mice/cage) and maintained under controlled condition of temperature (22 ± 1 °C) and light (12L: 12D) and provided standard mice feed and water ad libitum. Body weight and the basal water consumption of all groups were recorded before the start of the experiment and were monitored till the end of the study. The research was conducted with the approval of the Institute's Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA).

2.2. Drug

Melatonin (N-acetyl-5-methoxytryptamine) (Sigma-Aldrich, USA) was administered in drinking water to the melatonin treatment groups. Melatonin was initially dissolved in ethanol; 8 mg of melatonin was dissolved in 100 μl of alcohol, and the desired volume was obtained by adding drinking water. The percentage of alcohol in drinking water was approximately 0.1%. Night-time water consumption (12 h period of dark-phase) of the mice was measured continuously, and the amount was 3–3.5 ml, approximately. The melatonin dose was adjusted such that the mice received a dose equivalent to 10 mg/kg body weight. The daily water/drug uptakes were monitored throughout the length of the experiment. Since melatonin is considered to be a photo-labile chemical agent, all bottles used for drinking water were colored black and opaque. Selection of an optimal dose of melatonin was based on our previous study [26].

2.3. Experimental design

Mice were selected from inbred colonies and divided into 4 groups of 10 animals each; control, chronic mild stress (CMS), chronic mild stress + melatonin (CMS + MT) and melatonin only (MT). Melatonin was continuously administered nocturnally, through drinking water for four weeks along with the chronic stress regime. Sucrose consumption based anhedonia test was conducted before the start and on the termination of experiment. Behavioral paradigms were performed after the four-week time period, in the following order: spontaneous motor activity, novel object recognition test and the forced swim test.

2.4. Chronic stress protocol

Mice were exposed to different stressors like-cold, illumination (only during daytime), space reduction, cage tilt, wet bedding, no bedding, white noise, rat bedding and restraint stress. For the cold stress, mice were placed on a bedding of icepack and illumination stress was provided with a white lamp of 20 W/6500 K/1120 lm or 17,000 lx placed 10 cm over the cage. Native cage with a center partition (50% space reduction) was employed for the space reduction stress and a perforated tube with 10 cm height and 3 cm diameter ending in a cone of 2 cm height was used for the restraint stress. These variables were applied at three different time points during a day, first half (2 h stress 10:00 A.M–12:00 Noon IST), second half (2 h stress 2:00 P.M–4:00 P.M. IST) and an overnight stress (15 h stress 6:00 P.M to 9:00 A.M) as shown in Table 1.

2.5. Behavioral studies

All behavioral studies were conducted only during the light phase between 10 AM and 2 PM in a sound-attenuated behavioral room. The mice were handled by a single, unbiased experimenter. For the acclimatization, all mice were brought to the behavioral room five weeks prior to the behavior paradigms.

2.5.1. Sucrose Consumption Test

Mice were given a choice between 2% sucrose solution and water for a 6 h period during the daytime. The bottle positions were swapped 3 h after the start of test to avoid any position specific biasness. The test was conducted on day 0 (before the start of the stress regime) and day 28 (after the termination of the stress regime). The liquid intake was measured after the six-hour period, and the Percentage Sucrose Uptake (PSU) was calculated as given below.

$$\mathrm{PSU} = \left(\text{Sucrose Volume consumed on Day 28}/\text{Sucrose Volume Consumed on Day 0}\right) \times 100.$$

2.5.2. Spontaneous motor activity

The spontaneous behavioral activities of the mice were evaluated using an Opto-Varimex automated behavioral monitoring system (Version 4.93), a high-throughput monitoring system based on IR beams. The beams are 0.5 in. apart on the horizontal plane, which provides a high-resolution grid covering the 40 × 40 cm XY plane. The software provides the count of beam breaks by the mice, which were assessed in an aggregate value within every one-minute interval. The following parameters were observed and recorded: the

Table 1
One-week stress regime adopted for the chronic mild stress model. The same protocol was repeated for the next three weeks.

	1st half	2nd half	Overnight
Day1	Cold	Illumination	Space reduction
Day 2	Cage tilt	Wet bedding	No bedding
Day 3	White noise	Restrain stress	Rat bedding
Day 4	No bedding	Cold	Cage tilt
Day5	Illumination	White noise	Space reduction
Day6	Wet bedding	Cage tilt	No bedding
Day7	Restrain stress	No bedding	Rat bedding