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Astrocytes and the Modulation of Sleep

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Abstract

Astrocytes are being identified as having multiple roles in sleep. Initially they were shown to modulate the process of sleep homeostasis through the release of adenosine which acts on adenosine A1 receptors (A1R) to promote sleep drive. More recent studies indicate that the astrocyte also plays pivotal, sleep-dependent roles in “cleaning the brain” during sleep. This work indicates that a glymphatic pathway that critically relies on astrocytic aquaporin 4, is able to flush solutes from the brain and that deficits in this pathway may contribute to Alzheimer’s disease. Finally, astrocytes are known to play important metabolic roles and provide energy on demand to neurons through an astrocyte-neuron shuttle. Given that the time course of astrocytic function is orders of magnitude slower than that of the neuron, this non-neuronal cell is perfectly tuned to modulating slow, state dependent changes in the brain.

Introduction

More than a century ago Santiago Ramon Y Cajal proposed that astrocytes regulate the process of sleep by extending their processes into synapses during periods of sleep and retracting these processes during wakefulness [1]. It was not until 2009, however, that the first evidence emerged that sleep/wake cycles are modulated by these glial cells [2]. From that time the importance of astrocytes in sleep regulation has become increasingly clear. Here we review some of the emerging roles of astrocytes in brain function and recent studies on sleep-related behaviors of astrocytes. We discuss how astrocytes influence sleep homeostasis through the release of adenosine, how astrocytes contribute to the metabolic demands of neurons and how astrocytes play a vital role during sleep to the clearance of solutes from the brain

Sleep is controlled by two process - circadian oscillations and sleep homeostasis. It has been determined that the sleep homeostatic mechanism is regulated by the accumulation of adenosine [3]. One established piece of evidence in support of this notion is that coffee, which promotes wakefulness, contains caffeine, a well-known adenosine receptor antagonist [4].

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While all cell types are capable of releasing adenosine the source(s) of slow changes in adenosine that modulate sleep homeostasis were ill-defined. For example a train of action potentials leads to the co-release of ATP, along with the traditional neurotransmitter, which in the extracellular space is hydrolysed to adenosine. Is this pathway the source of this nucleoside that powerfully promotes sleep? Given that neurons and synaptic activity signal over time scales of milliseconds to seconds it is difficult to envision how the activity-dependent release of ATP, and as a consequence adenosine, would contribute to sleep homeostasis; however, astrocytes, which are not electrically excitable and signal over slower and more prolonged timescales [5] are more suited to regulating states of brain circuits and thus of contributing to the modulation of sleep.

Astrocytes, adenosine and sleep homeostasis

The classical view that astrocytes merely provide structural support was first challenged in 1994 when it was demonstrated that Ca^{2+} signals in astrocytes induced the release of the chemical transmitter glutamate, which in turn caused a slow modulation of neurons [6]. This demonstration paved the way for future studies to determine whether such glial-derived transmitters, or gliotransmitters, could regulate synaptic and circuits [7–13]. In 2005 a novel mouse model was developed in which astrocyte-specific expression of the SNARE domain of a vesicle protein was expressed to impair exocytosis [14]. When this molecule was expressed selectively in astrocytes the tonic adenosine-mediated presynaptic inhibition of excitatory synaptic transmission was relieved. Although it was well known that adenosine acts through adenosine 1 receptors (A1R) to cause presynaptic inhibition, the source of adenosine was assumed to be neuronal. However, the Pascual et al study clearly showed that astrocytes are a source of this presynaptic inhibition. Given the importance of adenosine in modulating sleep homeostasis, several laboratories became intrigued by the potential for the astrocyte as a contributor to the regulation of sleep/wake cycles.

Using the same astrocytic SNARE mouse a collaborative study from three laboratories demonstrated an important role for astrocytes in sleep homeostasis [2]. When astrocytic dnSNARE was expressed sleep homeostasis was impaired; for example, changes in the power of slow wave activity were attenuated as was compensatory changes in sleep time following sleep deprivation (Figure 1). These astrocyte-dependent changes were determined to be due to a reduced supply of adenosine acting on A1R.

Activation of astrocytes stimulates neuronal slow oscillations and sleep

It is known that, in the cortex, slow wave activity arises from synchronized slow oscillations, or up and down states, in cortical pyramidal neurons. To determine whether astrocytes regulate these processes two types of study were performed. First, whole cell recordings of pyramidal neuron activity were performed *in vivo* when dnSNARE was expressed in astrocytes [15]. As a consequence the power of slow oscillations was reduced (Figure 2), an effect that was identified as being due to reduced neuronal NMDA receptor activity. Second, astrocytes were optogenetically stimulated *in vivo* while the activity of associated neurons was recorded [16]. When astrocytes were stimulated neurons were found to transition to slow oscillations. In another related study astrocytes in the hypothalamus were optogenetically stimulated and the time spent asleep at night was consequently increased

[17]. Several labs using independent molecular genetic expression systems have thus been able to draw the same conclusion that astrocytes modulate sleep homeostasis by regulating slow oscillations.

Sleep deprivation and memory formation

In addition to the consequences of sleep deprivation on subsequent sleep need and intensity, prolonged wakefulness is known to impair memory consolidation. How? Given the importance of an astrocytic source of adenosine in modulating wakefulness-dependent sleep homeostasis we asked whether these glia might contribute to the impairment of memory formation. To address this question we trained mice prior to a period of sleep or enforced wakefulness and, 24 hours later, determined how well mice had consolidated their memories. As shown by others, sleep deprivation impairs memory consolidation. We thus asked whether astrocytic dnSNARE expression might permit memory consolidation even with an intervening period of sleep deprivation. Intriguingly, despite 6 hours of sleep deprivation mice expressing dnSNARE in astrocytes were able to form memories as effectively as control mice in which sleep was not experimentally interrupted [2]. We also probed a cellular analog of memory consolidation, late-long term potentiation (L-LTP). Prior sleep deprivation prevented L-LTP, and astrocytic dnSNARE expression overcame this impairing effect of lack of sleep [18].

How could astrocytes impact memory formation in this manner? Subsequent studies have shown that, following training, a period of cAMP elevation is required for the consolidation of memory [19]. Since A1R couple through the G protein G_i , which is known to reduce the activity of the enzyme adenylyl cyclase that gives rise to cAMP, it is logical to assume that prolonged wakefulness prevents the necessary rise of cAMP through this astrocytic control of A1Rs.

The importance of sleep for “cleaning” the brain

During the last few years an interesting observation has been made concerning the importance of sleep for the export of chemicals from the brain. *In vivo* microdialysis studies have shown that the levels of amyloid β ($A\beta$) rise in the interstitial fluid (ISF) of the CNS during wakefulness and that they decline during sleep [20]; moreover, the daily wakefulness-dependent changes in $A\beta$ decline during the progression of Alzheimer’s disease. Studies of the clearance rate of $A\beta$ from the CNS of patients with Alzheimer’s disease have shown that clearance is impaired during the disorder [21]. As a consequence there is great interest in the pathways that control $A\beta$ clearance and the way in which it is regulated in physiology and Alzheimer’s disease.

A novel pathway for this clearance pathway was recently proposed: the glymphatic pathway [22–25] (Figure 3). In effect this is considered a lymphatic-like pathway that is associated with the vasculature and astrocytes of the CNS. According to the glymphatic hypothesis solutes are cleared from the ISF during sleep by a process that requires astrocytes. Given the importance of astrocytes in sleep regulation it is perhaps not surprising that the solute exchange in the glymphatic pathway is regulated during sleep. During wakefulness there is little exchange between the CNS and the glymphatic pathway; however, during sleep solutes

from the ISF, including A β ₁₋₄₂, clear rapidly through the glymphatic pathway [26]. How wake/sleep regulate solute exchange in this pathway is not clear. What is clear is that one of the controlling factors is the importance of the water permeant channel aquaporin-4, since deletion of the gene coding for this channel impairs glymphatic clearance [26]. This observation has exciting implications for Alzheimer's disease: in Alzheimer's mouse models exchange of brain A β ₁₋₄₂ with the glymphatic pathway is reduced. The efflux of A β ₁₋₄₂ from the glymphatic pathway to the circulation is also impaired in these models, raising the potential that the astrocytic, sleep-dependent contribution to the glymphatic pathway is a locus of clearance impairment in Alzheimer's disease.

Structural and transcriptional changes in astrocytes during wakefulness

The effect of wakefulness extends beyond the control of gliotransmission and the glymphatic pathway. It has recently been shown that wakefulness regulates both structural and transcriptional aspects of astrocytes. Translating ribosome affinity purification (TRAP) approaches, which allow the selective determination of those RNAs being translated, were applied to astrocyte during different vigilance states [27]. Intriguingly 1.4% of the astrocyte transcripts were dependent on vigilance state: many were upregulated during wakefulness (both spontaneous and enforced wakefulness) and included transcripts relevant to cytoskeleton and process extension. Serial block scanning electron microscopy revealed that even a few hours of sleep deprivation led to the astrocytic processes extending closer to the synaptic cleft. These structural changes potentially represent the importance of positioning the astrocyte adjacent to the synapses for the clearance of neurotransmitter via astrocytic transporters, or for the supply of glial-derived signals to support synaptic plasticity.

Metabolic tuning and the astrocyte-neuron lactate shuttle (ANLS)

There is a long-standing hypothesis concerning the importance of astrocytes in providing lactate as an energy source for neurons [28]. Considerable data support this hypothesis whereby astrocytes take up glucose and metabolize it to lactate (providing energy to the astrocyte) and then shuttle the lactate to the neuron via monocarboxylate transporters, where lactate is converted to pyruvate as an aerobic energy source. A source of lactate has been shown to be pivotal for memory formation in the hippocampus [29].

In the study previously discussed concerning wakefulness and astrocytic gene expression it was shown that metabolic genes are regulated in these glial cells; for example, an astrocytic sleep gene that was identified is *Slc16a1* [27], which codes for the monocarboxylate transporter MCT1 that regulates the transport of lactate. In another study GFP-expressing astrocytes were enriched using fluorescence-activated cell sorting (FACS) and their gene expression profiles examined. In this study [30] mice were subjected to sleep deprivation and genes encoding proteins involved in the astrocyte-neuron lactate shuttle (ANLS) were also upregulated: these gene products include *Ldha*, GLUT1, α 2-subunit of Na/K ATPase, and GLT1.

That the ANLS is regulated by sleep provides the opportunity to match neuronal activity and metabolic demand with energy supply. Additionally, this pathway has potential implications for the regulation of orexinergic/hypocretin neurons of the lateral hypothalamus. It is known

that, under physiological glucose levels, the activity of the orexinergic/hypocretin neurons are regulated by the supply of lactate. Does this lactate arise from astrocytes? Do wake/sleep and the associated changes in expression of ANLS gene products in turn regulate the lactate delivery and resulting excitability of the orexinergic/hypocretin-containing neurons? And do these neurons go on to regulate wakefulness?

Sleep deprivation is a known risk factor that can cause breakthrough seizures in patients who have been pharmacologically controlled by anti-convulsants. Could this result from enhanced excitability caused by lactate delivery from the astrocyte to the neuron? In support of this concept it has been shown that pyramidal neurons are excited by lactate and, as discussed above, that this lactate is taken up into neurons via monocarboxylate transporters where it is converted to pyruvate. As a consequence, ATP-sensitive K^+ channels are inactivated, leading to increased excitability. Pharmacological inhibition of LDH, the enzyme responsible for converting lactate to pyruvate, hyperpolarizes the neurons, an effect that is rescued by exogenous addition of pyruvate. Furthermore, LDH inhibition reduces seizure frequency in animal models [31]. Interestingly a known anticonvulsant, stiripentol, has been shown to be an LDH inhibitor.

Conclusion

The work of the past few years has consolidated our early appreciation for the importance of astrocytes in regulating sleep homeostasis. Studies subsequent to those using dnSNARE mice have demonstrated that stimulation of astrocytes has direct influence on neuronal excitability and synaptic circuits. Wakefulness itself changes the transcripts levels expressed by astrocytes and regulates the extension of the processes of these glial cells; thus there is a view is developing of how integration of brain states by the astrocyte leads to feedback effects on neuronal circuits. Our understanding of how astrocytes and neurons interact with one another is still in its nascent stages, but it is fascinating to see a confluence of opinion from independent laboratories that the astrocyte influences and is influenced by vigilance state. It is even possible that the clearance from the brain of toxic amyloid is regulated through vigilant state-dependent interactions with astrocytes and the glymphatic pathway.

Acknowledgments

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Highlights

- Astrocytes promote sleep through the release of adenosine
- During sleep astrocytes clean the brain through the activity of a glymphatic pathway
- Neurons receive energy from astrocytes in the form of lactate

Figure 1.

Expression of dnSNARE in astrocytes leads to reduced sleep pressure. Cortical EEGs were performed on mice and the power of low frequency delta power (0.5–1.5Hz) was measured in dnSNARE and wild type animals. A) During a baseline day the power of low frequency SWA was less in astrocytic dnSNARE mice than in WT littermates. This differences was accentuated during the recovery period following sleep deprivation (B). These observations show the importance of the astrocyte in modulating this feature of sleep homeostasis. From Halassa et al., 2009

Figure 2.

Shorter up-states in astrocytic dnSNARE mice. A) Examples of up and down states in wild type and dnSNARE mice. B) Average upstates from WT and dnSNARE mice. C) Cumulative durations of up-states (left) and down states (rights). From Fellin et al 2009

Figure 3.

Summary diagram of the glymphatic pathway. Water is siphoned from a para-arteriole pathway (left) through astrocytic Aquaporin 4 and through convective bulk flow flushes solutes of the interstitial fluid to a paravascular efflux pathway. This pathway of brain cleaning is thought to have elevated activity during sleep. From Illes et al 2012