The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Goodnight, astrocyte: waking up to astroglial mechanisms in sleep

Ashley M. Ingiosi and Marcos G. Frank

Department of Translational Medicine and Physiology, Elson S. Floyd College of Medicine, Washington State University, Spokane, WA, USA

Keywords
astrocytes; brain; calcium; homeostasis; imaging; neurons; sleep; slow-wave activity; wake

Correspondence
M. G. Frank, Department of Translational Medicine and Physiology, Elson S. Floyd College of Medicine, Washington State University, 412 East Spokane Falls Boulevard, Spokane, WA 99202, USA
Tel: 509-368-6747
E-mail: marcos.frank@wsu.edu
and
A. M. Ingiosi, Department of Translational Medicine and Physiology, Elson S. Floyd College of Medicine, Washington State University, 412 East Spokane Falls Boulevard, Spokane, WA 99202, USA
Tel: 509-368-6527
E-mail: ashley.ingiosi@wsu.edu

(Received 6 January 2022, revised 16 February 2022, accepted 7 March 2022)

doi:10.1111/febs.16424

Astrocytes mediate many important aspects of neural homeostasis, but until recently, their role in sleep was largely unknown. The situation has dramatically changed in the last decade. The use of transgenic animals, optogenetics, chemogenetics, brain imaging and sophisticated molecular assays has led to exciting discoveries. Astrocytes dynamically change their activity across the sleep–wake cycle and may encode sleep need via changes in intracellular signalling pathways. Astrocytes also exocytose/secrete sleep-inducing molecules which modulate brain activity, sleep architecture and sleep regulation. Many of these observations have been made in mice and *Drosophila melanogaster*, indicating that astroglial sleep mechanisms are evolutionarily conserved. We review recent findings and discuss future directions.

Introduction

Sleep remains one of the great unsolved biological mysteries. Sleep is widely found in the animal kingdom and like the wing or eye, has evolved more than once. It is also a powerful drive that when deprived, can overwhelm other powerful drives like hunger and mating. These observations strongly suggest that sleep serves important and conserved biological functions. Yet in contrast to these other biological structures and

Abbreviations
AANAT1, arylalkylamine *N*-acetyltransferase 1; AdK, adenosine kinase; AQuA, astrocyte quantitative analysis; BDNF, brain-derived neurotrophic factor; Ca^{2+}, calcium; cAMP, cyclic adenosine monophosphate; CaMPARI2, calcium-modulated photoactivatable ratiometric integrator 2; dnSNARE, dominant-negative SNAP (soluble NSF attachment protein) receptor; DREADD, designer receptors exclusively activated by designer drugs; dTrpA1, *Drosophila*-transient receptor potential A1; EEG, electroencephalography; ER, endoplasmic reticulum; GECI, genetically encoded calcium indicator; GPCR, G-protein-coupled receptor; IL-1, interleukin-1; IP_3, inositol 1,4,5-trisphosphate; IP_3R2, inositol 1,4,5-trisphosphate receptor, type 2; KO, knockout; NREMS, non-rapid eye movement sleep; REMS, rapid eye movement sleep; SD, sleep deprivation; SMP, superior medial protocerebrum; *spz*, *spätzle*; STIM1, stromal interaction molecule 1; SWA, slow-wave activity; TNFα, tumour necrosis factor α; TyRII, tyramine receptor II; VLPO, ventrolateral preoptic nucleus; WT, wild-type.

drives, the adaptive functions of sleep remain a point of intense debate [1,2]. Whatever these functions may be, they are likely tied to two important mechanisms: circuits that transition the brain into sleep states conducive for that function and regulatory mechanisms that ensure that sleep time and intensity are maintained within adaptive ranges in service of that function. This is in keeping with what we know about other adaptive drives in organisms [3]. Therefore, resolving how the brain controls these intertwined mechanisms is key to understanding the deeper mystery of sleep.

The traditional view is that these mechanisms are neuronal. More specifically, that they arise from reciprocal feedback loops between wake-active and sleep-active neurons. This interaction governs transitions between brain states and homeostatic changes in sleep in response to sleep loss. An emerging and alternative view is that other brain cells might play a more central role in these processes than traditionally believed. In this article, we review recent findings supporting an emerging view that non-neuronal cells (astrocytes) may play key roles in these two critical aspects of sleep (for discussion of astrocytes and circadian regulation, see [4]).

Astrocytes and brain function

Astrocytes (which means 'star cells' in Greek) are one of three principal classes of glia in the mature mammalian brain – the others being oligodendrocytes and microglia. Astrocytes perform several support functions in the brain, including buffering ions, recycling neurotransmitters and regulating metabolism. Astrocytes are non-excitable, as they do not produce action potentials and respond linearly to current injections [5]. These observations led early investigators to conclude that astrocytes play no special or direct roles in brain activity or behaviour. This view, however, has evolved with the recognition that astrocytes influence neuronal activity through several different mechanisms including neurotransmitter uptake, ion transport and direct chemical signalling (i.e. gliotransmission). It is now increasingly clear that astrocytes more directly participate in neural processes thought to be solely neuronal in origin [6–9].

Astrocytes and sleep

The role of astrocytes in sleep provides one of the most dramatic examples of this changing view. Observations going back decades suggested that astrocytes might be important in sleep [10], but direct evidence for this idea has only accumulated in the last decade. These studies demonstrated that astroglial transport or gliotransmission of sleep-inducing molecules modulate sleep homeostasis *in vivo* [11–13]. Other studies showed that astroglial intracellular signalling pathways [e.g. calcium (Ca^{2+}) and cyclic adenosine monophosphate (cAMP)] modulate sleep time and sleep homeostasis [14–17]. Microscopic measurements of astroglial Ca^{2+} activity *in vivo* and *ex vivo* revealed that astrocyte Ca^{2+} levels change across the sleep–wake cycle in ways that suggest they encode sleep need [14,18]. These changes may be brain region-specific and do not appear to be simply passive responses to surrounding neuronal activity. We discuss these findings in more detail in the following sections (for a discussion of other interactions between astrocytes and sleep, see [19]).

Astroglial release of somnogenic substances: Retrospective and recent developments

Astrocytes *in vitro* secrete and/or exocytose a variety of somnogenic molecules that when introduced *in vivo* increase sleep time or intensity [i.e. non-rapid eye movement (NREM) slow-wave activity (SWA)]. These molecules include immune factors and cytokines [e.g. interleukin-1 (IL-1) and tumour necrosis factor (TNF) α], neurotrophins [e.g. brain-derived neurotrophin factor (BDNF)], prostaglandins (e.g. PGD2) and purines (e.g. adenosine) [20–25]. Astrocytes release these substances in response to neuronal signals, including classic neurotransmitters and purines acting via membrane bound receptors [21,26]. These findings support a mechanism by which neuronal signalling to astrocytes during wakefulness leads to the release of substances that can increase sleep amounts and intensity [10,21].

Prior to 2010, there was only indirect evidence supporting the presence of this mechanism *in vivo*. Cultured astrocytes often display many properties that do not exist *in vivo* or in brain slices [27,28]. Knockout (KO) mutant mice lacking the TNFα, IL-1 and purine type 2 X7 receptors have sleep phenotypes consistent with this mechanism (i.e. reduced NREM sleep amounts and SWA). However, because neurons and astrocytes both release these molecules, the precise source of the somnogen or its target cells (but see [29]) were unknown [30–32]. Determining the precise role of astrocyte signalling in mammalian sleep requires comparable, inducible and selective manipulations of these signalling pathways *in vivo*.

One promising approach in mammals includes targeted manipulations of astroglial sources of adenosine which is considered a principle mediator of sleep homeostasis [11]. One of these sources is the

gliotransmission of adenosine triphosphate (ATP), which is hydrolysed to adenosine extracellularly [9,33]. Certain forms of vesicle-mediated gliotransmission require the formation of a SNARE complex between vesicles and the target membrane in a manner similar to neuronal synaptic transmission [26,34] (but see [7,8]). The selective expression of a dominant-negative SNARE (dnSNARE) in astrocytes reduces extracellular accumulation of adenosine *in situ* and *in vivo* [33]. When combined with an astrocyte-specific inducible system (Tet-off), theoretically, gliotransmission can be turned on or off *in vivo* [33,35].

As shown by Halassa et al. [12], this strategy provided the first direct evidence *in vivo* that astroglial signalling influences mammalian sleep. The effects of suppressing gliotransmission had no effect on baseline sleep–wake amounts. However, this manipulation reduced several classic indices of sleep need (i.e. impacted sleep homeostasis). First, the normal accumulation of NREM SWA was reduced [12]. As the accumulation and discharge of NREM SWA reflects changes in sleep need, this suggested that sleep need was also reduced. This idea was confirmed by examining compensatory responses to sleep deprivation (SD) in dnSNARE mutant mice and wild-type (WT) controls. The normal compensatory increases in NREM sleep (NREMS) time, bout duration and NREM SWA after SD were attenuated in dnSNARE mutant mice. This appeared to be mediated by a reduction of adenosine A1 receptor activation, as the sleep changes in mutant mice could be phenocopied in WT mice by infusing (intracerebroventricularly) antagonists to the A1 (but not A2) receptor [12]. Follow-up studies *in situ* and *in vivo* demonstrated that the attenuation of slow waves in NREMS also involved a second gliotransmitter, D-serine [13]. Subsequent studies in the dnSNARE mutant further showed that normal changes in brain adenosine concentrations induced by SD [36] or inflammation (which also increases sleep time) [37] were also dependent on gliotransmission.

Studies using optogenetics provide further support for gliotransmission-mediated changes in sleep [38]. Pelluru et al. [39] expressed channelrhodopsin in astrocytes that envelop histaminergic neurons in the posterior hypothalamus. These neurons are known to promote wakefulness; thus, the idea being tested was that activation of astrocytes would increase sleep or sleep drive, presumably via gliotransmission of ATP and the subsequent activation of adenosine A1 receptors. Although ATP (or other gliotransmitters) were not measured, optogenetic stimulation transiently increased sleep time and NREM SWA at circadian times when rodents are mostly awake [39]. Complementary findings using a similar approach were then reported by Kim et al. [40] who instead expressed channelrhodopsin in astrocytes in a sleep-promoting region [the ventrolateral preoptic nucleus (VLPO) of the hypothalamus] in mice. Activation of astrocytes in the VLPO also increased sleep time during the circadian active phase. Additional analyses showed that ATP levels in the VLPO were higher after optogenetic stimulation, while other gliotransmitters and cytokines were unchanged. The underlying mechanism appeared to be adenosine, as VLPO ATP and adenosine levels were elevated during the normal sleep phase and pharmacological inhibition of the extracellular enzyme that hydrolyses ATP to adenosine reduced sleep time. The precise adenosine signalling pathway is unclear, as there is evidence for both A1 and A2 receptors in the VLPO, but the authors suggest that this reflects an A1-mediated disinhibition of VLPO neurons [40].

Collectively, the use of the dnSNARE mouse and optogenetics support a role for astrocyte gliotransmission, particularly of ATP, as an important mechanism in sleep expression and homeostasis. However, one caveat is that under certain conditions transgene expression in the dnSNARE mouse may also occur in neurons [41], but this appears to be the exception rather than the rule [12,42]. An additional caveat is that optogenetic activation of astrocytes in some cases may be non-physiological. Astrocytes do not densely express large cation pores like channelrhodopsin. Considering the very hyperpolarized state of these cells, when these experimentally expressed channels are suddenly opened, this leads to an inrush of cations unlikely to occur naturally. A third point is that optogenetic gliotransmission involves non-vesicular forms of gliotransmission (e.g. via hemichannels and purinergic receptors) [40]. The mechanisms governing non-vesicular gliotransmission under more natural conditions are incompletely understood, but they appear distinct from those involved in SNARE-mediated gliotransmission [43,44].

Astrocytes can also increase extracellular adenosine via changes in equilibrative transport. Equilibrative transport of adenosine occurs via gradient-based mechanisms that decrease extracellular adenosine as a function of cellular metabolism [11,45]. This is mediated by adenosine kinase (AdK) which reduces cytosolic concentrations of adenosine leading to passive transport of extracellular adenosine into cells. This results in a reduction of adenosine available to act on membrane-bound adenosine A1 receptors. Based on these observations, it was hypothesized that extracellular changes in adenosine necessary for sleep homeostasis are determined by AdK-mediated adenosine transport [11]. This hypothesis was tested by conditionally deleting AdK

from astrocytes *in vivo* which resulted in an increase of NREM SWA under baseline conditions and after SD. The excess adenosine also slowed the normal decay of NREM SWA during sleep, further supporting a role for AdK in sleep homeostasis. On the other hand, this mutation also increased SWA in baseline waking and even during enforced waking (SD) [46]. This is peculiar as changes in waking SWA (in contrast to NREM SWA) do not accumulate and discharge in a way proportionate to sleep drive. In contrast, SWA changes in the dnSNARE mutant were entirely restricted to NREMS [12]. This suggests that the AdK pathway is part of the sleep homeostat, but its precise role requires more investigation.

The exocytosis of sleep-inducing molecules by astrocytes is also reported in *Drosophila melanogaster*. Selective manipulation of the mammalian homologue of TNFα (Eiger) in astrocytes results in profound changes in sleep time and homeostasis [47]. As appears to be true in mammals, this reflects interactions between an astrocyte-derived ligand (Eiger) and a neuronally expressed receptor (Wengen). *Drosophila* astrocytes can also be activated by transport of extracellular cations into the cell. Astroglial activation via temperature-sensitive *Drosophila*-transient receptor potential A1 (dTrpA1) – a cation channel permeable to calcium and sodium – increases sleep duration [18]. This astroglial-mediated sleep increase is accompanied by decreased excitability of arousal-promoting large ventrolateral clock neurons (as determined by patch-clamp electrophysiology) and increased Ca^{2+} activity in sleep-promoting R5 ring neurons [18]. These results suggest that, as proposed for mammals, *Drosophila* astrocytes can increase sleep by inhibiting arousal-promoting neurons and exciting sleep-promoting neurons. The underlying mechanisms are similar to those in mammals in that they involve release of astrocyte-derived somnogens and changes in gradient-based transport of molecules.

New advances in exploring astroglial activity in sleep

A fundamental limitation in our understanding of astrocytes has been our inability to observe their behaviour in natural brain states. In contrast to neuronal activity which can be measured electrically [e.g. via electroencephalography (EEG)], astrocytes are not electrically excitable. Until recently, the activity of astrocytes *in vivo* could only be inferred based primarily on measurements *ex vivo* (e.g. morphological changes in fixed tissue, genes and proteins) or in reduced preparations *in vitro* or *in situ*. These studies provided important clues (reviewed in [19]), but it was not until the last few years that scientists could directly measure activity changes in astrocytes across the sleep–wake cycle. This was made possible by adopting *in vivo* Ca^{2+} imaging techniques developed in neurons, particularly one- and two-photon microscopy and fibre photometry imaging of fluorescent genetically encoded Ca^{2+} indicators (GECI) like GCaMP [48–50]. Since intracellular Ca^{2+} is a marker and mediator of astroglial activity and function using these tools, scientists could now examine astrocytes across the sleep–wake cycle and determine their role in sleep expression and regulation (Fig. 1; see Box 1).

Astroglial Ca^{2+} changes across the sleep–wake cycle

A key finding across independent laboratories is that astroglial activity changes with sleep and wakefulness. Overall, astroglial intracellular Ca^{2+} activity is highest during wakefulness and lowest during sleep, but this pattern varies in different brain regions [14–16,52]. For example, this general trend is reported in frontal cortex [14], barrel cortex [16], primary visual cortex [15], hippocampus and cerebellum [52], but not in the hypothalamus or brainstem [52]. These findings primarily reflect changes in GECI amplitudes (i.e. $\Delta F/F$ or ratios) and/or in the number or frequency of Ca^{2+} events (Fig. 1). Several laboratories also report dramatic spikes in astroglial activity during transitions from rapid eye movement sleep (REMS) to wakefulness [14,16,52]. The mechanisms governing these changes in astroglial activity, and their peaks, at REM-to-wakefulness transitions are unclear but may reflect global changes in neuromodulator concentrations that coincide with the sleep–wake cycle.

More fine-grained analyses revealed similar dynamic Ca^{2+} patterns inside individual astrocytes. Sleep–wake changes in Ca^{2+} event frequency and amplitude are compartmentalized within the cell, with the most dynamic changes occurring in the processes compared to the soma [14,16]. In addition, the temporal and spatial dimensions of astroglial Ca^{2+} activity differ across arousal states based on AQuA (Astrocyte Quantitative Analysis) [14,15] and regions-of-activity [16] analyses (Fig. 1). These two analytical techniques measure the spatiotemporal complexity of astroglial Ca^{2+} events which move and change shape within individual cells. The duration of individual Ca^{2+} events are longer during wakefulness compared to sleep, whereas the area (or size) of these events are more variable across arousal states [14–16]. These dimensions are also influenced by other factors including proximity to the soma and

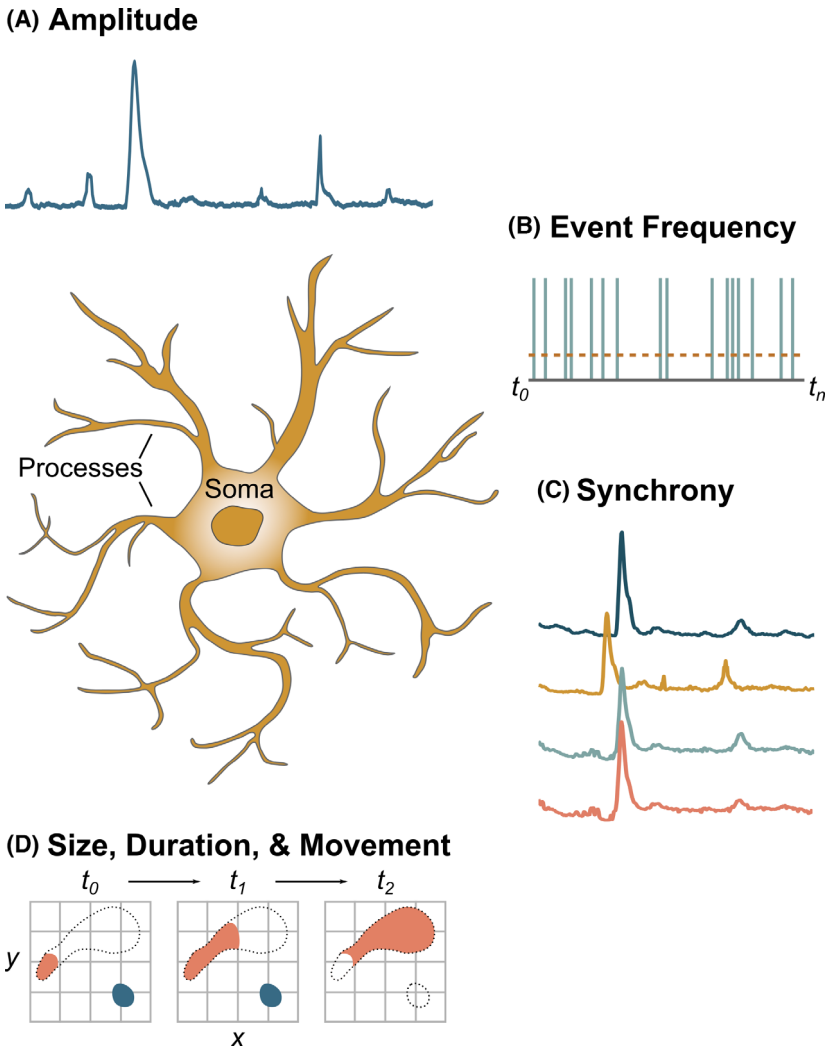


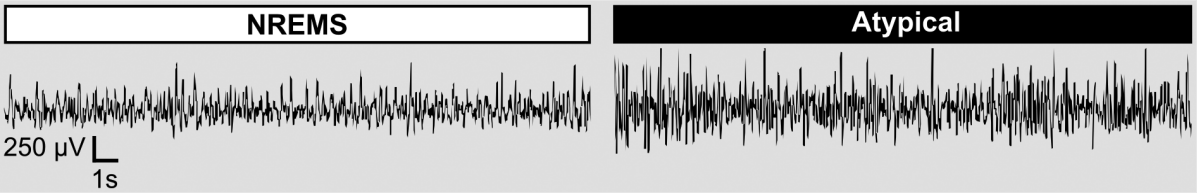
Fig. 1. Assessing astroglial Ca^{2+} activity. A variety of metrics have been used to study the intensity, frequency and spatiotemporal characteristics of astroglial Ca^{2+} activity across the sleep–wake cycle. (A) Amplitude describes intensity changes of fluorescent Ca^{2+} indicators as a proxy for changes in Ca^{2+} concentration. Amplitude is typically expressed as ΔF/F – change in fluorescence over a baseline fluorescent value (e.g. mean, median) – or *z*-score values. (B) More prominent deflections of Ca^{2+} activity from baseline levels can be defined as events based on a set of criteria and/or a threshold (dotted line). These events are then tallied for different brain states. (C) Synchrony can be determined by comparing patterns of Ca^{2+} activity across astrocytes, within individual astrocytes, or with a different cell type (e.g. neurons). (D) AQuA (Astrocyte Quantitative Analysis) and regions-of-activity analysis provide fine-grained measures of Ca^{2+} activity within individual astrocytes and networks. The size of Ca^{2+} events can be shown as the area or perimeter of individual events. Ca^{2+} events can also be described by the duration of individual events as well as if an event moves, grows or shrinks (in orange) or remains static (in blue) over time. These metrics can also be used to compare Ca^{2+} activity in distinct cellular compartments of the astrocytes (e.g. soma, processes). The functional implications for these various metrics are not completely understood (e.g. propagating vs. static events, long vs. short events), but they all change across the sleep–wake cycle and/or after sleep deprivation.

whether a Ca^{2+} event propagates/moves. For example, the size of propagating Ca^{2+} events that originate in the distal processes are larger than events that start closer to the soma, whereas static (i.e. non-propagating) Ca^{2+} events sizes are not impacted by somal proximity [14]. The significance of these brain state and regional differences in Ca^{2+} is unknown, but may be related to how different functions are compartmentalized in astrocytes. Astroglial distal processes form microdomains around synapses that appear to operate independently, while other processes may surround blood vessels [53,54].

A particularly intriguing finding is that sleep–wake changes in cortical astrocytes do not appear to be a

Box 1. Caveats in measuring neural activity and sleep in head restraint

Two-photon microscopy is used to image fine processes of astrocytes, but this requires animals to be head restrained to prevent movement of the imaging plane [14–16]. Care must be exercised in quantifying and identifying sleep in such conditions. For example, mice typically do not express normal amounts of REMS in head restraint. This is likely due to the loss of motor tone characteristic of REMS [51], which results in the mouse being abruptly suspended from its head. This often causes the mouse to wake up. Head restraint can also induce an atypical high-amplitude, high-frequency EEG pattern in mice [14,16] [see below for representative NREMS (left) and atypical (right) EEG traces from a mouse in head restraint] that is not observed in freely behaving mice or in mice instrumented with head-mounted miniscopes [14]. This peculiar brain state may reflect an inability to fully transition between wakefulness, NREMS and REMS while in head restraint.



passive response to surrounding neuronal activity. Synchronized activity across cortical astrocytes is maximal during wakefulness and at a nadir during NREMS [14]. The exact opposite pattern is observed in cortical neurons, which show their greatest synchrony during the slow thalamocortical oscillations typical of NREMS [55,56]. Dual Ca^{2+} imaging of neuronal somata and astroglial gliopil also revealed that the onset of Ca^{2} events in astrocytes and surrounding neurons is less synchronized during sleep compared to wakefulness. Astroglial Ca^{2+} events can also precede neuronal Ca^{2+} events which further suggests that astrocytes are not simply passively responding to neurons [16]. In addition, changes in astroglial Ca^{2+} event frequency can precede changes in the EEG, increasing during NREMS prior to the onset of wakefulness and decreasing during wakefulness before NREMS onset [15,16]. Collectively, these findings show that astrocytes are not always synchronized with neuronal activity and may even anticipate or drive changes in neuronal activity.

Astroglial Ca^{2+} and sleep homeostasis

As discussed earlier, astroglial sources of adenosine influence mammalian sleep homeostasis. What is less clear is how astrocytes regulate this process as a function of sleep need. According to one model (Fig. 2), astrocytes detect neuronal signals released during wakefulness, integrate those signals via changes in intracellular Ca^{2+} and, via negative feedback, dampen those waking signals resulting in increased NREM SWA and sleep time [57]. Two predictions of this model are that astroglial Ca^{2+} should rise and fall in proportion to sleep drive, and interruption of this process should reduce the accumulation of sleep need. These two predictions have been partially borne out in mice and *D. melanogaster*.

In vivo imaging of GECIs in mice showed that astroglial Ca^{2+} concentrations are highest at circadian times of high sleep need (i.e. first half of the sleep phase) and are elevated further after SD. They decline to their lowest levels by the end of the rest phase and/or after post-SD recovery sleep. These changes in astroglial Ca^{2+} are most pronounced in NREMS and parallel peaks and declines in NREM SWA [14]. SD also causes additional transient changes, including a reduction in cortical synchrony both within and across astrocytes and changes in the duration and area (i.e. size) of individual Ca^{2+} events inside individual astrocytes [14]. Similar changes in astrocytes are reported in *D. melanogaster*. SD increases Ca^{2+} concentrations in astroglial processes and somata within the superior medial protocerebrum (SMP) and antennal lobe of *Drosophila* as shown via *ex vivo* imaging of the GECI CaMPARI2 [18]. These data were confirmed by *in vivo* two-photon imaging of GCaMP6s in the SMP, which also showed that SD increases Ca^{2+} event frequency in astroglial processes.

Manipulating astroglial Ca^{2+}: Impact on sleep architecture and sleep homeostasis

A second prediction of the model shown in Figure 2 is that disrupting the regulation of astroglial Ca^{2+} should interfere with the normal expression of sleep and sleep homeostasis. This idea was recently investigated using different methods of enhancing or disrupting pathways

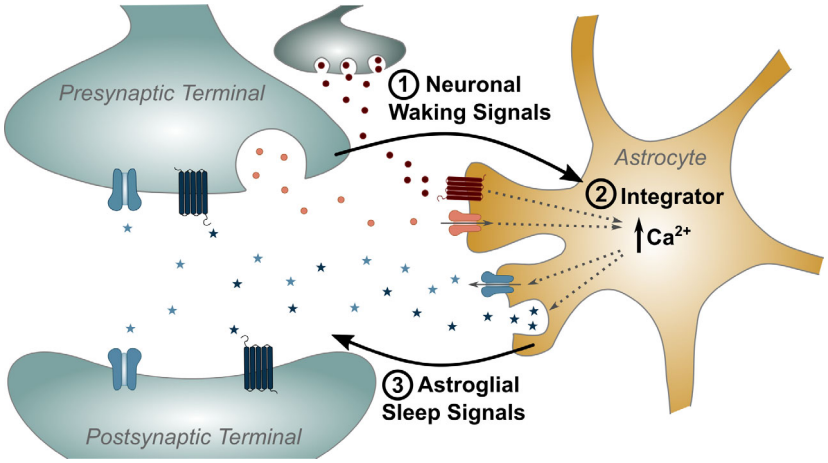


Fig. 2. A hypothesized neuronal–astroglial feedback model for sleep homeostasis. This model includes three main components: (1) neuronal waking signals that reflect sleep need, (2) an astroglial integrator of the neuronal waking signals and (3) astroglial-derived feedback molecules that reduce the waking signals to promote sleep. The neuronal waking signals may be neurotransmitters (shown in peach) or neuromodulators (shown in burgundy) that accumulate during wakefulness. These neurotransmitters and neuromodulators can interact with astroglial ion channels [18] or G-protein-coupled receptors [15,18] to change astroglial Ca^{2+} activity. Ca^{2+} is hypothesized to be part of an astroglial integrator of these neuronal waking signals. Astroglial Ca^{2+} changes with sleep need and plays a role in compensatory responses to sleep loss [14,18]. Ca^{2+} also plays a role in astroglial release of sleep-promoting substances [94,95]. Astroglial release of sleep signals (e.g. ATP, *spz*; shown in light blue and navy) occurs through gliotransmission and possibly other mechanisms [12,18,40]. This proposed neuronal–astroglial feedback model may occur ubiquitously in the brain and govern both local sleep (e.g. within the cortex) and global sleep (e.g. via interactions with canonical sleep–wake circuits). Additional possible mechanisms not shown here include metabolic interactions, ion buffering and glutamate uptake. Adapted from Ref. [96].

that regulate astroglial intracellular Ca^{2+} stores. The results of these experiments have produced complex and somewhat equivocal results.

Several laboratories examined sleep in transgenic mice bearing deletions of the inositol 1,4,5-trisphosphate (IP_3) receptor, type 2 (IP_3R2) [58] or its ligand IP_3 [59]. The IP_3 family of receptors (which include IP_3R1, IP_3R2 and IP_3R3) act downstream of G proteins and regulate the release of Ca^{2+} from endoplasmic reticulum (ER) intracellular stores [60,61]. Manipulation of this pathway, however, produces relatively subtle changes in sleep. Constitutive, global KO of IP_3R2 in IP_3R2 KO (*Itpr2*$^{-/-}$) mice fragments NREMS and reduces NREM SWA under baseline conditions [16]. Regions-of-activity analyses during NREMS showed that residual cortical astroglial Ca^{2+} activity manifests as longer (but smaller area) events [16]. There are no changes in REMS other than an increase in EEG theta activity. On the other hand, genetic reduction of IP_3 selectively in astrocytes (via inducible expression of IP_3 5-phosphatase – which metabolizes IP_3 to IP_2) has no effect on NREMS architecture or NREM SWA but instead increases time spent in REMS [59].

The discrepancy in these results may be due to several factors. First, different IP_3 receptors operate in different cellular regions of astrocytes [61]. IP_3R2 may preferentially govern Ca^{2+} signalling in the soma and proximal branches, whereas IP_3R1 instead modulates Ca^{2+} signalling in the more distal branches [61]. Therefore, constitutive KO of IP_3R2 may not impact Ca^{2+} signalling in distal processes which is the site of the most dynamic changes across the sleep–wake cycle. A second issue is a lack of cell-type specificity in the IP_3R2 KO mouse. IP_3R2 is also found in oligodendrocytes [62], heart [58], lung, liver, kidney [63], pancreas [64] and thymus [65]. Therefore, global knockout of IP_3R2 could impact sleep–wake behaviour outside of its role in central nervous system astrocytes.

On the other hand, targeted deletions of other proteins involved in the regulation of astroglial Ca^{2+} result in significant changes in sleep homeostasis. In mice, inducible KO of astroglial stromal interaction molecule 1 (STIM1) – an ER sensor that mediates replenishment of intracellular Ca^{2+} [66] – has no effect on baseline sleep–wake architecture. This manipulation, however, reduces the normal accumulation and discharge of sleep need after SD [14]. A role for astroglial Ca^{2+} in sleep homeostasis was also demonstrated in flies via targeted knockdown of a subunit (Ca-α1D) of the membrane-bound L-type voltage-gated Ca^{2+} channel. In flies, the L-type voltage-gated Ca^{2+} channel is another mechanism by which astrocytes regulate

cytoplasmic Ca^{2+} concentrations [67]. This specific L-type channel is activated at more hyperpolarized membrane potentials, like those found in astrocytes, compared to other voltage-gated Ca^{2+} channels [68]. Similar to results in the STIM1 KO mouse, astroglial knockdown of Ca-α1D in flies has minimal effects on baseline sleep while reducing compensatory changes in sleep after SD [18].

Additional signalling pathways in astrocytes

Progress has been made in determining related astroglial signalling pathways that modulate intracellular Ca^{2+} and sleep. This was investigated in mice via inducible manipulations of different G-protein-coupled receptors (GPCRs) with DREADDs (designer receptors exclusively activated by designer drugs). In astrocytes, the G_q/phospholipase C cascade is triggered by noradrenergic α1 receptors and muscarinic acetylcholine receptors [69–71] which increases intracellular Ca^{2+} via IP_3-mediated release of Ca^{2+} from ER stores [15,71–73] and ATP release [74]. The function of the G_i pathway in astrocytes is less clear. G_i activation also increases intracellular Ca^{2+} through IP_3-mediated pathways [15,71–73,75,76] but acts downstream of astroglial $GABA_B$ and mGluR3 receptors [71,75,77].

The results of these DREADD-based experiments suggest separate roles for different GPCRs. Cortical activation of G_i-DREADDs increases NREM SWA but not NREMS duration [15]. On the other hand, activating cortical astroglial G_q-DREADDs increases NREMS duration but does not affect NREM SWA [15]. Further analyses showed that each GPCR had different effects on astroglial Ca^{2+}. Activation of G_i-DREADDs increases Ca^{2+} event frequency. In contrast, G_q-DREADDs increase Ca^{2+} above baseline levels but lead to a transient increase in Ca^{2+} event frequency. This suggests that different astroglial signalling pathways and/or Ca^{2+} changes modulate distinct components of sleep, even when they converge on IP_3 Ca^{2+} signalling.

Complementary approaches have been used in flies. In flies, SD increases expression of the monoamine receptor tyramine receptor II (TyRII) [18]; a receptor that when activated increases astroglial Ca^{2+} [78]. Knockdown of astroglial TyRII has no effect on baseline sleep–wake architecture, but it suppresses SD-induced increases in astroglial Ca^{2+} and reduces recovery sleep duration post-SD [18]. Astroglial arylalkylamine N-acetyltransferase 1 (AANAT1) also appears to selectively influence sleep homeostasis in flies. AANAT1 catabolizes and inactivates monoamines, and its knockdown increases brain monoaminergic levels and increases compensatory sleep duration following SD [17]. An additional mechanism is the analogue to the mammalian sleep-inducing cytokine IL-1 – *spätzle* (*spz*). Previous work in mice found that astroglial IL-1 receptor 1 plays a role in homeostatic NREM SWA responses to SD [29]. In flies, *spz* activates Toll receptors in sleep-promoting R5 ring neurons, and neuronal Toll knockdown or astroglial *spz* knockdown suppresses SD-induced compensatory increases in sleep and Ca^{2+} increases in R5 ring neurons [18]. Additionally, astroglial RNA interference knockdown of *necrotic*, which prevents maturation of *spz* into its Toll-binding form, increases baseline sleep [79].

Discussion: Stargazing into the future

Scientists in the last decade have made great progress elucidating the role of astrocytes in sleep. The emerging picture is that astrocytes represent a parallel level of brain organization that is just as dynamic as neuronal networks. Astrocytes may also form a key component of the sleep homeostat by integrating waking signals and releasing somnogenic substances. These observations are reported in invertebrates and mammals, which suggests that these properties are evolutionarily conserved. There are, however, many unanswered questions and unexplored areas.

Astroglial mechanisms: Local, global or both?

Sleep can be discussed not only in terms of whole brain and organismal changes (i.e. 'global' sleep), but also in the context of smaller collections of nervous tissue (i.e. 'local' sleep [80,81]). One way to reconcile these ideas is that sleep may have its origins in simple networks [80,81], but as brains became more complex and differentiated, secondary systems that coordinated activity across the brain evolved. Astrocytes may explain both aspects of sleep.

Astrocytes are dispersed widely in sub-cortical and cortical brain areas [82], including regions known to trigger sleep and wakefulness [12]. Therefore, they may regulate brain activity by acting within specific neocortical circuits (i.e. local sleep) or via modulation of basal forebrain and hypothalamic sleep and arousal centres (i.e. global sleep) [80,83–85]. It is possible that astrocytes mediate both processes via local modulation of surrounding neurons. Astrocytes generally do not send long-ranging projections, as is true for mammalian neurons. Instead, astrocytes operate independently or within confined networks. For example, cortical astrocytes are arrayed in adjacent, but independent, domains surrounding different synapses. In

other parts of the adult brain, astrocytes can form local networks via gap junctions, but these also lack long-ranging efferent projections as found in neurons [86]. This suggests that astrocytes act locally rather than as part of a brain-wide interconnected network. Local influence, however, appears sufficient to gate activity of surrounding neurons within canonical 'executive' sleep–wake neuronal circuits (e.g. within the hypothalamus) as well as in sites of local sleep (e.g. the cortex). For example, manipulations of cortical astrocytes influence SWA [15], whole brain changes in astrocytes influence sleep architecture and EEG activity [12,14–18,46,47,79], and selective modulation of astrocytes in canonical sleep and wake centres influences sleep [39,40]. What then coordinates these independently operating modules? One possibility is that their activity is synchronized by global changes in neuromodulators. Astrocytes express membrane bound receptors for the major classes of neuromodulators that cycle with sleep and wakefulness (e.g. noradrenaline and acetylcholine) and are influenced by efferents from neuromodulator nuclei (e.g. the locus coeruleus) [70,87].

Cracking the code of astrocyte signalling

An important future direction is to determine whether astrocyte activity varies across different brain regions. More specifically, do astrocytes respond the same way to neuronal signals (e.g. wake-promoting neuromodulators) uniformly, or does this vary by brain region? If the latter turns out to be true, then what determines these differences in response? Perhaps they could be driven by the neuronal subtype (e.g. wake-promoting vs. sleep-promoting, excitatory vs. inhibitory) and/or by astroglial subtype. There are many subtypes of astrocytes and we are only beginning to understand the extent of their morphological, molecular and functional heterogeneity [88,89]. This level of complexity may also vary across and within animal genus and species [90,91]. Therefore, we may discover that different classes of astrocytes serve distinct roles in local and global sleep. This cellular heterogeneity may explain findings of regional astroglial Ca^{2+} activity differences across the sleep–wake cycle [52].

Concluding remarks

Scientists have made remarkable progress in understanding the role of glia in sleep. As is true for other neural systems, astrocytes do not appear to be bystanders, but active participants in sleep expression and regulation. Nevertheless, the study of astroglial sleep mechanisms is truly in its infancy, as a host of unanswered questions remain. For example, in addition to Ca^{2+}, what comprises the astroglial integrator of sleep need? Could this involve cascades of Ca^{2+} binding proteins that converge on the nucleus, or is the mechanism solely post-translational in nature? How does the role of astrocytes change across the lifespan? Sleep undergoes changes in expression and regulation during the perinatal period [92] and in senescence [93] – could these changes be influenced by astrocytes? What roles do astrocytes play in hypothesized functions of sleep, like memory consolidation and synaptic plasticity? These are but a few of the interesting questions awaiting future investigation.

Acknowledgements

This work was supported by National Institutes of Health grants K99 NS119293 (AMI), R01 MH099544 (MGF) and R01 NS114780 (MGF).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

AMI and MGF contributed equally to the writing and editing of this manuscript.

Data availability statement

Data sharing is not applicable to this article as no new data was generated for this review.

References

1 Rechtschaffen A. Current perspectives on the function of sleep. *Perspect Biol Med*. 1998;**41**:359–90.
2 Frank MG. In: Winkelman JW, Plante DT, editors. The functions of sleep in Foundations of Psychiatric Sleep Medicine. Cambridge: Cambridge University Press; 2010. p. 59–78.
3 Benington J. Sleep homeostasis and the function of sleep. *Sleep*. 2001;**23**:959–66.
4 Astiz M, Delgado-García LM, López-Mascaraque L. Astrocytes as essential time-keepers of the central pacemaker. *Glia*. 2021;**24**:21.
5 Verkhratsky A, Parpura V, Vardjan N, Zorec R. Physiology of astroglia. *Adv Exp Med Biol*. 2019;**1175**:45–91.
6 Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*. 2010;**72**:335–55.

7 Fiacco TA, Agulhon C, McCarthy KD. Sorting out astrocyte physiology from pharmacology. *Annu Rev Pharmacol Toxicol*. 2009;**49**:151–74.
8 Hamilton NB, Atwell D. Do astrocytes really exocytose neurotransmitters? *Nat Rev Neurosci*. 2010;**11**:227–38.
9 Halassa MM, Fellin T, Haydon PG. Tripartite synapses: roles for astrocytic purines in the control of synaptic physiology and behavior. *Neuropharmacology*. 2009;**57**:343–6.
10 Frank MG. Beyond the neuron: Astroglial regulation of mammalian sleep. *Curr Top Med Chem*. 2011;**11**:2452–6.
11 Greene RW, Bjorness TE, Suzuki A. The adenosine-mediated, neuronal-glial, homeostatic sleep response. *Curr Opin Neurobiol*. 2017;**44**:236–42.
12 Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, et al. Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron*. 2009;**61**:213–9.
13 Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, et al. Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci USA*. 2009;**106**:15037–42.
14 Ingiosi AM, Hayworth CR, Harvey DO, Singletary KG, Rempe MJ, Wisor JP, et al. A role for astroglial calcium in mammalian sleep and sleep regulation. *Curr Biol*. 2020;**30**:4373–4383.e7.
15 Vaidyanathan TV, Collard M, Yokoyama S, Reitman ME, Poskanzer KE. Cortical astrocytes independently regulate sleep depth and duration via separate GPCR pathways. *eLife*. 2021;**10**:e63329.
16 Bojarskaite L, Bjørnstad DM, Pettersen KH, Cunen C, Hermansen GH, Åbjørsbråten KS, et al. Astrocytic Ca^{2+} signaling is reduced during sleep and is involved in the regulation of slow wave sleep. *Nat Commun*. 2020;**11**:3240.
17 Davla S, Artiushin G, Li Y, Chitsaz D, Li S, Sehgal A, et al. AANAT1 functions in astrocytes to regulate sleep homeostasis. *eLife*. 2020;**9**:e53994.
18 Blum ID, Keleş MF, Baz E-S, Han E, Park K, Luu S, et al. Astroglial calcium signaling encodes sleep need in *Drosophila*. *bioRxiv*. 2020;**7**:4.
19 Frank MG. The role of glia in sleep regulation and function. *Handb Exp Pharmacol*. 2019;**253**:83–96.
20 Tobler I, Borbély AA, Schwyzer M, Fontana A. Interleukin-1 derived from astrocytes enhances slow wave activity in sleep EEG of the rat. *Eur J Pharmacol*. 1984;**104**:191–2.
21 Krueger JM. The role of cytokines in sleep regulation. *Curr Pharm Des*. 2008;**14**:3408–16.
22 Hayaishi O. Functional genomics of sleep and circadian rhythm: Invited review: molecular genetic studies on sleep-wake regulation, with special emphasis on the prostaglandin D2 system. *J Appl Physiol*. 2002;**92**:863–8.
23 Kushikata T, Fang J, Krueger JM. Brain-derived neurotrophic factor enhances spontaneous sleep in rats and rabbits. *AJP - Reg Integ Comp Physiol*. 1999;**276**:R1334–8.
24 Huang Z-L, Urade Y, Hayaishi O. Prostaglandins and adenosine in the regulation of sleep and wakefulness. *Curr Opin Pharmacol*. 2007;**7**:33–8.
25 Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C. A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *J Neurosci*. 2008;**28**:4088–95.
26 Kofuji P, Araque A. G-protein-coupled receptors in astrocyte-neuron communication. *Neuroscience*. 2021;**456**:71–84.
27 Yamamoto K, Miwa T, Ueno R, Hayaishi O. Muramyl dipeptide-elicited production of PGD2 from astrocytes in culture. *Biochem Biophys Res Comm*. 1988;**156**:882–8.
28 Inagaki N, Wada H. Histamine and prostanoid receptors on glial cells. *Glia*. 1994;**11**:102–9.
29 Ingiosi AM, Raymond RM Jr, Pavlova MN, Opp MR. Selective contributions of neuronal and astroglial interleukin-1 receptor 1 to the regulation of sleep. *Brain Behav Immun*. 2015;**48**:244–57.
30 Kapas L, Bohnet SG, Traynor TR, Majde JA, Szentirmai E, Magrath P, et al. Spontaneous and influenza virus-induced sleep are altered in TNF-{alpha} double-receptor deficient mice. *J Appl Physiol*. 2008;**105**:1187–98.
31 Baracchi F, Opp MR. Sleep-wake behavior and responses to sleep deprivation of mice lacking both interleukin-1[beta] receptor 1 and tumor necrosis factor-[alpha] receptor 1. *Brain Behav Immun*. 2008;**22**:982–93.
32 Krueger JM, Taishi P, De A, Davis CJ, Winters BD, Clinton J, et al. ATP and the purine type 2 X7 receptor affect sleep. *J Appl Physiol*. 2010;**586**:20.
33 Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, et al. Astrocytic purinergic signaling coordinates synaptic networks. *Science*. 2005;**310**:113–6.
34 Scales SJ, Bock JB, Scheller RH. The specifics of membrane fusion. *Nature*. 2000;**407**:144–6.
35 Morozov A, Kellendonk C, Simpson E, Tronche F. Using conditional mutagenesis to study the brain. *Biol Psychiat*. 2003;**54**:1125–33.
36 Schmitt LI, Sims RE, Dale N, Haydon PG. Wakefulness affects synaptic and network activity by increasing extracellular astrocyte-derived adenosine. *J Neurosci*. 2012;**32**:4417–25.
37 Nadjar A, Blutstein T, Aubert A, Laye S, Haydon PG. Astrocyte-derived adenosine modulates increased sleep pressure during inflammatory response. *Glia*. 2013;**61**:724–31.
38 Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of parkinsonian neural circuitry. *Science*. 2009;**324**:354–9.
39 Pelluru D, Konadhode RR, Bhat NR, Shiromani PJ. Optogenetic stimulation of astrocytes in the posterior

hypothalamus increases sleep at night in C57BL/6J mice. *Eur J Neuorsci*. 2016;**43**:1298–306.
40 Kim J-H, Choi I-S, Jeong J-Y, Jang I-S, Lee M-G, Suk K. Astrocytes in the ventrolateral preoptic area promote sleep. *J Neurosci*. 2020;**40**:8994–9011.
41 Fujita T, Chen MJ, Li B, Smith NA, Peng W, Sun W, et al. Neuronal transgene expression in dominant-Negative SNARE mice. *J Neurosci*. 2014;**34**:16594.
42 Papouin T, Dunphy JM, Tolman M, Dineley KT, Haydon PG. Septal cholinergic neuromodulation tunes the astrocyte-dependent gating of hippocampal NMDA receptors to wakefulness. *Neuron*. 2017;**94**:840–854.e7.
43 Orellana JA. Physiological functions of glial cell hemichannels. *Adv Exp Med Biol*. 2016;**949**:93–108.
44 Abudara V, Retamal MA, Del Rio R, Orellana JA. Synaptic functions of hemichannels and pannexons: A double-edged sword. *Front Mol Neurosci*. 2018;**11**:435.
45 Boison D. Adenosine kinase: exploitation for therapeutic gain. *Pharmacol Rev*. 2013;**65**:906.
46 Bjorness TE, Dale N, Mettlach G, Sonneborn A, Sahin B, Fienberg AA, et al. An adenosine-mediated glial-neuronal circuit for homeostatic sleep. *J Neurosci*. 2016;**36**:3709–21.
47 Vanderheyden WM, Goodman AG, Taylor RH, Frank MG, Van Dongen HPA, Gerstner JR. Astrocyte expression of the *Drosophila* TNF-alpha homologue, Eiger, regulates sleep in flies. *PLoS Genet*. 2018;**14**: e1007724.
48 Kawamoto EM, Vivar C, Camandola S. Physiology and pathology of calcium signaling in the brain. *Front Pharmacol*. 2012;**3**:61.
49 Guerra-Gomes S, Sousa N, Pinto L, Oliveira JF. Functional roles of astrocyte calcium elevations: from synapses to behavior. *Front Cell Neurosci*. 2018;**11**:427.
50 Lim EY, Ye L, Paukert M. Potential and realized impact of astroglia Ca2+ dynamics on circuit function and behavior. *Front Cell Neurosci*. 2021;**15**:22.
51 Fraigne JJ, Torontali ZA, Snow MB, Peever JH. REM sleep at its core – circuits, neurotransmitters, and pathophysiology. *Front Neurol*. 2015;**6**.
52 Tsunematsu T, Sakata S, Sanagi T, Tanaka KF, Matsui K. Region-specific and state-dependent astrocyte Ca^{2+} dynamics during the sleep-wake cycle in mice. *J Neurosci*. 2021;**2912**:20.
53 Khakh BS, McCarthy KD. Astrocyte calcium signaling: from observations to functions and the challenges therein. *Cold Spring Harbor Perspect Biol*. 2015;**7**:a020404.
54 Shigetomi E, Bushong EA, Haustein MD, Tong X, Jackson-Weaver O, Kracun S, et al. Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. *J General Physiol*. 2013;**141**:633–47.
55 Steriade M. Corticothalamic resonance, states of vigilance and mentation. *Neuroscience*. 2000;**101**:243–76.
56 Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, et al. Cortical firing and sleep homeostasis. *Neuron*. 2009;**63**:865–78.
57 Frank MG. In: Shaw PJ, Tafti M, Thorpy M, editors. Astroglial regulation of sleep in The Genetic Basis of Sleep & Sleep Disorders. London: Cambridge Press; 2013. p. 84–90.
58 Li X, Zima AV, Sheikh F, Blatter LA, Chen J. Endothelin-1-induced arrhythmogenic Ca2+ signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate(IP3)-receptor type 2-deficient mice. *Circ Res*. 2005;**96**:1274–81.
59 Foley J, Blutstein T, Lee S, Erneux C, Halassa MM, Haydon P. Astrocytic IP3/Ca2+ signaling modulates theta rhythm and REM sleep. *Front Neural Circuits*. 2017;**11**.
60 Xie AX, Petravicz J, McCarthy KD. Molecular approaches for manipulating astrocytic signaling in vivo. *Front Cell Neurosci*. 2015;**9**:44.
61 Sherwood MW, Arizono M, Panatier A, Mikoshiba K, Oliet SHR. Astrocytic IP(3)Rs: Beyond IP(3)R2. *Front Cell Neurosci*. 2021;**15**:695817.
62 Zeisel A, Muñoz-Manchado AB, Codeluppi S, Lönnerberg P, Manno GL, Juréus A, et al. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*. 2015;**347**:1138–42.
63 Fujino I, Yamada N, Miyawaki A, Hasegawa M, Furuichi T, Mikoshiba K. Differential expression of type 2 and type 3 inositol 1,4,5-trisphosphate receptor mRNAs in various mouse tissues: in situ hybridization study. *Cell Tissue Res*. 1995;**280**:201–10.
64 Orabi AI, Luo Y, Ahmad MU, Shah AU, Mannan Z, Wang D, et al. IP3 receptor type 2 deficiency is associated with a secretory defect in the pancreatic acinar cell and an accumulation of zymogen Granules. *PLoS One*. 2012;**7**:e48465.
65 Ouyang K, Leandro Gomez-Amaro R, Stachura DL, Tang H, Peng X, Fang X, et al. Loss of IP3R-dependent Ca2+ signalling in thymocytes leads to aberrant development and acute lymphoblastic leukemia. *Nat Commun*. 2014;**5**:4814.
66 Sampieri A, Santoyo K, Asanov A, Vaca L. Association of the IP3R to STIM1 provides a reduced intraluminal calcium microenvironment, resulting in enhanced store-operated calcium entry. *Sci Rep*. 2018;**8**:13252.
67 Parpura V, Grubišić V, Verkhratsky A. Ca2+ sources for the exocytotic release of glutamate from astrocytes. *Biochimica Et Biophysica Acta (BBA) - Mol Cell Res*. 2011;**1813**:984–91.
68 Lipscombe D, Helton TD, Xu W. L-type calcium channels: the low down. *J Neurophysiol*. 2004;**92**:2633–41.
69 Wahis J, Holt MG. Astrocytes, noradrenaline, α1-adrenoreceptors, and neuromodulation: evidence and unanswered questions. *Front Cell Neurosci*. 2021;**15**.

70 Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, et al. α1-Adrenergic receptors mediate coordinated Ca2+ signaling of cortical astrocytes in awake, behaving mice. *Cell Calcium*. 2013;**54**:387–94.
71 Durkee CA, Covelo A, Lines J, Kofuji P, Aguilar J, Araque A. G(i/o) protein-coupled receptors inhibit neurons but activate astrocytes and stimulate gliotransmission. *Glia*. 2019;**67**:1076–93.
72 Shen W, Chen S, Liu Y, Han P, Ma T, Zeng L-H. Chemogenetic manipulation of astrocytic activity: Is it possible to reveal the roles of astrocytes? *Biochem Pharmacol*. 2021;**186**:114457.
73 Chai H, Diaz-Castro B, Shigetomi E, Monte E, Octeau JC, Yu X, et al. Neural circuit-specialized astrocytes: transcriptomic, proteomic, morphological, and functional evidence. *Neuron*. 2017;**95**:531–549.e9.
74 Yang L, Qi Y, Yang Y. Astrocytes control food intake by inhibiting AGRP neuron activity via adenosine A1 receptors. *Cell Rep*. 2015;**11**:798–807.
75 Nagai J, Rajbhandari AK, Gangwani MR, Hachisuka A, Coppola G, Masmanidis SC, et al. Hyperactivity with disrupted attention by activation of an astrocyte synaptogenic cue. *Cell*. 2019;**177**:1280–1292.e20.
76 Mariotti L, Losi G, Sessolo M, Marcon I, Carmignoto G. The inhibitory neurotransmitter GABA evokes long-lasting Ca(2+) oscillations in cortical astrocytes. *Glia*. 2016;**64**:363–73.
77 Sun W, McConnell E, Pare JF, Xu Q, Chen M, Peng W, et al. Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. *Science*. 2013;**339**:197–200.
78 Bazargani N, Attwell D. Amines, astrocytes, and arousal. *Neuron*. 2017;**94**:228–31.
79 You S, Yu AM, Roberts MA, Joseph IJ, Jackson FR. Circadian regulation of the *Drosophila* astrocyte transcriptome. *PLoS Genet*. 2021;**17**:e1009790.
80 Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J. Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci*. 2008;**9**:910–9.
81 Krueger JM, Nguyen JT, Dykstra-Aiello CJ, Taishi P. Local sleep. *Sleep Med Rev*. 2019;**43**:14–21.
82 Zhang Q, Haydon PG. Roles for gliotransmission in the nervous system. *J Neural Transm*. 2005;**112**:121–5.
83 Strecker RE, Morairty S, Thakkar MM, Porkka-Heiskanen T, Basheer R, Dauphin LJ, et al. Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behav Brain Res*. 2000;**115**:183–204.
84 Szymusiak R, Gvilia I, McGinty D. Hypothalamic control of sleep. *Sleep Med Adv Sleep Med*. 2007;**8**:291–301.
85 Benington J, Heller HC. Restoration of brain energy metabolism as the function of sleep. *Prog Neurogibol*. 1995;**45**:347–60.
86 Oberheim NA, Goldman SA, Nedergaard M. Heterogeneity of astrocytic form and function. *Methods Mol Biol*. 2012;**814**:23–45.
87 Bekar LK, He W, Nedergaard M. (2008) Locus coeruleus alpha-adrenergic-mediated activation of cortical astrocytes in vivo. *Cereb Cortex*. 1991;**18**:2789–95.
88 Matias I, Morgado J, Gomes FCA. Astrocyte heterogeneity: Impact to brain aging and disease. *Front Aging Neurosci*. 2019;**11**:55.
89 Pestana F, Edwards-Faret G, Belgard TG, Martirosyan A, Holt MG. No longer underappreciated: The emerging concept of astrocyte heterogeneity in neuroscience. *Brain Sciences*. 2020;**10**:168.
90 Vasile F, Dossi E, Rouach N. Human astrocytes: structure and functions in the healthy brain. *Brain Struct Funct*. 2017;**222**:2017–29.
91 Verkhratsky A, Ho MS, Parpura V. In: Verkhratsky A, Ho MS, Zorec R, Parpura V, editors. Evolution of Neuroglia in Neuroglia in Neurodegenerative Diseases. Singapore: Springer Singapore; 2019. p. 15–44.
92 Frank MG. The ontogenesis of mammalian sleep: form and function. *Curr Sleep Med Rep*. 2020;**6**:267–79.
93 Ingiosi AM, Opp MR, Krueger JM. Sleep and immune function: glial contributions and consequences of aging. *Curr Opin Neurobiol*. 2013;**23**:806–11.
94 Lee J, Chun YE, Han KS, Lee J, Woo DH, Lee CJ. Ca (2+) Entry is Required for mechanical stimulation-induced ATP release from astrocyte. *Experi Neurobiol*. 2015;**24**:17–23.
95 Kawamata H, Ng SK, Diaz N, Burstein S, Morel L, Osgood A, et al. Abnormal intracellular calcium signaling and SNARE-dependent exocytosis contributes to SOD1G93A astrocyte-mediated toxicity in amyotrophic lateral sclerosis. *J Neurosci*. 2014;**34**:2331–48.
96 Frank MG. Astroglial regulation of sleep homeostasis. *Curr Opin Neurobiol*. 2013;**23**:812–818.