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Melatonin impaired acquisition but not expression of contextual fear in rats

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HIGHLIGHTS

- Systemic injection of melatonin impaired contextual fear conditioning.
- No effect of melatonin was observed on fear expression.
- The deficit of fear acquisition was not due to motor deficits or altered anxiety behavior.

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ABSTRACT

Melatonin has been shown to be involved in the processes that contribute to learning and memory. At present study, we tested the effects of exogenous melatonin (2.5 mg/kg) on contextual fear conditioning (experiment 1) and fear expression (experiment 2) in rats. Behavior procedure involved three training phases: habituation, fear conditioning and test. Melatonin was injected either 30 min before conditioning (experiment 1) or 30 min before testing (experiment 2). Results showed that rats injected melatonin 30 min before conditioning presented a significant lower freezing during both fear conditioning and test phases. Melatonin injected 30 min before testing was ineffective on fear expression. These results suggest that melatonin, at the dose applied in this study, impaired fear acquisition but not fear expression. These findings extend previous research on the melatonin effects on learning and memory.

1. Introduction

Melatonin is a pineal gland hormone synthesized and secreted at night in vertebrates. Besides its involvement in the regulation of circadian rhythms and sleep [5,6], melatonin is thought to be involved in modulating complex processes such as learning and memory [4], as well as it has shown to display antidepressant and anxiolytic properties in animal models [1,11,19]. Recent studies showed that melatonin induces spatial learning deficit [8], and impaires visuo-spatial performance in rats [30], however, other reports showed that melatonin enhances performance in a verbal association task [12]. Furthermore, it was reported that zebrafish has a better memory performance during the day than during the night and melatonin is necessary for the suppression of memory during the night [25].

Although the mechanisms for how melatonin induces these neurobehavioral effects are not entirely known, the effects of melatonin may be through the direct modulation of memory formation circuits [26]. In the brain, melatonin receptors [MT(1)/MT(2)] have been found in regions implicated in cognition and memory [7,34], such as the hippocampus [21]. In vitro studies have reported that melatonin may modulate neuronal firing in the hippocampus [22,33,35] and modify synaptic transmission between hippocampal neurons [11,13,32]. Excitingly, by using both in vitro physiological and in vivo behavioral approaches, a recent research showed that MT(2) receptor knockout mice demonstrates both a significantly reduced long-term potentiation (LTP) and impaired memory performance through the use of the elevated plus-maze paradigm (hippocampal dependent task) [16]. Thus, melatonin may regulate learning and memory through its influence on synaptic connections in central nervous system neurons.

In light of these studies, we designed three experiments to explore the effects of melatonin on learning and memory using a fear conditioning paradigm, a procedure that involves pairing an initially neutral conditioned stimulus (CS) such as a tone or context with an aversive unconditioned stimulus (US) like a foot-shock. After several pairings of these stimuli, the CS comes to elicit

Abbreviations: ANOVA, analysis of variance; CS, conditioned stimulus; US, unconditioned stimulus; i.p., intraperitoneally; LTP, long-term potentiation; AC–PKA, adenylyl cyclase–protein kinase A.

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conditioned fear responses such as defensive behavioral responses (e.g., freezing) and autonomic and endocrine activation. It is well established that context, but not tone, learning requires the hippocampus [15,20,31]. At present study, we examined the effects of melatonin administration on contextual fear conditioning (Experiment 1) and fear expression (Experiment 2), as well as locomotor activity and anxiety behavior (Experiment 3) in rats.

2. Material and methods

2.1. Subjects

Experimental procedures were performed on adult male Sprague-Dawley rats (270–310 g) obtained from the Laboratory Animal Center of Central South University, Changsha, Hunan, China. After arrival, the rats were housed one per cage at 25 °C and an appropriate level of humidity, with ad libitum access to food and water. A 12:12 light–dark cycle (lights on at 7 PM) was maintained, with all procedures occurred between 3:00 PM. and 6:00 PM. Prior to all behavioral procedures, the rats were handled daily for 1 week in order to eliminate handling stress as a confounding variable. Experiments were conducted according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*, and experimental protocols were approved by the animal care and use committee of Central South University.

2.2. Behavioral apparatus

Rats were trained and tested in a conditioning chamber enclosed in a sound-attenuating cabinet (Huaibei Zhenghua Biological Equipment Co. Ltd., Anhui, China). The 46 cm × 46 cm × 46 cm chamber was illuminated with a 8 W white house light mounted on the ceiling of the cabinet, and background noise and air exchange (60 dB) were provided by a ventilation fan mounted on the right wall of the cabinet. The floor of the chamber consisted of 23 stainless steel bars (6 mm in diameter) spaced 20 mm apart that were connected to a shock generator and scrambler for the delivery of foot-shock USs. The presentation and sequencing of all stimuli were controlled by a custom written computer program. Chambers were cleaned with 75% ethanol before each session.

2.3. Contextual fear conditioning and test

The behavioral procedure (Fig. 1A) involved three phases: habituation, fear conditioning, and testing. During habituation phase, rats were habituated to the conditioning chamber (context) for 20 min with no stimuli presented. Twenty-four hours later (fear conditioning phase), rats received a 1 s 0.4 mA footshock (US) beginning 3 min after being placed in the chamber. The training session consisted of 5 of these conditioned and unconditioned stimulus (context-US) pairings with an inter-trial interval of 60 s. The rats remained in the training box for 60 s following the last context-US pairing, after which they were returned to the home cages. Freezing was used as the measure of fear and is characterized by cessation of movement except that required for respiration every 2 s [3]. Freezing activity on each time block during which no footshock was presented was scored with a digital stopwatch from videotapes. Observers scoring freezing were blind to the treatments. Freezing is presented as the percent time spent freezing (time spent freezing/total time × 100). Twenty-four hours after fear conditioning (testing phase), rats were placed in the original training chamber for 5 min without any shock, and freezing behavior was scored during the entire duration of context exposure.

2.4. Open field test

Two additional groups, which respectively received the same melatonin or vehicle treatment as fear conditioning groups, were used to perform an open field test. The open field test was conducted 30 min after injection. The rats were placed in the center of the open field (46 cm × 46 cm × 46 cm) and allowed to explore the arena freely for 10 min. Rat location was tracked by an elevated video camera during the session. Total distance traveled was measured as a means to assess spontaneous locomotor activity. Also the time spent in the center versus the periphery of the field was measured as a means to assess anxiety. The field was thoroughly cleaned with 75% ethanol following each session.

2.5. Drugs

Melatonin was purchased from Sigma-Aldrich and was tested at doses of 2.5 mg/kg [2]. This compound was homogenized in small volume of 75% ethanol and further diluted in saline (vehicle) to the final volume immediately before administration. The final concentration of alcohol was <0.5%. Rats were administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. Vehicle-treated rats received the same volume via intraperitoneal injection.

2.6. Timing of melatonin injection

Melatonin or vehicle was injected 30 min before fear conditioning in Experiment 1 (Fig. 1A) or 30 min before testing in Experiment 2 (Fig. 2A). And in Experiment 3, rats received melatonin or vehicle 30 min before the open field test.

2.7. Statistical analyses

Percent freezing values during fear conditioning phases were analyzed using two-way repeated-measures analysis of variance (ANOVA). Percent freezing values during test phases were analyzed using Student's *t*-test. Additionally, Student's *t*-test was also used to analyze the significance in the open field test. All data were represented as mean ± SEM. Significant level was set at $p < 0.05$. Statistics were run on SPSS (Version 13; SPSS, Chicago, IL).

3. Results

3.1. Experiment 1: Effects of melatonin on contextual fear conditioning

During the contextual fear conditioning phase (Fig. 1B), a two-way repeated-measures ANOVA of percent freezing found there was a significant difference between groups (group, $F(1, 16) = 123.282$, $p < 0.001$; group × time block, $F(5, 80) = 39.175$, $p < 0.001$). Post hoc comparisons indicated that the melatonin group presented a significant lower freezing in the time blocks 4, 5 and 6 (all, $p < 0.01$). During the test phase (Fig. 1C), a significantly lower freezing was observed in the melatonin group in comparison to the vehicle group ($p < 0.01$). These data suggested that melatonin impaired the acquisition of contextual fear response.

3.2. Experiment 2: Effects of melatonin on fear expression

During the conditioning phase (Fig. 2B), there was a significant time block effect of percent freezing ($F(5, 90) = 151.406$, $p < 0.001$) but not a significant difference was observed between groups ($F(1, 18) = 0.020$, $p > 0.05$ and $F(5, 90) = 2.209$, $p > 0.05$, respectively), indicating that the two groups showed equivalent fear learning. During the test phase (Fig. 2C), there was no a significant group effect of