# Effects of Chronic Administration of Melatonin on Spatial Learning Ability and Long-term Potentiation in Lead-exposed and Control Rats[1]

XIU-JING CAO[*, #], MING WANG[*], WEI-HENG CHEN[*], DA-MIAO ZHU[*], JIA-QI SHE[*], AND DI-YUN RUAN[*, 2]

[*]*School of Life Science, University of Science and Technology of China, Hefei 230027, Anhui, China;* [#]*Department of Maternal and Child Health, Anhui Medical University, Hefei 230032, Anhui, China*

**Objective** To explore the changes in spatial learning performance and long-term potentiation (LTP) which is recognized as a component of the cellular basis of learning and memory in normal and lead-exposed rats after administration of melatonin (MT) for two months. **Methods** Experiment was performed in adult male Wistar rats (12 controls, 12 exposed to melatonin treatment, 10 exposed to lead and 10 exposed to lead and melatonin treatment). The lead-exposed rats received 0.2% lead acetate solution from their birth day while the control rats drank tap water. Melatonin (3 mg/kg) or vehicle was administered to the control and lead-exposed rats from the time of their weaning by gastric gavage each day for 60 days, depending on their groups. At the age of 81-90 days, all the animals were subjected to Morris water maze test and then used for extracellular recording of LTP in the dentate gyrus (DG) area of the hippocampus *in vivo*. **Results** Low dose of melatonin given from weaning for two months impaired LTP in the DG area of hippocampus and induced learning and memory deficit in the control rats. When melatonin was administered over a prolonged period to the lead-exposed rats, it exacerbated LTP impairment, learning and memory deficit induced by lead. **Conclusion** Melatonin is not suitable for normal and lead-exposed children.

**Key words:** Melatonin; Lead; Learning; Memory; Long-term potentiation (LTP)

## INTRODUCTION

Indoleamine melatonin (MT) is a pineal gland hormone involved in the induction of sleep and modulation of neuronal activity in response to diurnal and seasonal changes. Exogenous melatonin is readily absorbed by crossing the blood-brain barrier with ease and has been used in therapy for circadian disruption and insomnia. Melatonin alone or in combination with other ingredients has been used in a number of dietary supplements or functional foods. Although exogenous melatonin has been demonstrated to be effective in improving certain aspects of cognitive function in elderly people and spatial memory impairment induced by materials such as thinner and acute ethanol in rats[1-2], its effect on spatial learning and memory in healthy children is unclear. The first objective of this study is to explore the changes in spatial learning performance and long-term potentiation (LTP) which is recognized as a component of the cellular basis of learning and memory[3] in normal rats after administration of melatonin for two months from their weaning. The other objective is to detect the effects of two-month melatonin supplement on spatial learning performance and LTP on lead-exposed rats that received 0.2% lead acetate from the time of their birth. Lead pollution is a serious problem in China. The average blood lead concentration in Chinese children is 92.9 μg/L, and 33.8% of children have a blood lead concentration higher than 100 μg/L[4]. Although lead is potentially dangerous to people at any age, infants and very young children are particularly vulnerable to its neurotoxic effects including impairment in attention, memory and learning that persist in adulthood[5-6]. It is, therefore, important to understand the effect of melatonin on the impaired

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[2]Correspondence should be addressed to Di-Yun RUAN. Tel: 86-551-3606374. Fax: 86-551-3601443. E-mail: Ruandy@ustc.edu.cn

Biographical note of the first author: Xiu-Jing CAO, female, born in 1972, associate professor, majoring in developmental neurobiology.

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spatial learning ability of lead-exposed rats.

## MATERIALS AND METHODS

### *Experimental Animals and Treatment*

Pregnant Wistar rats were divided into control and lead-exposed groups. From the day of birth of pups (day 0), tap water drunk by the mothers of lead-exposed rats was replaced by 0.2% lead acetate (PbAc) solution. The pups were thus exposed to lead via mother's milk from birth to weaning (day 21). Mothers of rats in the control group drank distilled water. After weaning, all mothers and female pups in the two groups were taken away and the remaining male pups in the two groups drank the same solution as their mothers during their life. At this time, the control rats were divided into melatonin group receiving melatonin (3 mg/kg)[7-8] by gastric gavage per day for 60 days and control group receiving melatonin vehicle (0.1% alcohol in saline) instead of melatonin by gastric gavage for 60 days. The lead-exposed rats were also divided into lead-exposed group treated with melatonin (3 mg/kg) by gastric gavage per day for 60 days and lead-exposed group treated with melatonin vehicle by gastric gavage. The design of experiment is shown in Fig. 1. All the animals were maintained in a 12:12 light/dark cycle in a air-conditioned room at constant temperature (24±1℃). At the age of 81-90 days, all animals were subjected to Morris water maze test and then used for extracellular recording of LTP in the DG area of the hippocampus *in vivo*.

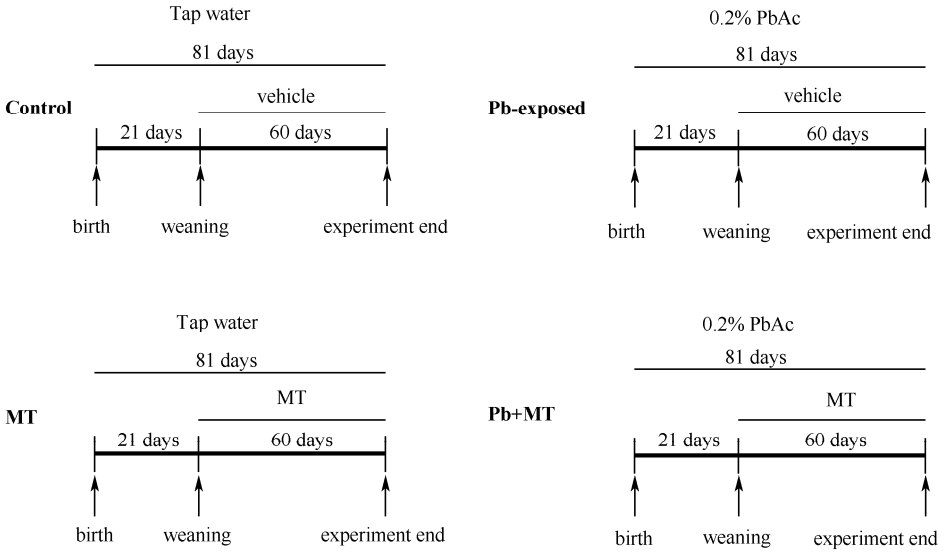


FIG.1. Scheme of experiment design.

Melatonin (Sigma) was dissolved in ethanol and further diluted in saline. The final concentration of alcohol was <0.1%.

### *Morris Water Maze Test*

To assess spatial learning ability of rats, their performance in the Morris Water Maze (MWM) test was evaluated. This test required the rats to escape from water by finding the hidden platform (10 cm in diameter) located at a fixed place within a round swimming pool (150 cm in diameter). The colorless platform, placed 2-3 cm under water in the southeast quadrant of the pool, was the only way for the rats to escape from the water. The maze was located in a quiet test room, surrounded by many visual cues external to the maze (e.g. the experimenter, rack, *etc.*), which were visible from within the pool and could be used by the rats for spatial orientation. Locations of these cues were unchanged throughout the testing. Movement of the animals was recorded with a TV camera located over the center of the pool and connected to a personal computer. Each individual rat was placed on the platform for 30 s before being gently released into the water at one of the four starting locations which was randomly selected. The animals were tested for their ability to remember the position of the platform on which they were allowed to rest for 30 s between trials. Each rat performed four consecutive trials each day for 6 consecutive days at its 81-90 days of age. Recording was automatically terminated as escape latency (time length required to reach the platform) when the animal found the target. If the rats could not find the platform within 120 s, they were placed on the platform by hand and allowed to remain there for the same period of time. Their escape latency was accepted as 120 s. During the inter-trial intervals, the

animals were kept in a dry cage for 60 s. The mean latency of finding the invisible platform was measured for individual animals on each day.

The day after the acquisition phase, a probe test was conducted by removing the platform. The rats were allowed to swim freely in the pool for 60 s. The time spent in the correct quadrant (target quadrant) containing the hidden platform, was recorded. The time spent in the target quadrant indicated the degree of memory consolidation taken place after learning.

*Stimulation and Recording*

After completion of Morris water maze test, all rats underwent electrophysiological recording *in vivo*. Rats were anaesthetized with urethane (1.8 g/kg) and their heads were fixed with a stereotaxic head holder. The skull was exposed and the animals' body temperature, heart rate and electrocardiogram were monitored. A concentric bipolar stimulating electrode (a wire, 250 μm in diameter, insulated with Teflon except for the cut tips) was placed in the medial perforant path (coordinating with the skull surface flat: 7.8 mm posterior to bregma, 4.3 mm lateral to the midline, 2.8 mm ventral). A glass micropipette recording electrode (a tip, 3-5 μm in diameter, 1-3 MΩ, coordinating: 3.8 mm posterior to bregma, 2.2 mm lateral to the midline) was lowered into the DG until the maximal response was observed. The glass micropipettes used for extracelluar recordings were filled with 2 mol/L Nacl.

Extracellular evoked responses were obtained from the dentate granule cells in response to electrical stimulation of the medial perforant path. Electrical stimulation was provided by a stimulator (SDQ-4, China) which was passed through an isolation unit (SS-102J, Nihon Kohden, Tokyo, Japan) to provide a constant current. Stimuli were given every 20 s. Extracellular field potentials were amplified using a Neurolog NL 104 amplifier (500×) and filtered (1 Hz to 3 KHz bandpass) with a Neurolog NL125 module (Neurolog units from Digtimer, Winsford, UK). Signals were transmitted through an analogue to digital interface (MCSP-1, Beijing, China) and finally connected to the computer where data collection, analysis and calculations were done.

*Long-term Potentiation*

The stimulus intensity selected for baseline measurements was adjusted to yield approximately 40% of excitatory postsynaptic potential (EPSP) maximal amplitude. After 10-minute recording with stimuli applied at 20 s intervals, a high frequency stimulus was applied (HFS: 250 Hz, 1 s). Post-tetanic recordings were performed for 1 h with a single pulse applied at a frequency of 0.05 Hz. All data were recorded by Igor program (version 4.0).

*Data Analysis*

Data are presented as $\bar{x} \pm s$. Differences in escape latencies between groups were analyzed by assessing variance for repeated measurements (ANOVAR) followed by Fisher's *post hoc* test for all groups. The amplitude of the rising phase slope of the EPSP was measured at a fixed latency (0.5 ms) from EPSP onset. The PS amplitude was measured by averaging the distance from the negative peak to the preceding and following positive peak. The value of LTP was described as:

LTP = the value of EPSP or PS after HFS induction/ the average value of EPSP or PS of baseline *100%

Comparisons among the four groups were analyzed by a repeated ANVOA. $P<0.05$ was considered statistically significant.

## RESULTS

*Morris Water Maze Test*

Although the latencies to reach the submerged platform decreased gradually in all groups during the 6-day training in Morris water maze test, the mean latency significantly prolonged in the melatonin group, lead-exposed group treated with melatonin and lead-exposed group compared with the control group ($P<0.05$). Furthermore, the lead-exposed rats treated with melatonin showed a longer escape latency than the lead-exposed rats not treated with melatonin (Fig. 2, $P<0.05$).

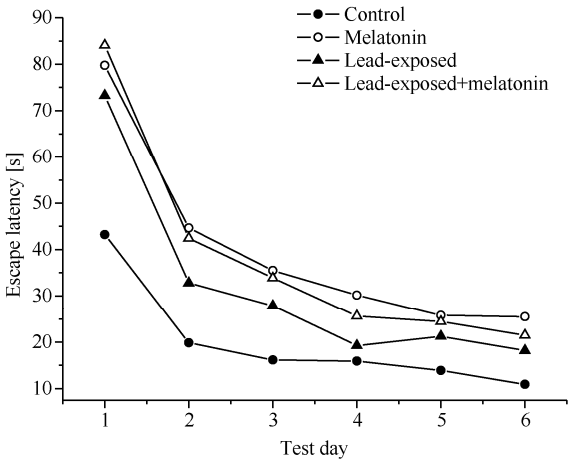


FIG. 2. Mean escape latency of the four trials per day for 6 days in Morris water maze (MWM) test.

In the probe trial, with the platform removed, all

rats in the other three groups spent a significantly shorter time in the correct quadrant than the control groups ($P$<0.05). Furthermore, the lead-exposed rats treated with melatonin spent a significantly shorter time than the lead-exposed rats not treated with melatonin (Table 1; $P$<0.05). Not only in the escape latency test, but also in the probe trial, the amplitudes of melatoin-induced impairments in the control rats were greater than those in the lead-exposed rats ($P$<0.05).

*Effect of Melatonin on LTP of Control Rats*

Fig. 3 shows the amplitudes of LTP on both EPSP slope (A) and PS amplitude (B) in the control and melatonin groups. In the control group ($n$=12), the amplitudes were 126%±5% and 220%±13%, respectively, when estimated from the EPSP slope and PS amplitude, which were significantly decreased to 1093%±3% (EPSP slope, $F$=14.6, $P$<0.05) and 179%±9% (PS amplitude, $F$=41.2, $P$<0.05) in the melatonin group ($n$=12).

TABLE 1

Effects of Melatonin and/or Lead Treatment on the Time Spent in the Correct Quadrant at the Probe Trial on Day 7

| Groups | | Time in the Correct Quadrant(s) |
|---|---|---|
| Control | ($n$=12) | 38±6 |
| Melatonin | ($n$=12) | 21±8[a] |
| Lead-exposed | ($n$=10) | 28±6[a] |
| Lead-exposed+melatonin | ($n$=10) | 22±6[a,b] |

*Note*. [a] $P$<0.05 *vs* control group, [b] $P$<0.05 *vs* lead-exposed group.

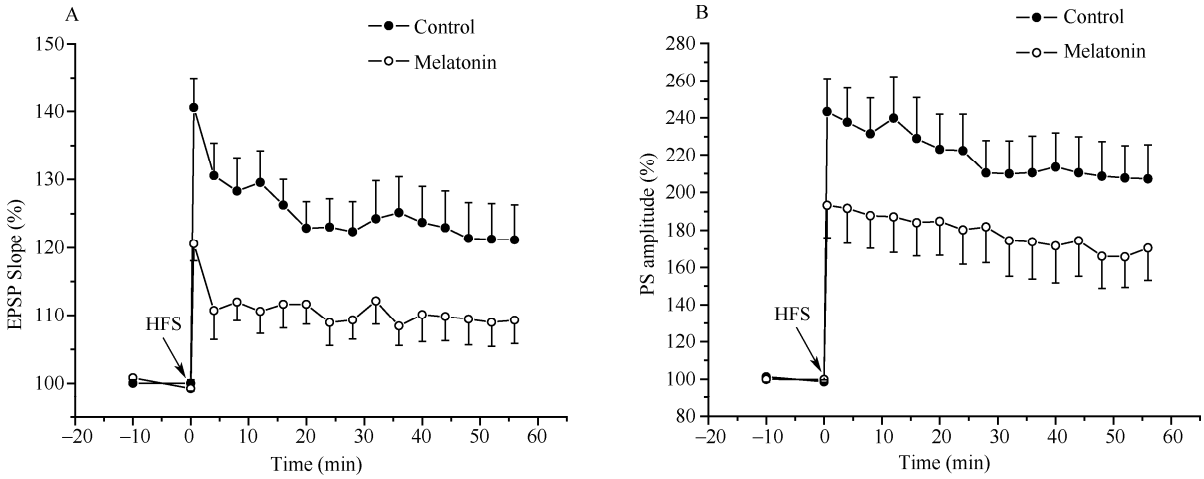


FIG. 3. Effects of melatonin application on LTP of the EPSP slope (A) and PS amplitude (B) in the control rats. Down arrow shows the application of HFS.

*Effect of Melatonin on LTP of the Lead-exposed Rats*

The amplitudes of LTP on the EPSP slope and PS amplitude in the control and lead-exposed and lead-exposed plus melatonin treatment groups are shown in Fig. 4. As shown in Fig. 4A, the amplitude of LTP on the EPSP slope in the control group was 126%±5%, which was significantly decreased to 113%±3% after lead exposure ($F$=20.2, $P$<0.05; *post-hoc* analysis, $P$<0.05 lead-exposed *vs* control) and further decreased to 105%±4% in the lead-exposed groups treated with melatonin ($P$<0.05 lead-exposed+MT *vs* lead-exposed). As shown in Fig. 4B, the amplitude of LTP on the EPSP slope in the control group was 220%±13%, which was significantly decreased to 196%±13% after lead exposure ($F$=18.1, $P$<0.05; *post-hoc* analysis, $P$<0.05 lead-exposed *vs* control) and further decreased to 180%±11% in the lead-exposed group treated with melatonin ($P$<0.05 lead-exposed +MT *vs* lead-exposed). There were no significant differences between the lead-exposed +MT and melatonin groups in the amplitudes of LTP on both EPSP and PS ($P$>0.05). The amplitudes of melatonin-induced impairments of both EPSP and PS LTP in the lead-exposed rats were lower than those in the control rats ($P$<0.05).

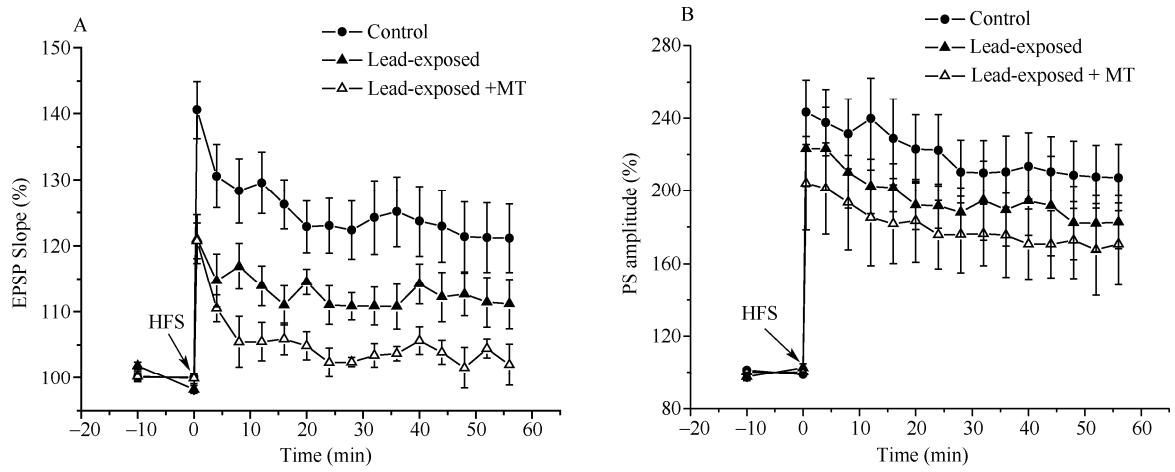


FIG. 4. Effects of chronic lead exposure (A) and melatonin (B) on LTP and PS amplitude in the lead-exposed rats. Down arrow shows the application of HFS.

## DISCUSSION

In this study, long-term administration of low dose melatonin from weaning impaired the long-term potentiation in the DG area of the hippocampus and induced learning and memory deficit in the intact control rats. When melatonin was chronically administered to the lead-exposed rats, it exacerbated the impaired LTP and learning and memory deficit induced by lead.

The findings that melatonin given at a dose of 3 mg/kg daily from weaning impaired long-term potentiation in the DG area of hippocampus in the control rats are in close agreement with those observed in the CA1 area[9-10]. Chronic administration of melatonin also impaired LTP in the DG area and the performance of spatial learning and memory in the control rats during their development. As LTP is recognized as one of the components of the cellular basis of learning and memory[3,11], deficit in performance of Morris water maze test may be closely related to the impairment of LTP. Furthermore, melatonin can inhibit the electrical activity of neurons in rat hippocampus[12-13], which may partly explain why melatonin impairs the spatial learning and memory. Previous experiments showed that melatonin-induced LTP impairment is closely related to GABAergic system as melatonin increases *in vivo* GABA accumulation in hypothalamus, cerebellum, cerebral cortex and pineal gland of rats and decreases excitability of guinea pig hippocampal neurons by potentiating the inhibitory postsynaptic potentials[14-15].

Lead, a kind of potent environmental neurotoxin, has been widely studied in recent years. Although lead-induced deficit in spatial learning ability varies from study to study[16-20], our results support the theory that chronic lead exposure impairs spatial learning ability. Impairment of the long-term potentiation has also been observed in lead-exposed rats, which is consistent with our previous finding[21-23].

This study showed that melatonin could exacerbate the performance deficit and lead-induced LTP impairment. However, the amplitudes of melatonin-induced deficits in performance of lead-exposed rats were lower than those in the control rats (Fig. 2 and Table 1). The amplitude of melatonin-induced LTP impairment in the lead-exposed rats was also lower than that in the control rats. The mechanism of control rats may be due to the increase of GABA accumulation and the potentiation of the inhibitory postsynaptic potentials[14-15]. The main factor related to the decreased LTP in lead-exposed rats is the inhibition of N-methyl-D- aspartate(NMDA) receptor[24-25]. Since $Pb^{2+}$ can inhibit GABAergic system[26], GABAergic transmission in lead-exposed rats was lower than that in the control rats. After administration of melatonin, the amplitude of increased GABAergic transmission was lower in the lead-exposed rats than in the control rats.

In conclusion, administration of 3 mg/kg melatonin for two months from weaning can impair the performance of lead-exposed and control rats. The amplitudes of melatonin- induced impairment of learning and memory and LTP in the control rats are more evident than those in the lead-exposed rats, suggesting that melatonin administration may exacerbate lead exposure-induced learning and memory deficit in rats. Melatonin should not be used as an ingredient of functional food or dietary supplement as it might do harm to the learning and memory of rats

during their development.

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