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Integrated Brain Circuits: Astrocytic Networks Modulate Neuronal Activity and Behavior

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Abstract

The past decade has seen an explosion of research on roles of neuron-astrocyte interactions in the control of brain function. We highlight recent studies performed on the tripartite synapse, the structure consisting of pre- and postsynaptic elements of the synapse and an associated astrocytic process. Astrocytes respond to neuronal activity and neuro-transmitters, through the activation of metabotropic receptors, and can release the gliotransmitters ATP, D-serine, and glutamate, which act on neurons. Astrocyte-derived ATP modulates synaptic transmission, either directly or through its metabolic product adenosine. D-serine modulates NMDA receptor function, whereas glia-derived glutamate can play important roles in relapse following withdrawal from drugs of abuse. Cell type–specific molecular genetics has allowed a new level of examination of the function of astrocytes in brain function and has revealed an important role of these glial cells that is mediated by adenosine accumulation in the control of sleep and in cognitive impairments that follow sleep deprivation.

Keywords

sleep; ATP; adenosine; NMDA; astrocyte; synapse

INTRODUCTION

In 1895 Santiago Ramóny Cajal proposed that astrocytes, the major subtype of glial cell in the brain, control sleep and waking states. He specifically proposed that astrocytic processes are electrical insulators that, when extended between neurons, act as circuit breakers to facilitate sleep but, when retracted, allow neuronal circuits to communicate, facilitating wakefulness (1). Now, more than a century after Cajal's proposal, we know that his intuition was, in part, correct; astrocytes have an intimate structural and functional association with neurons and, by virtue of their complex physiology, are able to modulate behaviors such as sleep (2).

DISCLOSURE STATEMENT

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Cajal was the first to systematically study astrocytes from a structural standpoint (1), and until very recently, our view of astrocytic morphology has been based both on Cajal's metal impregnation methods and on glial fibrillary acidic protein (GFAP) staining. Advances in cellular labeling and imaging technologies showed that astrocytic morphology is far more complicated than previously thought. By filling single astrocytes with fluorescent dyes, researchers in one study showed that GFAP staining reveals only 15% of the astrocytic volume and that astrocytes extend fine processes that occupy the surrounding neuropil (3). Astrocytes occupy nonoverlapping spatial territories in which a single astrocyte contacts hundreds of neuronal processes and multiple neuronal cell bodies (3, 4). The processes of one astrocyte contact tens of thousands of synapses, with more than 50% of hippocampal excitatory synapses, for example, being closely opposed to an astrocytic process (5) at a structure termed the tripartite synapse to recognize the structural and functional relationship between the astrocyte and the pre- and postsynaptic terminals (6). Astrocytes are also intimately associated with the cerebral microvasculature, onto which they extend several endfeet (7). By being strategically positioned between synapses and blood vessels, astrocytes are thought to be mediators of neurovascular coupling, the process by which neuronal activity is coupled to cerebral blood flow and the cellular substrate of functional brain imaging (8–12).

Astrocytes interact with neurons at multiple spatial and temporal scales. By controlling the ionic and metabolic environment of the neuropil, astrocytes can dramatically impact neuronal activity. In addition, astrocytes listen and talk to synapses via regulated pathways of transmitter release (13, 14). The use of molecular genetics has revolutionized the study of astrocytic physiology and, as we discuss below, has provided an unprecedented understanding of how these cells impact brain function at the levels of synapses, circuits, and behavior. The view that the brain is a collection of integrated circuits of astrocytes and neurons that control thought and behavior may not only enhance our understanding of this fascinating organ but also have many implications for the treatment of neurological and psychiatric disorders.

ASTROCYTIC MEMBRANE PROPERTIES SUPPORT NEURONAL PHYSIOLOGY

Although not equipped with the cellular machinery necessary for generating action potentials, an astrocyte exhibits changes in its electrical properties that are essential for supporting normal neuronal activity. Astrocytes express inward rectifier K^+ (K_{ir}) channels (15), which maintain the astrocytic membrane potential close to the equilibrium potential of K^+. Thus, when extracellular K^+ rises, K^+ ions flow into astrocytes through these inward rectifiers. Astrocyte-specific knockout of Kir4.1, the major K_{ir} channel in these glia, results in seizure activity and premature death (16). Astrocytes also express Ca^{2+}-activated potassium channels (BK channels), which allow for coupling of astrocytic Ca^{2+} signaling (discussed below) to the release of K^+ from astrocytic endfeet onto blood vessels (17).

Astrocytes express a number of electrogenic neurotransmitter transporters such as glutamate (18), GABA (19), and glycine (20). Not only is the uptake of transmitters necessary for maintaining the fidelity of synaptic transmission, but this process is part of a cycle that provides presynaptic terminals with a renewable source of these transmitters. For example, when glutamate is taken up by astrocytes, the enzyme glutamine synthetase (GS) converts it to glutamine (21). Astrocytes then release glutamine onto both glutamatergic and GABAergic terminals. Because GABAergic terminals have a limited glutamine store, pharmacological inhibition of GS results in failure of inhibitory synaptic transmission under conditions of elevated neuronal activity (22). One consequence of such failure can be the development of seizures (23, 24). Another can be the dysregulation of neuronal coding and

information transfer among networks; because GABAergic inhibition acts as an oscillatory timing signal for neuronal network activity (25–27), failure of inhibition profoundly affects information processing in the brain and is thought to contribute to a number of psychiatric states (28, 29). Thus, by being the sole supplier of glutamine to synapses, astrocytes have the potential to shape neuronal activity and information processing by controlling neuronal inhibition.

ASTROCYTES FORM METABOLIC NETWORKS THAT RESPOND TO EXTERNAL STIMULI

Astrocytes play essential roles in brain energy homeostasis and metabolism (30). These glial cells express transporters that mediate the uptake of glucose from cerebral microvessels. An important structural/signaling feature of astrocytes is their extensive intercellular coupling, mediated mainly by connexin 43– and connexin 30–containing gap junctions (31). Such gap junctions allow for the diffusion of glucose among many astrocytes (32), and the genetic ablation of these connexins profoundly impacts neuronal activity and metabolism.

Glucose, which is taken up by the endfeet of the astrocytes, can diffuse through the multicellular astrocytic network through gap junctions. This process is regulated because the diffusion of fluorescent glucose analogs among hippocampal astrocytes is enhanced by neuronal activity (32). This process is sensitive to AMPA receptor blockade, suggesting the involvement of postsynaptic mechanisms in recruiting astrocytic metabolic coupling. Under conditions of high neuronal activity and low metabolic supply, astrocytic metabolic coupling is essential for maintaining glutamatergic synaptic transmission. Delivering glucose specifically to a single astrocyte rescues synaptic activity when extracellular glucose is lowered, but this process is inhibited by astrocyte-specific genetic ablation of connexin 43 and connexin 30.

Following its uptake by astrocytes, glucose is either stored as glycogen or metabolized to lactate (33). Astrocytic glycogen metabolism is regulated by neuronal mechanisms. For example, under conditions of increased metabolic demand, noradrenaline activates astrocytic cAMP signaling, resulting in the breakdown of intra-cellular glycogen. The resulting glucose is further metabolized by astrocytes to lactate and is released to the extracellular space, where it is taken up by neurons (34). This metabolic relationship between neurons and astrocytes, known as the lactate shuttle, is a highly dynamic process that can be regulated by a number of physiological processes, including the sleep/wake cycle (30). Thus, as we discuss below, the function of astrocytes as modulators of sleep in mammals may link their metabolic functions to their neuromodulatory roles.

ASTROCYTES EXHIBIT Ca^{2+} EXCITABILITY

Astrocytes respond to transmitter spillover from nearby synaptic activity with an elevation of their Ca^{2+} level. Neuronally released transmitters can engage astrocytic metabotropic receptors, a subset of which couples through Gq to phospholipase C (PLC), resulting in the accumulation of diacylglycerol (DAG) and IP_3. IP_3 binds to its receptor and causes the release of Ca^{2+} from internal stores. The activation of this signaling pathway has now been demonstrated to occur in vivo in response to sensory stimulation. For example, stimulation of an individual mouse whisker causes Ca^{2+} signals in astrocytes located in the corresponding cortical barrel. This response, which has a latency of ~3 s, is dependent on activation of astrocytic metabotropic glutamate receptors (35). In the visual cortex of the ferret, astrocytic activity is driven by visual gratings that trigger the activity of nearby neurons, suggesting a spatial alignment between these two cell types (36). Interestingly, astrocytes show sharper tuning to orientation and spatial frequency than do neurons, and a

group of 2 to <10 astrocytes respond to a particular one-stimulus orientation, suggesting a network organization of visual cortical astrocytes (36). Because sensory tuning of cortical neurons is shaped by the interplay between excitation and inhibition (37), this experiment raises the possibility that astrocytes are tracking the output of these cortical pyramidal neurons rather than their input. Pharmacological agents that inhibit astrocytic Ca^{2+} signaling while leaving the neuronal response intact, such as increasing the level of isofluorane anesthesia, also inhibit the activity-dependent vascular response in the visual cortex (36). Interestingly, the astrocytic response is inhibited not by metabotropic glutamate receptor antagonists in the visual cortex of the ferret, but instead by glutamate transporter inhibitors, suggesting that the sensory-evoked response of astrocytes in the visual cortex is distinct from that in the barrel cortex. However, caution must be exerted in interpreting such an observation because the resulting increase in ambient glutamate may lead to desensitization of metabotropic receptors.

Similar to the findings from the visual cortex of the ferret, olfactory bulb astrocytes in the mouse respond to odor-evoked synaptic input, and their response coincides with the vascular response (38). These converging findings from different brain regions and different species strongly link astrocytic Ca^{2+} signaling to the activity-dependent neurovascular coupling. Interestingly, recent findings have shown decoupling of cerebral blood flow from local neuronal activity (39) under certain conditions, suggesting a nonneuronal mediator of the cerebrovascular signal and that under such conditions the vascular response plays an anticipatory role in brain state changes, rather than act as a follower for neuronal activity.

ASTROCYTES RELEASE CHEMICAL TRANSMITTERS TO MODULATE NEURONAL ACTIVITY

In addition to regulating neuronal signaling by controlling the ionic environment of the neuropil and controlling the supply of neuro-transmitters to synapses, astrocytes can directly activate neuronal receptors by releasing gliotransmitters. Gliotransmission is an umbrella term that includes the release of many chemical transmitters from astrocytes, including classical transmitters, peptides, chemokines, and cytokines, through a number of different mechanisms.

Exocytosis mediates one of the better-characterized pathways of gliotransmission. Regulated exocytosis is dependent on the docking and fusion of vesicles to the plasma membrane, which is mediated via the formation of the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) complex. Astrocytes express the core machinery proteins involved in forming the SNARE complex, such as synaptobrevin II (40, 41) [and its homolog cellubrevin (42)] and SNAP-23 (40, 41), and ancillary proteins to this complex, such as Munc 18 (40, 41), complexin 2 (40, 41), and synaptotagmin IV (43). Molecular genetic manipulations directed at the SNARE complex and to perturb synaptotagmin IV have shown their requirement for Ca^{2+}-regulated vesicular gliotransmission.

In cultured astrocytes, SNARE proteins colocalize with a number of vesicular organelles, including small vesicles positive for vesicular glutamate transporters (VGlut 1–3) (40–42), ATP-storing vesicles (44, 45), and D-serine-containing vesicles (46), suggesting the involvement of vesicular mechanisms in the release of these gliotransmitters. The size of these vesicular organelles ranges between 30 and 100 nm in diameter in cultured astrocytes, and immunoelectronmicroscopy (IEM) in situ has shown the existence of 30-nm clear astrocytic vesicles opposed to presynaptic terminals (47). This latter demonstration strongly supports the existence of a vesicular pathway of gliotransmission in the intact brain.

ASTROCYTES RELEASE GLUTAMATE, WHICH MODULATES NEURONAL NMDA RECEPTOR FUNCTION

Glutamate was the first gliotransmitter shown to be released by astrocytes. The first evidence for its release came from high-performance liquid chromatography (HPLC) in cultured astrocytes, where bradykinin-evoked Ca^{2+} signaling was followed by the release of glutamate from these cells (48). Astrocytic glutamate was also detected by cocultured neurons, where bradykinin evoked N-methyl-D-aspartate (NMDA)-dependent Ca^{2+} transients in neurons only in the presence of astrocytes. In addition to vesicular gliotransmission of glutamate, other pathways of glutamate gliotransmission have been proposed, including reversal of uptake by glutamate transporters (49), anion channel–mediated efflux (50), release by the glutamate/cystine antiporter (51), diffusion through ionotropic purinergic receptors (52), and hemichannel (connexin/pannexin)-mediated efflux (53). Although all pathways can mediate release of glutamate, it is extremely important to identify the conditions that activate these release pathways so that it will become possible to understand when they are utilized in the nervous system.

Although much of our discussion here focuses around Ca^{2+}-regulated exocytosis, we discuss one transporter, the cystine/glutamate transporter, as it has great relevance for addiction and brain tumors. Astrocytes express a cystine/glutamate transporter that is responsible for the uptake of cystine, which is subsequently used in the synthesis of glutathione. Following a subject's withdrawal from cocaine administration, the extracellular levels of glutamate in the nucleus accumbens fall due to reduced activity of this transporter, which exchanges glutamate for cystine. To determine whether the reduced activity of this transporter is important for relapse and reinstatement of drug-seeking behavior following subsequent exposure to cocaine, Kalivas's group experimentally activated this transporter while monitoring behavior. Either direct infusion of cystine or systemic administration of N-acetylcysteine, which activates the transporter and increases extracellular glutamate, prevented relapse (54).

The same cystine/glutamate transporter is receiving considerable scrutiny in the study of glioblastoma multiforme. Glioma cells release excess glutamate as a consequence of the activation of the cystine/glutamate transporter. Because glioma do not express Na^{+}-dependent glutamate transporters, which normally take up glutamate, the presence of gliomal cells leads to enhanced extracellular glutamate, which causes exocytotic death of neurons, which presumably helps clear space for tumor growth. Cystine uptake is rate limiting the production of glutathione. Sontheimer's group has investigated the importance of cystine/glutamate transport in tumor growth and found that inhibition of the transporter leads to a rapid depletion of glutathione and a reduction in tumor growth (55). On the basis of these results, clinical trials are under way, with the objectives of stimulating the transporter to prevent relapse and inhibiting the transporter as a treatment for brain tumors.

Ca^{2+}-regulated glutamatergic gliotransmission targets neuronal extrasynaptic NR2B-containing NMDA receptors, resulting in changes in neuronal excitability and modulating synaptic transmission. Two forms of modulation have been observed, discrete transient slow-inward currents (SICs) and a tonic modulation of synaptic transmission mediated through NMDA receptors. SICs have been observed in physiological conditions of normal extracellular Mg^{2+} in brain slices isolated from the thalamus, hippocampus, cortex, nucleus accumbens, and olfactory bulb (56–62). These events have distinct kinetics that distinguishes them from excitatory postsynaptic currents (EPSCs), and a unique pharmacological signature that is consistent with their nonneuronal origin (57). SICs are not blocked by tetrodotoxin (TTX) or perfusion with tetanus toxin (TeNT) at a concentration that results in inhibition of EPSCs (57). Whole-cell patch clamp of hippocampal pyramidal

neurons has shown that these events can occur synchronously in two neurons within 100 μm from one another, suggesting that a single astrocyte can synchronize a group of neurons it contacts via glutamatergic gliotransmission (4). Several groups have shown that, in addition to an increase in SICs, glutamatergic gliotransmission results in an increase in the frequency of EPSCs in nearby neurons (47, 63) or can tonically activate postsynaptic NMDA receptors (64). Astrocytic modulation of synaptic transmission is dependent on a vesicular mechanism because it is attenuated by the dialysis of the light chain of TeNT into individual astrocytes and because small-electron lucent vesicles are apposed to astrocytic membranes adjacent to presynaptic NR2B receptors that mediate the synaptic modulation (47).

THE DEVIL IS IN THE DETAILS: DISCREPANCIES IN THE FIELD

Although it is clear that glutamatergic gliotransmission occurs under physiological conditions in situ (57, 59), this research is now entering a phase in which it is necessary to understand the details of the process so that we may gain a thorough understanding of its function in vivo. There are several questions that need to be confronted in order to gain a more thorough appreciation of the significance of glutamatergic gliotransmission. Here we discuss three prominent concerns in the field.

First is the neurochemical concern: Because astrocytes metabolize glutamate to glutamine, there may be insufficient residual glutamate for gliotransmission. However, two arguments indicate that this is not the case: (*a*) Because astrocytes are living, there is sufficient residual glutamate for protein synthesis despite the conversion of glutamate to glutamine by the enzyme glutamine synthetase. (*b*) The K_m of vesicular glutamate transporters is lower than that of glutamine synthetase, providing the opportunity for glutamate transport into vesicles. Glutamine synthetase has a K_m of ~7.0 mM for glutamate (65). Vesicular glutamate transporters have K_ms of ~0.6–4.7 mM for glutamate (66–68), indicating that these molecules can package glutamate in astrocytic vesicles under physiological conditions.

Second, it is important to determine which molecules are expressed in astrocytes in vivo and whether they are sufficient to support gliotransmission. Numerous approaches have been used to study glutamate, and the majority support the idea that astrocytes contain the necessary machinery for glutamatergic gliotransmission. Studies performed in cell culture, in brain slices, with acutely isolated astrocytes, and with tissue sections provide compelling support for the presence of vesicular machinery for glutamatergic gliotransmission. Studies in cell culture have provided the highest resolution for the study of glutamatergic gliotransmission. To name a few, astrocytes both in vitro and in vivo express vesicular glutamate transporters (69, 70), SNARE proteins that are necessary for membrane fusion, as well as synaptotagmin IV (43, 69). Because of the concern that cultured cells may express aberrant proteins, considerable work has been performed in vivo and with freshly isolated astrocytes. Such research is in agreement with the detection of the message (based on RT-PCR) and the protein. However, in contrast, two studies, each using microarrays, have not detected the message for vesicular glutamate transporters (71, 72). The reason for this apparent discrepancy is not clear. However, it is important to appreciate some of the concerns about the interpretation of microarray data. One pressing issue is the lack of consensus in data acquired by commercially available microarray platforms (73) and the high variability in relative gene expression profiles obtained from different laboratories performing seemingly identical experiments (74). This issue is particularly important in light of the microarray data, which report a lack of enrichment of Entpd2, a 5′ ectonucleotidase gene, in astrocytes (71), disagreeing with decades of biochemical and immunohistochemical findings that show that this protein is expressed primarily by astrocytes (75). Because microarray studies set cut-off criteria for the number of copies, a gene must be considered “enriched” in a specific cell type, and such studies run the risk of overlooking genes with a

relatively low abundance. Certain neuronal genes, such as β-neurexin, have low message abundance but long-lived protein expression, which makes them particularly resistant to RNAi-mediated gene knockdown (76). In contrast, the more sensitive method of RT-PCR has detected these messages. However, RT-PCR can also be criticized, although in contrast to microarrays, on the basis of its high sensitivity and potential to amplify contaminating mRNA. Perhaps the most critical observation is based on the use of IEM, which demonstrates the presence of VG-LUT on astrocytic vesicles adjacent to synapses (69). Further work is required to determine the reasons for such discrepancies.

A third concern is that astrocytic Ca^{2+} signals regulate the dilatation/constriction of microvessels as well as gliotransmission. A priori one would envision that it is important to have layers of control mechanisms that would allow differential signaling to these two targets—the vasculature and synapses. Considerable evidence supports the presence of localized Ca^{2+} signals within a subregion of an individual astrocyte. Such microdomain Ca^{2+} signals would permit local synaptic modulation without regulating the vasculature, which requires a Ca^{2+} signal to propagate along the process of the astrocyte that links to the endfoot. When a cell-wide Ca^{2+} signal that will regulate the vasculature is evoked, will gliotransmission necessarily be activated? Perhaps not. Although a wealth of experimental data in tissue culture and in brain slice preparations supports a model in which astrocytic Ca^{2+} signaling is necessary and sufficient for glutamate release, recent studies show that there are conditions in which an astrocytic Ca^{2+} signal does not initiate gliotransmission. Despite the ability of astrocytic flash photolysis of caged Ca^{2+} and caged IP_3 to evoke SICs (57) and increases in the frequency of EPSCs (63) in nearby neurons, a recent study has shown that the regulation of this process is more complex than initially appreciated because not all agonist-induced Ca^{2+} signals trigger glutamatergic gliotransmission (61) (see below for a discussion).

Molecular genetic techniques have been used to express novel G protein–coupled proteins (GPCRs) in astrocytes to ask whether their selective activation triggers glutamatergic gliotransmission (77). One of these receptors, MrgA1, is normally expressed by dorsal root ganglion neurons but not expressed in the central nervous system (CNS). Expression of this receptor in astrocytes results in Ca^{2+} transients in these glia in response to the peptide ligand FLRFa. However, despite a robust volume-averaged Ca^{2+} signal, astrocyte-dependent modulation of neighboring neurons was not detected. The use of molecular genetics to introduce a foreign receptor into astrocytes for the study of receptor-activated gliotransmission is highly innovative but is not without potential concerns. For example, the expression of a foreign receptor, RASSL, in astrocytes by the same group caused hydrocephalus, even in the absence of the foreign ligand (78). Whether the expression of the foreign receptor MrgA1, which was used to activate astrocytic Ca^{2+} signals, perturbed the physiological state of the astrocyte is not known. However, it is of concern that this publication does not report the use of doxycycline to prevent transgene expression during development, raising the possibility that the inability of this laboratory to reproduce their earlier results and those of others may be a product of the experimental approach. Further support for this possibility is that, in the same study, photolysis of caged IP_3 was shown capable of evoking gliotransmission.

An alternative interpretation is that there is more to a Ca^{2+} signal than just its amplitude. Support for this possibility is provided by the work of Khakh's laboratory, which has shown a differential coupling between distinct astrocytic receptors and gliotransmission. Although the activation of two astrocyte-enriched receptors, PAR-1 and P2YR, resulted in Ca^{2+} signals of similar amplitudes but slightly different kinetics, only PAR-1 receptor activation triggered SICs in nearby neurons (61). Evidence for an astrocytic origin of this process is provided by dialysis of the Ca^{2+} chelator BAPTA into the astrocytic syncytium, which

inhibits PAR-1-induced SICs. The optical activation of surface-expressed ion channels in astrocytes can evoke the release of ATP from astrocytes in vivo, raising the possibility that astrocytes use different sources of Ca^{2+} to support different modes of gliotransmission. The discovery that astrocytes express a number of TRP channels (79) supports the possibility that influx of Ca^{2+} from the extracellular space may support certain forms of gliotransmission. These studies raise the exciting possibility of distinct astrocytic Ca^{2+} signals, ones that couple with gliotransmission and others that may mediate other processes such as the control of the vasculature.

ASTROCYTES RELEASE D-SERINE, WHICH MODULATES NEURONAL NMDA RECEPTOR FUNCTION

NMDA receptor gating is regulated by several signals. Although glutamate binding is essential for NMDA receptor activation, ion permeation requires coincident depolarization. This coincident-detector property of the NMDA receptor is dependent on Mg^{2+} ions blocking the receptor pore at negative membrane potentials. In addition, receptor activity is regulated by a coagonist binding site, the so-called glycine binding site (80). In many brain areas D-serine is an endogenous ligand for this site. D-serine is synthesized by the enzyme serine racemase, which is expressed predominantly by astrocytes (81). Astrocytes release D-serine-containing vesicles in a Ca^{2+}-dependent manner (46), and this form of gliotransmission is thought to regulate NMDA receptor function and synaptic plasticity (82). Long-term depression (LTD) and long-term potentiation (LTP) are two opposing forms of synaptic plasticity requiring different degrees of NMDA receptor activation. The innovative use of the hypothalamus as a model for astrocyte-neuron interaction has provided considerable insight into the role of D-serine in synaptic plasticity. For example, in the supraoptic nucleus, astrocytic ensheathment of synapses is reduced during lactation (82). As a consequence, less D-serine is provided to NMDA receptors, resulting in a switch in the ability of a neuronal stimulus to induce plasticity. Virgin rodents with a higher degree of astrocytic coverage of synapses and, thus, relatively high synaptic D-serine levels exhibit LTP, whereas lactating rodents with reduced synaptic coverage by astrocytes, D-serine levels, and NMDA receptor activation exhibit LTD. Thus, the degree of astrocyte-induced D-serine-dependent coactivation of the NMDA receptor confers metaplasticity to the synapse. Given that astrocytic processes are highly dynamic and capable of extending and retracting on the timescale of minutes (83, 84), an exciting possibility is that this form of astrocyte-induced metaplasticity is a widespread process in the CNS.

THE RELEASE OF ATP BY ASTROCYTES

Shortly after the observation that astrocytes can release glutamate in culture, several groups demonstrated that ATP can also be released from astrocytes and that it mediates coupling between astrocytes at least in culture. In culture and in vivo, elevation of the Ca^{2+} signal within one astrocyte can lead to a Ca^{2+} wave that propagates through the coupled glial network. High-resolution cell culture studies showed that ATP is the signal mediating this Ca^{2+} wave (85). Indeed, considerable evidence shows that ATP is a significant extracellular signaling molecule that is utilized by astrocytes to signal with one another as well as to neurons. Below, we discuss astrocytic purinergic release mechanisms, roles of purines in synaptic modulation, as well as the recently discovered role of an astrocytic ATP metabolic product, adenosine, in the modulation of sleep phenotypes.

Incubation of astrocyte cultures with styryl dye results in labeling of a recycling pool of vesicles that include lysosomes (44, 86). Although traditionally thought of as organelles of degradation, lysosomes of a number of cells, including melanocytes and hematopoietic cells, are secretion competent (87). In one study, lysosomes were imaged in living astrocytes by

transfecting an EGFP-tagged version of the lysosomal marker CD-63. This fusion protein, colocalized with a number of styryl dyes, including FM1-43 and FM2-10, but did not colocalize with vesicular glutamate transporters, suggesting that these organelles do not support glutamatergic gliotransmission (44). Astrocytic stimulation by ATP or glutamate resulted in partial destaining of these CD-63^{+}, FM2-10-labeled puncta, suggesting a kiss-and-run mode of fusion for lysosomes. Furthermore, incubating astrocytes with the cell-permeable Ca^{2+} chelator BAPTA-AM inhibited lysosomal fusion, suggesting a Ca^{2+}-dependent mechanism for lysosomal exocytosis by astrocytes. In another study, astrocytes were labeled with FM4-64 in an activity-independent manner, and the resulting puncta colocalized with the lysosomal marker lysotracker and with a number of vesicular SNARE proteins, including synaptobrevin II and cellubrevin. This study also showed a Ca^{2+}-dependent mode of exocytosis of astrocytic lysosomes (86).

What, if any, sort of gliotransmission do lysosomes support? Biochemical and imaging experiments show that these organelles are highly enriched in ATP. Subcellular fractionation experiments revealed that the highest levels of ATP are found in the fractions containing the lysosomal markers LAMP-1 and β-hexosaminidase. In contrast, confocal imaging showed that incubation of astrocytes with Mant-ATP, a fluorescent ATP analog, results in colocalization with EGFP-CD63 and with FM2-10 (44). Interestingly, astrocytes appear to be highly enriched in *Bloc1s1*, a gene involved in lysosome biogenesis (71), raising the possibility that astrocytes require a constant generation of this organelle for continuous release of purines.

A previous study has shown that ATP is enriched in astrocytic dense-core vesicles that are positive for secreteogranin II (45). This study also showed that the release of ATP from astrocytes is Ca^{2+} dependent and is inhibited by TeNT. Thus, the evidence is strong for a Ca^{2+}-dependent, vesicular pathway of ATP release from astrocytes. However, whether such release is predominantly mediated by lysosomes or dense-core vesicles is still not clear. The recent discovery of the vesicular nucleotide transporter (VNUT) (88) and its enrichment in brain astrocytes may be helpful in answering this important question and others related to the mechanism of vesicular ATP release by astrocytes.

PHYSIOLOGICAL CONSEQUENCES OF ASTROCYTIC PURINES ON SYNAPSES AND EXCITABILITY

In hypothalamic slices, astrocytic release of ATP is necessary and sufficient for noradrenaline-dependent synaptic potentiation (89). Hypothalamic astrocytes express α1-adrenergic receptors, and in response to adrenergic input they release ATP onto nearby magnocellular neurosecretory neurons. Activation of P2X7 receptors on these neurons, as a result, causes the enhancement of AMPA receptor surface expression and an increase in miniature excitatory postsynaptic current (mEPSC) amplitude. In contrast, studies in the mammalian retina demonstrated the role of astrocytic purinergic signaling in suppressing neuronal activity (90). Using retinal whole-mounts, Newman showed that stimulation of photoreceptors using light causes glial Ca^{2+} signaling (91) and that such Ca^{2+} signals lead to ATP release from Muller cells (92). Patch clamp recordings showed that the resulting actions on ganglion cells are mediated by adenosine acting through adenosine 1 (A1) receptors to suppress neuronal activity. Both studies demonstrated ATP release, but one study showed that ATP has direct actions, whereas the latter showed that this gliotransmitter acts through a metabolite, adenosine. It is unclear whether there is an absence of ectonucleotidases in the hypothalamus or whether there is a tight spatial association of glial ATP release sites and neuronal P2X7 receptors to allow direct actions of ATP in this system.

Similar suppressive actions of glia-derived adenosine have been observed in the hippocampus (93–95). Hippocampal astrocytes release ATP, which, following its degradation by extra-cellular nucleotidases to adenosine, causes an A1-dependent presynaptic inhibition of synaptic transmission. Because astrocytes express receptors and use signaling pathways that are shared with neurons, it is difficult to use pharmacological manipulations to discern the role of these glia in the modulation of neuronal physiology. However, the use of glia-specific toxins (95) and the selective loading of the Ca^{2+} chelator BAPTA into astrocytes (94) have shown that the aforementioned suppressive actions of adenosine on hippocampal synaptic transmission are of glial origin.

PURINERGIC GLIOTRANSMISSION MODULATES SYNAPTIC NETWORKS

In search of an understanding of the importance of gliotransmission, we developed conditional astrocyte-specific molecular genetic manipulations based on our initial cell culture studies so that we could study the importance of gliotransmission in situ and in vivo. Several studies have shown that astrocytes express SNARE proteins that are required for membrane-membrane fusion. In cultures the overexpression of the cytoplasmic SNARE domain of synaptobrevin (in the absence of the vesicular tail) led to reduced glutamate release from astrocytes. We applied this perturbation in situ and in vivo through the development of a tet-off astrocyte-specific mouse that expresses dnSNARE only in astrocytes. Molecular genetic inhibition of SNARE-dependent gliotransmission resulted in the discovery that ATP is a major gliotransmitter in vivo (2). In our initial studies we were surprised to find that the magnitude of Schaffer collateral CA1 synaptic transmission is enhanced when astrocytes express dnSNARE. Fortuitously, we discovered that the well-known tonic A1 receptor–mediated presynaptic inhibition of excitatory synaptic transmission is mediated by adenosine derived from an astrocytic source (93).

Astrocytic specificity of this manipulation was validated in vitro (93) and in vivo (2). The observation that SNAP-23 appears in the cytoplasmic fraction when dnSNARE is expressed in astrocytes confirmed the hypothesis that this molecular manipulation targets vesicular gliotransmission. One concern about this approach is that it should selectively perturb one specific pathway of membrane trafficking—the regulated release of gliotransmitter—and not impact trafficking of receptors, channels, transporters, or enzymes to the membrane. Previous studies examining the specificity of SNARE domain interactions indicate that specificity should be achieved with this approach because SNARE domains compete in a highly SNARE protein–specific manner. This possibility was borne out by control experiments showing that dnSNARE expression does not affect astrocytic membrane physiology, including K^+ buffering, glutamate transport activity, and agonist-dependent Ca^{2+} signaling. Moreover, ectonucleotidases that are responsible for the hydrolysis of ATP to adenosine are intact, as the exogenous addition of ATP fully reconstitutes A1-dependent signaling in transgenic mice expressing dnSNARE.

Hippocampal slices derived from animals expressing dnSNARE in astrocytes (dnSNARE animals) during postnatal life following weaning show enhanced basal synaptic transmission. Luciferin/luciferase bioluminescence studies showed that astrocytic dnSNARE expression leads to reduced extracellular ATP as well as adenosine and that in wild-type mice the inhibition of ectonucleotidases leads to enhanced ATP-dependent signaling. Because exogenous ATP can reconstitute the A1-dependent signaling in transgenic mice, we concluded that the tonic source of adenosine that is responsible for A1 receptor activation is astrocytic ATP. This is an important observation because it was previously thought that adenosine is derived from a metabolic source that is cell type independent: During activity adenosine accumulates intracellularly and exits into the extracellular space through equilibrative nucleotide transporters. In actuality, under physiological conditions adenosine

is derived from astrocytic ATP that is released into the extracellular space, and under hypoxic conditions the metabolic source predominates (96).

In addition to this tonic control of neuronal A1 receptor functions, astrocytic Ca^{2+} signaling enhances the vesicular pathway of ATP gliotransmission. Tetanic stimulation of a subset of CA3-CA1 synapses leads to A1 receptor–dependent heterosynaptic depression of neighboring (untetanized) synapses (97) that requires the recruitment of astrocytes, which provide adenosine (93). Thus, as the activity of neurons waxes and wanes, astrocytes have the potential to dynamically modulate neuronal network function.

PURINERGIC GLIOTRANSMISSION MODULATES SLEEP

A1 receptor function regulates a number of mammalian behaviors including sleep (98). Sleep is a fundamental behavior that is universal among multicellular animals (99). Evidence suggests that if an organism has a nervous system, then that nervous system must sleep (100). Although modulated by the circadian clock, which entrains many physiological processes to salient environmental cues, such as the light/dark cycle (101), sleep is additionally controlled by a homeostatic process (also known as Process S), which is completely dissociable from circadian control. The fact that, when prevented, sleep is subsequently compensated suggests that important processes take place during this state, and may be the strongest argument against the null hypothesis of sleep function (that sleep has evolved as an inactive state to hide organisms from their predators).

The molecular and cellular process underlying sleep homeostasis has been under investigation for more than 90 years. This investigation started with transfer experiments in which cerebrospinal fluid (CSF) extracts and cerebral venous blood from sleep-deprived animals were injected into control animals to determine whether they could induce sleep. Although these experiments were unsuccessful at identifying endogenous sleep factors, they were essential in establishing that such factors are generated locally within the brain (102).

More recently, studies have implicated adenosine as an endogenous sleep factor. Porkka-Heiskanen and colleagues have determined that adenosine levels vary with sleep propensity; during wakefulness, adenosine levels progressively increase, whereas during sleep they subside. Antagonizing adenosine (both A1 and A2A receptors) promotes wakefulness (103), and injecting adenosine or its agonists into the brain promotes sleep (104). Thus, adenosine may be not only a sleep factor but also a mediator of the homeostatic sleep response.

Criteria for identifying sleep were initially developed for the study of mammals and thus are both behavioral and electrophysiological (105). During sleep, organisms exhibit an increased threshold to sensory stimulation (99), and in mammals, specific changes occur in the electroencephalogram (EEG) (106). On the basis of a combination of these criteria, mammalian sleep can be subdivided into rapid-eye-movement (REM) sleep and nonrapid-eye-movement (NREM) sleep.

During NREM sleep, the EEG is dominated by slow rhythms (<5.0 Hz) (100, 107, 108) whose power positively correlates with the accumulation of sleep pressure. The longer the animal is kept awake, the larger and more frequent these slower rhythms become in subsequent sleep (105, 109, 110); this phenomenon is referred to as the slow-wave activity (SWA) of the EEG and is thought to be an electrophysiological marker of sleep pressure.

The first clue to the involvement of purinergic gliotransmission in sleep regulation was that animals exhibit a blunting of their SWA when dnSNARE is expressed in astrocytes (2). One concern with the inactivation of a tonic A1 receptor–dependent pathway is that such inactivation may lead to neurodegeneration given the powerful neuroprotective role of this

signaling pathway (111). However, this is not the case in this system because all sleep phenotypes are reversed when transgene expression is subsequently turned off in the same animals.

When animals were subjected to 6 h of sleep deprivation, their homeostatic response (both the electrophysiological and behavioral components) was intact only when normal gliotransmission was allowed to occur. The reversible inhibition of gliotransmission attenuated the increase in SWA and the increase in total sleep time that follows a period of sleep deprivation. Interestingly, when animals were sleep deprived after novel object recognition (NOR) training, a task whose memory is sensitive to the effects of sleep pressure, NOR memory was intact when purinergic gliotransmission was blocked. This suggests that cognitive impairment following short-term sleep loss is an active process and is dependent on astrocytic purinergic signaling.

These behavioral effects that were perturbed by astrocytic dnSNARE expression were fully reconstituted in wild-type mice in which we introduced the A1 receptor antagonist CPT. Using osmotic minipumps to administer CPT intracerebroventricularly, we similarly found reduced SWA, reduced responses to sleep deprivation, as well as the maintenance of NOR memory following sleep deprivation. Further support for astrocytic adenosine signaling in vivo is provided by recent studies using optogenetics in vivo. The expression and activation of the blue light–sensitive cation channel channelrhodopsin-2 (ChR-2) in astrocytes of the subthalamic nucleus (STN) result in a robust suppression of nearby neuronal spiking in vivo (112). Astrocyte-induced neuronal suppression has an onset of ~400 ms and an offset of ~800 ms following optical activation, consistent with the possibility that astrocytic ChR-2 activation results in the release of ATP and its degradation to adenosine to suppress nearby spiking (112).

The role of astrocytic gliotransmission in sleep modulation is the first experimental demonstration of active glial involvement in mammalian behavior. Because adenosinergic gliotransmission operates over significantly longer timescales than does synaptic transmission, it is an ideal cellular candidate for mechanisms underlying the control of slowly evolving behaviors such as sleep. The accumulation of adenosine and/or its downstream A1-mediated effects in thalamocortical circuits during wake-fulness over the course of wakefulness may promote the generation of slow oscillations in these structures. Interestingly, adenosine accumulation in the cortex exerts negative feedback on brainstem circuits involved in promoting wake-fulness (113). These findings support the possibility that cortical activity is an important determinant of global vigilance state and provide a possible link between local sleep and global sleep. Because slow oscillations can occur in quiet wakefulness (114) and because many cortical regions appear to generate this rhythm, the involvement of a certain number of thalamocortical circuits in this rhythm may promote the switch from wakefulness to sleep.

Theoretical and empirical studies suggest that the increase in the genesis and spread of cortical slow waves is dependent on the increase in synaptic strength of the thalamocortical system (107). Biochemical and electrophysiological recordings of rats in vivo show that wakefulness is associated with net synaptic potentiation, whereas sleep is associated with net synaptic depression (115). One hypothesis of sleep function suggests that one of the primary functions of sleep is homeostatic synaptic downscaling, in which the overall strength of synapses is decreased while preserving relative synaptic weights (100). Synaptic downscaling may be essential for a system such as the brain, which, when engaged in sensory processing (during wake), exhibits a progressive strengthening of its connections. Wake-dependent increase in synaptic strength would increase energy and space demand, requiring a process to offset synaptic potentiation. Thus, sleep may have evolved to scale

synapses back and allow the brain to function as a learning/ adaptive machine in the face of space and energy constraints. Additionally, it may have evolved to offset the effects of increased excitability of neural circuits, which if left unchecked may result in epileptiform activity. Given that sleep deprivation is a known precipitator of seizures in patients with epileptic disorders (116), this hypothesis may have therapeutic implications.

ASTROCYTES IN NEUROLOGICAL DISEASES

Astrocytes become “reactive” in response to a number of brain insults, including trauma, infection, epilepsy, and neurodegeneration (117). Reactive astrocytes are characterized by well-described morphological changes, but less understood physiological alterations. Whether the reactive astrocyte phenotype is a single biological state or a family of related changes is not clear.

A major feature of reactive astrocytes is the upregulation of the intermediate filament proteins GFAP and vimentin. This upregulation is associated with thickening of the main processes of the astrocyte (astrocytic hypertrophy) (118). Genetic ablation of these astrocytic intermediate filaments results in exacerbation of neuronal injury following middle cerebral artery occlusion, suggesting that the upregulation of these molecules is beneficial in the context of certain brain insults (119). Status epilepticus (SE) causes the loss of astrocytic domains and the overlapping of astrocytic processes (120). This last morphological feature is not seen in the context of other brain injuries such as neurodegenerative disorders.

Certain studies suggest that reactive astrocytosis is accompanied by astrocytic proliferation, and recent cell fate mapping studies suggest that adult astrocytes are able to divide following brain injury (121). Inhibition of astrocytic proliferation using molecular genetics results in a poorer outcome of acute injury models, including forebrain stab wound (122), traumatic brain injury (123), and stab or crush spinal cord injury (124). Introducing an NMDA receptor antagonist into the forebrain in the stab wound model ameliorated its severity, suggesting that astrocytic proliferation is important for maintaining glutamate homeostasis (122). In another set of studies, the molecular ablation of astrocytic signal transducer and activator of transcription 3 (STAT3) resulted in the impairment of astrocytic reactivity, astrocytic migration toward injury, and the worsening of SCI functional outcome (125). These studies demonstrate that astrocytic reactivity and proliferation are beneficial in the context of certain models of acute brain injury.

Depending on the nature of the insult, astrocytic reactivity can be associated with physiological alterations of glutamate uptake, K^+ buffering, and/or H_2O homeostasis (126). Both epilepsy and neurodegenerative disorders such as Alzheimer’s disease appear to be associated with an alteration in astrocytic glutamate uptake and K^+ buffering. The vascular β-amyloid load in mouse models of Alzheimer’s disease correlates with the loss of K_{ir}, BK, and aquaporin 4 channels in astrocytes (127). Furthermore, these channels are reduced in postmortem brains of patients with moderate to severe vascular amyloid deposition (127). Postmortem tissues derived from patients with Alzheimer’s disease show evidence for reduced astrocytic glutamate transporter expression and reduced glutamate uptake by biochemical assays. It will be important to investigate whether reversing these molecular alterations in astrocytes slows the progression of Alzheimer’s disease in animals models and ultimately in humans.

Until recently, the neurodevelopmental disorder Rett syndrome (RTT) was thought to be a result of cell-autonomous, lack-of-function expression of the transcriptional factor methyl-CpG binding protein 2 (MeCP2) in neurons. However, a careful study by Ballas et al. (128) revealed that this transcription factor is expressed in brain astrocytes, albeit at lower levels than in neurons. Furthermore, wild-type neurons exhibit aberrant dendritic morphology and

synapse numbers when cocultured with MeCP2-deficient astrocytes, reminiscent of morphological abnormalities of RTT. These changes are recaptured when wild-type neurons are incubated in MeCP2-deficient astrocyte-conditioned medium, suggesting that astrocytes release a soluble factor to support neuronal development, which is inhibited by the RTT mutation. The growing list of astrocytic factors important for synaptogenesis, including thrombospondins and cholesterol (129), suggests that disruption of this important astrocytic function contributes to a number of neurodevelopmental disorders.

Do changes in gliotransmission contribute to the development of neurological disorders? A growing body of evidence suggests that astrocytic Ca^{2+} signaling is enhanced in the context of certain neurological diseases (130, 131). For example, in mouse models of Alzheimer's disease, astrocytes appear to exhibit higher resting levels of Ca^{2+} and to generate synchronous Ca^{2+} oscillations (130). In addition, following pilocarpine-induced SE, astrocytic Ca^{2+} signaling is also enhanced; this enhancement lasts for days after the termination of SE (131). The pharmacological antagonism of both mGluR5 and NR2B provides protection from the delayed neuronal death that occurs following SE, suggesting that enhanced Ca^{2+} signaling in astrocytes results in enhanced glutamatergic gliotransmission, which targets NR2B-containing NMDA receptors (discussed above). The activation of extrasynaptic NR2B-containing NMDA receptors stimulates CREB dephosphorylation and neuronal death (132). Thus, enhanced astrocytic Ca^{2+} promotes neuronal death via glutamatergic gliotransmission. This conclusion is supported by the observation that the loading of cortical astrocytes with a Ca^{2+} chelator is neuroprotective in the context of SE (131). Whether molecular manipulations that target astrocytic Ca^{2+} signaling promote neuronal survival following SE is an open question and an active area of investigation.

ASTROCYTES IN PSYCHIATRIC DISEASES

In contrast to neurological disorders, which show glial upregulation of intermediate filament proteins, two psychiatric disorders, namely major depressive disorder (MDD) and schizophrenia, are characterized by a decrease in GFAP expression (and perhaps a decrease in glial cell number) in the prefrontal cortex, as revealed by postmortem studies (133). Whether these changes are causal in the context of these disorders or a consequence of the illness is not clear.

Studies have shown altered gene expression of GFAP, glutamate transporters, and GS in MDD (134). Chronic unpredictable stress (CUS) of rodents, an animal model of depression, mimics the human MDD changes in astrocytic gene expression (134, 135). Certain functional consequences of these gene expression changes result from the disruption of astrocytic support of GABAergic transmission. For example, ex vivo nuclear magnetic resonance (NMR) reveals reduced metabolic cycling of glutamate in the prefrontal cortex of rats undergoing CUS. Riluzole, a chemical that facilitates glutamate uptake by astrocytes, reverses CUS-induced anhedonia and helplessness, suggesting that boosting the glutamate/glutamine cycle can be therapeutic in the context of MDD. Support for this hypothesis comes from studies showing that patients with MDD exhibit reduced cortical GABA levels, similar to rats undergoing CUS (136), and that normalizing GABA levels in these individuals correlates with their clinical improvement. Thus, it appears that a dysregulation of astrocytic support of GABAergic transmission contributes to the pathophysiology of MDD and that this process may provide novel therapeutic targets for the treatment of this debilitating state. Because one of the leading hypotheses regarding the pathophysiology of schizophrenia suggests a disruption in the function of a subclass of inhibitory interneurons (namely the parvalbumin-positive phenotype of interneurons), which leads to a dysregulation of γ oscillations and related temporal coding (137), it would be intriguing to

investigate whether the astrocytic support of GABAergic transmission (22) is important for the generation of fast-cortical rhythms and whether its disruption can contribute to schizophrenia endophenotypes.

Do changes in gliotransmission contribute to psychiatric disorders? Although the answer to this important question is not known, it is intriguing that cycloserine, an analog of the gliotransmitter D-serine, is used as a therapeutic agent in schizophrenia (138). In a previous perspective, we speculated that a hypoactive astrocyte phenotype may exist in certain psychiatric disorders, which would result in the attenuation of astrocytic activation of neuronal NMDA receptors (139). Because a hypo-NMDA phenotype is thought to be a final common pathway of the pathophysiology of schizophrenia, disrupted gliotransmission may also contribute to this debilitating disorder. Future experiments will test this hypothesis and, more importantly, provide a framework for the role of astrocytes in psychiatric disorders.

Glossary

Astrocyte	the dominant subclass of nonneuronal glial cell of the nervous system
Gliotransmission	the release of chemical transmitters from glial cells, with particular reference to astrocytes
SNARE	soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor
NMDA	N-methyl-D-aspartate
Ectonucleotidases	cell surface–expressed enzymes that stimulate the hydrolysis of ATP to adenosine
Photolysis	an organic molecule is attached to a bioactive molecule to yield a biologically inactive product. Absorption of a UV photon leads to the photolysis of this molecule, yielding the biologically active species
A1	adenosine 1
Heterosynaptic depression	the ability of the activity of one synapse to lead to the depression of a neighboring independent synapse
Sleep homeostat	sleep is regulated by a circadian oscillator (which controls timing) and a sleep homeostat, which integrates the period of prior wakefulness and provides the drive for sleep
REM	rapid eye movement
NREM	nonrapid eye movement
Slow-wave activity (SWA)	low-frequency activity in the range of 0.5 to 4 Hz detected by EEG recordings during NREM sleep

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SUMMARY POINTS

1. Astrocytes are physically associated with synapses and provide the structural substrate for reciprocal chemical signaling between neurons and glia.
2. Astrocytes provide important metabolic support to the neuron that is required to maintain physiological properties of neuronal networks.
3. The astrocyte is chemically excitable and expresses a plethora of receptors that allow the detection of neuronal activity and induce second messenger signaling within these glial cells.
4. Chemical gliotransmitters can be released from astrocytes through a variety of mechanisms. These pathways lead to the release of glutamate, D-serine, and ATP.
5. Astrocyte-derived glutamate plays important roles in behavioral responses to cocaine and the growth of glioblastoma. D-serine modulates NMDA receptor function, whereas ATP and its metabolic product adenosine modulate synaptic transmission and promote sleep drive.

FUTURE ISSUES

1. How do astrocytes and gliotransmission modulate inhibitory synaptic transmission?
2. Could astrocytes act as therapeutic targets in which one hijacks the glial signaling pathways to modulate and repair defective synaptic transmission, circuit function, and behavior?
3. What is the range of behaviors for which astrocytes modulate neuronal network activity?
4. How do astrocytes integrate the period of wakefulness to promote sleep drive?