Exp Biomed Res 2025; 8(3): 188-202 **https://doi.org/ 10.30714/j-ebr.2025.248**

Experimental Biomedical Research *Original article*

In vivo investigation of the effects of light/dark cycle changes on synaptic plasticity in the dentate gyrus of the rat hippocampus

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ABSTRACT

Aim: To investigate the impact of alterations in the light-dark cycle on the activity of dentate gyrus neurons within the hippocampus.

Methods: The Light/Dark cycle was implemented in controlled environments equipped with automated lighting systems, maintaining a consistent 12-hour duration for each phase. Wistar Albino male rats were categorized into two groups. The light group was exposed to light from 08:00 to 20:00 followed by darkness, while the dark group remained in darkness from 08:00 to 20:00 and was exposed to light during the subsequent 12 hours. All periods were examined concurrently at the same time of day on the 30th experimental day.

Results: There was no statistical difference in the slopes of excitatory postsynaptic potentials (EPSP) and the amplitudes of population spikes (PS) across varying stimulation intensities ($p>0.05$), with the exception of the 1.5 mA intensity ($p=0.04$). The stimulus facilitation index for the EPSP slope was significantly greater in the light group compared to the dark group at 120, 140, and 160 ms ($p<0.05$). The enhancement observed in the night group relative to baseline values during the PTP, induction, and maintenance periods was significantly lower than that in the light group ($p<0.001$).

Conclusion: The results suggest that variations in light-dark frequency can influence the electrical characteristics of dentate gyrus neurons, indicating the presence of an endogenous timing mechanism within the hippocampus that may regulate hippocampal Long-Term Potentiation (LTP).

Keywords: Long-term potentiation, paired-pulse facilitation, hippocampus, light-dark, circadian rhythm.

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Received: 2025-04-13 / Revisions: 2025-05-05
Accepted: 2025-05-11 / Published: 2025-07-01

1. Introduction

The hippocampus primarily facilitates spatial learning and remembering. Alterations in the pre- and post-synaptic neurons are crucial for the development of learning and memory. AMPA and NMDA receptors, ligand-gated ion channels for the excitatory neurotransmitter glutamate, are crucial in synaptic attrition throughout several brain regions, particularly the hippocampus [1].

Glutamate serves as the primary excitatory neurotransmitter in the central nervous system and plays a crucial role in regulating synaptic plasticity. AMPA and NMDA receptors are ion channels that are activated by glutamate binding and are crucial for synaptic plasticity mechanisms. AMPA receptors facilitate fast excitatory synaptic transmission, whereas NMDA receptors govern long-term

modifications of synaptic plasticity by permitting calcium ions to enter the cell. The activation of NMDA receptors is essential for the generation of long-term potentiation (LTP), a significant mechanism of synaptic plasticity [2-4]. Recent studies have highlighted the importance of circadian rhythms in modulating hippocampal function, particularly affecting synaptic plasticity [3-7]. Circadian regulation has been shown to influence the expression and function of both AMPA and NMDA receptors in the hippocampus, leading to variations in synaptic strength throughout the day. Evidence suggests that disruptions in circadian rhythms can impair hippocampal plasticity, potentially through dysregulation of glutamate receptor activity. Melatonin, the key hormone regulating circadian rhythms, plays a critical role in modulating synaptic plasticity [1, 8-11]. Studies have indicated that melatonin may influence glutamate receptor expression and function, particularly through its action on NMDA receptors. By regulating calcium influx and signaling pathways associated with NMDA receptor activation, melatonin helps maintain optimal synaptic function and protect against excitotoxicity, which could otherwise result from excessive glutamate receptor activation [9, 10, 12]. A theoretical framework for understanding the interaction between NMDA and AMPA receptors and circadian rhythms can be proposed based on existing literature. During periods of high melatonin levels (such as at night), NMDA receptor activity may be modulated to reduce excitatory neurotransmission and prevent overstimulation of synapses, promoting restorative processes. Conversely, during the day, when melatonin levels are lower, heightened NMDA receptor activity could facilitate synaptic strengthening and LTP, enhancing cognitive functions such as learning and memory [2, 13-15].

Numerous biochemical, physiological, and behavioral factors exhibit a circadian rhythm in humans. Circadian rhythms are biological processes that recur in about 24-hour cycles, governing the sleep-wake cycle, hormone secretion, body temperature, dietary patterns, and several other physiological functions in the human body [4, 7, 11, 16]. The rhythm is regulated by central pacemakers located in the suprachiasmatic nucleus (SCN) within the hypothalamus [16]. The SCN is situated superior to the optic chiasm in the brainstem and serves as the principal regulator of circadian rhythms. The SCN regulates the body's circadian clock in alignment with external light/dark cycles and responds to environmental alterations [5]. The primary regulator of rhythm is the light/dark cycle in the external environment. The SCN takes visual impulses from the retina, turns them into biochemical and electrical signals, and transfers them to other brain areas and the body. This mechanism regulates optimal physiological processes by aligning the body's circadian clock with the ambient light/dark cycle [17]. The correlation between melatonin release and the light/dark cycle indicates that this hormone functions as an endogenous synchronizer. Melatonin is a hormone produced by the pineal gland, with its release rate elevated during nocturnal hours. Melatonin is crucial for regulating the sleep-wake cycle and synchronizing circadian rhythms. The secretion of melatonin is elevated during periods of darkness and inhibited by light exposure, facilitating the body's adaptation to circadian rhythms [18, 19].

Research indicates that the expression of certain NMDA receptor subtypes in hippocampus cells is elevated in rats treated with melatonin. Moreover, modification of the light-dark cycle in rats impairs the intrinsic melatonin secretion rhythm [11, 20]. The fluctuation of

melatonin levels in the bloodstream at various times of the day is anticipated to correspondingly affect the quantity of hippocampus glutamate receptors. The correlation between receptor density in the hippocampus and melatonin levels remains unexamined; nevertheless, endogenous excitatory post-synaptic potentials of glutamate, indicative of receptor levels, may provide insight into this matter. Research indicating the existence of melatonin receptors in the hippocampus and their association with the light-dark cycle requires a thorough investigation of the connection between the light/dark cycle and hippocampal synaptic responses.

This study sought to examine the impact of alterations in the light-dark cycle on neuronal activity in the dentate gyrus of the hippocampus.

2. Materials and methods

2.1. Study design, settings, and sample

Sixteen mature male Wistar Albino rats, aged 150-180 days, were utilized for this investigation. This age range was chosen because it represents young adult rats with mature hippocampal circuitry, providing optimal conditions for assessing synaptic plasticity without the confounding effects of developmental immaturity or age-related decline. The rats were randomly assigned to two groups: the light group and the dark group, with n = 8 in each group. The light group was exposed to light from 08:00 to 20:00 followed by darkness, while the dark group remained in darkness from 08:00 to 20:00 and was exposed to light during the subsequent 12 hours. To facilitate daily electrophysiological recordings from a single experimental animal, one rat from the light group was housed in its cage each day for the initial 8 days of the investigation. Beginning on the ninth day, the dark group was confined to their own cages, one each day. Commencing on the thirtieth day, a rat was introduced to the Department of Physiology, and electrophysiological recordings were conducted daily. Each rat was permitted to remain in the light/dark cycle for 30 days across both groups. To regulate the light/dark cycle, we used a controlled environment where the light intensity during the light phase was set to 300 lux, a level that ensures proper entrainment of circadian rhythms while avoiding overstimulation. The lighting used during the light phase consisted of full-spectrum white LED lights, which include a balanced range of wavelengths with minimal blue light. This is important as blue light can significantly impact circadian rhythms and neuronal activity. By maintaining minimal blue light exposure, we aimed to avoid interference with normal hippocampal function. During the dark phase, the environment was kept completely dark to ensure no light contamination that could disrupt the circadian cycle. The rats used in the study were obtained from Hakan Çetinsaya Experimental and Clinical Research Center (ERU DEKAM) and the light-dark cycle change was created in special rooms there.

2.2. Stimulation and recording

Rats were administered urethane (1.5 g/kg, intraperitoneally) for sedation and subsequently placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). A bipolar tungsten electrode (stainless steel, Teflon-coated, 127 μm, insulated except at the tip) was utilized to stimulate the right medial perforating route (AP: -8.0, ML: 4.2, DV: 2.3 mm from bregma). The stimulation electrode was connected to the output of the isolator (SS-201J Isolator, Nihon Kohden, Tokyo, Japan), which in turn was linked to the stimulator (SEN 3201 Electronic Stimulator, Nihon Kohden, Tokyo, Japan). A glass micropipette (Borosilicate, outer diameter: 1.5 mm, length: 10 cm, World Precision Instruments) filled with 3M NaCl (tip resistance:

2–10 MΩ) was utilized to record excitatory postsynaptic potential (EPSP) fields of the ipsilateral dentate gyrus (in mm: AP: -3.5, ML: 2.14, DV: 3.3 mm from the dura mater were introduced into the granule cell layer. An Ag-AgCl disk electrode was subcutaneously implanted in the neck to serve as a reference electrode [6, 20].

The active and reference electrodes were connected to the amplifier (VCC600 single-blade epithelial voltage/current clamp device, Physiological Instruments, Harvard Apparatus, Holliston, MA, USA) through a head-stage. A Faraday cage provided comprehensive protection for the entire system. The recording depth was systematically adjusted in 0.1 mm increments to induce a broad positive excitatory postsynaptic potential (EPSP), followed by a subsequent negative population spike (PS). After documenting the standard response at the final depth, the stimulation electrode was adjusted to enhance the PS amplitude in response to perforating route stimulation. The Scope application (ADInstruments, Colorado Springs, CO, USA) was employed for stimulation control and data recording [6, 20]. Monophasic stimuli of 10V and 0.175 ms were generated by a computer A/D board (Powerlab/8SP, ADInstruments, Colorado Springs, CO, USA) and triggered a stimulator connected to the isolator. Biological signals underwent amplification by a factor of 1000 in a pre-amplifier with a bandwidth of 0.1 to 10 kHz. The waveform was digitized in real-time at a frequency of 40 kHz over a duration of 20 ms, displayed on a computer monitor, and archived using “Scope” for further analysis [6, 20].

2.3. Input – Output (I/O) Curve

Fifteen minutes post-electrode placement, stimulation and monophasic electrical currents were administered every 20 seconds for 175 milliseconds to generate an input-output (I/O) curve. Stimulation current was administered within the range of 0.1 to 1.5 mA. For each current value, three evoked responses were averaged. The correlation between stimulus intensity and EPSP slope or PS amplitude was characterized by a sigmoidal curve. The stimulus intensity corresponding to half of the maximal response was identified from this curve. The test stimulus intensity established for the experimental animals ranged from 0.15 to 0.6 mA. Test stimuli established for Paired-Pulse Facilitation (PPF) and Long-Term Potentiation (LTP) experiments were utilized [6, 20].

2.4. Paired-Pulse Facilitation (PPF)

Paired-Pulse Facilitation was measured by taking the evoked response to pairs of stimuli given to the perforating path at 20, 30, 40, 50, 60, 70, 80, 90 and 100 ms intervals, 20 s apart. Three responses were averaged for each pair. The ratio of population spike (PS) amplitudes (second PS amplitude/first PS amplitude x 100, stimulus pair index) was calculated [6, 20].

2.5. Long-Term Potentiation (LTP)

After a 10-minute baseline recording of excitatory postsynaptic potential (EPSP), four tetanic (100 Hz) stimulations were given at 5-minute intervals at 50, 55, 60 and 65 minutes to create LTP. Following the last tetanic stimulus, the test stimulus was applied every 30 seconds until the 120th minute [6, 20].

2.6. Statistical analysis

The average values of EPSP slopes and PS amplitudes during the basal period (the initial 10 minutes of recording prior to the administration of high-frequency stimulation (HFS)) were assessed as 100% for each rat. The slope of each subsequent EPSP and the amplitude of the PS were expressed as a percentage of the calculated value. Data were presented as percentage change relative to the baseline period. The slope of the EPSP was determined by measuring the amplitude change between 20% and 80% of the

voltage difference from the onset of the EPSP to the onset of the waveform. The PS amplitude was quantified from the positive peak to the negative peak. Four-time intervals were selected for the statistical evaluation of slope and amplitude, and the average values for these periods were calculated. The Mann-Whitney U test was employed to compare slope and amplitude data across groups for each period. Values were presented as mean ± standard deviation, with probability values below 0.05 deemed significant.

mA to 1.5 mA, while Table 1 provides the statistical analysis of the data. No substantial difference was seen in the EPSP slopes and PS amplitudes across all stimulation intensities ($p>0.05$), with the exception of the 1.5 mA intensity ($p=0.04$). The results indicate that the neurons in the dentate gyrus, which are activated by afferent stimulation in both groups, and the neurons that produce action potentials upon crossing the threshold exhibit comparable electrophysiological characteristics (Table 1, Figure 1).

Table 1. Statistical analysis of EPSP slope and PS amplitudes recorded from dentate gyrus neurons of light and dark group rats against 8 different stimulus intensities ranging from 0.1 mA to 1.5 mA.

Stimulus (mA)	Light Group	Dark Group	P-value
EPSP Slope			
0.1	22.7 (−3.5–48.9)	30 (−13.2–73.2)	0.23
0.3	35.1 (2.5–67.7)	42.2 (8.2–76.2)	0.21
0.5	39.9 (8.7–71.1)	48.4 (19.2–77.6)	0.10
0.7	46.9 (13.3–80.5)	50.1 (18.3–81.9)	0.56
0.9	50.7 (12.9–88.5)	55.9 (23.9–87.9)	0.39
1.1	50.3 (13.7–86.9)	58.5 (24.3–92.7)	0.17
1.3	49.3 (7.1–91.5)	55 (24.6–85.4)	0.36
1.5	53.6 (12.4–94.8)	57.2 (26.4–88.0)	0.56
PS Amplitude			
0.1	3.9 (−9.1–16.9)	11.2 (−29.2–51.6)	0.15
0.3	23.3 (−20.1–66.7)	19.9 (−24.5–64.3)	0.64
0.5	48.3 (−11.7–108.9)	32 (−15.6–79.6)	0.08
0.7	66.4 (−1.8–134.6)	49.5 (−9.3–108.3)	0.12
0.9	83.9 (−1.0–168.8)	61.9 (−4.7–128.5)	0.09
1.1	91.8 (−2.4–186.0)	69.1 (−4.7–142.9)	0.12
1.3	100.9 (−5.7–207.5)	73.9 (−5.7–153.5)	0.09
1.5	108.8 (−8.4–226.0)	72.9 (−14.7–160.5)	0.04*

*Values: Median (Min–Max). EPSP: Excitatory postsynaptic potentials, PS: Population spike, *: p<0.05.*

3. Results

3.1. Input / Output (I/O) Outcomes: Figure 1 presents the alterations in EPSP slope and PS amplitudes seen in dentate gyrus neurons of rats from both the light and dark groups, across eight distinct stimulation intensities ranging from 0.1

3.2. Paired-Pulse Facilitation (PPF) Outcomes: The alterations in the EPSP slope and PS amplitudes observed in the dentate gyrus neurons of the light and dark group rats, across eight distinct test stimuli with stimulus pair intervals varying from 20 ms to 160 ms, are presented in figure 2, while the statistical

analysis of the data is detailed in table 2. The slope of the EPSP responses to the second stimulus in the pair did not increase across varying stimulation interval values in both groups. Consequently, no reduction in EPSP slopes was noted. The stimulus facilitation index for the EPSP slope was significantly greater in the light group compared to the dark group at 120, 140, and 160 ms ($p<0.05$). The amplitude of PS responses to the second stimulus in the pair increased in both groups across varying stimulation interval values. Consequently, a reduction in PS amplitudes was noted. The most substantial facilitation in both groups occurred with a stimulus pair interval of 80 ms. Facilitation at the 80 ms interval was observed to be 38% in the light group and 11% in the dark group. Comparison of the PPF between the two groups revealed significantly higher values in the light group than in the dark group at 60, 80, 100, 120, 140, and 160 ms. The findings indicate that dentate gyrus neurons, innervated by afferent stimulation in the dark group, exhibited lower amplitude responses to the second stimulus in pairs applied at medium and long intervals (Table 2, Figure 2).

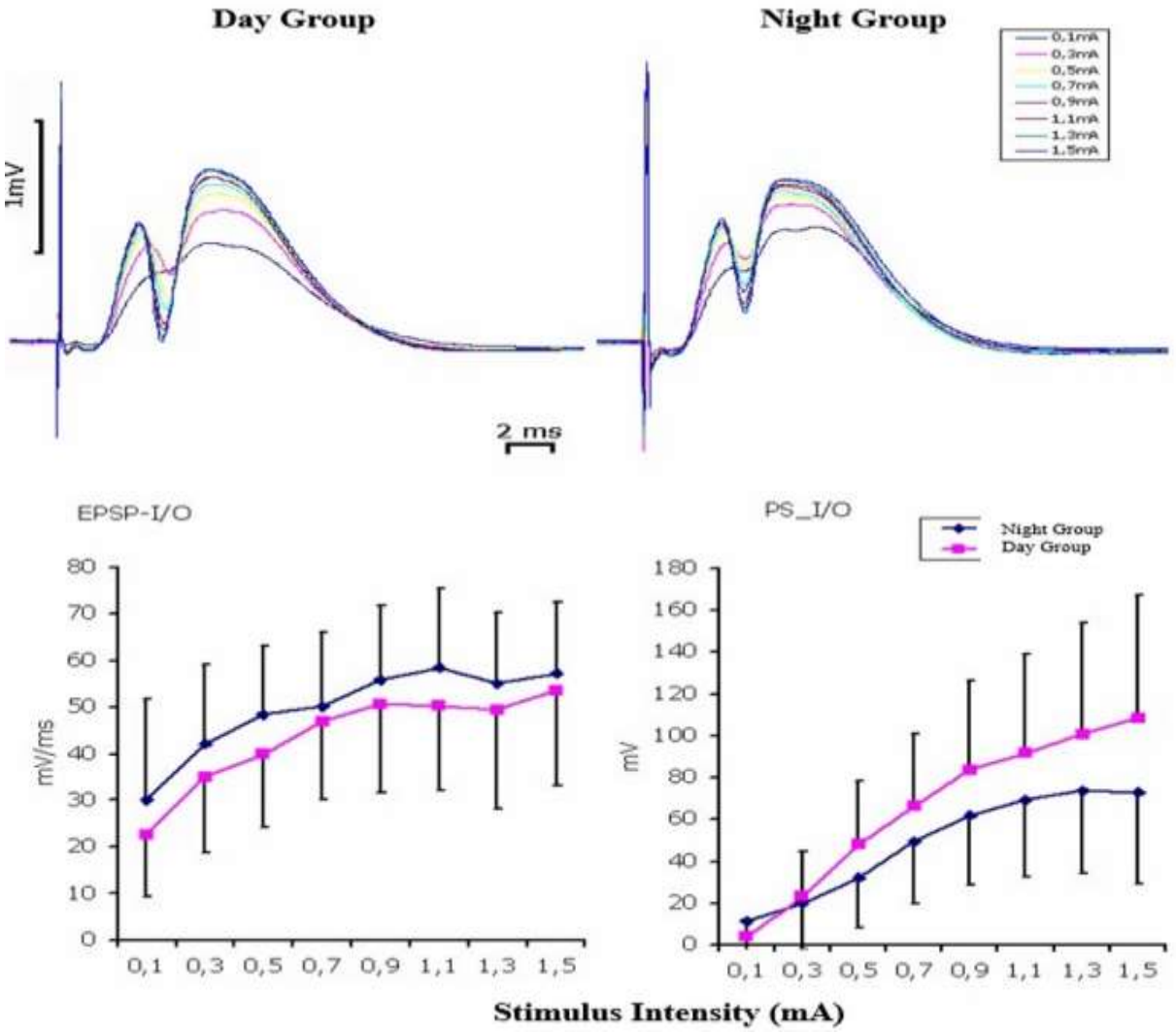

Figure 1. Variations in EPSP slope and PS amplitudes recorded from dentate gyrus neurons of light and dark rat groups across eight distinct stimulus intensities, ranging from 0.1 mA to 1.5 mA (EPSP: Excitatory postsynaptic potentials, PS: Population spike).

Table 2. Statistical analysis of EPSP slopes and PS amplitudes recorded from dentate gyrus neurons of light and dark group rats against 8 separate test stimulus pairs with stimulus pair intervals ranging from 20 ms to 160 ms.

Interval (ms)	Light Group	Dark Group	P-value
EPSP Slope			
20	0.76 (0.34–1.18)	0.77 (0.45–1.09)	0.39
40	0.90 (0.76–1.04)	0.86 (0.52–1.20)	0.20
60	0.92 (0.78–1.06)	0.96 (0.74–1.18)	0.18
80	0.97 (0.79–1.15)	0.94 (0.76–1.12)	0.26
100	0.96 (0.74–1.18)	0.90 (0.72–1.08)	0.12
120	0.94 (0.80–1.08)	0.87 (0.75–0.99)	0.01*
140	0.94 (0.72–1.16)	0.86 (0.76–0.96)	0.03*
160	0.91 (0.75–1.07)	0.84 (0.72–0.96)	0.02*
PS Amplitude			
20	0.20 (−0.22–0.62)	0.10 (0.02–0.18)	0.10
40	0.69 (−0.13–1.51)	0.52 (0.24–0.80)	0.13
60	1.27 (0.29–2.25)	0.90 (0.68–1.12)	0.03*
80	1.38 (0.64–2.12)	1.11 (0.61–1.61)	0.04*
100	1.35 (0.57–2.13)	0.98 (0.64–1.32)	0.01*
120	1.17 (0.45–1.89)	0.91 (0.57–1.25)	0.04*
140	1.02 (0.26–1.78)	0.76 (0.52–1.00)	0.04*
160	1.01 (0.39–1.63)	0.71 (0.49–0.93)	0.01*

*Values: Median (Min–Max). EPSP: Excitatory postsynaptic potentials, PS: Population spike, *: p<0.05.*

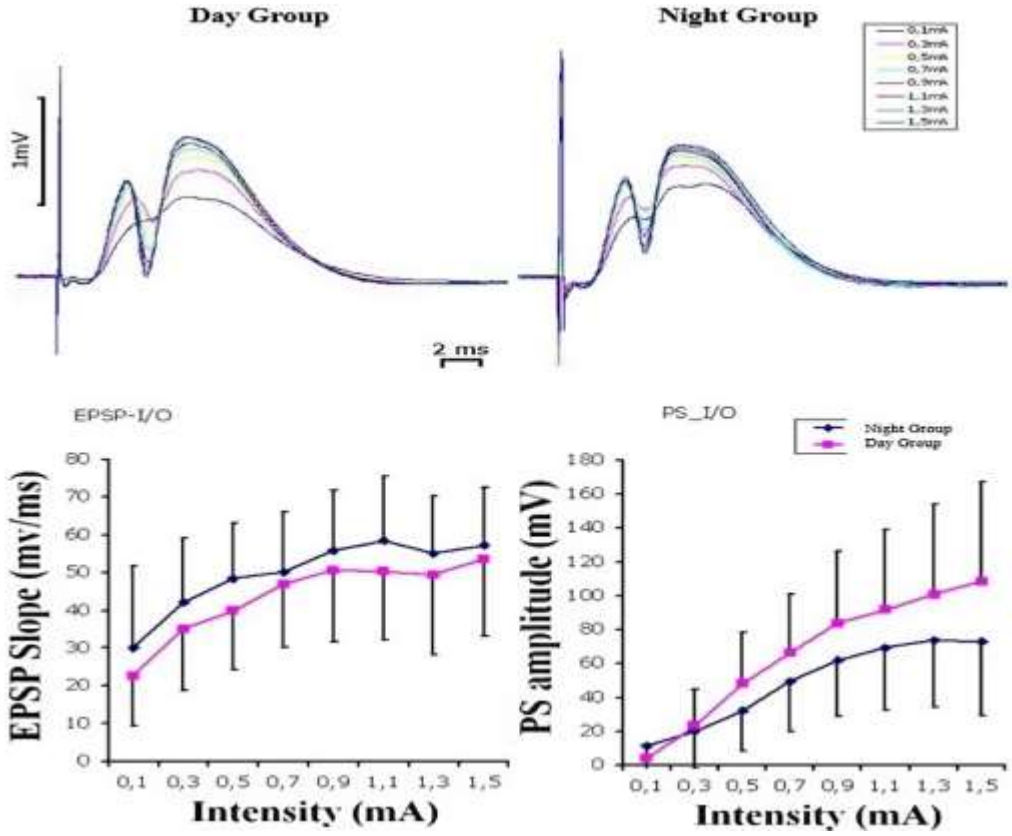

Figure 2. Illustrates the input-output connection of field potentials recorded from dentate gyrus neurons in rats from both light and dark groups, across eight distinct stimulus intensities ranging from 20 ms to 160 ms (I/O: Input – Output, EPSP: Excitatory postsynaptic potentials, PS: Population spike).

Table 3. Average values of field potentials recorded in four different time periods in light and dark groups.

Term	**Baseline**		**PTP**		**Induction**		**Maintenance**	
Time (Minute)	0-15 min		15-20 min		30-35 min		85-90 min	
Group	Light	Dark	Light	Dark	Light	Dark	Light	Dark
PS Amplitude								
Mean	100.23	101.94	219.77	154.31	234.15	163.26	195.59	158.55
SD	6.17	5.53	58.11	59.25	62.97	68.87	47.10	45.37
P value		0.068		0.000*		0.000*		0.000*
EPSP Slope								
Mean	99.39	98.18	136.58	108.68	139.62	120.99	119.54	100.44
SD	4.65	4.95	18.33	19.91	18.38	16.18	18.41	11.95
P value		0.056		0.000*		0.000*		0.000*

*PTP: Post-Tetanic Potentiation, EPSP: Excitatory postsynaptic potentials, PS: Population spike, *: p<0.05.*

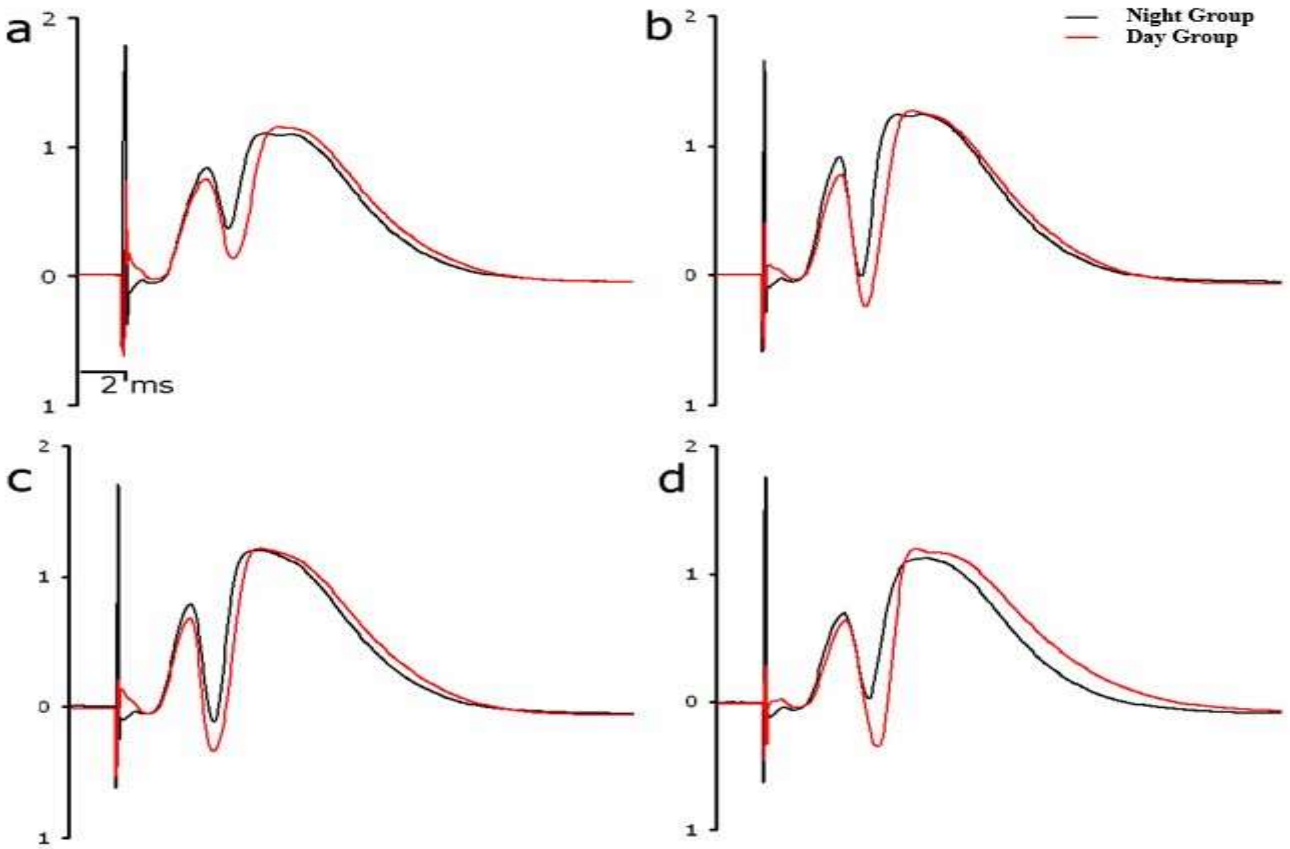

Figure 3. Average traces of field potentials recorded from the dentate gyri of light and dark groups over four time periods. The baseline is illustrated in “a”, post-tetanic augmentation is depicted in “b”, the induction phase is represented in “c”, and the maintenance period is indicated in “d”. The Y-axis represents millivolts (mV).

3.4. High-Frequency Stimulation (HFS) Outcomes: Four-time intervals were selected for statistical evaluations, and the average percentages of the EPSP slope and PS amplitude values of the responses obtained in the HFS experimental protocol compared to the baseline were given.

3.5. Analysis by time intervals

The average responses of the light and dark groups, recorded during the four selected time intervals of the HFS protocol (baseline, Post-Tetanic Potentiation (PTP), induction, and maintenance), are presented in Figure 3, while the statistical evaluation results are detailed in Table 3. The results indicate that the enhancement observed in the dark group relative to baseline values during the PTP, induction, and maintenance periods was significantly lower than that in the light group ($p<0.001$) (Table 3, Figure 3).

4. Discussion

In this study, it was found that the EPSP slope and PS amplitudes recorded from the dentate gyrus neurons of the day and night group rats against 8 different stimulus intensities ranging from 0.1 mA to 1.5 mA did not show a statistical difference between the groups, except for the 1.5 mA intensity. These findings show that in both groups, the neurons innervated by afferent stimulation in the dentate gyrus and the neurons that generate action potentials by reaching the threshold value have similar electrophysiological properties. It was found that the facilitation index (PPF) in the EPSP slope and PS amplitudes recorded from the dentate gyrus neurons of the night and day group rats against 8 separate test stimuli with stimulus pair intervals ranging from 20 ms to 160 ms did not show a statistical difference between the groups. These findings revealed that neurons innervated by afferent stimulation in the dentate gyrus in both groups showed similar facilitation to the second of the stimulation pairs applied with short intervals.

Our findings on the modulation of synaptic plasticity in the dentate gyrus by light/dark cycle alterations are consistent with previous studies investigating the role of circadian rhythms in hippocampal function. Several studies have demonstrated that melatonin, a key regulator of circadian rhythms, plays a critical role in hippocampal plasticity through its influence on synaptic strength and neuronal excitability [9, 21, 22]. Ang et al. showed that chronic disruptions of the light/dark cycle, such as exposure to constant light or constant darkness, negatively impacted synaptic plasticity in the hippocampus, particularly reducing the efficiency of LTP [22]. Our study further supports this by showing significant differences in the stimulus facilitation index between light and dark groups, highlighting the importance of proper light regulation. Comparatively, melatonin's role in stabilizing neuronal activity has been shown to counteract the adverse effects of circadian misalignment in other studies, suggesting that endogenous circadian timing mechanisms, possibly mediated by melatonin, are essential for maintaining optimal hippocampal function [9, 21]. By comparing our results with these findings, we contribute to the understanding of how light/dark cycles and circadian rhythms interact with hippocampal synaptic plasticity.

This investigation revealed that the EPSP slope and PS amplitudes recorded from the dentate gyrus neurons of rats in both the light and dark groups did not exhibit a statistically significant difference across eight stimulus intensities, ranging from 0.1 mA to 1.5 mA, with the exception of the 1.5 mA intensity. The findings indicate that in both groups, the neurons innervated by afferent stimulation in the dentate gyrus and the neurons that produce action

potentials upon attaining the threshold have comparable electrical characteristics. The facilitation index (PPF) of the EPSP slope and PS amplitudes recorded from dentate gyrus neurons of the light and dark group rats, tested with eight distinct stimuli at pair intervals of 20 ms to 160 ms, exhibited no statistically significant difference between the groups. The findings indicated that neurons in the dentate gyrus, innervated by afferent stimulation in both groups, exhibited comparable facilitation in response to the second stimulus pair delivered at short intervals.

Numerous in vivo investigations indicate that the hippocampus excitability rhythm exhibits diurnal modulation [4, 7, 23, 24]. While the function of the suprachiasmatic nucleus in these rhythms remains incompletely understood, it has been proposed that synaptic plasticity in the hippocampus may exhibit variations at different times of the day [4, 7]. Reports indicate that the PS amplitude following high-frequency stimulation (1X100 Hz) is, on average, 70% greater during the nocturnal phase compared to the diurnal phase [25]. In a comparable in vivo investigation done in the rat hippocampus, LTP volumes reaching their zenith at night were observed, therefore confirming this daily cycle [26]. All these investigations furnish significant evidence that long-term potentiation exhibits rhythmicity [18, 26-28]. Zen et al. demonstrated in their study on mice that alterations in the 24-hour light/dark cycle did not influence learning and memory capabilities; instead, shorter cycles might enhance these cognitive processes [29]. Some studies also report no variations in long-term potentiation between day and night. The variation in outcomes may stem from the species and/or techniques employed in the investigations. Hippocampal excitability rhythms can endure even in brain slice preparations. In CA3 neurons, depolarization-induced excitability and the amplitude of high voltage-activated calcium currents fluctuate diurnally, generally reaching their zenith at night. This daily fluctuation suggests that inherent biological processes in the hippocampus may be affected by circadian rhythms, influencing the overall excitability and functionality of hippocampal neurons [30]. Our study revealed that the facilitation index in the EPSP slope and PS amplitudes recorded from dentate gyrus neurons of both light and dark group rats, across eight distinct test stimuli with stimulus pair intervals ranging from 20 ms to 160 ms, exhibited no statistically significant difference between the groups. The findings indicated that neurons in the dentate gyrus, innervated by afferent stimulation in both groups, exhibited comparable facilitation in response to the second stimulus pair delivered at short intervals. The significance of calcium current in stimulus pair facilitation is recognized. The variation in calcium current based on the time of day and the absence of differences in stimulus pair facilitation among groups in our study can be attributed to the fact that all trials were completed at the same time daily.

The diurnal pattern seen in PS-LTP responses from C3H rats secreting melatonin was considered endogenous and likely circadian in origin. This study found that the diurnal variation persisted in rats maintained in a Dark/Dark cycle. In a study of rats with monitored free activity cycles, it was shown that whether they were euthanized during the subjective night or day phase, the hippocampal portions exhibited larger PS-LTP responses during the night phase compared to the day phase. The sustained rhythm in rats maintained in a Dark/Dark cycle indicates a robust endogenous timing mechanism governing the rhythm in PS-LTP [31, 32]. Our investigation revealed that the EPSP slope and PS amplitudes recorded from dentate gyrus

neurons of light and dark group rats, across eight distinct stimulus intensities ranging from 0.1 mA to 1.5 mA, exhibited no significant difference between the groups, with the exception of the 1.5 mA intensity. The findings may result from the studies being done at the same time of day during the light phase, together with the phase-resetting impact of the associated light. In an optimal circadian rhythm research, measurements must be conducted at various intervals, and variations in magnitude should be shown.

In a research, rats subjected to a light/dark cycle were killed during the light phase, and evoked responses in hippocampal slices were recorded at night [33]. If the isolated hippocampus slice has oscillatory characteristics, the timing of its preparation may be less critical than the timing of the LTP recording. Furthermore, despite hippocampal slices being prepared during the day and recordings conducted at night, the potentiated evoked responses were seen to be similar to those recorded both during the day and night. The PS-LTPs of rats euthanized during the day and measured at night were comparable to those of the dark group, exhibiting greater values and a more gradual decline over time [34]. Control slices were recorded during the late daylight period and were found to maintain typical daytime dimensions and kinetics. The findings indicate that hippocampus slices are not static entities throughout preparation and that alterations persist from day to night in vitro [33].

Research using two distinct rat species, one that produces melatonin and one that does not, indicates the potential function of melatonin as a signaling molecule crucial for the preservation of hippocampal rhythms [8]. Melatonin is commonly considered a crucial chemical in the circadian system, tasked with transmitting regularly produced temporal information. It affects several physiological processes via high-affinity receptors that are extensively located throughout the nervous system, including the hippocampus, subiculum, and entorhinal cortex [35, 36]. Electrophysiological investigations of CA1 neurons have shown that melatonin enhances the spontaneous firing rate while suppressing GABA-A receptor-mediated activities. Furthermore, previous studies have demonstrated that melatonin can modulate synaptic plasticity in hippocampal neurons [4, 37, 38]. These findings suggest that the cycles of long-term potentiation (LTP) may be governed by the circadian regularity of melatonin release. The continued presence of these cycles in C57 rats, which lack melatonin secretion, indicates that the function of melatonin in long-term potentiation and learning requires more comprehensive examination [10]. Although melatonin may significantly influence hippocampal physiology and synaptic plasticity, it is probably not the only mechanism contributing to the observed cycles. Additional variables may potentially contribute to the modulation of these processes.

Circadian rhythms in hippocampus functioning are affected by many variables that vary throughout the day. These elements may exert both direct and indirect influences. The circadian system is affected by animal activity levels, and changes in these levels can influence hippocampus function. In vivo research shown that long-term potentiation (LTP) evaluated during the active nocturnal phase of the rat was superior to measurements taken during sleep or wakefulness without activity. Researchers have proposed that cholinergic activation may account for this phenomenon [39-41].

Oscillations in the suprachiasmatic nucleus (SCN) or other cerebral areas may exert long-term impacts on the rhythmicity of hippocampal neurons, but this phenomenon is more challenging to elucidate. A study revealed that

the expression of circadian CLOCK genes mPer1 and mPer2 mRNAs reached its zenith at night and exhibited rhythmicity [42]. Furthermore, several in vivo investigations have indicated diurnal variations in adenosine production within the hippocampal dentate gyrus and the inhibition of adenosine receptors, suggesting that adenosine modulates hippocampal synaptic transmission [43]. Cortisol levels in the bloodstream exhibit a pronounced diurnal cycle. Research indicates that cortisol exerts regulatory effects on hippocampus physiology and LTP [3, 44]. Joel et al. observed that in vivo PS-LTP responses were elevated at night in control mice, but this result was inverted in adrenalectomized animals [45].

4.1. Conclusions

This study investigated how changes in the light/dark cycle affect hippocampal synaptic plasticity, specifically within the dentate gyrus, using electrophysiological models such as paired pulse facilitation and long-term potentiation. Our findings suggest that diurnal variations have a significant influence on synaptic activity, with the dark group showing reduced synaptic efficacy compared to the light group. This disparity may indicate that alterations in light exposure can negatively impact mechanisms underlying learning and memory. Importantly, the observed differences in synaptic potentiation and the lack of enhancement in paired pulse facilitation in the dark group suggest that the timing of light exposure might influence both the induction and maintenance of synaptic plasticity. This could potentially impair cognitive processes regulated by the hippocampus, such as memory consolidation.

Our results highlight the need for further exploration into how circadian rhythms and light exposure influence hippocampal functioning, which could have significant implications for understanding sleep-related cognitive deficits, and possibly for therapeutic interventions targeting circadian disruption. Future studies should aim to elucidate the mechanisms by which these light-induced alterations in hippocampal plasticity contribute to cognitive dysfunction.

Funding: *The authors received no financial support for the research, authorship, and/or publication of this article.*

Conflict of Interest: *The authors declared no conflict of interest.*

Ethical Statement: *This experiment was carried out after receiving approval from the Erciyes University Animal Experiments Ethics Committee (Approval date: 05.12.2006 and no: 01/461).*

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