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Circadian disruption through light-dark cycle alteration induced Alzheimer's disease-like pathology in mice

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Research Article

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Abstract

Background

Circadian disruption (CD) has emerged as a critical factor compromising human health in contemporary society. Increasing evidence suggests that disturbances in circadian rhythms are involved in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD). The hyperphosphorylation of tau and the deposition of amyloid-β (Aβ) are recognized as major pathological hallmarks of AD. In this study, we aimed to explore the impact of long-term CD on AD-like pathological changes and to explore the underlying molecular mechanisms using a mouse model.

Methods

To mimic the CD experienced by shift workers, mice were subjected to lighting conditions involving repeated reversals of the light-dark cycle. In this study, Western blotting and immunohistochemical analyses were used to evaluate AD-like pathological changes in the hippocampus following CD. For elucidating the underlying mechanisms, we assessed circadian expression patterns of clock genes and major neurotransmitters, activation of microglia and astrocytes, and alterations of tight junction proteins within the hippocampus.

Results

Our findings demonstrated that Light-dark cycle disruption triggered CD in mice, and then CD led to Aβ protein deposition and tau hyperphosphorylation. CD significantly disrupted the circadian expression profiles of hippocampal clock genes and major neurotransmitters, induced microglial and astrocytic activation, and decreased the expression of the tight junction proteins zonula occludens-1 and occludin in the hippocampus.

Conclusion

These results suggest that CD induces AD-like pathological changes in mice, potentially mediated by dysregulated circadian oscillations of clock genes, neuroinflammation, blood-brain barrier impairment, and disturbed neurotransmitter expression in the hippocampus. Collectively, this study underscores the importance of circadian stability for brain health, and highlights the necessity for deeper exploration into the connection between AD and CD.

Background

Alzheimer's disease (AD) is a permanent neurodegenerative disease marked clinically by cognitive impairments, such as memory impairment and aphasia. In 2019, around 50 million people worldwide

were impacted by AD and other forms of dementia, with China accounting for 13 million cases, or approximately 25.5% of the global total. As the world faces an increasingly aging population, the number of affected individuals is projected to rise to 150 million by 2050. [1, 2] The pathogenesis of AD is widely believed to stem from the interaction between internal factors, such as genetic predisposition and aging, and external environmental factors, including physical and chemical hazards and lifestyle changes. The hallmark pathological features of AD include deposition of amyloid-β (Aβ) plaque, neurofibrillary tangles (NFTs) constituted by hyperphosphorylated tau protein, and neuronal loss in the brain. Pathophysiological processes underlying AD involve synaptic damage, mitochondrial dysfunction, neuroinflammation, hormonal alterations, and neurotransmitter imbalances. [3, 4] Recent studies have shown that circadian and sleep cycle dysregulation play important roles in promoting Aβ deposition and tau hyperphosphorylation. [5] Therefore, environmental health hazards, such as circadian disruption (CD) and sleep dysregulation, could significantly elevate the risk of developing AD.

Circadian rhythms have evolved over time to enable living organisms to adapt to environmental cues, including light, temperature, and feeding patterns. These approximately 24-h cycles govern numerous behavioral and physiological processes in most living organisms. [6, 7] Research in mammalian models has demonstrated that circadian rhythms are essential for memory formation and consolidation. [8] On a molecular scale, circadian rhythms are governed by transcription and translation feedback loops involving "core" clock genes. These include basic helix-loop-helix ARNT-like protein 1 (*Bmal1*) and circadian locomotor output cycles kaput (*Clock*), which encode activators, as well as period circadian regulator (*Per*) *1*, *Per2*, cryptochrome (*Cry*) *1*, and *Cry2*, which code for transcriptional repressors. This core clockwork operates not only in the central pacemaker of the mammalian hypothalamus—the suprachiasmatic nucleus—but also in almost all cell types, including astrocytes and neurons. [9, 10] In physiological states, endogenous circadian rhythms are synchronized with external environmental cues, primarily light, to maintain alignment with the day-night cycle. However, exposure to atypical work schedules, such as shift work, night work, or frequent travel across time zones, can desynchronize endogenous rhythms from the external environment, leading to adverse effects on physiological and behavioral functions. [11, 12]

Many studies have identified CD and sleep deprivation as common features of AD. As single-cell oscillators within an organism, neurons of the central nervous system (CNS) coordinate with specific brain regions to regulate activities such as body temperature, memory formation and maintenance, and melatonin secretion. [13] Increasing evidence suggests that synaptic plasticity, particular neuronal firing frequency, may be regulated by the circadian clock. Disruption of circadian oscillations during cellular memory consolidation has been shown to impair the persistence of hippocampus-dependent memory. [14] Chronic CD, acting as a long-term stressor, may impair hippocampal memory through pathological mechanisms, including neuroinflammation, neuronal loss, neurotransmitter dysregulation, and synaptic degradation, potentially contributing to the neuropathological progression of AD. [15, 16] Neuroinflammation, broadly defined as inflammation of the CNS involving activation of astrocytes and microglia, is increasingly recognized as a contributor to cognitive decline. Furthermore, as neurotransmitters are critical mediators of neuronal communication, impairments in their uptake and

storage can lead to cognitive dysfunction. [17] Although CD and sleep disturbances are often regarded as late manifestations of AD, growing evidence implies that CD may precede the onset of clinical symptoms. [18] The present study revealed an association rather than a direct causal relationship regarding CD and AD, and further research is needed to elucidate this link.

This study intended to explore the causal relationship regarding CD and AD-like pathological changes. Following 90 consecutive days of circadian reversal, the expression levels of Aβ and tau proteins within the mouse hippocampus were measured to assess AD-like pathological alterations. To further examine the pathological events associated with neuroinflammation, we evaluated the activation states of astrocytes and microglia. Additionally, we explored the mechanisms underlying the development of AD pathological changes by assessing the effects of CD on tight junction proteins and the circadian oscillations of clock genes as well as major neurotransmitters.

Methods

Animals and light-dark conditions

Seventy-two 5-week-old male C57BL/6J mice (19–21 g) were obtained from GemPharmatech Co. Ltd (Nanjing, China). The mice were fed in the specific pathogen-free animal facility at the Institute of Environmental Medicine and Occupational Medicine, Academy of Military Medicine, Tianjin Academy of Military Sciences. The room conditions were maintained at a temperature of 20–23°C and 40–60% humidity, with food and water available *ad libitum*. After an interval of acclimatization, the mice were randomly assigned to either the control group or the circadian disruption (CD) group. Mice in the control group were maintained under a standard light-dark cycle (light: 8:00–20:00; dark: 20:00–8:00) throughout the experiment. In the CD group, mice were subjected to a reversed light-dark schedule: for the first 3 days, the light cycle was inverted (dark: 8:00–20:00; light: 20:00–8:00), followed by reversion to the normal schedule (light: 8:00–20:00; dark: 20:00–8:00) for the subsequent 3 day. This 6-day cycle was repeated 15 times for a total duration of 90 days. Upon completion of the modeling, the hippocampuses were dissected and stored at − 80°C for further analyses. The research was approved by the Animal Ethics Committee of the Tianjin Institute of Environmental Medicine and Occupational Medicine (IACUC of AMMS-04-2021-010).

Western blotting

The hippocampal tissue was lysed in radioimmunoprecipitation assay lysis buffer (Solarbio, Beijing, China), and the total protein concentration was determined using a bicinchoninic acid quantification kit (Solarbio, Beijing, China). Equal amounts of protein were separated by polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes. Primary antibodies (Santa Cruz Biotechnology, Shanghai, China) were used: mouse anti-β-amyloid (B-4) (1:500), mouse anti-TauC (1:500), rabbit anti-phospho-tau (Ser396) (PS396; 1:500), rabbit anti-PS404 (1:500), rabbit anti-glial

fibrillary acidic protein (GFAP; 1:500), rabbit anti-ionized calcium-binding adaptor molecule 1 (IBA1; 1:1000), mouse anti-claudin 1 (1:1000), mouse anti-zonula occludens-1 (ZO-1; 1:500), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:10,000). ImmunoPure Goat Anti-Mouse IgG (H + L) or horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (H + L) (1:10,000) served as secondary antibodies. Protein band optical density (OD) was measured using Amersham Imager 680 software (GE Healthcare, USA). Relative protein expression was determined via normalizing the OD of each target protein to that of GAPDH.

Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was performed as previously described, and the detailed procedures can be found in the supplementary data file.[19] The sequences of qRT-PCR primers are shown in supplementary Table 1.

Immunohistochemistry analyses

Brain tissues were paraffin-embedded and coronally sectioned at a thickness of 4 μm. Sections were deparaffinized and then rehydrated through a graded ethanol series. Antigenic retrieval was performed using bovine serum albumin (BSA) blocking solution. Endogenous peroxidase activity was blocked through incubating sections in 3% hydrogen peroxide for 25 min at 20–24°C, followed by triple rinsing using phosphate-buffered saline (PBS). Sections were incubated overnight at 4°C with monoclonal anti-Aβ (1:500) or anti-tau (1:1000) antibodies (Servicebio, China). After washing three times with PBS, sections were incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:300; Servicebio, China) for 50 min at 20–24°C. Following four PBS washes, 3,3´-diaminodbenzidine chromogenic substrate (Servicebio, China) was applied until a brownish-yellow coloration appeared under the microscope. The sections were dehydrated and then sealed with neutral gum after being counterstained with hematoxylin for 1 min. Stained sections were detected using a NIKON microscope (E100, Shanghai, China). For each group, five fields per section were randomly selected and imaged at 400× magnification under consistent field conditions. The ImageJ software was employed to analyze the absorbance of positive staining.

Immunofluorescence analyses

The brain tissues were sectioned coronally with a thickness of 4 μm after being embedded in paraffin. Sections were dewaxed, rehydrated by a graded ethanol series, and blocked with 5% BSA to prevent nonspecific binding. Following triple rinsing with PBS, sections were incubated using monoclonal anti-IBA1 antibody (1:800, Servicebio, China) or monoclonal anti-GFAP antibody (1:500, Servicebio, China) overnight at 4°C. After incubation, sections were rinsed thrice with PBS and then incubated with fluorescently labeled secondary antibodies (1:400, Servicebio, China) for 1 h at 20–24°C. Following thrice additional PBS washes, 4',6-diamidino-2-phenylindole (DAPI; Servicebio, China) was employed to counterstain the sections. Images were captured via a fluorescence microscope (NIKON ECLIPSE C1,

Shanghai, China). For each group, four fields per section were randomly selected and captured at 400× magnification under consistent conditions. The fluorescence intensity was analyzed and quantified by ImageJ software.

Ultra-high performance liquid chromatography-mass spectrometry metabolomics (UHPLC-MS)

UHPLC-MS was carried out following a reported method, and the detailed procedures are also presented in the supplementary data file.[20]

Statistical analyses

All measurement data are showed as mean ± standard deviation (SD). Statistical analyses were carried out with SPSS software (version 25.0; IBM, Armonk, NY, USA). Two - group comparisons were performed using Student's *t*-test. Differences in the expression levels of clock genes and neurotransmitters were evaluated through one- or two-way analysis of variance (ANOVA) followed by post hoc Least Significant Difference (LSD) tests. GraphPad Prism 8 software was used to generate figures and tables. A P value < 0.05 was considered statistically significant.

Results

CD induced AD-like pathological changes

Aβ deposition in the hippocampus caused by CD

To assess CD exposure effects on Aβ protein deposition, we measured the relative levels of hippocampal amyloid precursor protein (APP) and Aβ using western blotting and immunohistochemistry. In the CD group, APP and Aβ protein levels were significantly higher than the control group (Fig. 1A, 1C, 1D). To further evaluate the expression and distribution of Aβ in the hippocampus, we performed immunohistochemical analysis. In comparison with the control group, Aβ expression was notably higher in the hippocampus of CD-exposed mice, as indicated by increased OD (Fig. 1B, 1E). Overall, the immunohistochemistry findings were consistent with the western blotting results.

Tau phosphorylation in hippocampus caused by CD

Similarly, to assess the impact of CD exposure on Tau phosphorylation, western blotting and immunohistochemistry were used to assay total Tau5 protein (t-Tau) and phosphorylated Tau (p-Tau) at the Tau396 and Tau404 sites in the hippocampus. Both immunohistochemical and western blotting assays showed that the expression level of hippocampal Tau5 the CD group was markedly increased in comparison to the control group (Fig. 2A, 2B, 2D, 2F). Western blotting assay findings further revealed

that the expression levels of Tau phosphorylated at the Tau396 and Tau404 sites were also significantly elevated in the CD group (Fig. 2A, 2C, 2D). These results suggest that CD exposure induces AD-like pathological changes in wild-type mice.

CD altered the expression profiles of core clock genes in the mouse hippocampus

To explore CD effects on clock gene expression profiles in the mouse hippocampus, we examined 24-h expression levels of clock genes. Under normal light conditions, one-way ANOVA showed that time had a significant influence on the circadian profiles of *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, NR1D1 or nuclear receptor subfamily 1 group D member 1 (*Rev-erbα*), *Rev-erbβ*, D-site albumin promoter binding protein (*Dbp*), and Circadian associated repressor of transcription (*Ciart*) mRNA ($P < 0.05$). The R^2 values of the fitted cosine curves for all clock genes studied exceeded the significance threshold ($R^2 > 0.25$). Thus, based on both the *P* and R^2 values, robust circadian rhythms of clock genes were observed in the hippocampus of control mice (Fig. 3, Supplementary Table 2). Under reversed light conditions, one-way ANOVA revealed that the 24-h profiles of *Bmal1*, *Per2*, *Cry1*, *Rev-erbα*, *Dbp*, and *Ciart* were still significantly influenced by time ($P < 0.05$), whereas *Clock*, *Per1* and *Rev-erbβ* showed no significant time-dependent variation ($P > 0.05$). The R^2 values and amplitudes of the fitted cosine curves were generally low, indicating that the oscillations in mRNA levels were markedly weakened. Among these, the R^2 values for *Bmal1*, *Per2*, *Cry1*, and *Ciart* exceeded the threshold ($R^2 > 0.25$), whereas *Rev-erbα* and *Dbp* did not. Thus, based on the combination of *P*-values and R^2 values, *Bmal1*, *Per2*, *Cry1*, and *Ciart* retained circadian rhythms, while the rhythmicity of *Clock, Per1, Rev-erbα, Rev-erbβ*, and *Dbp* was lost in the hippocampus of CD mice (Fig. 3, Supplementary Table 2). These results suggest that CD exposure impairs the circadian oscillations of hippocampal clock genes. Alterations in the transcriptional profiles of these clock genes may disrupt brain homeostasis and contribute to the exacerbation of AD-like pathological changes.

CD induced astrogliosis and microglia activation in the mouse hippocampus

Glial cell activation is a key pathological feature of neurodegenerative diseases, including AD. [21] To assess microglial and astrocyte activation, we measured expression levels of the astrocyte marker GFAP as well as the microglia activation marker IBA1. Western blotting results showed that GFAP and IBA1 protein levels were significantly increased in the CD mice compared with the control group (Fig. 4A–C). Consistently, immunofluorescence staining revealed enhanced activation, morphological changes, and significantly increased expression of GFAP and IBA1 in astrocytes and microglia, respectively, compared with the control group (Fig. 4D–G). Overall, these results suggest that CD exposure induces astrocyte and microglia activation, which may exacerbate AD-like pathological changes.

CD altered the expression of tight junction proteins in the mouse hippocampus

In order to investigate the impact of CD on the permeability of the hippocampal blood-brain barrier (BBB), we measured the expression of major tight junction - related genes via RT - PCR and analyzed the levels of corresponding proteins through western blotting. In comparison to the control group, the mRNA expression levels of ZO-1, Occludin, Claudin-1, and Claudin-5 were significantly decreased in the hippocampus of CD mice (Fig. 5A–D). Consistently, western blotting results confirmed that CD exposure led to reduced expression of ZO-1 and Occludin proteins within the hippocampus (Fig. 5E–G). These results suggest that CD disrupts tight junction integrity, thereby increasing BBB permeability and potentially aggravating AD-like pathological changes.

CD induced abnormal expression of hippocampal neurotransmitters in mice

The brain's functional activity depends on the transduction of signals by neurotransmitters between different types of neurons and glial cells. Neurotransmitters are intricately involved in multiple cerebral functions, encompassing memory, learning, emotion, and movement. [22] We used targeted UHPLC-MS to measure major neurotransmitter concentrations in the hippocampus every 4 h throughout a 24-h period. The concentration curves of dopamine (DA), serotonin (5-HT), acetylcholine (Ach), glutamate (Glu), and γ-aminobutyric acid (GABA) over 24 h in hippocampal samples were depicted in Fig. 6. In comparison to the control group, DA, 5-HT, and ACh levels were decreased, while Glu and GABA levels were increased in the CD group (Supplementary Fig. 1A-E). Differences in metabolite concentrations between the groups are further illustrated in the heat map (Supplementary Fig. 2). We assessed the circadian rhythms of the five neurotransmitters over a 24-h period using cosine analysis. In the control group, DA, Ach, and Glu displayed circadian rhythmicity, while GABA and 5-HT did not (Fig. 6, Supplementary Table 3). In contrast, the CD group exhibited loss of circadian rhythmicity in all five neurotransmitters (DA, 5-HT, Ach, Glu, and GABA) (Fig. 6, Supplementary Table 3). Thus, CD disrupts circadian oscillations of major hippocampal neurotransmitters, which may impair hippocampal neural conduction, learning, and memory.

Discussion

With the rapid development of the global economy, CD has become a common phenomenon in modern society. In this study, we simulated CD, a condition frequently encountered in human work or daily life, by reversing the light and dark environments around the mice. The results showed that CD led to Aβ deposition and Tau hyperphosphorylation in mouse hippocampus, indicating that CD may raise the risk of AD. Additionally, CD induced abnormal expression of hippocampal clock genes involved in circadian rhythm regulation, disrupted the expression of neurotransmitters related to signal transduction, triggered neuroinflammatory changes associated with cognitive impairment, and caused dysfunction of tight

junctions in the BBB. Together, these findings highlight a novel pathway through which CD contributes to the onset or progression of AD, accompanied by dysregulated circadian oscillations of clock genes, neuroinflammation, BBB damage, and disturbed neurotransmitter expression.

While many studies have emphasized that CD and sleep disturbances signify common clinical features among individuals with AD, these factors may also exacerbate cognitive impairment. Animal model studies have similarly suggested a potential pathological link between circadian rhythm disruption and AD. [23] However, the precise role of circadian clock disruption in mediating the pathological changes associated with AD following CD remains an open question in the research field. Clock genes are crucial for driving and maintaining circadian rhythms. Chronobiology research has demonstrated that endogenous circadian oscillators regulate learning and memory through neurotransmitter signaling and synaptic remodeling, ultimately affecting cognitive function. [24] Environmental factors such as dietary restrictions, jet lag, shift work, and irregular living habits can all lead to CD in the body. [25] In this study, we found that CD resulted in Aβ deposition and Tau hyperphosphorylation in the hippocampus, accompanied by altered clock gene expression levels, suggesting that circadian clock dysfunction may be one of the factors causing AD-like pathological changes. Studies have found that the expression and distribution of Aβ and Tau protein in the brain have a circadian rhythm. [26] Therefore, CD may cause a sharp increase and aggregation of these proteins, accelerating the process of neurodegeneration in AD. In addition, it has been shown that Presenilin-2 normally cleaves APP, whose expression is activated by *Clock* and *Bmal1*. [27] This indicates that CD and alterations in transcription/translation feedback loops may also impact amyloid clearance. Relevant studies have pointed out that there may be a connection between the abnormal expression of Bmal1 following CD and the abnormal phosphorylation of Tau protein, with which the results of this study are consistent. [28] Therefore, light therapy could become a promising approach to impede the further exacerbation of AD-like pathological changes and may help slow the progression of neurodegenerative diseases.

Recent research has recognized neuroinflammation as the third significant pathological hallmark of AD, following Aβ deposition and NFTs, with the inflammatory response is pivotal in the onset and progression of the disease. [29] Neuroinflammation in the brain predominantly emanates from activated microglia and reactive astrocytes. In this study, we show that CD can induce the over-expression of markers of reactive microglia (Iba1) and astrocytes (GFAP). Clock genes can directly regulate neuroinflammation, and the disorder of clock genes will lead to the obvious activation of astrocytes. Evidence suggests that neuroinflammation accelerates the progression of amyloid and tau lesions, which are further exacerbated by amyloid plaques and Tau tangles. [30] Clinical studies have also shown that microglia activation is positively correlated with Aβ and tau load in patients with AD. GFAP, a marker of astrocyte activation, is associated with amyloidosis, and its expression correlates with Aβ plaque density. [31] In addition, recent findings suggest that destruction of BBB integrity in AD occurs years before the onset of symptoms of cognitive impairment. [32] Our findings indicated that CD disrupted the BBB by reducing the expression of tight junction proteins (occludin and ZO-1). The change in the permeability of the BBB in the hippocampus may lead to the entry of immune cells and pro-inflammatory factors from the periphery into the central nervous system, which can further accelerate the progression

of cognitive dysfunction. [33] Under normal physiological conditions, an intact BBB facilitates the clearance of Aβ and prevents the transfer of peripheral Aβ into the brain, thus avoiding toxic accumulation. However, when BBB permeability is increased, Aβ clearance is impaired, leading to Aβ accumulation and NFTs. [34] Therefore, neuroinflammation and BBB disruption play crucial roles in the pathophysiology CD, contributing to AD-like pathological changes in mice.

Brain function relies on the communication via signals between glial cells and different types of neurons, which is primarily mediated by neurotransmitters. These neurotransmitters can be excitatory, such as Glu, Ach, and DA, or inhibitory, such as GABA, glycine, and 5-HT. Neurotransmitters are actively involved in various brain functions, including movement, emotion, learning, and memory. [35] Studies have shown that the content of neurotransmitters and the expression of related genes and proteins show an approximately 24-hour periodic fluctuation. Light is an important environmental factor in regulating the circadian rhythm of neurotransmitters. Light signals are transmitted to the SCN through the retinohypothalamic tract, and then the expression of clock genes is regulated, indirectly affecting the rhythm of neurotransmitters. [36] Numerous studies have demonstrated that dysregulation of neurotransmitters, including DA, 5-HT, Ach, Glu, and GABA, is related to cognitive impairment in AD. [37] The present study demonstrates that CD disrupts the circadian expression of major neurotransmitters. Ach and DA are key excitatory neurotransmitters involved in learning, memory, and other higher cognitive functions. Recent clinical data indicate that patients with AD exhibit severe deficiencies of both Ach and DA. Additionally, a decrease in 5-HT precursor 5-hydroxytryptophan (5-HTP) has shown a positive correlation with cognitive impairment in AD. [38, 39] These findings align with our observation that neurotransmitter levels are significantly reduced in the brains of mice exposed to CD. In addition, the imbalance of neurotransmitters such as Glu and GABA may promote the phosphorylation of Tau protein by activating related signaling pathways including the calcium signaling pathway and the glycogen synthase kinase-3β pathway, accelerate the formation of NFTs, and further damage the structure and function of neurons. [40] Therefore, CD may contribute to or exacerbate AD-like pathological changes by disrupting the normal regulation of neurotransmitter expression.

However, this study does have certain limitations. First, male mice were used to construct a CD model, and previous studies have confirmed significant sex differences in the regulatory mechanisms of the circadian system, which may limit the model's generalizability to female subjects. Additionally, although this study revealed that CD may induce AD-like pathological changes in mice through dysregulation of core clock genes' circadian rhythms (such as *Bmal1* and *Per1*), the specific roles of clock genes with region-specific expression in different brain areas (e.g., the suprachiasmatic nucleus vs. the hippocampus) in regulating AD pathological cascades remain unclear. This scientific question warrants systematic investigation in future research using tissue-specific gene knockout models and single-cell RNA sequencing.

Conclusions

In conclusion, repeated light-dark reversal induces hippocampal Aβ deposition and tau hyperphosphorylation, accompanied by the dysregulation of circadian oscillations in clock genes in mice. Additionally, CD either induces or exacerbates AD-like pathological changes by activating microglia and astrocytes, disrupting the integrity of the BBB, and causing dysregulation of neurotransmitter expression. With the increasing incidence of neurodegenerative diseases, deeper insights into AD pathogenesis and risk factors may facilitate the development of targeted interventions for vulnerable populations.

Abbreviations

5-HT: Serotonin

Aβ: Amyloid-beta

Ach: Acetylcholine

ANOVA: Analysis Of Variance

APP: Amyloid Precursor Protein

BBB: Blood Brain Barrier

Bmal1: Basic Helix-Loop-Helix ARNT-Like Protein 1

BSA: Bovine Serum Albumin

CD: Circadian Disruption

Ciart: Circadian associated repressor of transcription

Clock: Circadian Locomotor Output Cycles Kaput

CNS Central Nervous System

Cry1/2: Cryptochrome 1/2

DA: Dopamine

DAPI: 4',6-Diamidino-2-Phenylindole

Dbp: D-Site Albumin Promoter Binding Protein

GABA: γ-Aminobutyric Acid

GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

GFAP: Glial Fibrillary Acidic Protein

Glu: Glutamate

HRP Horseradish Peroxidase

IBA1: Ionized Calcium-Binding Adapter Molecule 1

PBS: Phosphate-Buffered Saline

Per1/2: Period Circadian Regulator ½

Rev-erbα: NR1D1 or Nuclear Receptor Subfamily 1 Group D Member 1

RT-PCR: Reverse Transcription-Polymerase Chain Reaction

SD: Standard Deviation

Tau5: Tau Protein (total)

Tau396: Phosphorylated Tau at Ser396

Tau404: Phosphorylated Tau at Ser404

ZT: Zeitgeber Time (the time relative to the light/dark cycle, where ZT0 marks the light onset)

ZO-1: Zonula Occludens-1 (tight junction protein)

LSD: Least Significant Difference (statistical test)

UHPLC-MS: Ultra-high performance liquid chromatography-mass spectrometry metabolomics

Declarations

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of the Tianjin Institute of Environmental Medicine and Occupational Medicine.

Consent for publication

All authors have approved the publication of this article.

Availability of data and materials

Data will be made available on request.

Competing interests

The authors have no conflicts of interest to declare.

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Author Contributions

Honglian Yang and Guojie Zhao contributed to the conception, study design, experimental operation, data analysis, paper writing and critical revision. Yue Lu, Kefeng Ma, Xiaojun She and Xiujie Gao contributed to the experimental operation, data collection. Yingwen Zhu and Xiang Ji contributed to microscope and data analyses. Ying Wang contributed to the conception, study design and paper revision. Honglian Yang and Bo Cui supervised the project. All authors read and approved the final manuscript for publication.

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Figures

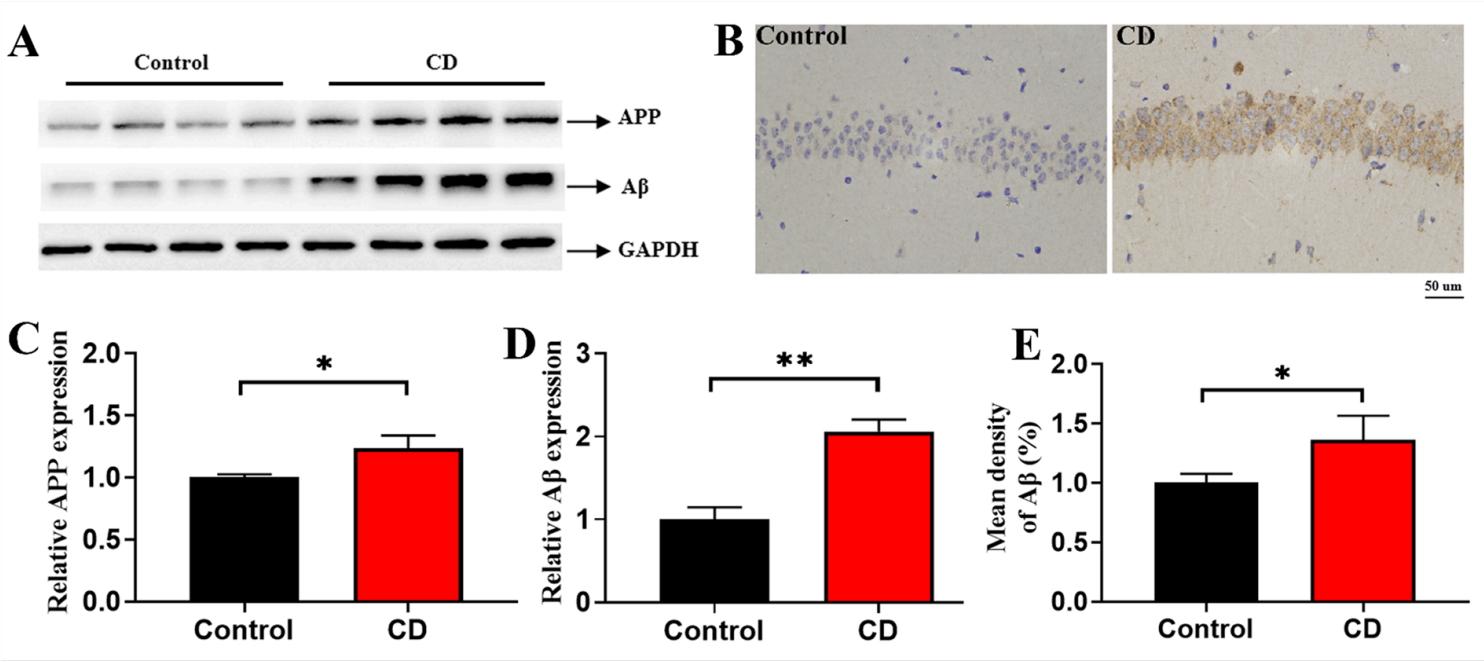


Figure 1

Expression of Aβ protein assessed by western blotting and immunohistochemistry.

(A, C, D) Western blot analysis showed that CD increased the levels of APP and Aβ proteins in the hippocampus (two-sample *t*-test). (B, E) Immunohistochemistry demonstrated that CD significantly increased the optical density of Aβ protein in the hippocampus (two-sample *t*-test). GAPDH was used as an internal reference protein. Data are expressed as mean ± SD, **P*< 0.05, ***P* < 0.01 (n = 4 per group).

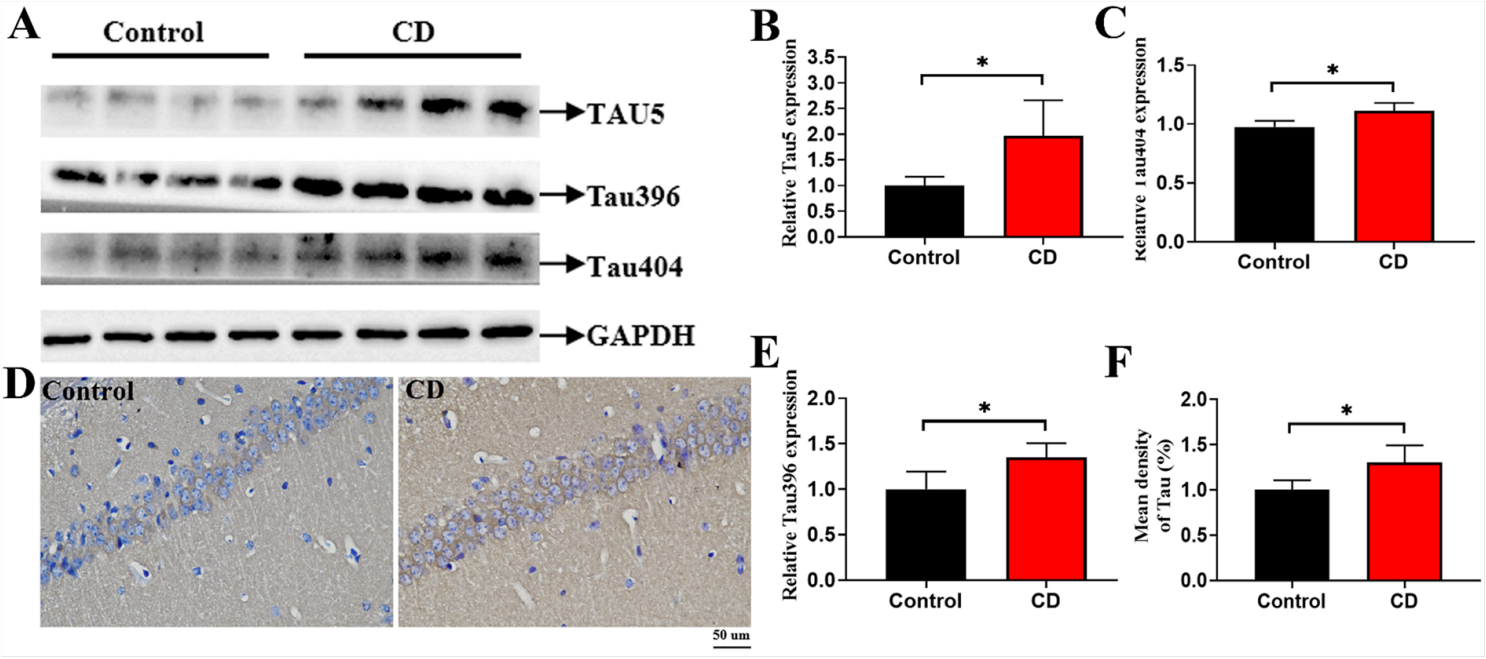


Figure 2

Expression and Phosphorylation of Tau protein by western blotting and immunohistochemistry.

(A, B, D, F) Western blot and immunohistochemistry analyses showed that CD increased Tau5 protein expression in the hippocampus of mice (two-sample *t*-test). (A, C, E) Western blot results indicated that CD induced hyperphosphorylation of tau at sites Tau396 and Tau404 in the hippocampus of mice (two-sample *t*-test). GAPDH was used as an internal reference protein. Data were expressed as mean ± SD, *P < 0.05 (n = 4 per group).

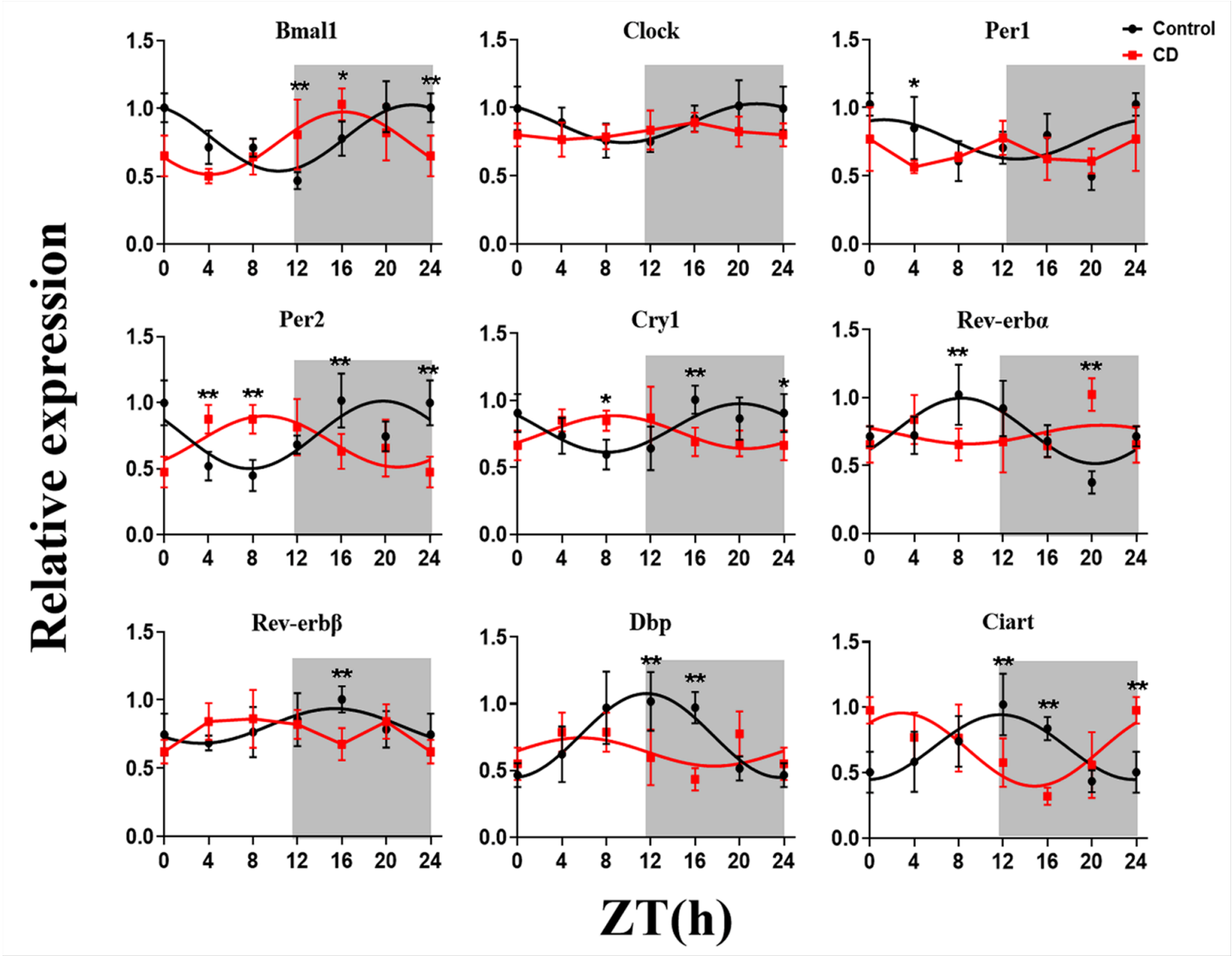


Figure 3

Transcriptional profiles of circadian clock genes in the hippocampus. The expression levels of clock genes are presented as the mean ± SD of five animals per time point. The values at ZT0 are replicated at ZT24. Asterisks indicate significant differences between Control and CD mice, tested by two-way ANOVA followed by LSD multiple comparison test (*$P < 0.05$, **$P < 0.01$). The X-axis represent the ZT, and the shaded areas indicate the dark period.

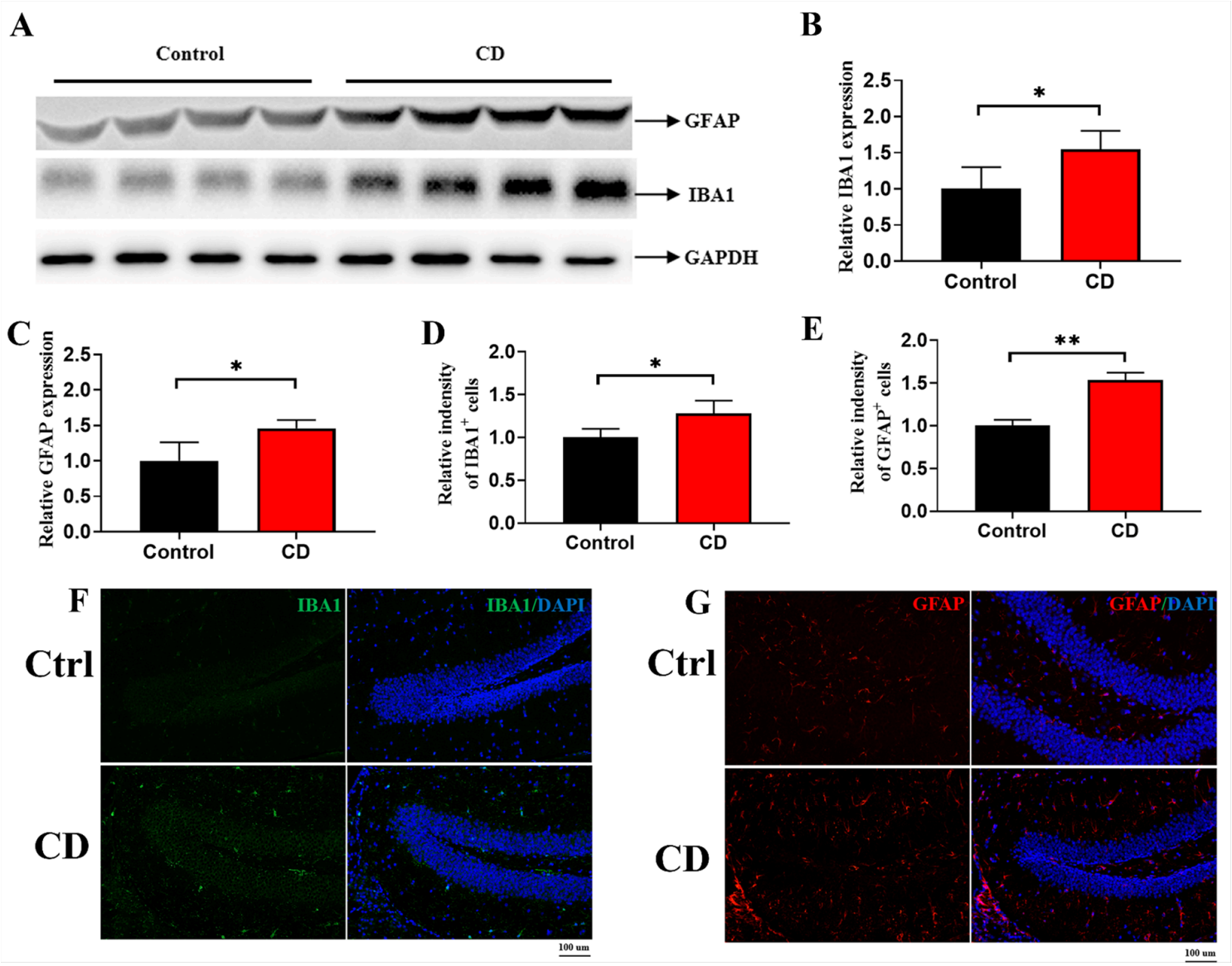


Figure 4

Expression of glial cell markers assessed by western blotting and immunofluorescence.

(A–C) Western blot analysis showed that CD increased GFAP and IBA1 protein expression in the hippocampus of mice (two-sample *t*-test). (D–G) Immunofluorescence analysis showed that CD induced activation of microglia and astrocytes in the hippocampus of mice (two-sample *t*-test). GAPDH was used as an internal reference protein. Data were presented as mean ± SD, **P* < 0.05, ***P*< 0.01 (n = 4 per group).

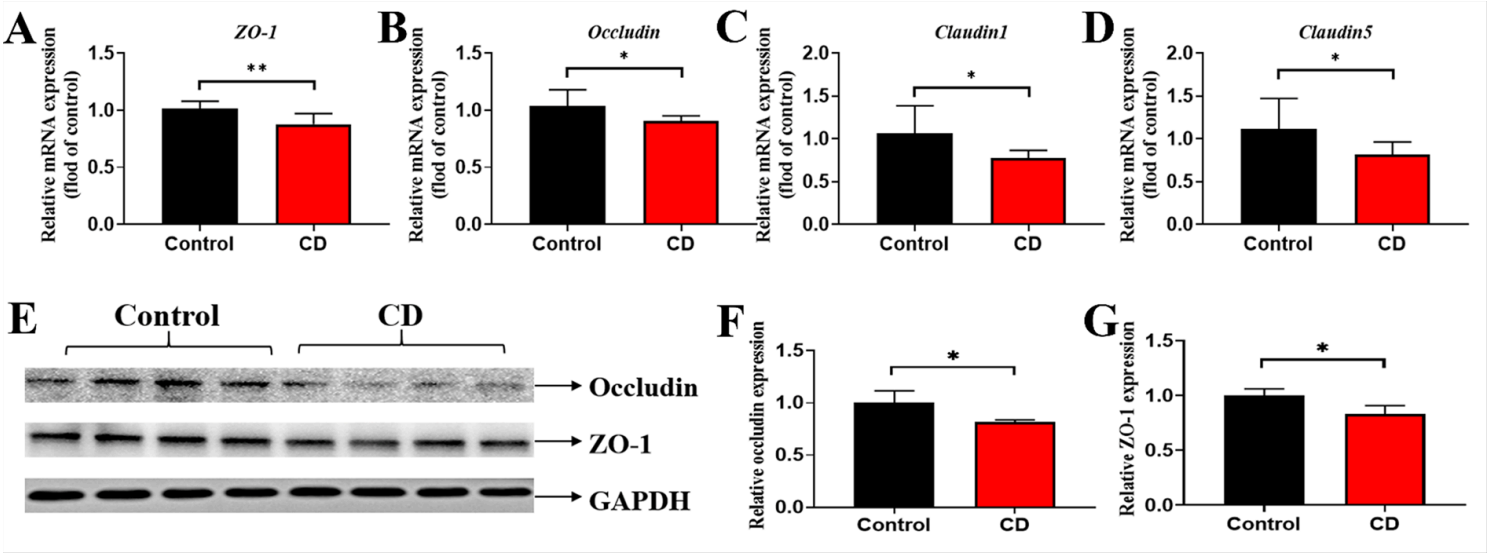


Figure 5

Expression of tight junction genes and proteins was detected using RT-PCR and western blotting.

(A–D) RT-PCR analysis showed that CD reduced the expression of tight junction genes in the hippocampus of mice (two-sample *t*-test, n = 6 per group). (E–G) Western blot analysis showed that CD decreased the expression of tight junction proteins in the hippocampus of mice (two-sample *t*-test, n = 4 per group). GAPDH was used as the internal reference protein. Data are expressed as mean ± SD, **P* < 0.05, ***P* < 0.01.

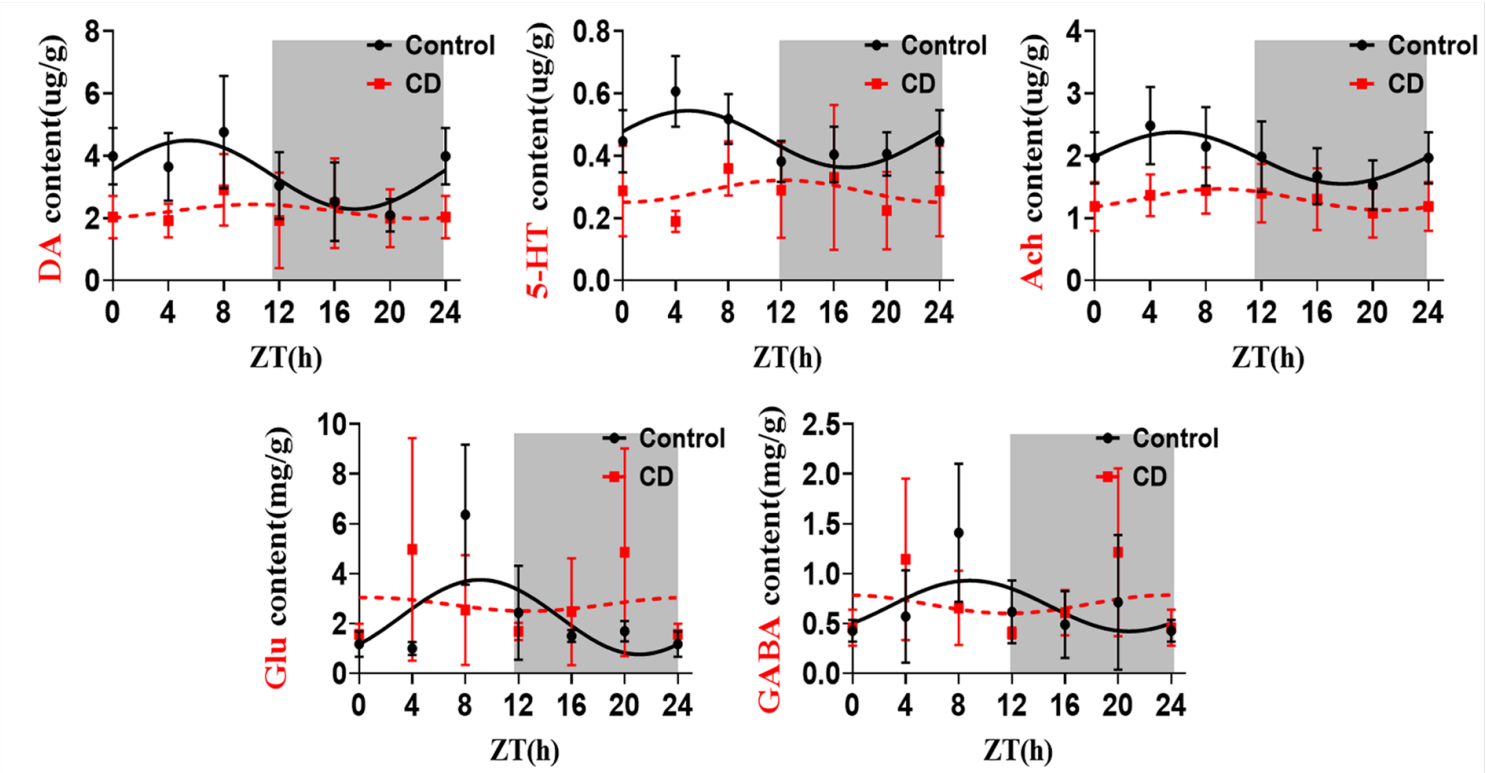


Figure 6

Transcriptional profiles of major neurotransmitters in the hippocampus. The expression levels of neurotransmitters was presented as the mean ± SD of six animals per time point. The values at ZT0 are replicated at ZT24. The X-axis represents the ZT, and the shaded areas indicate the dark period.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx