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The function of GABA in the suprachiasmatic nucleus and its effect on circadian rhythm

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Abstract. Gamma-aminobutyric acid (GABA) is one of the neuron transmitters commonly found in mammals. Previous works have proved the inhibitory nature of GABA in many activities it partakes in. However, recent studies have found that GABA could act as an excitatory factor in certain neural pathways especially in the suprachiasmatic nucleus (SCN) region of the hypothalamus. Circadian clock or circadian rhythm is critical among organisms' reaction and regulation to the time of day which helps the organisms maintain homeostasis to the changing weather and seasons. This process can be found in nearly all kingdoms of organisms, particularly which is regulated by the SCN region of the hypothalamus in mammals. In recent years, the potential link between GABA and sleep or circadian rhythm became a new topic among many researchers. Studies have found that GABA has an important effect on circadian rhythm. The excitatory or inhibitory effects of GABA in the SCN is dependent on the concentration of intercellular chloride ion, exposure to day-light and several other factors. GABA can also regulate the concentration level of intercellular ion in the SCN and thus achieve the self-regulating GABAergic neurons. The coupling of the circadian rhythms which is the essential process to synchronize the neuron cell firing rate is also mediated by GABA.

Keywords: GABA, suprachiasmatic nucleus, circadian rhythm, neural science, molecular mechanics.

1. Introduction

Gamma-aminobutyric acid (GABA) is a neurotransmitter, a chemical messenger in the mammalian central nervous system and has good water solubility and thermal stability. It usually acts as an inhibitor to multiple neural pathways, reducing the excitability of neuron cells, slowing down the reflect time of many brain functions. Outside the nervous system, GABA can also be found in immune cells and peripheral tissues including kidneys, liver, lungs, pancreas, immune system and the corresponding bioactive properties of GABA are: antidepressant, sedative, antihypertensive, antidiabetic, anticancer and immune system enhancer. Like other neurons, GABAergic cells also have distinct receptors for GABA to convey the signals. There are two types known GABA receptors: $GABA_A$, which is part of a ligand-gated ion channel complex and $GABA_B$, which are G protein-coupled receptors that can open or close ion channels with the intermediaries. GABA can also be found in plants. Evidence suggests it can act as a signaling messenger in plants.

Suprachiasmatic nucleus (SCN), is a small region of the brain in the hypothalamus, located directly above the optic chiasm. Which is the principal circadian pacemaker in mammals, responsible for

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generating and controlling circadian rhythms. The circadian rhythm can regulate multiple aspects of the whole body, including metabolic activities, sleep, body temperature, immune function, physical activity and digestive activity. The circadian clock is directly regulated by the BMAL and CLOCK gene in the SCN neurons, and receive inputs from retinal ganglion cells in the eyes which are photosensitive to lights to adjust the body circadian clock to the environment. In the past decades many studies on GABA and SCN were conducted and they found that the SCN is a network of cells containing γ-amino butyric acid (GABA). GABA synthesis and transporter proteins as well as GABA receptors are expressed in more than 90% of SCN neurons. So there is a clear connection between GABA and SCN, or to put it in other ways, GABA should have a connection to the circadian clock. This review will discuss what we know about the connection between GABA and the circadian clock.

2. GABA's excitatory function in the SCN

According to the various studies conducted in the late 20th century and early 2000s, the typical function of GABA is mostly inhibitory, reducing the sensitivity of central nervous system and help the organism to recover from fear, stress or anxiety. Yet around the 2010s many studies (shown in the following) have shown evidence that GABA could act as an excitatory factor in SCN regulatory pathways. For the past few years, the effects of GABA in the SCN region have been studied and yet the results remain controversial. GABA's main function includes both excitatory and inhibitory effects. Whether GABA networks are excitatory or inhibitory has been shown in models experimentally, depending on the intracellular chloride concentration [1-3]. This indicates GABA could be regulating the ion concentration in the brain, thus perform an excitatory function in the SCN region. Farrant M. and Nusser Z.'s work has shown that the endogenous excitatory responses of SCN to GABA might play a crucial role in regulating phase shifts to light and nonphotic phase shifts within the SCN. The impact of GABA in the suprachiasmatic nucleus (SCN) is mainly facilitated by two key types of receptors: $GABA_A$ receptors and $GABA_B$ receptors (referred to as $GABA_A$Rs and $GABA_B$Rs, respectively). $GABA_A$Rs are one type of ligand-gated chloride ion channels, and their subunit compositions influence their pharmacological characteristics, subcellular distribution, and circadian regulation within the SCN [4,5]. While $GABA_B$Rs are metabotropic G-protein-coupled receptors located on presynaptic, postsynaptic, and extrasynaptic membranes [6,7]. GABA can influence the SCN pacemaker's ability to adjust its phase in response to light by interacting with both $GABA_A$Rs and $GABA_B$Rs. [8-13]. The circadian pacemaker interprets the duration of the daily light period as a signal of seasonal changes, with longer light periods in summer and shorter ones in winter [14]. Notably, cation-chloride cotransporters in the SCN may react to variations in the photoperiod. Farajnia S. et al. demonstrated that extended exposure to daylight increases the proportion of SCN neurons that respond to GABA. [15]. Further supporting this point, Liu, Chen et al.'s work suggest that longer daylight exposure enhances the excitatory effect of GABA in the SCN, thereby increasing the SCN's response to light stimulation [16].

GABA's excitatory function has been discovered in the past, specifically during the early development of brain. GABA regulates the proliferation of neural progenitor cells; the migration, differentiation and the elongation of neurites and the formation of synapses. It is only in recent years Liu, Chen et al. found that GABA has excitatory effects in adult brain, and GABA may act as an excitatory factor for the SCN under the right conditions, enhancing its performance in response to light and playing a vital role in regulating phase shifts to light.

3. GABA's inhibitory function in the SCN

We discussed the excitatory effects that GABA has in the SCN region. However, because GABA is the major inhibitory transmitter in brain. GABA's function in the nervous system should still be mostly inhibitory, which are supported by many studies. The main function of GABA in the SCN region shows no difference, that is to say GABA inhibits certain transmission pathways in the SCN region thus inhibits the regulation of the circadian clock in most situations, resulting in the mammal's prolonged reaction to the light-dark cycle. Wagner, S. et al. concludes with the following statement that the firing rates of clock cells are inhibited by GABA at all circadian times [17]. Wagner, S. et al. conducted experiments

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by adding 100 μM GABA to cell culture media for either 1 hour or 6 hours at different circadian cycle phases. They observed that all clock cells (n > 50) exhibited complete inhibition of neuronal firing, regardless of the circadian phase, suggesting that individual clock cells do not show varying sensitivity to GABA's acute inhibitory effects. The only distinction between the 1-hour and 6-hour GABA treatments was that the duration of inhibition matched the length of exposure. Additionally, Tillman L. et al. found that GABA acts as an inhibitor in the SCN, diminishing its sensitivity to light stimuli. GABA serves as an inhibitory neurotransmitter at night, lowering neuronal firing rates, while during the day, it acts as an excitatory neurotransmitter, increasing firing rates [18]. The firing rate of neurons directly reflects their activity. Neurons with higher firing rates respond more quickly to stimuli, while those with lower firing rates respond more slowly. And regarding the regulation of circadian rhythms, neurons maintain a high firing rate during the day when light stimuli are strong and low firing rate at night when light stimuli are nearly absent. This mechanism is not only logical and intuitive but also consistent with the data recorded in these studies.

To conclude this section, shifting the overall circadian clock in the SCN, GABA could act as a responder to light, which could both be excitatory and inhibitory. Yet by acting on receptors to regulate the SCN is merely small part of the functions performed by GABA in the central nervous system. GABA still mainly regulates the nervous system by changing the activity of ion channels on the cell membrane. In the SCN, GABA introduces the change in the concentration level of chloride ions, thus changes the firing rate of the neuron cells, and further affects the circadian clock regulation pathways, leading to the prolonged jet-lag effects.

4. GABA mediates the ion equilibrium of SCN

Neural signals rely heavily on the correct extracellular ion concentration level, and the nervous system utilizes multiple regulatory molecules to ensure ion equilibrium to maintain the ideal neural activity. When combined with GABA, GABA receptor A will result in the influx of chloride ion ([Cl−]) and cause the membrane to enter the hyperpolarization status. In this status, the neuron cells will require a much higher stimulation to reach the threshold potential, which is the basic mechanism for the inhibitory effect of GABA on the GABAergic cells. Yet in the brain, there are more ion channels which is within the effect range of GABA, including the cation-Cl-cotransporter (CCC) NKCC1and KCC2.

The polarity of GABAergic neurons is influenced by the GABA equilibrium potential (EGABA), which is modulated by the intracellular concentration of chloride ions ([Cl−]i) [19]. In the case of suprachiasmatic nucleus (SCN) neurons, elevated levels of [Cl−]i result in a more positive EGABA, thereby facilitating depolarization through GABA signaling. Conversely, reduced [Cl−]i leads to a more negative EGABA, which induces inhibitory hyperpolarization [19]. Nonetheless, the precise mechanisms underlying GABAergic signaling remain ambiguous, and there is ongoing discourse regarding the classification of GABA as either an excitatory or inhibitory neurotransmitter [20]. The regulation of intracellular chloride ion concentration is predominantly governed by two cation-chloride cotransporters: NKCC1 and KCC2 [21]. NKCC1 facilitates the influx of chloride ions into the cell by utilizing the sodium gradient established by the Na+/K+/ATPase, whereas KCC2 is responsible for the efflux of chloride ions from mature neurons [22]. During neuronal development, there is a decrease in NKCC1 expression coupled with an increase in KCC2 expression, which results in elevated chloride ion concentrations in immature neurons and diminished levels in mature neurons. Consequently, as neurons progress in maturation, EGABA transitions from a depolarizing to a hyperpolarizing state, underscoring the critical roles of KCC2 and NKCC1 as regulators of GABA-mediated hyperpolarization and as essential components for inhibiting synapses in the adult mammalian brain [23,24]. The interplay between KCC2 and NKCC1 is crucial for maintaining intracellular chloride ion concentrations, which is fundamental in determining neuronal polarity [19]. In the early stages of development, immature neurons exhibit high chloride ion concentrations and demonstrate depolarizing responses upon activation, primarily attributable to elevated NKCC1 expression [23]. As neuronal maturation occurs, the expression of NKCC1 declines while that of KCC2 rises, leading to reduced chloride ion levels and a transition from excitatory to inhibitory responses [23]. This highlights the important function of the

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coordinated action of NKCC1 and KCC2 in regulating neuronal polarity [24]. Furthermore, McNeill JK 4th et al. suggests that NKCC1 levels may be influenced by the duration of light exposure, potentially providing a regulatory pathway for animals undergoing seasonal behavioral changes or entering hibernation [25].

5. Coupling and decoupling

The neurons sometimes do not act as individual cells, one chunk of the brain or one region can behave as a whole, the neurons they contain could be in action in sync, this is the result of coupling. The SCN is a typical region that has been observed to have this coupling effect, which is important for the circadian clock to function. In some *in vitro* culturing experiments for sliced SCN, these cells seem to be able to maintain the circadian rhythm. But when separated to single cell cultures the circadian clock is out of sync, which is different from *in vivo* studies and whole/sliced SCN culturing, this indicates there must be some synchronization methods for the SCN *in vivo* and GABA could play an important part in it. Vasoactive intestinal peptide (VIP) has been found to play a critical role in the synchronization of suprachiasmatic nucleus (SCN) neurons [26]. GABA, however, has a more complex role, with studies suggesting it can either synchronize or desynchronize SCN neurons [27,28]. Itri et al. demonstrated that exogenous vasoactive intestinal peptide (VIP) enhances gamma-aminobutyric acid (GABA) secretion from suprachiasmatic nucleus (SCN) neurons, suggesting a potential relationship between VIP and GABA [29]. And Evans J.A. et al. established that GABA counteracts VIP-mediated synchrony under steady-state conditions, while simultaneously facilitating resynchronization during long-day-induced antiphase conditions [30]. Their research revealed bimodal patterns of spontaneous impulse activity in the dorsal and ventral SCN following a six-hour shift in the light schedule. Subsequent analysis of SCN slices indicated the presence of a fast-resetting oscillator in the ventral SCN and a slow-resetting oscillator in the dorsal SCN. Experiments utilizing the GABAA antagonist bicuculline yielded results consistent with those observed in the slice-cutting experiments; specifically, brief applications of bicuculline at various phases of the circadian cycle resulted in increased electrical activity in the ventral SCN, while unexpectedly decreasing activity in the dorsal SCN.

GABA is implicated in the transmission of phase information between the oscillators of the ventral and dorsal SCN. In the dorsal SCN, GABA functions as an excitatory neurotransmitter, whereas it exerts an inhibitory effect on neuronal activity in the ventral SCN. Albus et al. hypothesize that this differential action contributes to asymmetric coupling between the SCN regions, with the ventral SCN exerting a more pronounced phase-shifting influence on the dorsal SCN than the reverse. In light of this asymmetry and the pivotal role of GABA in phase regulation, a novel model has been proposed for further investigation [31]. The GABAergic cells in SCN could regulate the activity of GABA receptors in response to light stimulation, thus changing the activity of GABAergic ion channel. In this process, GABA can affect the synchronization (coupling and decoupling) and polarization of SCN, and then regulate the circadian rhythm in the cell or the whole SCN region.

6. Conclusion

This brief review discussed the role of GABA in the regulation of circadian clock. Although GABA has a mostly inhibitory function in the nervous system, it does show some excitatory properties in the SCN region within the pathways regulated by circadian rhythm. With proper conditions, light exposure and extracellular/intercellular ion concentration levels can make GABA perform an excitatory effect on the SCN, increase the SCN's sensitivity to light stimuli, enhance the firing rate of the cells in the SCN region, and thus help the body to adjust to shifts in circadian rhythm. Yet when the circadian clock is in the "night phase", GABA will act as an inhibitory neuron transmitter as it is seen in most other regions in brain, decreasing the firing rate of the cells in SCN. This peculiar behavior should be the result of the change in the expression levels of certain circadian-related genes such as BMAL and CLOCK. We have observed the GABA receptors' effect on cation-chloride cotransporters NKCC1 and KCC2, but how this change in the ion channels is affected by the circadian clock genes is still unclear, which could be further investigated.

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Based on the current understanding of circadian rhythm, we can confirm the SCN regulates sleep and wakefulness. However, the details regarding the fundamental regulation pathways are still unclear. The mammalian nervous system is complex both in the structural and functional aspects. It is not sufficient to use independent studies as evidence to conclude the GABA's effect on the circadian rhythm to understand the roles of GABA in the circadian clock, which is needed to identify the specific neuronal networks and the interlink between these and the output pathway of the SCN. Gene insertion and editing could be useful in this scenario but to ensure the model animals about surviving long enough to perform a thorough examine on these pathways is a hard roadblock to bypass. In recent years, a self-inactivating rabies virus method is developed to provide life-long genetic and functional access to neural circuits [32]. Combined with the steadily improving *in vivo* imaging of neural activity including the methods like two-photon microscopy and fluorescence microscopy [33], we can expect future researchers to implement these new methods to provide a rich result of GABA's role in the SCN and its effect on the circadian clock, providing insights for new treatments to related diseases and pushing the frontier towards the ultimate goal of uncovering the functions of the human brain.

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