The role of astrocyte in the circadian rhythm

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Abstract. The circadian system can be found in nearly all mammalian organs and cells. The maintenance of circadian rhythms is related to the health of human life. Destroying circadian rhythms has a strong correlation with the emergence of many diseases, such as neurological diseases and cardiovascular diseases. Astrocytes are the most common type of cell in the human central nervous system. In recent years, the autonomous regulatory role of astrocytes in the circadian rhythm of the SCN has received increasing attention. This article aims to briefly introduce the role of SCN astrocytes in maintaining circadian rhythm from three aspects: gene expression of astrocytes, regulation of neurotransmitters by astrocytes, and plasticity of astrocytes. At the same time, this article also reviews the relationship between astrocyte activation and circadian rhythm disorders as a neurological disease, and several drugs targeting astrocytes for the treatment of nervous system diseases related to circadian rhythm disorders were proposed to highlight the potential of targeting SCN astrocytes in the treatment of improving circadian rhythm disorders. Finally, this article summarizes current strategies, future challenges, and therapeutic prospects for astrocyte-targeted therapy to improve circadian rhythm disorders. This review aims to highlight SCN astrocytes' effect on maintaining the circadian rhythm and their related mechanisms and provide a theoretical basis for the future proposal of targeted treatment strategies with astrocytes.

Keywords: Circadian rhythm, astrocyte, mechanistic pathway, medication.

1. Introduction

Halberg first used the term circadian rhythm to describe the biological oscillations in animals that occur about every 24 hours (h) and are related to the earth's daily rotation cycle. This endogenous rhythm has been observed in other organisms besides animals, such as plants and microorganisms. In the process of biological development, the circadian rhythm makes the biological behavior process consistent with external time, which indicates that changes in the external environment can influence and regulate the behavior of organisms to some extent [1, 2]. The maintenance of circadian rhythm is related to the health of human life. Many prevalent chronic diseases in modern society, such as severe depression and cardiovascular diseases, are closely related to circadian rhythm disorder or interruption of internal clock operation [3]. The circadian system can be found in nearly all mammalian organs and cells. The hypothalamic superchiasmatic nucleus (SCN), a major circadian pacemaker, houses the central clock. The SCN governs the peripheral clock of some local rhythms and functions as a gatekeeper to regulate the timing of cellular responses to external inputs [4].

The largest type of glial cells and those with the greatest distribution in the mammalian brain are astrocytes. They extend and fill the space between the cell body and the processes of the nerve cells [5]. While having previously been associated with preserving tissue homeostasis, it is becoming increasingly clear that astrocytes actively participate in the control of behavior and physiology through changing neuronal circuits, including the adjustment of blood pressure, respiration, fluid balance, reproduction, and glucose homeostasis. In the SCN, astrocytes primarily influence behavior and physiology by controlling the circadian rhythm. Most cells in the body use a transcriptional/post-translational feedback loop (TTFL) to regulate timekeeping, in which the Period and Cryptochrome proteins influence the transcription factors BMAL1 and CLOCK to inhibit their own expression. Clock genes have rhythmic expression in the astrocytes of the SCN. These genes, which are present in the majority of mammalian organs, permit rhythmicity in cells that may differ from the SCN [6]. Brancaccio's experimental evidence showed that astrocytes are capable of starting and maintaining

complicated mammalian behavior on their own [7]. These results show that astrocytes are being recognized as significant SCN circadian clock regulators.

This article aims to introduce the pathway mechanism by which astrocytes maintain normal circadian rhythm of the body in SCN, mainly focusing on the three aspects of rhythm gene expression, neuro-regulation, and astrocyte plasticity, and paying attention to the relationship between astrocytes and diseases caused by circadian rhythm. Finally, the article summarizes the current strategy of astrocyte targeted therapy to improve circadian rhythm disorder and the challenges and treatment prospects in the future.

2. Astrocytes maintain a normal circadian rhythm

2.1. Expression of astrocyte circadian rhythm genes

The basic molecular mechanism for the production and maintenance of circadian rhythm depends on the transcription/translation dynamics of a group of clock genes controlled by a negative feedback loop. These constant cycles of molecular change, which have a cycle of about 24 hours, don't respond to external inputs [8]. The heterodimer formed by the expression of Clock and Baml1 genes is a key component in the negative feedback loop. When it binds to E-box regulatory regions, it can function as a transcription factor and promote the expression of clock genes Cryptochrome (Cry) and Period (Per), ultimately inhibiting the activation of Clock-Bmal1 [3]. Like SCN neurons, SCN astrocytes also exhibit rhythmic oscillations at the molecular level; unlike SCN neurons, the peak time of Cry1 expression and Ca2+ release in SCN astrocytes is almost opposite to the peak time of SCN neurons during the daytime. The circadian rhythms of SCN neurons and the rhythmic oscillations of astrocytes together make up the stable biological clock of the entire organism due to their complimentary nature.

Baml1 is one of the core clock genes that is necessary for maintaining circadian rhythms. Olga Barca-Mayo's research shows that knockout of the Bmal1 gene in astrocytes leads to diurnal rhythm disorders in mice, mainly manifested by changes in circadian activity patterns, and their cognitive abilities are also affected to a certain extent [9]. And Marco Brancaccio et al. have shown specific expression of the cry gene in astrocytes can help mice that have no rhythm regain rhythm, which means Cry1 gene complementation in SCN astrocytes can initiate and maintain circadian rhythm activities in mammals by enlisting potential SCN neural networks [7]. The above experimental results demonstrate the clock-related genes' expression in astrocytes can play a positive role in maintaining circadian rhythms.

2.2. Regulation of SCN astrocytes on neurotransmitters

2.2.1. Glutamate-mediated circadian rhythm maintenance

Glutamate is the brain's most abundant free amino acid and its primary excitatory neurotransmitter involved in neural signal transmission. However, excessive glutamate in the extracellular and synaptic regions can lead to overexcitation and neuronal death. Therefore, extracellular space must rapidly clear any glutamate that is not used during synaptic transmission. Astrocytes have a limited ability to absorb and release glutamate, which helps to control glutamate levels and maintain stability in the surrounding environment of the nervous system. The regulation of glutamate by astrocytes involves three processes: glutamate uptake, glutamate metabolism, and glutamate release (Figure 1). Na+ independent and Na+ dependent glutamate transporters are the two main types of glutamate transporters that facilitate glutamate uptake, the latter being also called excitatory amino acid transporters (EAATs). The glutamate levels in synaptic spaces are mainly regulated by EAATs. Each time a cell ingests one glutamate, it cooperatively transports three sodium ions, while one potassium ion is discharged outside the cell. At the same time, compared to other cell types, the abundant adenosine triphosphate (ATP) in astrocytes also provides the possibility for their sustained uptake of glutamate. Glutamic acid ingested from synaptic gaps is metabolized in astrocytes through two pathways: one is to convert glutamic acid into glutamine through glutamine synthetase. Glutamine

can be released out of the astrocyte through the neutral Na+ dependent transporter SN1 and enter neurons to participate in neurotransmitter synthesis; another way is by oxidizing glutamic acid to form α- Ketoglutarate can participate in the TCA cycle and then produce a large amount of ATP. When the extracellular glutamic acid concentration is high, it is beneficial for the oxidative metabolic pathway of glutamic acid to proceed, thereby producing more ATP and further enhancing the uptake ability of astrocytes. Glutamate can also be temporarily stored in astrocytes after ingestion and released by the cells at appropriate times. There are generally two types of release mechanisms: Ca2+ dependent exocytosis and many other Ca2+ dependent or independent mechanisms, among which Ca2+ independent mechanisms include the dual pore domain potassium channel TREK-1 and others [10].

Astrocytes can regulate extracellular glutamate concentration through the above mechanisms. As a neurotransmitter, astrocytes can activate SCN neurons during the day and inhibit SCN neurons at night, affecting the rearrangement of circadian rhythms. According to Brancaccio's research, dorsal SCN neurons express NMDA receptors (NMDARs) (NR2C), one of the processes through which SCN neurons gauge glutamate levels. The concentration change of glutamate produced by astrocytes can be perceived by NR2C, which makes NR2C specifically participate in the regulation of SCN circadian rhythm. The study dampened circular complications in the SCN and prolonged its cycle by selectively suppressing NR2C [11].

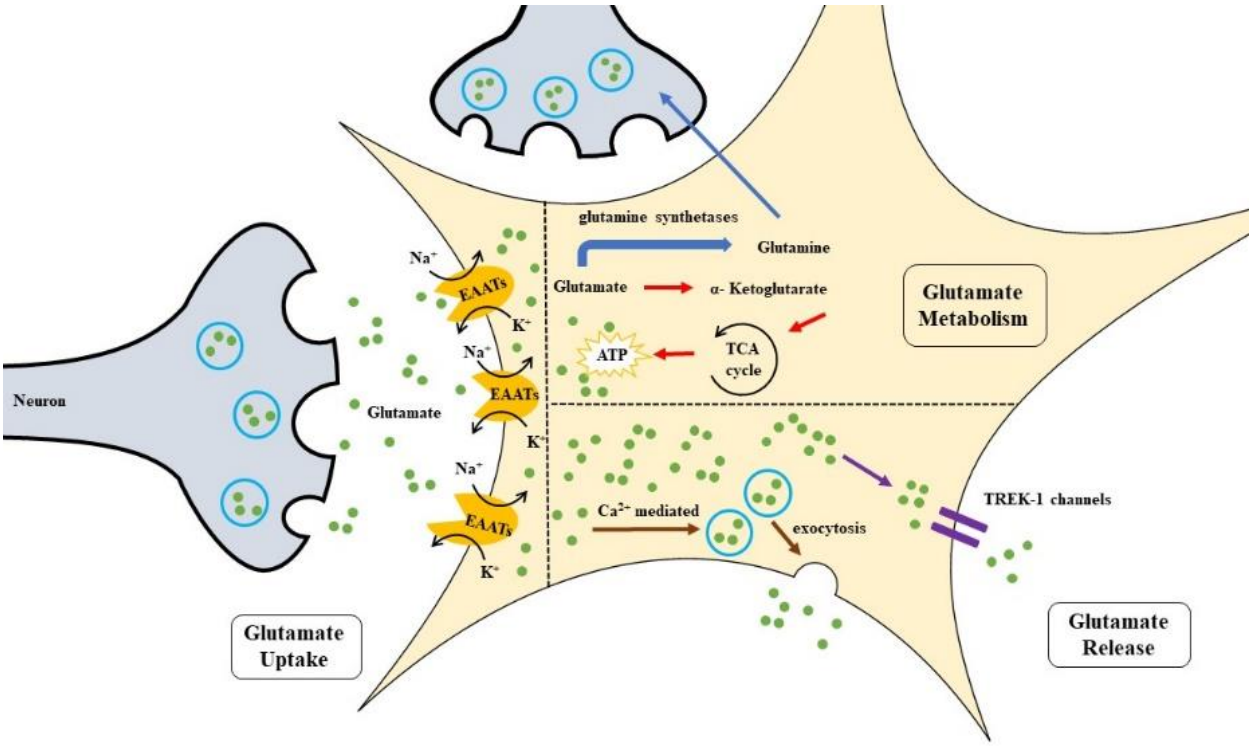

Figure 1. Schematic diagram of the regulatory effect of astrocytes on glutamate [10]

2.2.2. γ-aminobutyric acid (GABA) mediated circadian rhythm maintenance

The neuronal networks of the various areas of the brain depend on GABA, a significant inhibitory neurotransmitter. Notably, GABA is expressed by practically all SCN neurons. Although SCN neurons are the source of GABA production, astrocytes are primarily responsible for the daily rhythm of GABA absorption, which regulates the circadian rhythm dynamics of significant amounts of GABA in extracellular space [12]. The cellular interactions between SCNs are also crucial for the stability and unity of the rhythmic biological clock. The daily oscillations of GABA synthesis, transportation, and release have an impact on the coupling process between SCNs on separate sides, which also necessitates GABA mediation. GABA energy signal transduction is essential for the dorsal and ventral SCNs to oscillate evenly. By analyzing the PER2: LUC rhythm of SCN slices, Evans et al. demonstrated GABA mediates the dorsal ventral coupling of long-day coding in SCN. They

arrived at the conclusion GABA has a state-dependent influence on how circadian rhythms' synchronization [13].

2.3. Plasticity of astrocytes

High levels of structural plasticity are present in astrocytes in the SCN. Endocytosis is the mechanism by which the plasma membrane undergoes these astrocytic structural changes. The factors that lead to the structural plasticity of astrocytes and the mechanism that leads to the establishment of circadian rhythm are still unknown [14].

The SCN plays a vital part in regulating the circadian rhythm of the entire nervous system by regulating the behavior of secondary circadian oscillators in other brain regions. In the molecular sense, the hippocampus exhibits circadian rhythm fluctuations in several synaptic excitability-related genes and proteins. For instance, during the circadian rhythm's dark phase, the expression of Per2 is substantially enhanced in the pyramidal and radiative layers of the CA1 region of the hippocampus. At the cellular level, the astrocyte coverage of postsynaptic density decreases in the dark period of the circadian clock. These structural alterations show how astrocyte plasticity contributes to circadian rhythms [15]. The SCN's outer shell expresses a plastic neural protein known as doublecortin-like (DCL). Astrocytes that express DCL specifically surround AVP-positive cells in the SCN's outer layer. According to Coomans' research, mice with DCL knockdown had a shortened circadian period and a weaker rhythm. At the same time, they react to changes in the light-dark cycle more quickly than WT mice—roughly twice as quickly. The hypothesis that a subset of astrocytes' plasticity regulates circadian behavior serves as further evidence of the astrocytes' expanding importance in timekeeping [14].

3. Association between SCN astrocytes and disease induced by rhythm disorders

Astrocyte activation has been associated with increased production of the cytoskeletal protein glial fibrillary acidic protein, is a frequent response to brain damage that can range from neurodegeneration to trauma. There are many different astrocyte activation phenotypes, and astrocytes triggered by various stimuli have distinctive transcriptional patterns that may be connected to various phenotypes. Although it is unknown how circadian clock disturbance and astrocyte stimulation interact, they are two common and ubiquitous characteristics of neurological disorders [16].

When the primary circadian gene, Bmal1, is absent, all circadian clock activities are lost. This deletion can also lead to astrocyte activation and proliferation, which may enhance oxidative stress and inflammation. According to research by Lananna Brian V. et al., the BMAL1 protein synergistically controls astrogliosis through less potent non-cell-autonomous neuronal signaling and a cellular autonomic pathway [16]. The type of the inciting stimulus determines the reactive phenotypic polarization. Astrocyte reactivity is incredibly heterogeneous, not just one response to brain injury or trauma. A1 and A2 are two different functional phenotypes that quiescent astrocytes can adopt, according to research published in 2017 by Liddelow and Barres [17]. A2s has a proliferative function that results in the creation of glial scars, the elimination of debris, and the restoration of the blood-brain barrier while adhering to the typical reactive astrocyte spectrum. In addition to suppressing processes essential to promoting neuronal survival and synaptogenesis, neurotoxic A1 reactive astrocytes have proinflammatory features and are linked to neurodegeneration and persistent neuropathic pain. In actuality, BMAL1-deficient astrocytes spontaneously polarize into an A1-like phenotype. Additional investigation into this mode of cellular autoregulation leads to the hypothesis that BMAL1 expression blocks glutathione transferase signaling to prevent proinflammatory astrocyte responses [16].

4. Current therapeutics target astrocytes

Circadian rhythm disorder refers to the internal biological clock of an organism that is not synchronized with the environmental light cycle, resulting in sleep disorders, mental disorders, and other issues. Currently, the treatment methods for circadian rhythm disorders mainly include drug treatment and non-drug treatment [18]. Current studies have shown that astrocytes are crucial to the control of circadian rhythms. Therefore, some drugs targeting astrocytes have been proposed and are expected to become new strategies for treating circadian rhythm disorders [19]. However, there is currently no specific drug that has been proven to directly target astrocytes to treat circadian rhythm disorders. Due to the strong correlation between circadian rhythm disorders and neurological diseases, targeted astrocyte therapy can be used to improve neurological diseases and indirectly improve circadian rhythm disorders. Currently known related drugs and their therapeutic mechanisms are shown in Table 1. In the future, more research on targeting astrocytes will be needed, and more effective treatment options will be explored in combination with clinical practice. In summary, the treatment of circadian rhythm disorders requires a comprehensive consideration of medication, non-medication, and a healthy lifestyle. In the future, drugs targeting astrocytes are also expected to become a new treatment strategy.

Table 1. Existing drugs targeting astrocytes for the treatment of neurological diseases

Drug	Targeted therapeutic effect	Experimental evidence
Fingolimod[20]	Fingolimod can alleviate inflammatory reactions and damage in nervous system diseases by inhibiting the proinflammatory state of astrocytes.	After treatment with Fingolimod, the expression level of proinflammatory substances in IFN-γ activated astrocytes decreased, while Fingolimod can also inhibit activation of NF- κB signaling pathway and expression of its downstream effector genes.
Bushi[21]	By inhibiting the Ca2+transport channel TRPV4 in the anterior spinal cord of astrocytes, which causes calcium ion reactions, it reduces the communication between neurons and astrocytes, thereby inhibiting neuropathic pain.	Using Bushi to treat neuropathic pain in a mouse model can significantly reduce its behavioral responses and sensory thresholds and has shown good results in pharmacokinetic and safety tests in vivo.
Siponimod[22]	Siponimod can induce activation of the Nrf2 signaling pathway and inhibit the NF-κB signal pathway to alleviate astrocyte-induced neuronal damage and neurodegenerative diseases.	Siponimod can promote Nrf2 nuclear translocation and ARE gene expression, while reducing NF-κB nuclear translocation and downstream effector gene expression further combat astrocyte induced neuronal damage.

5. Challenges and treatment prospects

Recent advances in technology have made it possible to discover new roles for astrocytes in both health and disease, indicating their possibilities as targets for drug therapy. Hong Yun Lee summarized current astrocyte-targeted therapy strategies, mainly including: 1) regulating astrocyte function using genetic techniques, RNA interference, or CRISPR/Cas9 techniques to treat neurological diseases. 2) Stem cell technology is used to replace astrocytes that have lost their function due to neurological damage and restore their normal function. 3) Using drugs to interfere with astrocyte metabolic pathways, such as natural compounds such as mannitol and glycyrrhizin, can treat neurological diseases by improving astrocyte energy metabolism and oxidative stress status. 4) Improve the protective effect of astrocytes on neuronal apoptosis through immune regulation, as astrocytes may lose this protective effect in certain neurological diseases [23]. Astrocytes are critical regulators of circadian rhythms. The in-depth study of the function and physiological mechanisms of astrocytes can provide new ideas and methods for the treatment of circadian rhythm disorders and related diseases. A number of studies are now being undertaken on the involvement of astrocytes in nervous system illnesses and treatment strategies, especially in the field of neurodegenerative diseases,

and certain progress has been made. However, a core challenge in targeting astrocyte therapy is the existence of functional subsets that have a clear role in health and disease and that exhibit significant differences between regions of the central nervous system, diseases, or disease states. In the future, it is expected to improve or cure neurological diseases through the use of genetic engineering and stem cell technology, the development of novel medications, and their use in immune modulation, thereby ultimately improving the treatment of circadian rhythm disorders [23].

6. Conclusion

Much attention has been paid to the function of astrocytes in regulating circadian rhythms. Astrocytes have a powerful circadian clock function, and the circadian rhythm oscillations of SCN neurons and SCN astrocytes complement each other to form a stable circadian rhythm. Destroying and restoring clock gene expression in astrocytes can exhibit surprising phenotypes. Astrocytes can communicate with SCN neurons by sensing and regulating extracellular neurotransmitters. At the glycine level, astrocytes have complete regulatory pathways from uptake to metabolism to release. GABA is essential for the conjugation of dorsal and ventral SCNs. The decrease in the expression of neural plasticity proteins and the weakening of the circadian rhythm in mice suggest the increasing importance of astrocyte plasticity in timing. At the same time, astrocyte activation is a significant marker of brain damage in neurological diseases. Although there are currently no drugs targeting astrocytes to directly treat circadian rhythm disorders, many studies have demonstrated that targeting astrocytes can be used to improve neurological diseases. The current challenge in targeting astrocytes is primarily the specificity of astrocyte subsets, which exhibit significant differences between regions of the central nervous system, diseases, or disease states. In the future, with in-depth research on the functions and physiological mechanisms of astrocytes, it is expected to improve or cure neurological diseases through the use of genetic engineering and stem cell technology, the introduction of novel medications, and their use in regulating the immune system, thereby achieving the goal of improving circadian rhythm disorders.

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