

## Developmental effect of light deprivation on synaptic plasticity of rats' hippocampus: implications for melatonin

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### ABSTRACT

**Objective(s):** There are few reports have demonstrated the effect of a change-in-light experience on the structure and function of hippocampus. A change-in-light experience also affects the circadian pattern of melatonin secretion. This study aimed to investigate developmental effect of exogenous melatonin on synaptic plasticity of hippocampus of light deprived rats.

**Materials and Methods:** The effects of intracerebroventricular (ICV) injection of 2µg/5µl melatonin was evaluated on the basic and tetanized field excitatory post-synaptic potentials (fEPSPs) recorded in the hippocampal CA3-CA1 pathway of normal light-reared (LR) and dark-reared (DR) rats at 2, 4, and 6 weeks of age. Using RT-PCR and western blotting, developmental changes in the expression of melatonin receptors, MT1 and MT2, in the hippocampus were also evaluated.

**Results:** The amplitude of basic responses decreased across age in the LR rats. While light deprivation increased the amplitude of baseline fEPSPs, it decreased the degree of potentiation in post-tetanus responses. Melatonin injection also increased the amplitude of fEPSPs and suppressed the induction of long-term potentiation in both LR and DR rats. The expression of melatonin receptors increased in the hippocampus during brain development, and dark rearing reversed the expression patterns of both receptors.

**Conclusion:** Although melatonin changed basic and tetanized responses of CA1 neurons across age during critical period of brain development, the pattern of its effects did not match the expression pattern of melatonin receptors in the hippocampus. Thus, the effects of melatonin on hippocampal neuronal responses may be exerted through other ways, like intercellular molecules and nuclear hormone receptors.

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### Introduction

Environmental signals in addition to spontaneous activity of neurons cause the maturation of cortical neuronal circuits during critical period of brain development (1). Change in these signals during brain development deeply affects the structure and function of neurons (2). Among environmental signals, visual signals play a pivotal role in mammals' brain development (3). Many studies have shown the effects of change in visual experience on visual cortex function and development (4-6). It has been shown that change in visual experience impairs synaptic plasticity - a key mechanism for learning and memory - of mammals' visual cortical circuits. It has been reported that light deprivation differently affects synaptic plasticity in layer IV and white matter of rat visual cortex *in vitro* (7). Findings have also shown that monocular

deprivation reduces the visual evoked potentials (VEP) of neurons in rat visual cortex (8).

It is believed that the hippocampus is a necessary region for some aspects of learning and memory, and the mechanisms of synaptic plasticity, long-term potentiation (LTP), and depression (LTD) are well studied in this region (9). After processing in the neocortex, a part of sensory inputs goes to the hippocampus and makes memories (10). The mammalian's visual cortex supplies an elementary sensory input to the hippocampus, and thus, a comparable synaptic plasticity is observable in the visual cortex and the hippocampus (11). Therefore, the hippocampus, like sensory cortices, may undergo a period of postnatal development (12); however, few studies have revealed the effects of reducing environmental signals (13) and environmental enrichment (14) on the maturation of hippocampal circuits.

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Change in visual signal inputs strongly influences circadian rhythms and also leads to a wide range of physiological alterations, especially change in secretion of the pineal gland hormone, melatonin, which occurs in the dark phase of circadian rhythms and is intensely suppressed by light (15). On the other hand, light deprivation increases melatonin secretion (16). In mammals, melatonin acts through two membrane-bound G-protein-coupled receptors, MT1 and MT2, occurring throughout the brain (17), especially in the hippocampus (18, 19). Expression of melatonin membrane receptors changes during normal aging (20) and also at different phases of circadian rhythms (21). There are limited studies with conflicting results that have shown the facilitatory (22, 23) and inhibitory (24, 25) effects of melatonin on the synaptic plasticity of different neural circuits. It has also been shown that melatonin suppresses the synaptic plasticity of CA1 neurons (26). The first part of the present study evaluates the effects of intracerebroventricular (ICV) injection of melatonin on the synaptic plasticity of hippocampal CA1 neurons in normal light reared (LR) and dark reared (DR) rats at 2, 4, and 6 weeks of age. The second part examines the synergic effects of aging and dark rearing during critical period of brain development on the expression of both melatonin receptors in rat hippocampus.

## Materials and Methods

### **Animal housing and ethics**

This experimental study was conducted on male Wistar rats kept in a standard animal house (temperature  $22\pm 2$  °C, and air humidity  $55\pm 5\%$ ) and given free access to food and water. The animals were housed in either 12-hr light/dark cycles (light reared; LR) or complete darkness (dark reared; DR) from birth to the end of the experiments. All experiments were approved by the Kashan University of Medical Sciences Ethics Committee and were carried out in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes.

### **In vivo electrophysiology**

This part of the study was carried out on 3 groups of 2, 4 and 6 weeks age LR (2WLR, 4WLR, and 6WLR;  $n=16$  for each) and DR animals (2WDR, 4WDR, and 6WDR;  $n=16$  for each). Stimulating the Schaffer's collateral neurons, field excitatory post-synaptic potentials (fEPSP) were recorded from neurons of the CA1 area of the hippocampus as previously described (26). Briefly, the rats were anesthetized with urethane (1.5 g/kg, IP) and placed in a stereotaxic apparatus (Stoelting, USA). The DR animals were anesthetized in dark house with their eyes tightly covered. All experiments were performed at the same time (8 A.M.) for the LR and

the DR animals. After removing tissue from above the skull, three small holes (1 mm diameter) were drilled into the skull to allow the insertion of stimulating electrode (4.2 mm posterior to the bregma, 3.8 mm lateral to the midline), recording electrode (3.4 mm posterior to the bregma, 2.5 mm lateral to the midline), and microinfusion cannula (1.5 mm lateral to the bregma) into the brain. Electrodes were made from Teflon-coated stainless steel wire (0.008 inch outside diameter, A-M systems, USA) exposed only at the tip (tip separation approximately 0.10 mm). The bipolar stimulating electrode was lowered 2.8 mm from the dural surface to reach the Schaffer's collaterals, and the monopolar recording electrode was advanced through the CA1 stratum radiatum (2.5 mm from the dural surface). The cannula was built with a 26-gauge stainless steel needle coupled to a polyethylene tube (0.065 inch diameter). The polyethylene tube was attached to a 10  $\mu$ l Hamilton syringe. The cannula was lowered 4 mm to implant in the lateral cerebral ventricle. Melatonin (2  $\mu$ g) or vehicle (saline and a small amount of ethanol) was injected in a 5  $\mu$ l volume over a 5 min period via a Hamilton syringe driven by the experimenter. Melatonin was dissolved in ethanol and then added to saline. It has been previously shown that the small amount of ethanol used to prepare the melatonin has no effect on electrophysiological recordings (26). All coordinates were based on a stereotaxic atlas (27) and adapted to the animals according to bregma-lambda distance when necessary. Using computer software (Neurotrace, WSI, IR Iran), constant current stimulation was programmed and delivered to a stimulator/isolator unit (WPI-A365, USA). fEPSPs were recorded from the stratum radiatum in the CA1 area of the hippocampus in response to stimulation (two sweeps/min at 30 sec intervals) of the ipsilateral Schaffer's collateral-commissural pathway. Paired pulse facilitation signified that the electrodes were correctly positioned. Using a range of stimulus currents, an input-output curve was drawn. Then, stimulation intensity was adjusted to elicit maximum fEPSP amplitude of 60%. Baseline fEPSPs were recorded for a 30-min period and averaged for comparison with post-microinfusion and post-tetanus recordings. The fEPSPs were amplified by a preamplifier (Electromodule, WSI, IR Iran), filtered at 1–3000 Hz, digitized (10 points/ms), and stored for offline analysis using "Potentialize" software (WSI, IR Iran). Drug (melatonin; Sigma-Aldrich, USA) or vehicle (sodium chloride) injections were then applied and followed by recordings at 30 sec intervals for an additional 30 min. Then, LTP was induced by high-frequency stimulation (HFS) at 100 Hz (10 bursts of 10 stimuli, 0.2 ms stimulus duration, and 10 sec inter-burst intervals), and recording was continued for at least 2 hr. Data was presented as

**Table 1.** Primers used for RT-PCR

Gene	Sequence (5'-3')	Annealing temperature (°C)	Product size (bp)	Cycles	NCBI Reference Sequence
HPRT	F GGTCCATTCTATGACTGTAGATTTT	60	172	33	NM_012583.2
	R CAATCAAGACGTTCTTTCCAGTT				
MT1	F CTGAGTGTCAATTGGCTCGGT	59.5	262	30	NM_053676.2
	R AATGGAAAACCACCAGGGCA				
MT2	F CAGACAGCCAGCACCCAATA	58.5	146	33	NM_001100641.1
	R AGGCGTAGCTTTCTCTCAGC				

mean $\pm$ SEM and normalized by comparing the post-microinfusion and post-tetanus fEPSPs with the pre-microinfusion amplitude of fEPSPs as 100%.

### Tissue collection and storage

The next part of the study was carried out on another 3 groups of the LR (2WLR, 4WLR, and 6WLR; n=6 for each) and DR animals (2WDR, 4WDR, and 6WDR; n=6 for each). The animals were deeply anesthetized and sacrificed by decapitation, brains were removed rapidly, and hippocampi were dissected immediately, placed in liquid nitrogen, and finally stored at -80 °C until use. The anesthetizing and decapitation of DR rats were performed in dark house.

### RT-PCR

Using the RT-PCR technique, the relative expression of target mRNAs was evaluated. Total RNA of each hippocampus was extracted with an RNA extraction kit (peqGOLD RNA Pure, Peqlab Co, Germany) and assayed by spectrophotometer (BioPhotometer Plus, Eppendorf Co, Germany). In the next step, 1  $\mu$ g RNA from each sample was converted to cDNA according to standard protocol mentioned in the kit (Roche, Germany). Then, using specific primer sets (Table 1), target sequences were amplified with a thermocycler (peqSTAR 96X, Peqlab Co, Germany). Using gradient PCR and serial dilution of the cDNA sample, PCR was optimized for each primer as shown in Table 1. The PCR protocol was: initial denaturation at 94 °C for 2 min followed by denaturation at 94 °C for 30 sec, annealing at variable primer-specific temperatures for 45 sec, and extension at 72 °C for 45 sec. A further 7 min final extension at 72 °C was considered for all genes. The PCR products were identified by agarose gel electrophoresis (2% W/V, stained with ethidium bromide), observed, and photographed under an ultraviolet light with the gel documentation system (UV Tech, UK). Finally, the bands were analyzed by ImageJ software (Version 1.48, NIH, USA), and ratios of each target gene were calculated to that of Hypoxanthine-guanine phosphoribosyltransferase

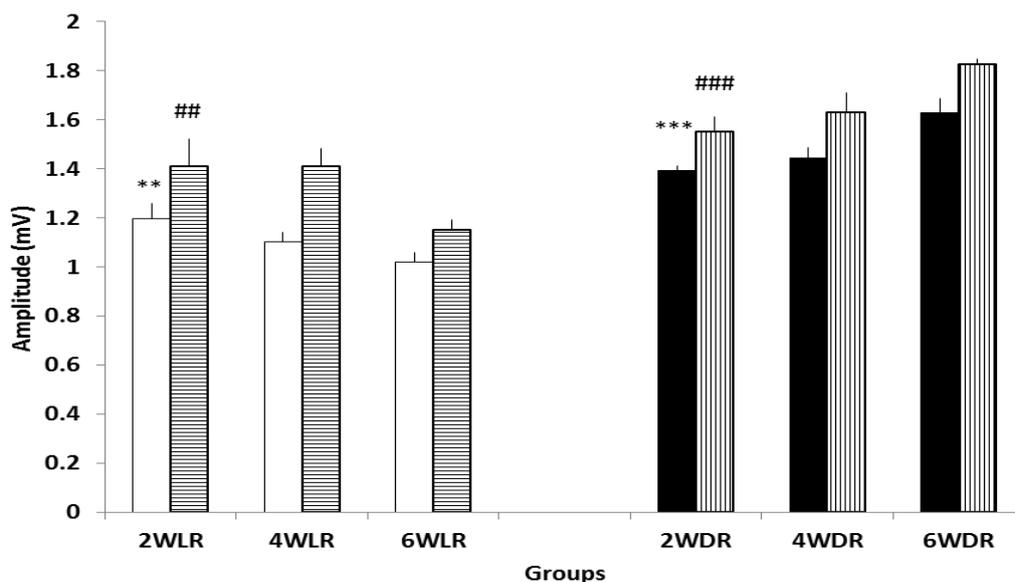
(HPRT) (as housekeeper gene) and then reported as semi-quantitative expressions of the samples.

### Immunoblotting

The protein content of each hippocampus was extracted by radioimmunoprecipitation assay buffer (RIPA buffer) with an antiprotease cocktail (Roche, Germany). Total protein quantification was performed using Bradford assay. Western blotting was also performed as described hereafter (28). Briefly, 50  $\mu$ g of sample proteins were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis at 120 V for 2 hr, blotted onto a PVDF membrane (Roche, Germany) with the semi-dry transfer technique at 10 V for 45 min, and incubated with different antibodies directed against  $\beta$  actin (1:5000; Abcam, ab8227, USA), MT1 (1:500; Santa Cruz, sc-13186, USA), and MT2 (1:200; Santa Cruz, sc-13174, USA), and then diluted in 5% milk PBS (phosphate buffered saline)-Tween 20 blocking buffer for 12 hr at 4 °C. After that, the blots were washed in Tris buffered saline with Tween (TBST) for 30 min and incubated with secondary antibodies (1:3000, horseradish peroxidase-conjugated goat anti-rabbit, ab6721, or rabbit anti-goat, ab6741, antibodies from Abcam, USA) for 1 hr at room temperature. Labeling and development were carried out with the enhanced chemiluminescence (ECL) western blot detection system (AceGlow, Peqlab Co, Germany). Densitometry was carried out using ImageJ software (Version 1.48, NIH, USA), and each band intensity was normalized against the band intensity of  $\beta$  actin as the structural protein.

### Data analysis

The statistical significance among amplitudes of fEPSPs in different groups was estimated by two-way ANOVA; however, the potentiation was interpreted as LTP if the amplitude of fEPSP was increased at least 20% post-tetanus. Multivariate ANOVA was performed on the data of relative expression of genes and proteins. Tukey's *post hoc* test was applied to the data when needed. All data was expressed as Mean $\pm$ SEM. Statistical significance was defined as  $P < 0.05$ .



**Figure 1.** Effects of melatonin injection on amplitude of field excitatory post-synaptic potentials (fEPSPs) recorded from the CA1 area of hippocampi of light reared (LR) and dark reared (DR) rats at different ages. The amplitude size of fEPSPs decreased by about 15% across age (\*\* $P<0.01$ , between 2WLR and 6WLR groups, white bars). Dark rearing strikingly reversed the effect of age on the baseline activity of synapses and caused an approximately 15% increase in the amplitude size of fEPSPs (\*\* $P<0.001$ , between 2WDR and 6WDR groups, black bars). Moreover, a statistical difference was observed in the mean amplitude of the LR animals when compared to their age-matched DR animals ( $P<0.001$ ). The intracerebroventricular (ICV) injection of melatonin (shaded bars) increased the amplitude of fEPSPs recorded from the CA1 area of the hippocampus of both LR and DR rats of all ages when compared to their vehicle-injected counterparts ( $P<0.05$ , simple bars). Statistical comparison revealed a difference between 2WLR and 6WLR melatonin-infused groups (## $P<0.01$ , horizontally shaded bars) and between 2WDR and 6WDR melatonin-infused groups (### $P<0.001$ , vertically shaded bars)

## Results

### Characteristics of synaptic transmission in the Schaffer's collateral to the CA1 pathway

The effects of dark rearing and melatonin on pre- and post-HFS responses of the CA1 neurons of the rat hippocampus were evaluated through *in vivo* recordings. Data analysis indicated that both treatments deeply affected basic synaptic transmission and post-HFS responses of the rats' CA1 neurons ( $F_{17,5748}=21.419$ ;  $P<0.0001$ ).

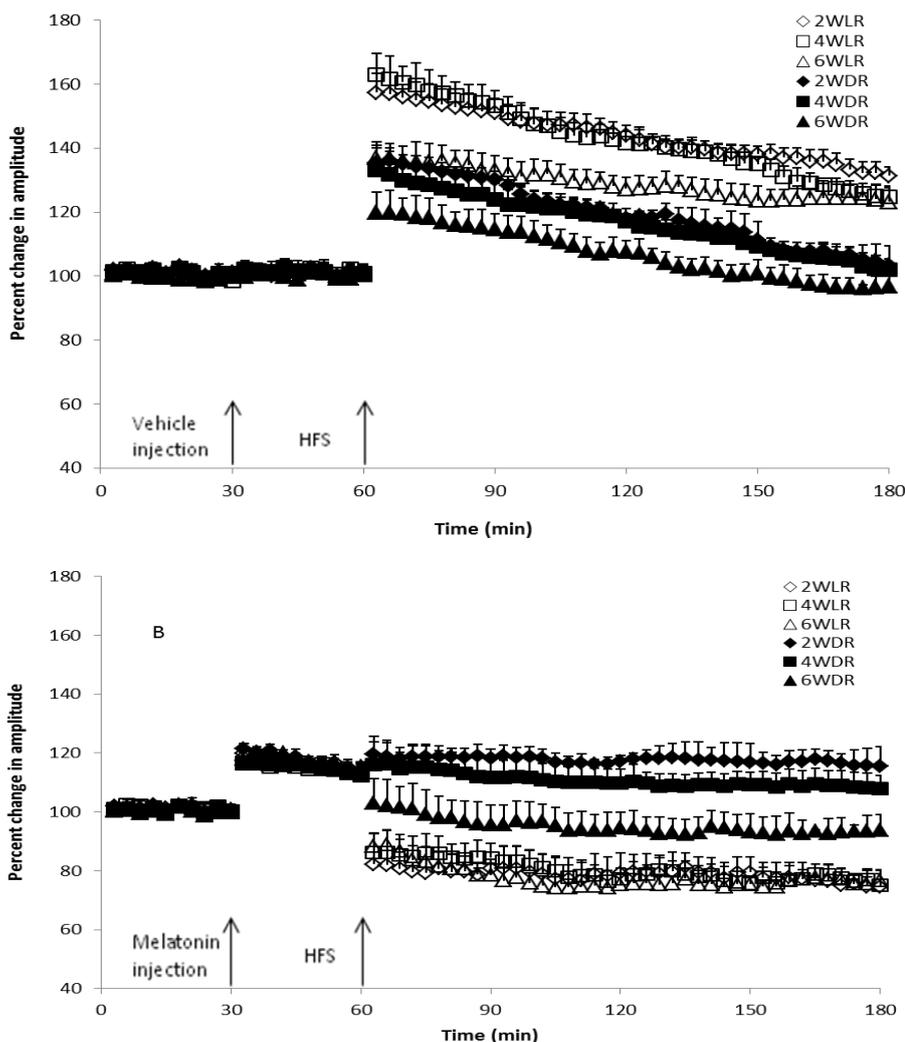
### Baseline responses

Figure 1 shows the effects of the melatonin injection on the amplitude of fEPSPs recorded from the CA1 area of the hippocampus of LR and DR rats at different ages. Data analysis showed that the amplitude size of fEPSPs recorded in the CA1 neurons of the normally LR rats decreased considerably across age from  $1.12\pm0.06$  mV in the 2WLR rats to  $1.02\pm0.04$  mV in the 6WLR rats ( $P<0.01$ ). As shown in Figure 1, dark rearing strikingly reversed the effect of age on the baseline activity of synapses where the mean amplitude of the fEPSPs increased from  $1.39\pm0.02$  mV in the 2WDR animals to  $1.63\pm0.06$  mV in the 6WDR animals ( $P<0.001$ ). A statistical difference was also observed between the mean amplitude of the LR animals when compared with their age-matched DR animals ( $P<0.001$ ). ICV injection of vehicle had no effect on the amplitude of fEPSPs in all groups, but

surprisingly, ICV injection of melatonin increased the amplitude of fEPSPs of both the DR (about 12%) and LR (about 20%) rats of all ages ( $P<0.05$ ). Comparison of the data showed that the post-microinfusion amplitude of fEPSPs had a decreasing manner in the LR rats (horizontally shaded bars in Figure 1) where mean amplitude was  $1.41\pm0.11$  mV and  $1.15\pm0.04$  mV in the 2WLR and the 6WLR animals, respectively ( $P<0.01$ ). An increasing manner was also observed in the post-microinfusion amplitude of fEPSPs recorded from the hippocampus of DR rats (vertically shaded bars in Figure 1), so the mean amplitude was  $1.55\pm0.06$  mV in the 2WDR group and increased to  $1.83\pm0.02$  mV in the 6WDR rats ( $P<0.001$ ).

### LTP induction in the Schaffer collateral to the CA1 pathway

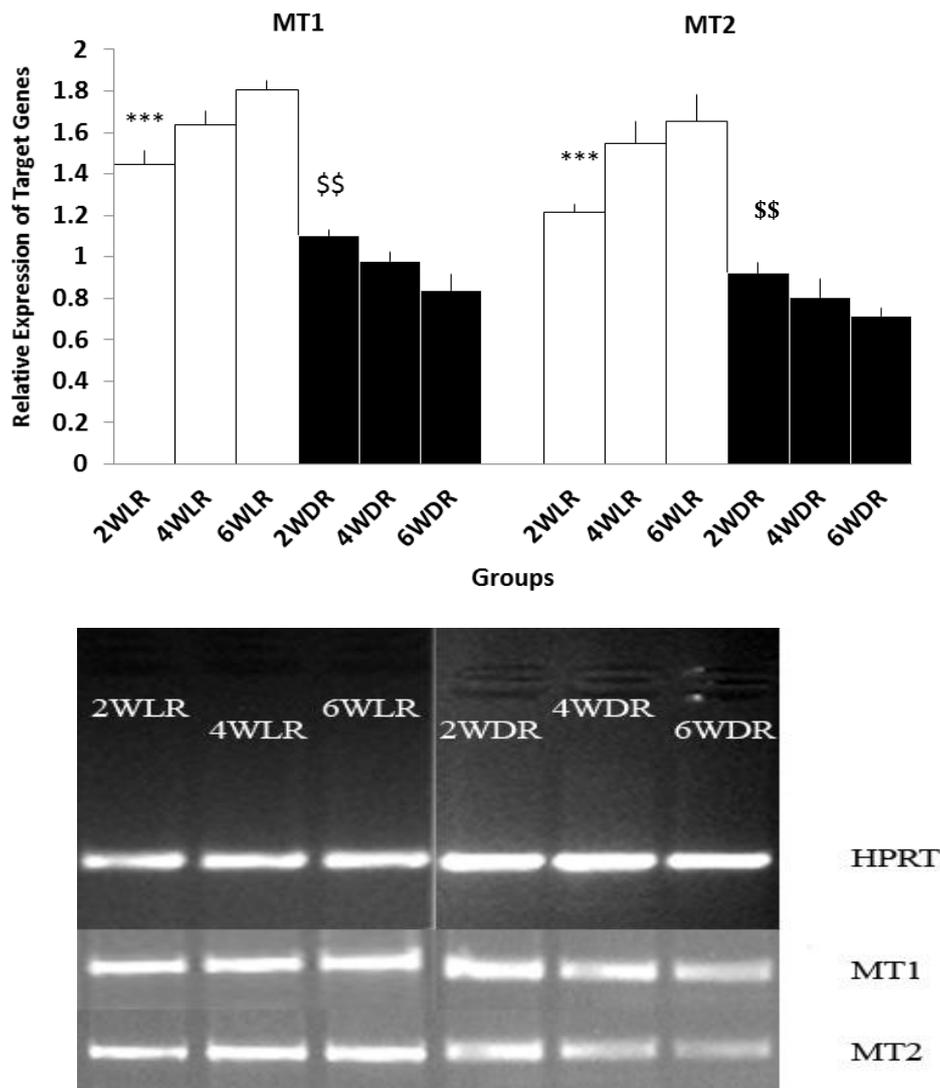
Stimulation of the Schaffer's collaterals caused induction of a prominent and maintained LTP in the fEPSPs recorded in all groups of the vehicle-injected LR rats ( $P<0.001$ ). The highest potentiation was observed in the 2WLR rats ( $57.31\pm6.16\%$ , open diamonds in Figure 2-A) and the lowest was recorded in the 6WLR rats ( $36.99\pm4.98\%$ , open triangles in Figure 2-A). There was a statistical difference in the post-HFS potentiation between the 2WLR and 6WLR groups ( $P<0.01$ ). However, the difference in post-HFS potentiation between the 2WLR and 4WLR groups was not notable. Figure 2-A also demonstrates that dark rearing deeply affected



**Figure 2.** Long-term potentiation (LTP) induction by high-frequency stimulation (HFS) on field excitatory post-synaptic potentials (fEPSPs) recorded in the CA1 area of the hippocampus of vehicle- (A) or melatonin-injected (B) rats at different postnatal light rearing and ages. A- HFS induced LTP in the fEPSPs of the CA1 area of all vehicle-treated light reared (LR) and dark reared (DR) groups ( $P < 0.001$ ). The potentiation was highest in the 2WLR rats and decreased over age ( $P < 0.01$  between the 2WLR and 6WLR groups). The same gradual decreasing pattern was also seen in the DR rats ( $P < 0.01$  between the 2WDR and 6WDR groups). Comparison of the induced LTP between the LR rats and their age-matched DR groups indicated that dark rearing effectively reduces synaptic plasticity ( $P < 0.01$ ). B- Intracerebroventricular (ICV) application of melatonin prominently inhibited induction of LTP in the LR rats, so that HFS of the Schaffer collaterals caused the depression of amplitude size of fEPSPs of about 20% in all LR rats ( $P < 0.01$ ). The inhibitory effects of melatonin injection on the potentiation of tetanized fEPSPs of the DR hippocampus were less prominent than on their age-matched LR counterparts ( $P < 0.01$ ), and they had an age-dependent pattern. The most potentiated response in the 2WDR rats ( $19.58 \pm 6.08\%$ ) was decreased to  $16.09 \pm 7.71\%$  and  $2.98 \pm 4.93\%$  in the 4WDR and 6WDR rats, respectively. Although statistical analysis demonstrated a significant difference in potentiated responses between the 2WDR and the 6WDR groups ( $P < 0.01$ ) and also between the 4WDR and the 6WDR groups ( $P < 0.01$ ), there was no significant difference in post-HFS responses between the 2WDR and the 4WDR rats.

LTP induction in CA1 neurons, where the amount of potentiation was statistically lower in DR rats in comparison with LR animals of similar ages ( $P < 0.01$ ). The developmental decrease in degree of LTP was also visible in the DR animals, so that the highest LTP was observed in the 2WDR rats ( $35.42 \pm 5.39\%$ ) and diminished to  $33.28 \pm 6.72\%$  and  $19.95 \pm 6.28\%$  in the 4WDR and 6WDR rats, respectively ( $P < 0.01$  between 2WDR and 6WDR). Figure 2-B illustrates the time course of pre- and post-HFS recordings in the CA1 area of the hippocampus of the melatonin-injected LR and DR animals. As shown in this Figure, ICV

injection of melatonin inhibited LTP induction in the CA1 area of the hippocampus of LR rats, prominently. High frequency stimulation of the Schaffer's collaterals in the presence of melatonin caused a depression of amplitude size in fEPSPs of about 20% in LR rats ( $P < 0.01$ ). *Post hoc* tests showed that there was no significant difference in post-HFS between all melatonin-injected LR rats. Melatonin injection also prevented the potentiation of tetanized fEPSPs of the DR hippocampi; however, compared to their age-matched LR counterparts, it was less prominent in all groups ( $P < 0.01$ ). It should be

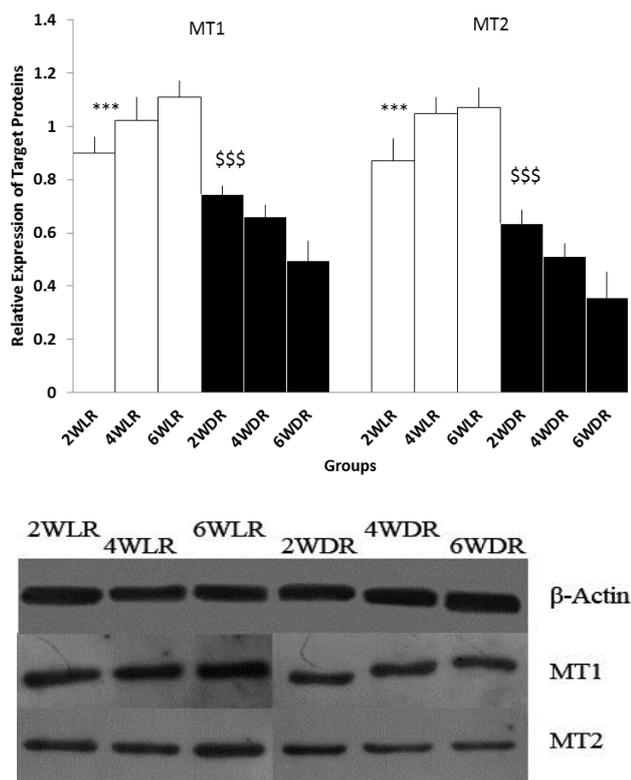


**Figure 3.** Effect of postnatal dark rearing on age-related mRNA expression of melatonin receptors in rat hippocampus. Expression of MT1 showed a significant increase from  $1.45 \pm 0.07$  in the 2WLR rats to  $1.81 \pm 0.04$  in the 6WLR animals ( $***P < 0.001$ ). The mRNA of another melatonin receptor, MT2, also increased about 36% across age ( $***P < 0.001$  between 2WLR and 6WLR groups). Postnatal dark rearing reversed the expression pattern of both melatonin receptors. The relative expression of MT1 and MT2 in the 2WDR rats ( $1.10 \pm 0.03$  and  $0.93 \pm 0.05$ , respectively) decreased across age to  $0.84 \pm 0.07$  ( $$$P < 0.01$ ) and  $0.71 \pm 0.04$  ( $$$P < 0.01$ ) in the hippocampus of the 6WDR animals, respectively. Data analysis also showed that the mRNA expression of melatonin receptors was suppressed by dark rearing when the light reared (LR) rats were compared with the age-matched dark reared (DR) ones ( $P < 0.001$  for all comparisons)

mentioned that melatonin-induced inhibition of post-tetanus potentiation had an age-dependent manner in the DR rats. The most potentiated response in the 2WDR rats ( $19.58 \pm 6.08\%$ , closed diamonds in Figure 2-B) was decreased to  $16.09 \pm 7.71\%$  and  $2.98 \pm 4.93\%$  in the 4WDR (closed squares in Figure 2-B) and 6WDR (closed triangles in Figure 2-B) rats, respectively. Although statistical analysis demonstrated a significant difference between potentiated responses in the 2WDR and the 6WDR rats ( $P < 0.01$ ) and also between the 4WDR and the 6WDR groups ( $P < 0.01$ ), there was no significant difference in post-HFS responses between the 2WDR and the 4WDR rats.

#### Expression of mRNA of melatonin receptors

Using the RT-PCR technique, the expression of the mRNAs encoding two receptors of melatonin (MT1 and MT2) was measured in this study. Multivariate ANOVA revealed a considerable difference in relative expression of the mRNAs between the LR and DR testing groups ( $F_{5,30} = 19.479$ ;  $P < 0.0001$ ). Figure 3 demonstrates the effect of postnatal dark rearing on age-related mRNA expression of melatonin receptors in rat hippocampus. Relative mRNA expression of the MT1 receptor increased from  $1.45 \pm 0.07$  in the 2WLR rats to  $1.81 \pm 0.04$  in the 6WLR animals ( $P < 0.001$ ). Statistical analysis showed that the difference in the expression of MT1 between the 2WLR and 4WLR groups and between the 4WLR and 6WLR groups



**Figure 4.** Expression of hippocampal melatonin receptors in the light reared (LR) and dark reared (DR) rats across age. A developmental increase (by about 32%) in the expression of MT1 and MT2 receptors was found in the LR animals from 2 to 6 weeks postnatal (\*\* $P < 0.001$  for both comparison). Expression of melatonin receptors has a decreasing pattern across age in the hippocampus of the DR rats, where expression of the MT1 decreased from  $0.84 \pm 0.03$  in the 2WDRs to  $0.56 \pm 0.04$  in the 6WDR rats ( $$$$P < 0.001$ ). Moreover, expression of the MT2 decreased from  $0.73 \pm 0.05$  in the 2WDRs to  $0.35 \pm 0.03$  in the 6WDR rats ( $SSSP < 0.001$ ). Comparing the data taken from age-matched groups showed that visual deprivation significantly suppressed the expression of both melatonin receptors ( $P < 0.001$  for all comparisons)

was not significant. The mRNA of another melatonin receptor, MT2, also displayed an increasing pattern of expression with age in the hippocampus of LR animals, where the relative expression of MT2 was  $1.22 \pm 0.04$  in the 2WLR rats and increased to  $1.65 \pm 0.12$  in the hippocampus of the 6WLR animals ( $P < 0.001$ ). No significant difference was observed in the expression of MT2 between the 2WLR and 4WLR rats or between the 4WLR and 6WLR groups. Keeping animals in complete darkness reversed the expression pattern of both melatonin receptors in the hippocampus of rats (Figure 3, black bars). The relative expression of MT1 and MT2 in the 2WDR rats ( $1.10 \pm 0.03$  and  $0.93 \pm 0.05$ , respectively) decreased across age to  $0.84 \pm 0.07$  ( $P < 0.01$ ) and  $0.71 \pm 0.04$  ( $P < 0.01$ ), respectively, in the hippocampus of the 6WDR animals. The relative expression of both melatonin receptors in the DRrats' hippocampus was lower than their age-matched counterparts ( $P < 0.001$  for all comparisons). Statistical analysis showed that

the difference in the expression of both melatonin receptors between the 2WDR and 4WDR animals and between the 4WDR and 6WDR groups was not significant.

**Protein expression of melatonin receptors**

Using the western blotting method, the relative expression of target proteins of melatonin receptors was evaluated. Figure 4 shows the synergistic effect of age and sensory experience on the expression of both melatonin receptors in the rat hippocampus. Multivariate analysis of variance showed a significant difference in the expression of target proteins between the LR and DR testing groups ( $F_{5,30} = 27.631$ ;  $P < 0.0001$ ). As illustrated in Figure 4, a developmental increase (by about 36%) in the expression of both melatonin receptors was found in the LR animals (light bars); however, the changes were noticeable ( $P < 0.001$ ) only in the 6WLR rats when compared with the 2WLR animals. Light deprivation reversed postnatal alteration in the expression of both melatonin receptors (black bars), where expression of the MT1 decreased from  $0.84 \pm 0.03$  in the 2WDRs to  $0.56 \pm 0.04$  in the 6WDR rats ( $P < 0.001$ ). Moreover, expression of the MT2 decreased from  $0.73 \pm 0.05$  in the 2WDRs to  $0.35 \pm 0.03$  in the 6WDR rats ( $P < 0.001$ ). Statistical analysis showed that the difference in the expression of both melatonin receptors between the 2WDR and 4WDR rats and between the 4WDR and 6WDR animals was not significant. For both melatonin receptors, the statistical comparison of data from three groups of the LR and DR animals indicated that protein expression was significantly higher in all groups of LRs ( $P < 0.001$ ).

**Discussion**

The present study evaluated the developmental effects of ICV-injected melatonin on the synaptic plasticity of hippocampal CA1 neurons in LR and dark reared rats. The results demonstrated that the amplitude size of fEPSPs recorded in the CA1 neurons of the normally LR rats decreased considerably during postnatal development, and light deprivation reversed the developmental decrease in basic excitatory synaptic transmission. Although HFS induced LTP in the CA1 area of the rat hippocampus, dark rearing resulted in a diminished synaptic plasticity across aging during critical periods in brain development.

As previously described, there are only a few studies that have shown the effects of change in visual experience on hippocampal synaptic plasticity, but numerous studies have demonstrated the effects of change in visual experience on electrophysiological properties of visual cortical circuits (29, 30). The mammalian's visual cortex supplies an essential sensory input to the hippocampus, and synaptic

plasticity in the visual cortex parallels hippocampal excitability potentiation, so that theta-burst stimulation (TBS) of lateral geniculate nucleus (LGN) increases the amplitude of population spikes recorded from dentate gyrus (11). In the preliminary work of this study, it was revealed that dark rearing enhances the amplitude of fEPSPs recorded from the CA1 area of rats and inhibits induction of LTP in the Schaffer's collateral to the CA1 pathway (26). It was also shown that light deprivation differently influenced basic synaptic activity and the occurrence of LTP in both dentate gyrus and CA1 area of the rat hippocampus (13).

It seems that synaptic transmission is determined by a balance between excitation and inhibition. During brain development, the expression of AMPA receptors increases in glutamatergic synapses (31), but because of change in the composition of its subunits, AMPA receptor-dependent excitatory post-synaptic currents decrease across development (32). Moreover, the composition of N-Methyl-D-aspartate (NMDA) receptor subunits changes prominently during critical period of postnatal development. NR2A and NR2B subunits are predominant NMDARs subunits in the forebrain (33). At birth, the expression of NR2B in many parts of the brain, including the hippocampus and neocortex, is much more than NR2A, and when brain is matured, the expression of NR2A is more than that of NR2B (34). An increased NR2A/NR2B ratio leads to decline in NMDA receptor kinetics (33). In addition, the structure and function of GABAergic neurons were changed during brain development (35), and therefore, these neurons have an excitatory action in early postnatal development of rat hippocampus, and take on an inhibitory role at maturation (36). Altogether, diminished AMPA- and NMDA-dependent excitatory post-synaptic currents and the maturation of GABAergic neurons are possible causes of the decreasing pattern in the pre- and post-HFS amplitude of CA1 neurons. It has been shown that change in visual experience induces an imbalance between excitatory and inhibitory neurotransmissions in the visual cortex. Dark rearing increases both AMPA receptor subunits GLUR1(32) and GLUR2 (4) in the visual cortex. Increased expression of the GluR1 subunit elevates miniature excitatory post-synaptic currents (mEPSC) in mouse visual cortex (32). The composition of NMDA receptors also changes the visual cortex of DR animals. It has been demonstrated that the NR2A/NR2B ratio decreases the visual cortex of DR rats (37). Findings have revealed that complete darkness inhibits age-dependent increase in GABAergic inputs in the visual cortex (38). The current study also showed that dark rearing decreases post-HFS responses in CA1 area circuits age-dependently. Results of some studies have demonstrated that dark rearing reduces the

synaptic plasticity of the neural circuit in the visual cortex (39, 40). Some types of hippocampal LTP, like LTP in the Schaffer's collateral pathway, is NMDA receptors dependent (41). In this form of LTP, because of calcium accumulation and increased post-synaptic potentials, the magnesium blockade of NMDARs is removed and LTP is developed (42). It is proved that both NR2A and NR2B subunits are fundamental for induction of LTP in the CA1 area of the hippocampus (43). Furthermore, there is a correlation between the decline of developmental plasticity in the visual cortex and the reduction in the expression of the NR2B subunit in layer IV of the visual cortex (44). Dark rearing may induce an imbalance between excitatory and inhibitory neurotransmissions in hippocampal circuits and may delay maturation of neurons in this area.

Results of the current study revealed that melatonin increases the amplitude size of fEPSPs in both LR and DR rats. In addition, the ICV injection of melatonin inhibited LTP induction in both LR and DR rats. This inhibition was noticeable in the LR rats, so that tetanizing the Schaffer's collaterals of LR rats elicited depression at all postnatal ages. The current study also evaluated the expression of both melatonin receptors in rat hippocampus. Expression of MT1 and MT2 in the hippocampus of normally LR rats increased during postnatal development, and as expected, the expression of melatonin receptors decreased in rearing rats in complete darkness.

Melatonin, the neurohormone of the pineal gland, has a circadian pattern of secretion; its production occurs during the dark phase and is intensely suppressed by light. The classic receptors of melatonin, MT1 and MT2, belong to the family of G-protein-coupled receptors and are localized in different areas of the brain (15), especially in the hippocampus (18). Using RT-PCR, it has been previously demonstrated that both melatonin receptors are expressed in different areas of the rat hippocampus (45). The expression of melatonin receptors across age in different organs has been evaluated in some studies. Previous findings showed that the expression of melatonin receptors in the suprachiasmatic nucleus (SCN) decreases after birth, remains stable for approximately 1 month, and increases again at puberty to reach birth values in adults (46). In another study, it was shown that the expression of MT1 receptor in the SCN of Syrian hamsters decreases from postnatal day 0 (P0) to P60 (47). Also, the results have demonstrated that the expression of both melatonin receptors in rats' eyes has a decreasing pattern from P14 to P60, and the expression of MT2 decreases more than the expression of the MT1 receptor (48). Melatonin regulates neuronal activity via regulation of the circadian rhythms of neurotransmitters and their receptors (49). It has been illustrated that melatonin regulates the circadian

rhythms of gamma-aminobutyric acid (GABA) and glutamate (50) and dopamine secretion (51) in mouse striatum. Melatonin can also regulate neural survival and activity by affecting the intracellular molecules and messengers like brain derived neurotrophic factor (BDNF) (52), nitric oxide (53), calmodulin (54), and calcitonin (55). A recent study demonstrated that chronic melatonin treatment reduces synaptic inhibition in the hippocampus of a mouse model with Down syndrome via increasing the density and/or activity of glutamatergic synapses, which leads to a full recovery of LTP induction by TBS in the CA1 area of these animals (56). It has also been shown that melatonin enhances the firing rate of CA1 area neurons and induces post-synaptic facilitation in this area of the hippocampus (45). On the other hand, studies have found that melatonin treatment has no effect on basic neuronal responses (57) or that it reduces the amplitude of evoked responses (24, 58). These regulatory actions of melatonin on neuronal activity may be mediated by both of melatonin receptors, MT1 and MT2 (57). The MT2 receptors knockout mice show altered hippocampal LTP induced by TBS in CA1 area circuits (59). The inhibitory effects of melatonin on LTP induction in the CA1 area of the hippocampus are prevented by MT2 receptor antagonists (57). Melatonin inhibits both glutamate and NMDA-induced excitation in rat striatum (60). Because LTP in the NMDA receptor is mediated in the CA1 area of the hippocampus (41), it can be concluded that melatonin may inhibit LTP induction in CA1 area neurons via inhibiting NMDA receptors.

### Conclusion

In this study the pattern of expression of both melatonin receptors in LR and DR rats during the postnatal critical period of development did not match the pattern of changes in the responses of CA1 area neurons before or after melatonin microinjection. Although melatonin changed basic and tetanized responses of hippocampal neurons, it can be concluded that the effects of melatonin on hippocampal neuronal responses may be exerted through other ways, like intercellular molecules and nuclear hormone receptors.

### Conflict of interest

The authors have not personal or financial competing of interest.

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