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Glial Cell Regulation of Rhythmic Behavior

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Abstract

Brain glial cells, in particular astrocytes and microglia, secrete signaling molecules that regulate glia–glia or glia–neuron communication and synaptic activity. While much is known about roles of glial cells in nervous system development, we are only beginning to understand the physiological functions of such cells in the adult brain. Studies in vertebrate and invertebrate models, in particular mice and *Drosophila*, have revealed roles of glia–neuron communication in the modulation of complex behavior. This chapter emphasizes recent evidence from studies of rodents and *Drosophila* that highlight the importance of glial cells and similarities or differences in the neural circuits regulating circadian rhythms and sleep in the two models. The chapter discusses cellular, molecular, and genetic approaches that have been useful in these models for understanding how glia–neuron communication contributes to the regulation of rhythmic behavior.

1. INTRODUCTION

There is accumulating evidence that brain glial cells have critical roles in modulating synaptic transmission, plasticity, and behavior, in addition to their well-characterized functions in synapse development and neurodegeneration (Brown & Neher, 2014; Clarke & Barres, 2013; Stork, Bernardos, & Freeman, 2012). Studies in both vertebrate and invertebrate models support such physiological roles of glial cells as summarized in recent reviews (Araque et al., 2014; Frank, 2013; Halassa & Haydon, 2010; Haydon, Blendy, Moss, & Jackson, 2009; Jackson, 2011; Jackson & Haydon, 2008; Salter & Beggs, 2014; Zwarts, Van Eijs, & Callaerts, 2014). For example, mammalian astrocytes physiologically regulate neuronal circuits in the adult brain that control neuronal excitability (Clasadonte & Haydon, 2012), cognitive state (Lee et al., 2014), sleep and plasticity (Halassa et al., 2009), and responses to drugs of addiction (McIver, Muccigrosso, & Haydon, 2012; Turner, Ecke, Briand, Haydon, & Blendy, 2013). Similarly, recent studies in *Drosophila* have implicated glial cell function in the regulation of neuronal excitability (Melom & Littleton, 2013; Rusan, Kingsford, & Tanouye, 2014), vision (Borycz, Borycz, Loubani, & Meinertzhagen, 2002; Chaturvedi, Reddig, & Li, 2014; Stuart, Borycz, & Meinertzhagen, 2007), circadian behavior (Ng, Tangredi, & Jackson, 2011; Suh & Jackson, 2007), sleep (Chen et al., 2014; Seugnet et al., 2011), behavioral sensitivity to drugs (Bainton et al., 2005), and olfaction (Liu et al., 2014). Although glial cells regulate development and modulate many different physiological processes, a comprehensive summary of that literature is beyond the scope of

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this chapter. Rather, this chapter highlights recent evidence revealing roles of glial cells in the neural circuits regulating circadian rhythms and sleep of *Drosophila* and mammals, providing details about the genetic strategies employed in such studies. Those recent studies are summarized in several sections of this chapter. Sections at the end of the chapter describe *Drosophila* molecular genetic methods that have been useful to our lab for the identification of glial cell types and glia–neuron interactions that regulate circadian behavior.

2. STUDIES OF GLIAL CELL FUNCTION IN CIRCADIAN BEHAVIOR AND SLEEP

2.1. Glia and circadian behavior

Examination of neurotrophins, cytokines, and gliotransmitters such as ATP suggest that glia–neuron communication is important within the mammalian circadian neural circuitry (reviewed in section 3). Whereas little is known about *Drosophila* circadian gliotransmitters, there is ample evidence for roles of fly glial cells in circadian rhythmicity. Several studies have documented rhythmic expression of clock proteins and other neural proteins (e.g., PER, TIM, Ebony, CREB2, Na^+ /K^+ -ATPase) in glial cells of the adult *Drosophila* brain (Damulewicz, Rosato, & Pyza, 2013; Suh & Jackson, 2007; Tanenhaus, Zhang, & Yin, 2012; Zerr, Hall, Rosbash, & Siwicki, 1990). The use of genetic techniques in *Drosophila* has revealed that glial cells are also critical elements of the neural circuits regulating circadian behavior and sleep (Chen et al., 2014; Ng et al., 2011; Seugnet et al., 2011). The first direct evidence of a role for fly glial cells in behavioral rhythmicity came from the analysis of Ebony (Suh & Jackson, 2007), a glial-specific non-ribosomal peptide synthetase which functions in recycling of aminergic neurotransmitters (Borycz et al., 2002; Hovemann et al., 1998; Richardt et al., 2003). Studies of Suh and Jackson (2007) revealed that Ebony exhibits a circadian abundance rhythm in glial cells of the adult brain that is not dependent on pigment dispersing factor (PDF), an important circadian neurotransmitter (Renn, Park, Rosbash, Hall, & Taghert, 1999). The authors showed that many of the Ebony-containing glial cells also rhythmically express PER and TIM clock proteins, indicative of oscillator function, and consistent with older reports demonstrating the presence of PER/ TIM-based clocks in adult glia (Zerr et al., 1990). Cell-specific genetic rescue studies demonstrated that Ebony is required in glial cells for normal behavioral rhythmicity. A later analysis indicated that most Ebony-containing cells of the central fly brain are astrocyte-like glia (F. S. Ng & F. R.Jackson, unpublished results), implicating this glial class in rhythmicity. Studies of Ebony are described in greater detail in an earlier review (Jackson, 2011).

The advantage of the *Drosophila* model is the availability of sophisticated genetic methods. For example, the use of cell type-specific, conditional genetic manipulations in recent studies has demonstrated that adult fly glial cells can physiologically modulate circadian behavior (Ng et al., 2011). Those studies utilized the Gal4/UAS binary expression system to manipulate glial cell function conditionally in adult flies; they demonstrated that expression of a bacterial sodium channel (NaChBac) or a dominant-negative, temperature-sensitive dynamin (shi^{ts}, to perturb exocytosis/endocytosis) resulted in arrhythmic locomotor activity. The same studies showed that conditional, pan-glial knockdown of the endoplasmic

reticulum (ER) calcium (Ca^{2+})-regulating factor SERCA (a Ca^{2+} ATPase) leads to arrhythmic behavior, indicating that intracellular Ca^{2+} stores are important for normal behavioral rhythmicity. Using glia class-selective Gal4 drivers, Ng et al. (2011) showed that conditional, adult expression of *shi*^{ts} in astrocytes caused arrhythmic locomotor activity, whereas flies expressing the temperature-sensitive factor in other glial classes had normal free-running rhythms. Thus, similar to mammals, fly astrocytes are important regulators of adult behavior.

An important finding from these studies was that *shi*^{ts} -mediated perturbations had reversible effects on rhythmicity: rhythms were eliminated at a restrictive temperature of 30 °C but returned—in phase with that of the entrained condition—when the temperature was reduced to 24 °C. Reversibility and lack of a phase shift strongly suggests that glial cells modulate circadian output without affecting the clock itself. Indeed, Ng et al. showed that conditional adult inhibition of glial cell function, using pan-glial expression of *shi*^{ts} at high temperature, does not affect the neuronal molecular clock, nor viability of neurons and astrocytes, but leads to decreased PDF immunoreactivity in the axonal projections of the small lateral ventral (s-LNv) neurons. PDF is required for morning activity in a light/dark (LD) cycle and normal free-running behavior in DD. It coordinates the pacemaker cell population (Lin, Stormo, & Taghert, 2004) and stimulates the DN1p clock neurons to effect pacemaker output (Seluzicki et al., 2014). As with circadian behavior, the effect of *shi*^{ts} expression on PDF was reversible, and normal levels of PDF were observed when flies were transferred to the permissive temperature after being maintained at high temperature for 2–3 days. In unpublished studies, it has also been shown that the astrocyte-specific knockdown of a glial factor predicted to block exocytosis leads to arrhythmic behavior and decreased PDF in the s-LNv neuronal projections (F. S. Ng, S. Sengupta, & F. R. Jackson, unpublished results). The aggregate of these behavioral and molecular results suggests that astrocytes of the adult fly brain secrete at least one factor via vesicular release mechanisms to physiologically regulate circadian neuronal output (Fig. 1A). *In vivo* glial perturbation studies have not been reported for mammalian circadian behavior, but a similar analysis of mouse astrocytes has revealed an important role of this cell type in regulating sleep.

2.2. Mammalian astrocytes and the sleep homeostat

Although genetic manipulations, *in vivo*, have only begun to reveal glia– neuron interactions important for the circadian circuitry, similar studies in mice have yielded a deep understanding of the glial modulation of sleep. In pioneering studies, Philip Haydon and colleagues (Halassa et al., 2009; Schmitt, Sims, Dale, & Haydon, 2012) have examined the mechanisms underlying the astrocytic control of sleep homeostasis, a conserved compensatory process that regulates sleep amount. It has long been known that adenosine (ADO) accumulation is associated with sleep pressure, but only recently has it been shown that the likely source of ADO is the metabolism of ATP that is released from astrocytes via SNARE-dependent exocytosis. These studies utilized mouse genetics and cell-specific expression of a dominant-negative SNARE (dnSNARE)—which inhibits exocytosis (Pascual et al., 2005)—to selectively decrease vesicle-mediated gliotransmission in astrocytes. In one study, it was shown that blocking SNARE-dependent gliotransmission from astrocytes—conditionally in the adult mouse brain—decreased the slow wave

component of non-REM sleep and the rebound sleep that normally arises after sleep deprivation (SD; Halassa et al., 2009). Treatment of wild-type mice with an ADO A1 receptor antagonist mimicked the effects of dnSNARE expression, suggesting that ATP released from astrocytes is converted to ADO which then acts on neuronal A1 receptors to modulate sleep pressure. Consistent with this model, the sleep-suppressing effects of an A1 receptor antagonist were eliminated in mice with astrocytic expression of dnSNARE, presumably due to decreased astrocyte-derived ATP/ADO. Remarkably, memory deficits in a novel object recognition paradigm that normally occur with SD were absent in the dnSNARE mice, suggesting that astrocyte-derived gliotransmitters produce the deficit.

Although it seemed likely that sleep pressure was a consequence of increased extracellular ADO, derived from astrocytic ATP, there were no measurements of astrocyte-derived ADO during wakefulness or in sleep-deprived animals until recently. To conduct such measurements, Schmitt et al. (2012) employed both slice and *in vivo* recording methods in wild-type and dnSNARE mice. In an initial set of experiments, it was shown that ADO A1 receptor-mediated inhibition of hippocampal neurons (ADO tone) changed over the course of the sleep–wake cycle, with tone being highest during wakefulness, consistent with accumulating ADO. Using the same indirect measurement of ADO, the investigators then showed that wakefulness-dependent increases in ADO tone required for vesicular release from astrocytes, i.e., tone was low during wakefulness in animals with astrocyte-specific expression of dnSNARE. Finally, Schmitt et al. (2012) used biosensors to perform direct measurements of ADO in hippocampal slices of wild-type and dnSNARE mice obtained from animals during the waking state or after SD. These studies demonstrated that ADO accumulation during wakefulness or after SD is dependent on astrocyte vesicular release mechanisms, consistent with the idea that ADO is derived from astrocytes (presumably by extracellular conversion of released ATP). Use of the dnSNARE mouse model in a subsequent study demonstrated that the astrocytic activation of the ADO A1 receptor leads to increased phosphorylation of the NMDA receptor NR2B subunit with consequent effects on NR2B surface expression and neuronal excitability (Deng, Terunuma, Fellin, Moss, & Haydon, 2011). This provides a model for how accumulation of astrocyte-derived ADO during wakefulness or SD leads to changes in neurons that constitute the sleep homeostat.

Although *Drosophila* is a good genetic model for behavioral studies, not surprisingly, there may be mechanistic differences between flies and mammals in the regulation of sleep or circadian behavior. For example, an ADO receptor is expressed in *Drosophila* (Dolezelova, Nothacker, Civelli, Bryant, & Zurovec, 2007), but it does not appear to be essential for sleep. *Drosophila* ADO receptor mutants have normal baseline sleep and homeostatic responses to SD (Wu et al., 2009). As mentioned previously, flies lack neuronal P2X (ATP) receptors (Fountain, 2013), and thus astrocyte–neuron purinergic signaling may not be important for sleep or circadian behavior in this model. However, ADO signaling via the fly ADO receptor may contribute to *Drosophila* learning and memory (Knight et al., 2010).

2.3. Glia–neuron communication regulates *Drosophila* sleep

Similar to mammals, there is evidence that glial cells modulate sleep in *Drosophila*. The fruit fly is an established model for the analysis of sleep homeostasis and arousal (Andretic

& Shaw, 2005; Cirelli & Bushey, 2008; Hendricks et al., 2000; Ho & Sehgal, 2005; Shaw, Cirelli, Greenspan, & Tononi, 2000), and recent studies have documented roles of defined classes of neurons as well as glial cells in the neural circuits regulating these processes. Genetic analysis in this model has demonstrated roles of GABAergic and aminergic circuits in these processes (Agosto et al., 2008; Andretic, van Swinderen, & Greenspan, 2005; Kume, Kume, Park, Hirsh, & Jackson, 2005; Yuan, Joiner, & Sehgal, 2006), similar to vertebrates. Indeed, there is evidence for homology between the arthropod central complex and the vertebrate basal ganglia as well as the associated circuits regulating controlling sleep and arousal in arthropods and vertebrates (Strausfeld & Hirth, 2013). In this context, it is known that dopaminergic neurons regulating arousal project to the Fan-shaped body of the fly central complex (cc) (Liu, Liu, Kodama, Driscoll, & Wu, 2012; Ueno et al., 2012) and that a GTPase (Crossveinless) expressed in the cc is critical for sleep homeostasis (Donlea, Pimentel, & Miesenbock, 2014). Whereas sleep homeostasis is beginning to be understood, there is little known about the circadian control of sleep. Therefore, it is of interest that a mutant called *wide awake* (*wake*) alters the clock regulation of sleep (Liu et al., 2014). WAKE is required within a subset of the PDF neurons (the l-LNv cells) where it interacts with the GABA-A receptor to modulate excitability of these neurons. As a consequence of WAKE deficits, flies sleep less and exhibit a delayed onset of sleep. These studies are consistent with earlier work that demonstrated a role of the GABA-A receptor, the l-LNv neurons, and PDF release in the regulation of arousal and sleep onset (Parisky et al., 2008).

Two recent studies have documented glia–neuron interactions that regulate sleep in *Drosophila* (Chen et al., 2014; Seugnet et al., 2011). Seugnet et al. characterized a gene known as *Bunched* (*Bun*) that is upregulated by SD. *Bun* is known to negatively regulate Notch signaling, and these investigators showed that mutants of the gene have a reduced homeostatic response to sleep loss (i.e., less compensatory sleep than wild type in response to SD). They also demonstrated that normal sleep homeostasis requires *Bun* within the mushroom body, the fly hippocampal analog that has previously been implicated in control of sleep (Joiner, Crocker, White, & Sehgal, 2006; Pitman, McGill, Keegan, & Allada, 2006). Following up on the finding that *Bun* regulates Notch signaling, Seugnet et al. analyzed roles of Notch and Delta (a Notch ligand) in sleep homeostasis, finding that decreased Delta function suppressed the sleep homeostasis phenotype of *Bun* mutants, whereas overexpression of Delta in the mushroom body eliminated sleep homeostasis. Similarly, Notch gain-of-function mutations blocked the homeostatic response. Importantly, it was shown that Delta is localized to mushroom body neurons while the intracellular domain of Notch could be detected in glial processes surrounding the neuropil and cell bodies of the brain cortex. These results suggest that glia–neuron signaling within the mushroom body contributes to sleep homeostasis. Notch-mediated glia–neuron signaling may regulate other behaviors as well; for example, it was previously shown that fringe, a Delta-Notch signaling regulator controls expression of the fly glial EAAT1 glutamate transporter and larval locomotor activity (Stacey et al., 2010).

A different type of glia–neuron interaction contributes to the regulation of sleep amount in *Drosophila* (Chen et al., 2014). Chen et al. utilized a proteomics approach—two-dimensional gel analysis coupled with mass spectrometry—to examine changes in the fly

Sleepless (*sss*) mutant, which was previously shown to have reduced sleep (Koh et al., 2008). That analysis showed that the fly GABA transaminase (GABAT), known to be localized to neuropil-associated glia, is increased in the *sss* mutant. Concomitantly, brain GABA amounts are decreased in *sss* and increased in *gabat* mutants. These investigators showed that the loss of GABAT promotes sleep, consistent with increased GABA, in contrast to the *sss* mutant which has decreased sleep. Strikingly, *gabat* mutations suppress the sleep phenotype of *sss* flies. As SSS protein is known to be localized to neurons, this result indicates a genetic interaction involving glia–neuron communication. The authors demonstrate that GABAergic expression of SSS can rescue an *sss* mutant and that pan-glial expression of GABAT disrupts the ability of a *gabat* mutation to rescue *sss* (presumably, by driving high GABAT levels in glia). All of this evidence identifies a glia-GABAergic neuron signaling mechanism that regulates sleep amount. As a side note, recent astrocyte profiling studies (see later section 4.2) indicate that *gabat* mRNA exhibits enriched expression in fly astrocytes (Y. Huang & F. R. Jackson, unpublished results). Thus, fly GABAergic neurons may interact with astrocytes to regulate sleep. It is of related interest that astrocytes of the mouse neocortex release ATP—via vesicular and Ca^{2+}-mediated processes—to regulate GABAergic inhibitory as well as excitatory neurons (Lalo et al., 2014), although, to our knowledge, this particular glia–neuron interaction is not known to be important for the regulation of sleep.

2.4. Microglial clocks, synaptic strength, and sleep

Mammalian microglia, which migrate into the brain from peripheral tissues, are known to be important for immune surveillance in the nervous system, but they are also required for the refinement of neuronal connectivity and the regulation of behavioral plasticity (Salter & Beggs, 2014). Similar to immune cells and astrocytes, it was recently shown that adult mammalian microglia contain PER-based circadian clocks (Hayashi et al., 2013). This was established by acutely isolating microglia from the mouse cortex, using magnetic cell sorting methods, and thus it is not known whether the microglial clock is completely autonomous or requires neuronal interactions, similar to the astrocyte clock (Prolo, Takahashi, & Herzog, 2005). Nonetheless, microglia rhythmically secrete a protease (Cathepsin S, CatS), as a consequence of clock-regulated transcription of the CatS mRNA, and the CatS protease promotes ATP-induced microglial process extension while inhibiting miniature excitatory postsynaptic currents (mEPSCs) of cortical neurons (Hayashi et al., 2013). Although wild-type mice exhibit low-amplitude diurnal rhythms in cortical neuron spine density and mEPSCs, these rhythms are lacking in CatS null mice. These null mice also have increased locomotor activity and reduced sleep, as measured by the power of the EEG delta wave. These results indicate that a CatS-mediated effect on cortical neuronal plasticity regulates synaptic strength, activity, and sleep.

3. POTENTIAL CIRCADIAN GLIA-NEURON SIGNALING MOLECULES

3.1. Glial clocks and ATP rhythms

Previous studies have described the presence of PER-based molecular oscillators in *Drosophila* glia (Ng et al., 2011; Suh & Jackson, 2007; Zerr et al., 1990) mammalian astrocytes (Prolo et al., 2005), and mammalian microglia (Hayashi et al., 2013). Although

little is known about *Drosophila* gliotransmitters, studies in mammals have documented a clock-regulated secretion of several glial factors; in the case of certain rhythmically secreted glial proteins, there is evidence that they function *in vivo* to regulate circadian behavior (Burkeen, Womac, Earnest, & Zoran, 2011; Hayashi et al., 2013; Kramer et al., 2001; Marpegan et al., 2011; Womac, Burkeen, Neuendorff, Earnest, & Zoran, 2009). In addition, it has been shown that vasointestinal polypeptide (VIP) and other clock-modulating neuropeptides as well as certain rhythmic immune and growth factors can act on astrocytes to regulate their morphology and/or endogenous circadian clocks (Cavadini et al., 2007; Duhart et al., 2013; Girardet et al., 2013; Keller et al., 2009; Lopez et al., 2014; Marpegan, Krall, & Herzog, 2009; Mou, Peterson, & Prosser, 2009; Petrzilka, Taraborrelli, Cavadini, Fontana, & Birchler, 2009). These studies have documented a bidirectional communication between clock neurons and glial cells that is important for circadian function (last reviewed in Jackson, 2011).

Several studies have documented the circadian release of adenosine triphosphate (ATP) from astrocyte glial cells and examined the intracellular mechanisms regulating the circadian release of this purine (Burkeen et al., 2011; Marpegan et al., 2011; Womac et al., 2009). Circadian release of ATP is of interest as this purine and a metabolic product (ADO) are capable of acting on neuronal receptors to regulate excitability (Haydon, 2012). In this section, we summarize research that has examined the mechanisms regulating the circadian release of astrocytic ATP. Receptors for ATP are not expressed in the *Drosophila* nervous system (Fountain, 2013), and nothing is known about release of this purine from fly glial cells; thus, we focus here on recent work that examined rhythmic ATP release from mammalian astrocytes.

Two studies have characterized astrocytic ATP release rhythms, with the goal of understanding the intracellular mechanisms regulating release of the purine (Burkeen et al., 2011; Marpegan et al., 2011). Marpegan et al. (2011) demonstrated that individual wild-type astrocytes exhibit rhythms in ATP release, consistent with earlier studies that examined ATP rhythms in cultured astrocyte populations or tissue slices of the suprachiasmatic nuclei (SCN; Womac et al., 2009). Additionally, Marpegan et al. employed genetic approaches to show that ATP rhythms are eliminated in most astrocyte cultures that are mutant for *Bmal1*, *Clock*, *Cry*, or *Per*, indicative of circadian control. Using available mouse genetic models, the investigators asked whether glial exocytosis or Ins(1,4,5)P3 (IP3)-liberated stores of intracellular (ER) calcium (Ca^{2+}) were required for the ATP release rhythm. Whereas astrocytes expressing a dnSNARE (described previously)—to inhibit glial exocytosis (gliotransmission)—had normal ATP rhythms, the majority of cultures with decreased IP3 signaling were arrhythmic. These results suggest a requirement for intracellular Ca^{2+} but not SNARE-dependent exocytosis in rhythmic glial ATP release; interestingly, they contrast with studies of sleep, which demonstrate that both Ca^{2+} signaling and glial exocytosis are required for the ATP/ADO-mediated regulation of sleep homeostasis (Halassa et al., 2009; Schmitt et al., 2012). Thus, ATP may be released from astrocytes via two mechanisms, one relevant for sleep and the other for circadian regulation. As discussed by Marpegan et al., astrocytic circadian ATP release may occur through hemichannels or by another mechanism.

Burkeen et al. (2011) extended earlier studies that documented rhythms in extracellular ATP in mixed astrocyte/neuron SCN2.2 cultures, primary cortical astrocyte cultures, and SCN tissue slices (Womac et al., 2009). Based on the premise that glial ATP release is a Ca^{2+}-mediated process, the investigators examined rhythmicity in intracellular Ca^{2+} stores in SCN2.2 cultures (predominantly astrocytes) and then manipulated Ca^{2+} levels to determine if the ATP rhythm was regulated by this divalent cation. Both cytosolic and mitochondrial Ca^{2+} exhibited rhythms, but they were not in phase with one another. Interestingly, the rhythm in cytosolic calcium was also out of phase with the ATP rhythm, whereas mitochondrial Ca^{2+} levels were highest at the time of peak extracellular ATP. Both the intracellular Ca^{2+} and extracellular ATP rhythms were eliminated by BAPTA, a Ca^{2+} chelator, demonstrating a requirement for the cation. This finding is consistent with a role for IP3 in ATP rhythmicity (Marpegan et al., 2011) since that signaling molecule stimulates release of Ca^{2+} from the ER. Burkeen et al. also demonstrated that an inhibitor of the mitochondrial Ca^{2+} uniporter (Ru360) significantly decreases extracellular ATP, indicative of a requirement for mitochondrial stores of the cation in production and/or release of the purine.

The studies of Marpegan et al. (2011) and Burkeen et al. (2011) both point to Ca^{2+} as an important modulator of the ATP rhythm, although one study emphasizes a mitochondrial Ca^{2+} requirement. A simple model that integrates findings from the two papers postulates that Ca^{2+} is rhythmically released from the ER in response to IP3 and that it drives mitochondrial uptake of the cation. It has been established that there is bidirectional communication between the ER and mitochondrial compartments and that IP3 increases Ca^{2+} levels in the mitochondrial matrix, presumably by stimulating the release of ER Ca^{2+} (Hajnoczky, Csordas, Krishnamurthy, & Szalai, 2000) Thus, decreased IP3 signaling may limit the amount of cytosolic Ca^{2+} that can be sequestered by mitochondria, thus short-circuiting the mitochondrial Ca^{2+} rhythm and eliminating the peak in extracellular ATP accumulation. This model does not, however, explain the antiphase relationship of the cytosolic and mitochondrial Ca^{2+} rhythms, as shown by Burkeen et al., but if correct, it suggests there is a time lag built into the mechanism that results in later accumulation of Ca^{2+} in mitochondria. The rhythm in extracellular ATP accumulation may simply reflect a rhythm in ATP production, if the purine is released from the cell as it is synthesized. To our knowledge, however, an intracellular ATP rhythm has not been reported.

3.2. Glial neurotrophic factors and cytokines in circadian behavior

It has not been directly demonstrated that the circadian clocks of astrocytes or rhythmic accumulation of extracellular ATP influence mammalian behavior. However, certain astrocyte- or microglial-derived neurotrophins and cytokines can influence circadian rhythms. The first such signaling molecule to be identified was transforming growth factor alpha (TGFα; Kramer et al., 2001; Li, Sankrithi, & Davis, 2002). In studies of hamster locomotor activity rhythms, it was shown that TGFα is rhythmically expressed in the SCN and that infusion into the third ventricle reversibly inhibits locomotor activity (Kramer et al., 2001). The same procedure alters the timing of sleep episodes. Furthermore, *waved-2* mice, which lack the receptor for TGFα (EGFR), have abnormally high activity during the day (when mice normally sleep), consistent with a normal inhibition of activity by TGFα. The

mutants also fail to acutely respond to light (they lack masking), indicating that TGFα functions in output and for light reception. TGFα is expressed in ganglion cells and Muller glia of the retina, perhaps accounting for the lack of light response in the mutant. More interesting, it was subsequently shown that TGFα expression in the SCN is predominant in astrocytes (Li et al., 2002), strongly suggesting a role for this glial cell type in circadian output (Fig. 1B), similar to the role of astrocytes in *Drosophila* (Ng et al., 2011). To our knowledge, neurotrophic factors and cytokines have not been studied as possible glial signaling molecules in *Drosophila* circadian behavior although much is known about their functions in fly development and immunity.

Cytokines (e.g., TNFα, TNFβ, and TGFβ)—normally thought of as immune factors—have been implicated in recent studies in behavioral functions including activity-triggered synaptic plasticity (Salter & Beggs, 2014). For example, TGFβ-1 deficient or TGFβ-1 receptor (CX_3CR1) null mice have deficits in hippocampal neurogenesis and hippocampal-dependent learning and memory (Koeglsperger et al., 2013). Interestingly, the TGFβ-1 deficit causes a loss of both astrocytic glutamate transporters (GLAST and GLT-1), indicating that the effects on learning may be mediated by astrocytes. With regard to circadian behavior, the cytokines TNFα and TNFβ or an immune insult (LPS) can modulate clock gene expression of SCN tissues, SCN astrocytes, cultured fibroblasts, and immortalized neurons (Cavadini et al., 2007; Duhart et al., 2013; Lopez et al., 2014; Paladino, Mul Fedele, Duhart, Marpegan, & Golombek, 2014). In response to TNFα, astrocytes release cytokines (CCL2, TNFα, and IL-6) into the medium, one of which (TNFα) can produce phase delays of the circadian clock in SCN brain slices or *in vivo* in a TNF receptor-dependent manner (Duhart et al., 2013; Paladino et al., 2014). It is known that TNFα and other cytokines are released from immune cells according to a circadian rhythm (Keller et al., 2009) in response to an inflammatory insult, but it has not been shown directly that TNFα is rhythmically released from astrocytes of the SCN. Figure 1B illustrates known and possible interactions among SCN astrocytes, pacemaker neurons, and clock output neurons that are relevant for circadian behavior.

Brain-derived neurotrophic factor (BDNF) and its receptor (TrkB) are also present in the SCN, and it has been suggested that the trophic factor may be localized to a population of SCN glial cells, although it is predominantly found in neurons within the brain region (Liang, Sohrabji, Miranda, Earnest, & Earnest, 1998). This is in agreement with other studies demonstrating expression of BDNF in basal forebrain astrocytes (Wu, Friedman, & Dreyfus, 2004) and microglia (Coull et al., 2005; Parkhurst et al., 2013), the latter cell type releasing the growth factor to regulate learning-dependent synaptogenesis or pain sensitivity. Furthermore, BDNF mRNA and protein exhibit circadian rhythms in abundance within the SCN, with protein levels peaking in the early subjective night, a time of peak light-induced phase delays for the circadian clock (Liang, Walline, & Earnest, 1998). More interesting, BDNF has been implicated in light-induced phase shifting of the rodent clock. Decreased BDNF in heterozygous mutant mice or infusion of the factor into the SCN in rats decreased or eliminated light-induced phase shifts (Liang, Allen, & Earnest, 2000). A similar result was obtained with infusion of a kinase inhibitor predicted to affect TrkB receptor activity (Liang et al., 2000). TrkB receptor inactivation is also known to eliminate diurnal rhythms in

astroglial coverage of VIP neurons (Girardet et al., 2013), and this might account, in part, for the effect of the kinase inhibitor on resetting. All of these results suggest it may be worthwhile to revisit the localization of BDNF in SCN glial cells and ask whether the factor is rhythmically released from such cells.

3.3. Secreted molecules mediating glia–neuron communication in *Drosophila*

As mentioned previously, circadian gliotransmitters have not been identified in *Drosophila* although fly glial cells are known to secrete many factors that regulate embryonic neural development (Cafferty & Auld, 2007; Silies & Klambt, 2011). Similarly, developing neurons secrete growth factors that regulate the morphogenesis of fly glial astrocytes (Stork, Sheehan, Tasdemir-Yilmaz, & Freeman, 2014). Recent studies, however, have identified factors, including Maverick (Mav) and Wingless (Wg) that are required for postembryonic glia-synapse communication. Mav, a TGFβ ligand and bone morphogenetic protein (BMP) signaling molecule, is secreted from peripheral glia and acts on a muscle receptor to activate retrograde signaling at the fly larval neuromuscular junction and stimulate growth of synapses (Fuentes-Medel et al., 2012). Wg, a well-characterized intercellular signaling molecule, is also secreted from peripheral glia associated with the fly larval neuromuscular junction to regulate the size and the organization of muscle glutamate receptor clusters (Kerr et al., 2014). Interestingly, neuronal components of BMP signaling, including Mav, also regulate the LNv clock neurons to modulate circadian period (Beckwith et al., 2013).

Insights about secreted glial factors that regulate rhythmic behavior may be revealed through gene expression profiling studies of fly glial cells. Indeed, recent profiling studies of fly astrocytes (described in a later section 4.2) have identified a number of predicted secreted factors, and at least one of them is required for wild-type levels of adult locomotor activity and the normal diurnal distribution of activity (S. Sengupta, Y. Huang, F. S. Ng, & F. R.Jackson, unpublished results). Selective glial knockdown of this factor is associated with abnormally high nighttime activity, perhaps due to an effect on behavioral arousal. Genetic screens are currently underway to identify additional genes with astrocyte-enriched expression that are required for normal circadian behavior. Those screens are expected to identify novel gliotransmitters and other secreted factors that are important for glia–neuron communication within the circadian neural circuitry.

4. MOLECULAR GENETIC STRATEGIES FOR STUDYING THE GLIAL REGULATION OF *DROSOPHILA* RHYTHMS

Genetic methods have been important in the mouse and *Drosophila* models for the study of glia–neuron communication that is relevant for circadian rhythmicity or sleep (Halassa et al., 2009; Marpegan et al., 2011; Ng et al., 2011). In the sections later, we briefly highlight *Drosophila* molecular genetic strategies that our laboratory and others have found useful for the study of mechanisms underlying this type of intercellular signaling. The molecular and genetic toolkits available in *Drosophila* make this model particularly attractive for genetic analyses of behavior.

4.1. Glia-selective genetic perturbation methods

Earlier sections of this chapter have alluded to Gal4 enhancer/promoter drivers that permit *in vivo* expression of UAS transgenes in thousands of different spatial and temporal patterns. The work of several laboratories has identified Gal4 drivers selective for many different types of adult glial cells including astrocytes (reviewed in Edwards & Meinertzhagen, 2010; Stork et al., 2012). Pan-glial drivers (e.g., repo-Gal4), astrocyte-selective drivers (e.g., alrm-Gal4; Doherty, Logan, Tasdemir, & Freeman, 2009), and many others are listed in table 1 of Stork et al. (2012). Such drivers will be critical for glial-selective RNAi-based genetic screens (see later). A number of these drivers are not completely specific for glia, but they can be combined with neuronal Gal80 transgenes (e.g., elav-Gal80) to drive expression in glia while inhibiting Gal4 activity (with Gal80) in neurons. In studies of circadian behavior, our laboratory has used a number of selective Gal4 drivers to express UAS transgenes encoding dominant-negative factors or ion channels for genetic perturbation of adult fly glial cells.

In addition to the Gal4/UAS system, so-called enhancer trap (ET)-FLP recombinase strains permit loss or gain of Gal80 expression such that Gal4 activity occurs within or is excluded from the overlap of particular ET-FLP and Gal4 expression patterns (Bohm et al., 2010). This may be a powerful approach for expression of UAS transgenes in a subset of glial cells of a particular class. It has already proved to be quite useful in mapping particular neuronal circuits of the fly brain. Other available genetic methods include the so-called Mosaic analysis with a repressible cell marker (Luo, 2007; Viktorin, 2014), which can be employed to produce small numbers of mutant neurons or glia (Stork et al., 2014) in an otherwise wild-type nervous system, or the FlyBow labeling system (analogous to mammalian Brainbow) which allows stochastic multicolor labeling of cells, *in vivo*, for circuit analysis (Shimosako, Hadjieconomou, & Salecker, 2014). The latter methods will be useful for generating glial mosaics or examining the morphology of individual glial cells that express a behavior-altering factor.

4.2. Glial expression profiling and cell-specific targeted genetic screens

Forward genetic approaches have been utilized in *Drosophila* for decades to identify factors important for neural development and behavior. Combined with known lists of glial-expressed genes, such an approach will be powerful for defining factors that mediate glia–neuron interactions. To that end, cell-specific expression profiling methods will be useful for defining genes expressed in adult astrocytes and other glial classes. Studies of mouse glial cells, for example, have used transcriptional profiling methods to establish lists of glial-expressed genes (Cahoy et al., 2008; Lovatt et al., 2007). Furthermore, a method known as translating ribosome affinity purification (TRAP) has been developed in mice and flies (Heiman et al., 2008; Huang, Ainsley, Reijmers, &Jackson, 2013; Thomas et al., 2012) and that method allows immunopurification (IP) and RNA-seq analysis of ribosome-bound mRNAs from selected cell types. We have utilized TRAP-RNA-seq methods to examine circadian translation in a genome-wide manner (Huang et al., 2013) and to analyze fly *dbt* mRNAs (Huang, McNeil, & Jackson, 2014), which encode an important PER-modifying kinase (Price et al., 1998). Recently, we have profiled adult astrocytes using a glial-selective Gal4 driver to define RNAs that exhibit enriched expression in fly astrocytes (Y. Huang &

F. R. Jackson, unpublished results). See Appendix A for a detailed protocol that has been employed for TRAP profiling of glial cells in our lab.

Expression profiling of fly astrocytes has identified more than 1000 enriched mRNAs, and at least one-third of them have mammalian homologs known to be expressed in mouse astrocytes. For example, the previously mentioned GABAT and the GABA transporter are approximately fourfold enriched in astrocytes relative to total RNA from the head lysates used for ribosome IP. The fly *Csas* RNA, previously not reported to be expressed in glia, is ~sixfold enriched in astrocytes compared to total RNA. Of note, it encodes a cytidine monophosphate-sialic acid synthetase, required for sialylation of neuronal ion channels (Islam et al., 2013). Perhaps, it functions similarly in astrocytes. Among other enriched astrocyte RNAs are those encoding vesicular release factors or putatively secreted proteins which may function in glia–neuron signaling. Genetic screens, using RNAi-based glia-selective knockdown procedures, have identified an astrocyte-enriched gene that encodes a secreted factor required for the normal circadian distribution of locomotor activity (see Section 3.3). TRAP-RNA-seq methods are now being used to define rhythmically expressed glial RNAs (Y. Huang, S. You, & F. R.Jackson, unpublished results); these may contribute to the circadian control of glia–neuron communication.

4.3. Glial microRNAs as regulators of rhythmicity

There is renewed interest in the posttranscriptional and translational control of circadian behavior, based on studies demonstrating the importance of translational control in clock function (Bradley, Narayanan, & Rosbash, 2012; Cao et al., 2013; Kojima et al., 2007; Lim & Allada, 2013; Lim et al., 2011; Zhang, Ling, Yuan, Dubruille, & Emery, 2013) and in the clock regulation of translation (Green et al., 2007; Huang et al., 2013; Huang, Genova, Roberts, & Jackson, 2007; Jouffe et al., 2013). In *Drosophila*, our laboratory has used TRAP technology was used to examine circadian mRNA translation in a genome-wide manner. That analysis identified hundreds of rhythmically translated mRNAs in adult clock cells that are currently under investigation. As certain noncoding microRNAs(miRNAs) are known to be important for the translational regulation of many mRNAs, it is of interest to ask whether miRNAs function in glia to modulate circadian behavior. It is known,for example, that certain miRNAs show circadian rhythms in expression and/or are important for circadian behavior (Cheng & Obrietan, 2007; Kojima, Gatfield, Esau, & Green, 2010; Luo & Sehgal, 2012; Vodala et al., 2012; Yang, Lee, Padgett, & Edery, 2008).

We have begun to study roles of glial miRNAs in rhythmicity using transgenic inhibitors of miRNAs called “miR-sponges” (Ebert, Neilson, & Sharp, 2007). This technology was developed for inhibition of miRNAs in cultured cells and later adapted for use in *Drosophila* (Loya, Lu, Van, & Fulga, 2009), for which conditional and cell-specific expression methods can be utilized. The miR-sponges function as complementary oligomers which sop up miRNAs to prevent binding to their cognate mRNA targets; they consequently result in the upregulation of translation for most messages. Transgenic strains expressing miR-sponges representing nearly all *Drosophila* miRNAs have now been developed; these strains each carry a single UAS–miR-sponge that can be expressed in a cell-specific manner using an appropriate *Drosophila* Gal4 driver.

In recent studies, we have used the miR-sponge strains to ask which glial miRNAs are important for the circadian regulation of locomotor activity rhythms. At present, 146 different miR-sponges have been examined, and more than 20 have been identified that cause arrhythmic behavior and/or altered circadian period when expressed in a pan-glial manner (S. You & F. R. Jackson, unpublished results). The use of these miR-sponge strains with glial-selective Gal4 drivers (e.g., those specific for astrocytes) and in conditional expression experiments (using heat induction of sponge expression) will permit identification of those required in adult astrocytes for normal behavior. The knockout or overexpression of individual miRNAs (Bejarano et al., 2012; Szuplewski et al., 2012)—which often results in target degradation— together with TRAP-based profiling methods can be employed to identify putative target RNAs. Characterization of such targets will define genes expressed in glial cells that can modulate circadian behavior. Along with other genetic approaches, such studies are expected to yield novel insights about glia–neuron interactions within the neural circuits regulating behavior.

APPENDIX A. PROTOCOL FOR TRAP PROFILING OF FLY GLIAL CELLS

Immunoprecipitations of tagged ribosomes are performed using third-instar larvae or head tissues from adults expressing UAS-EGFP-L10a under the control of alrm-Gal4 or another astrocyte-selective driver. Control larvae or adults carry the UAS-EGFP-L10a transgene but not a Gal4 driver.

	10 ml	**50 ml**
Homogenization buffer		
20 m*M* HEPES (pH 7.3)	200 µl 1 *M*	1 ml
150 m*M* KCl	750 µl 2 *M*	3.75 ml
5 m*M* $MgCl_2$	50 µl 1 *M*	250 µl
Water	9.1 ml	45.5 ml
Add right before use:		
Protease inhibitors (allow to dissolve)	1 mini tablet	
0.5 m*M* DTT	5 µl 1 *M*	
RNasin	10 µl	
100 µg/ml CHX	10 µl	
0.15M KCl IP wash buffer		
20 m*M* HEPES (pH 7.3)	200 µl 1 *M*	1 ml
5 m*M* $MgCl_2$	50 µl 1 *M*	250 µl
150 m*M* KCl	750 µl 2 *M*	3.75 ml
1% Ipegal CA-630	1 ml 10%	5 ml
Water	8.1 ml	40.5 ml
Add right before use:		
0.5 m*M* DTT	5 µl 1 *M*	25 µl
RNasin (add if needed)	10 µl	µl

	10 ml	50 ml
100 μg/ml CHX	10 μl	μl
0.35 M KCl IP wash buffer		
20 m*M* HEPES (pH 7.3)	200 μl 1 *M*	1 ml
5 m*M* MgCl2	50 μl 1 *M*	250 μl
350 m*M* KCl	1.75 ml 2 *M*	8.75 ml
1% Ipegal CA-630	1 ml 10%	5 ml
Water	7.1 ml	35.5 ml
Add right before use:		
0.5 m*M* DTT	5 μl 1 *M*	25 μl
100 μg/ml CHX	10 μl	50 μl

Stock cycloheximide (*CHX*): 1000×: 100 mg/ml in MeOH, freshly made.

DHPC (1,2-Diheptanoyl-sn-glycero-3-phosphocholine): Prepare DHPC by adding 1.38 ml H_2O to 200 mg (300 m*M*, 10×); let sit on ice for at least 1 h. Mix by swirling gently. Do not vortex.

A.1. Bead preparation

Upon receiving the Invitrogen Dynabeads Antibody Coupling Kit, add 3 ml of DMF to the bottle containing 60 mg of Dynabeads so that every 50 μl suspension contains 1 mg of beads. The suspension can be stored at 4 °C for months.

1. Aliquot the appropriate amount of beads[1] into a 2-ml nonstick tube and place on the magnetic rack for 4–5 min. Remove DMF.
2. Wash beads 3 × with 1 ml RNAse-free PBS.
3. Prepare beads according to the instructions of the Invitrogen Dynabeads Antibody Coupling Kit using 10 μg purified 19C8 antibody/mg beads. Instead of 37 °C, incubate the beads with the antibody for 2 days at 4 °C with gentle mixing.
4. After 2 days, add IgG-free BSA to a final concentration of 3% (w/v). Incubate overnight with gentle mixing.
5. Prepare beads according to the kit instructions.
6. After finishing the washes using the kit buffers, wash an additional 3× in 1 ml 0.15 *M* KCl Wash Buffer with RNasin added.
7. Resuspend beads in an appropriate amount of 0.15 *M* KCl Wash Buffer with 30 mM DHPC. Aliquot as necessary into 2-ml nonstick tubes. Store beads on ice until tissue samples are ready.

[1]Depending on the strength and expression pattern of the *Drosophila* glial Gal4 driver, the amount of GFP-tagged ribosomes varies; thus, the amount of beads used should be adjusted to achieve optimal results (using too much bead preparation causes increased background). This should be empirically optimized by including both “UAS alone” and “no insertion” negative controls.

A.2. Sample preparation

Samples (fly heads, dissected brains, or larvae[2]) can be fresh or frozen. See footnotes concerning different types of fly samples. If samples are frozen, store at −80 °C, transfer on dry ice if necessary. Never allow samples to thaw until after adding homogenization buffer.

1. Add appropriate amount of homogenization buffer[3] to sample collected in a 1.5-ml tube. Homogenize immediately with a plastic pestle. Centrifuge at 20,000 ×*g* for 20 min at 4 °C.
2. Carefully transfer the supernatant to a new 1.5-ml tube using a long, thin Western gel loading tip (very thin tip for easier avoidance of floating lipid aggregates), do your best to avoid any junk and get very clear lysate.
3. Add one-eighth volume of 10% IGEPAL CA-630 and one-eighth volume of 300 mM DHPC to the cleared lysate, mix by gently inverting the vial a few times, and let it sit on ice for 5 min.
4. Centrifuge at 20,000 ×*g* for 20 min at 4 °C.
5. Carefully transfer the supernatant to the 2-ml tube containing prepared antibody-coupled beads. Again, use a long, thin Western gel loading tip to avoid floating lipid aggregates and get very clear lysate.

A.3. Immunoprecipitation

1. Invert the 2-ml tube containing lysate and antibody-coupled beads gently to disperse the beads.
2. Incubate at 4 °C for 1 h with end-over-end rotation.
3. Briefly spin tubes down, collect beads with magnet for 2 min, and save supernatant.
4. Wash 6 × with 1-ml 0.35 *M* KCl IP wash buffer.
5. Resuspend in 200 μl-H_2O.

A.4. RNA extraction for RNA-seq library preparation

1. Add 3 × sample volume Trizol (600 μl) to each sample.
2. Mix and incubate at RT for 5 min.
3. Add 160 μl (0.8 × sample volume) $CHCl_3$.
4. Cap tubes securely and shake vigorously by hand for 15 s.
5. Incubate at room temperature for 5 min.
6. Centrifuge at 11,000 rpm for 15 min at 4 °C.

[2]If working with larvae, the gut needs to be removed before homogenization. Inclusion of gut tissues will cause significant RNA degradation.
[3]In our experience with adult heads, 100 μl homogenization buffer per 100 heads works well.

7. Withdraw upper aqueous phase (~500 μl) to a new 2.0-ml nonstick tube.
8. Precipitate by adding 50 μl (1/10 volume) 3 *M* NaAc, pH 5.2 (salt precipitate), 2-μl linear acrylamide (5 mg/ml), and 550 μl isopropanol (isovolume).
9. Incubate overnight (or longer) at −80 °C.
10. Spin at maximum speed, 20 min, 4 °C.
11. Wash 2× with 1 ml freshly made, ice-cold 80% EtOH, centrifuging 5 min at maximum speed, 4 °C between each wash.
12. Air dry pellet. Resuspend in 10 μl H_2O.
13. Prepare cDNA libraries for RNA-seq analysis according to standard procedures.

A.5. Reagents

1 *M* HEPES	USB	16,924 1LT
2 *M* KCl	Ambion	AM9640G
1 *M* $MgCl_2$	Ambion	AM9530G
Water	Ambion	AM9932
Protease inhibitors	Roche	11,836,170,001
DTT	Sigma	D9779-5G
Super RNasin	Ambion	AM2696
Cyclohexamide	Sigma	C7698-5G
Ipegal CA-630	USB	19,626 500 ml
Methanol	Fisher	A453-500
DHPC	Avanti	850306P
2-ml Nonstick tube	Ambion	AM12475
1.5-ml Nonstick tube	Ambion	AM12450
Dimethylformamide	Amresco	0464-500ML
Dynamag-2	Invitrogen	123.21D
RNAse-free PBS	Ambion	AM9625
Dynabeads Ab Kit	Invitrogen	143.11D
19C8 antibody	MACF	Purified form
Rotator-revolver	Labnet	H5600-Revolver
IgG-free BSA	Jackson	001-000-162
Trizol	Invitrogen	10,296-028
Chloroform	Amresco	0757-950ML
3 *M* NaAc	Ambion	AM9740
Linear acrylamide	Ambion	AM9520
Isopropanol	Amresco	0918-4L
Ethanol	Fisher	BP2818-500

REFERENCES

Agosto J, Choi JC, Parisky KM, Stilwell G, Rosbash M, Griffith LC. Modulation of GABAA receptor desensitization uncouples sleep onset and maintenance in Drosophila. Nature Neuroscience. 2008; 11:354–359. [PubMed: 18223647]

Andretic R, Shaw PJ. Essentials of sleep recordings in Drosophila: Moving beyond sleep time. Methods in Enzymology. 2005; 393:759–772. [PubMed: 15817323]

Andretic R, van Swinderen B, Greenspan RJ. Dopaminergic modulation of arousal in Drosophila. Current Biology. 2005; 15:1165–1175. [PubMed: 16005288]

Araque A, Carmignoto G, Haydon PG, Oliet SH, Robitaille R, Volterra A. Gliotransmitters travel in time and space. Neuron. 2014; 81:728–739. [PubMed: 24559669]

Bainton RJ, Tsai LT, Schwabe T, DeSalvo M, Gaul U, Heberlein U. moody Encodes two GPCRs that regulate cocaine behaviors and blood–brain barrier permeability in Drosophila. Cell. 2005; 123:145–156. [PubMed: 16213219]

Beckwith EJ, Gorostiza EA, Berni J, Rezaval C, Perez-Santangelo A, Nadra AD, et al. Circadian period integrates network information through activation of the BMP signaling pathway. PLoS Biology. 2013; 11:e1001733. [PubMed: 24339749]

Bejarano F, Bortolamiol-Becet D, Dai Q, Sun K, Saj A, Chou YT, et al. A genome-wide transgenic resource for conditional expression of Drosophila micro-RNAs. Development. 2012; 139:2821–2831. [PubMed: 22745315]

Bohm RA, Welch WP, Goodnight LK, Cox LW, Henry LG, Gunter TC, et al. A genetic mosaic approach for neural circuit mapping in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:16378–16383. [PubMed: 20810922]

Borycz J, Borycz JA, Loubani M, Meinertzhagen IA. tan and ebony genes regulate a novel pathway for transmitter metabolism at fly photoreceptor terminals. Journal of Neuroscience. 2002; 22:10549–10557. [PubMed: 12486147]

Bradley S, Narayanan S, Rosbash M. NAT1/DAP5/p97 and atypical translational control in the Drosophila circadian oscillator. Genetics. 2012; 192:943–957. [PubMed: 22904033]

Brown GC, Neher JJ. Microglial phagocytosis of live neurons. Nature Reviews. Neuroscience. 2014; 15:209–216. [PubMed: 24646669]

Burkeen JF, Womac AD, Earnest DJ, Zoran MJ. Mitochondrial calcium signaling mediates rhythmic extracellular ATP accumulation in suprachiasmatic nucleus astrocytes. Journal of Neuroscience. 2011; 31:8432–8440. [PubMed: 21653847]

Cafferty P, Auld VJ. No pun intended: Future directions in invertebrate glial cell migration studies. Neuron Glia Biology. 2007; 3:45–54. [PubMed: 18634577]

Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, Christopherson KS, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: A new resource for understanding brain development and function. Journal of Neuroscience. 2008; 28:264–278. [PubMed: 18171944]

Cao R, Robinson B, Xu H, Gkogkas C, Khoutorsky A, Alain T, et al. Translational control of entrainment and synchrony of the suprachiasmatic circadian clock by mTOR/4E-BP1 signaling. Neuron. 2013; 79:712–724. [PubMed: 23972597]

Cavadini G, Petrzilka S, Kohler P, Jud C, Tobler I, Birchler T, et al. TNF-alpha suppresses the expression of clock genes by interfering with E-box-mediated transcription. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:12843–12848. [PubMed: 17646651]

Chaturvedi R, Reddig K, Li HS. Long-distance mechanism of neurotransmitter recycling mediated by glial network facilitates visual function in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:2812–2817. [PubMed: 24550312]

Chen WF, Maguire S, Sowcik M, Luo W, Koh K, Sehgal A. A neuron–glia interaction involving GABA transaminase contributes to sleep loss in sleepless mutants. Molecular Psychiatry. 2014 http://dx.doi.org/10.1038/mp.2014.11.

Cheng HY, Obrietan K. Revealing a role of microRNAs in the regulation of the biological clock. Cell Cycle. 2007; 6:3034–3035. [PubMed: 18075311]

Cirelli C, Bushey D. Sleep and wakefulness in Drosophila melanogaster. The Annals of the New York Academy of Sciences. 2008; 1129:323–329. [PubMed: 18591491]

Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. Nature Reviews. Neuroscience. 2013; 14:311–321. [PubMed: 23595014]

Clasadonte, J.; Haydon, PG. Astrocytes and epilepsy. In: Noebels, JL.; Avoli, M.; Rogawski, MA.; Olsen, RW.; Delgado-Escueta, AV., editors. Jasper's basic mechanisms of the epilepsies [Internet]. 4th ed.. Bethesda, MD: National Center for Biotechnology Information; 2012.

Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005; 438:1017–1021. [PubMed: 16355225]

Damulewicz M, Rosato E, Pyza E. Circadian regulation of the Na^+/K^+-ATPase alpha subunit in the visual system is mediated by the pacemaker and by retina photoreceptors in Drosophila melanogaster. PLoS One. 2013; 8:e73690. [PubMed: 24040028]

Deng Q, Terunuma M, Fellin T, Moss SJ, Haydon PG. Astrocytic activation of A1 receptors regulates the surface expression of NMDA receptors through a Src kinase dependent pathway. Glia. 2011; 59:1084–1093. [PubMed: 21544869]

Doherty J, Logan MA, Tasdemir OE, Freeman MR. Ensheathing glia function as phagocytes in the adult Drosophila brain. Journal of Neuroscience. 2009; 29:4768–4781. [PubMed: 19369546]

Dolezelova E, Nothacker HP, Civelli O, Bryant PJ, Zurovec M. A Drosophila adenosine receptor activates cAMP and calcium signaling. Insect Biochemistry and Molecular Biology. 2007; 37:318–329. [PubMed: 17368195]

Donlea JM, Pimentel D, Miesenbock G. Neuronal machinery of sleep homeostasis in Drosophila. Neuron. 2014; 81:860–872. [PubMed: 24559676]

Duhart JM, Leone MJ, Paladino N, Evans JA, Castanon-Cervantes O, Davidson AJ, et al. Suprachiasmatic astrocytes modulate the circadian clock in response to TNF-alpha. Journal of Immunology. 2013; 191:4656–4664.

Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: Competitive inhibitors of small RNAs in mammalian cells. Nature Methods. 2007; 4:721–726. [PubMed: 17694064]

Edwards TN, Meinertzhagen IA. The functional organisation of glia in the adult brain of Drosophila and other insects. Progress in Neurobiology. 2010; 90:471–497. [PubMed: 20109517]

Fountain SJ. Primitive ATP-activated P2X receptors: Discovery, function and pharmacology. Frontiers in Cellular Neuroscience. 2013; 7:247. [PubMed: 24367292]

Frank MG. Astroglial regulation of sleep homeostasis. Current Opinion in Neurobiology. 2013; 23:812–818. [PubMed: 23518138]

Fuentes-Medel Y, Ashley J, Barria R, Maloney R, Freeman M, Budnik V. Integration of a retrograde signal during synapse formation by glia-secreted TGF-beta ligand. Current Biology. 2012; 22:1831–1838. [PubMed: 22959350]

Girardet C, Lebrun B, Cabirol-Pol MJ, Tardivel C, Francois-Bellan AM, Becquet D, et al. Brain-derived neurotrophic factor/TrkB signaling regulates daily astroglial plasticity in the suprachiasmatic nucleus: Electron-microscopic evidence in mouse. Glia. 2013; 61:1172–1177. [PubMed: 23640807]

Green CB, Douris N, Kojima S, Strayer CA, Fogerty J, Lourim D, et al. Loss of Nocturnin, a circadian deadenylase, confers resistance to hepatic steatosis and diet-induced obesity. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:9888–9893. [PubMed: 17517647]

Hajnoczky G, Csordas G, Krishnamurthy R, Szalai G. Mitochondrial calcium signaling driven by the IP3 receptor. Journal of Bioenergetics and Biomembranes. 2000; 32:15–25. [PubMed: 11768758]

Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, et al. Astro-cytic modulation of sleep homeostasis and cognitive consequences of sleep loss. Neuron. 2009; 61:213–219. [PubMed: 19186164]

Halassa MM, Haydon PG. Integrated brain circuits: Astrocytic networks modulate neuronal activity and behavior. Annual Review of Physiology. 2010; 72:335–355.

Hayashi Y, Koyanagi S, Kusunose N, Okada R, Wu Z, Tozaki-Saitoh H, et al. The intrinsic microglial molecular clock controls synaptic strength via the circa-dian expression of cathepsin S. Scientific Reports. 2013; 3:2744. [PubMed: 24067868]

Haydon, PG. Purinergic signaling. In: Brady, ST.; Siegel, GJ.; Alberts, RW.; Price, DL., editors. Basic neurochemistry. New York: Academic Press; 2012. p. 377-389.

Haydon PG, Blendy J, Moss SJ, Jackson FR. Astrocytic control of synaptic transmission and plasticity: A target for drugs of abuse? Neuropharmacology. 2009; 56:83–90. [PubMed: 18647612]

Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE, et al. A translational profiling approach for the molecular characterization of CNS cell types. Cell. 2008; 135:738–748. [PubMed: 19013281]

Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A, et al. Rest in Drosophila is a sleep-like state. Neuron. 2000; 25:129–138. [PubMed: 10707978]

Ho KS, Sehgal A. Drosophila melanogaster: An insect model for fundamental studies of sleep. Methods in Enzymology. 2005; 393:772–793. [PubMed: 15817324]

Hovemann BT, Ryseck RP, Walldorf U, Stortkuhl KF, Dietzel ID, Dessen E. The Drosophila ebony gene is closely related to microbial peptide synthetases and shows specific cuticle and nervous system expression. Gene. 1998; 221:1–9. [PubMed: 9852943]

Huang Y, Ainsley JA, Reijmers LG, Jackson FR. Translational profiling of clock cells reveals circadianly synchronized protein synthesis. PLoS Biology. 2013; 11:e1001703. [PubMed: 24348200]

Huang Y, Genova G, Roberts M, Jackson FR. The LARK RNA-binding protein selectively regulates the circadian eclosion rhythm by controlling E74 protein expression. PLoS One. 2007; 2:e1107. [PubMed: 17971870]

Huang Y, McNeil GP, Jackson FR. Translational regulation of the DOUBLETIME/CKI delta/epsilon kinase by LARK contributes to circadian period modulation. PLoS Genetics. 2014; 10:e1004536. [PubMed: 25211129]

Islam R, Nakamura M, Scott H, Repnikova E, Carnahan M, Pandey D, et al. The role of Drosophila cytidine monophosphate-sialic acid synthetase in the nervous system. Journal of Neuroscience. 2013; 33:12306–12315. [PubMed: 23884937]

Jackson FR. Glial cell modulation of circadian rhythms. Glia. 2011; 59:1341–1350. [PubMed: 21732426]

Jackson FR, Haydon PG. Glial cell regulation of neurotransmission and behavior in Drosophila. Neuron Glia Biology. 2008; 4:11–17. [PubMed: 18950546]

Joiner WJ, Crocker A, White BH, Sehgal A. Sleep in Drosophila is regulated by adult mushroom bodies. Nature. 2006; 441:757–760. [PubMed: 16760980]

Jouffe C, Cretenet G, Symul L, Martin E, Atger F, Naef F, et al. The circadian clock coordinates ribosome biogenesis. PLoS Biology. 2013; 11:e1001455. [PubMed: 23300384]

Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, et al. A circadian clock in macrophages controls inflammatory immune responses. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:21407–21412. [PubMed: 19955445]

Kerr KS, Fuentes-Medel Y, Brewer C, Barria R, Ashley J, Abruzzi KC, et al. Glial wingless/Wnt regulates glutamate receptor clustering and synaptic physiology at the Drosophila neuromuscular junction. Journal of Neuroscience. 2014; 34:2910–2920. [PubMed: 24553932]

Knight D, Harvey PJ, Iliadi KG, Klose MK, Iliadi N, Dolezelova E, et al. Equilibrative nucleoside transporter 2 regulates associative learning and synaptic function in Drosophila. Journal of Neuroscience. 2010; 30:5047–5057. [PubMed: 20371825]

Koeglsperger T, Li S, Brenneis C, Saulnier JL, Mayo L, Carrier Y, et al. Impaired glutamate recycling and GluN2B-mediated neuronal calcium overload in mice lacking TGF-beta1 in the CNS. Glia. 2013; 61:985–1002. [PubMed: 23536313]

Koh K, Joiner WJ, Wu MN, Yue Z, Smith CJ, Sehgal A. Identification of SLEEPLESS, a sleep-promoting factor. Science. 2008; 321:372–376. [PubMed: 18635795]

Kojima S, Gatfield D, Esau CC, Green CB. MicroRNA-122 modulates the rhythmic expression profile of the circadian deadenylase Nocturnin in mouse liver. PLoS One. 2010; 5:e11264. [PubMed: 20582318]

Kojima S, Matsumoto K, Hirose M, Shimada M, Nagano M, Shigeyoshi Y, et al. LARK activates posttranscriptional expression of an essential mammalian clock protein, PERIOD1. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:1859–1864. [PubMed: 17264215]

Kramer A, Yang FC, Snodgrass P, Li X, Scammell TE, Davis FC, et al. Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. Science. 2001; 294:2511–2515. [PubMed: 11752569]

Kume K, Kume S, Park SK, Hirsh J, Jackson FR. Dopamine is a regulator of arousal in the fruit fly. Journal of Neuroscience. 2005; 25:7377–7384. [PubMed: 16093388]

Lalo U, Palygin O, Rasooli-Nejad S, Andrew J, Haydon PG, Pankratov Y. Exocytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex. PLoS Biology. 2014; 12:e1001747. [PubMed: 24409095]

Lee HS, Ghetti A, Pinto-Duarte A, Wang X, Dziewczapolski G, Galimi F, et al. Astrocytes contribute to gamma oscillations and recognition memory. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:E3343–E3352. [PubMed: 25071179]

Li X, Sankrithi N, Davis FC. Transforming growth factor-alpha is expressed in astrocytes of the suprachiasmatic nucleus in hamster: Role of glial cells in circadian clocks. Neuroreport. 2002; 13:2143–2147. [PubMed: 12438943]

Liang FQ, Allen G, Earnest D. Role of brain-derived neurotrophic factor in the circadian regulation of the suprachiasmatic pacemaker by light. Journal of Neuroscience. 2000; 20:2978–2987. [PubMed: 10751450]

Liang FQ, Sohrabji F, Miranda R, Earnest B, Earnest D. Expression of brain-derived neurotrophic factor and its cognate receptor, TrkB, in the rat suprachiasmatic nucleus. Experimental Neurology. 1998; 151:184–193. [PubMed: 9628753]

Liang FQ, Walline R, Earnest DJ. Circadian rhythm of brain-derived neurotrophic factor in the rat suprachiasmatic nucleus. Neuroscience Letters. 1998; 242:89–92. [PubMed: 9533401]

Lim C, Allada R. ATAXIN-2 activates PERIOD translation to sustain circadian rhythms in Drosophila. Science. 2013; 340:875–879. [PubMed: 23687047]

Lim C, Lee J, Choi C, Kilman VL, Kim J, Park SM, et al. The novel gene twenty-four defines acritical translational step in the Drosophila clock. Nature. 2011; 470:399–403. [PubMed: 21331043]

Lin Y, Stormo GD, Taghert PH. The neuropeptide pigment-dispersing factor coordinates pacemaker interactions in the Drosophila circadian system. Journal of Neuroscience. 2004; 24:7951–7957. [PubMed: 15356209]

Liu S, Lamaze A, Liu Q, Tabuchi M, Yang Y, Fowler M, et al. WIDE AWAKE mediates the circadian timing of sleep onset. Neuron. 2014; 82:151–166. [PubMed: 24631345]

Liu Q, Liu S, Kodama L, Driscoll MR, Wu MN. Two dopaminergic neurons signal to the dorsal fan-shaped body to promote wakefulness in Drosophila. Current Biology. 2012; 22:2114–2123. [PubMed: 23022067]

Liu H, Zhou B, Yan W, Lei Z, Zhao X, Zhang K, et al. Astrocyte-like glial cells physiologically regulate olfactory processing through the modification of ORN-PN synaptic strength in *Drosophila*. European Journal of Neuroscience. 2014; 40:2744–2754. [PubMed: 24964821]

Lopez M, Meier D, Muller A, Franken P, Fujita J, Fontana A. Tumor necrosis factor and transforming growth factor beta regulate clock genes by controlling the expression of the cold inducible RNA-binding protein (CIRBP). Journal of Biological Chemistry. 2014; 289:2736–2744. [PubMed: 24337574]

Lovatt D, Sonnewald U, Waagepetersen HS, Schousboe A, He W, Lin JH, et al. The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. Journal of Neuroscience. 2007; 27:12255–12266. [PubMed: 17989291]

Loya CM, Lu CS, Van VD, Fulga TA. Transgenic microRNA inhibition with spatiotemporal specificity in intact organisms. Nature Methods. 2009; 6:897–903. [PubMed: 19915559]

Luo L. Single-neuron labeling using the genetic MARCM method. CSH Protocols. 2007; 2007 pdb.prot4789.

Luo W, Sehgal A. Regulation of circadian behavioral output via a MicroRNA-JAK/STAT circuit. Cell. 2012; 148:765–779. [PubMed: 22305007]

Marpegan L, Krall TJ, Herzog ED. Vasoactive intestinal polypeptide entrains circadian rhythms in astrocytes. Journal of Biological Rhythms. 2009; 24:135–143. [PubMed: 19346450]

Marpegan L, Swanstrom AE, Chung K, Simon T, Haydon PG, Khan SK, et al. Circadian regulation of ATP release in astrocytes. Journal of Neuroscience. 2011; 31:8342–8350. [PubMed: 21653839]

McIver SR, Muccigrosso MM, Haydon PG. The effect of doxycycline on alcohol consumption and sensitivity: Consideration for inducible transgenic mouse models. Experimental Biology and Medicine (Maywood, N.J.). 2012; 237:1129–1133.

Melom JE, Littleton JT. Mutation of a NCKX eliminates glial microdomain calcium oscillations and enhances seizure susceptibility. Journal of Neuroscience. 2013; 33:1169–1178. [PubMed: 23325253]

Mou X, Peterson CB, Prosser RA. Tissue-type plasminogen activator-plasmin-BDNF modulate glutamate-induced phase-shifts of the mouse suprachiasmatic circadian clock in vitro. European Journal of Neuroscience. 2009; 30:1451–1460. [PubMed: 19811533]

Ng FS, Tangredi MM, Jackson FR. Glial cells physiologically modulate clock neurons and circadian behavior in a calcium-dependent manner. Current Biology. 2011; 21:625–634. [PubMed: 21497088]

Paladino N, Mul Fedele ML, Duhart JM, Marpegan L, Golombek DA. Modulation of mammalian circadian rhythms by tumor necrosis factor-alpha. Chronobiology International. 2014; 31:668–679. [PubMed: 24527954]

Parisky KM, Agosto J, Pulver SR, Shang Y, Kuklin E, Hodge JJ, et al. PDF cells are a GABA-responsive wake-promoting component of the Drosophila sleep circuit. Neuron. 2008; 60:672–682. [PubMed: 19038223]

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR III, Lafaille JJ, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell. 2013; 155:1596–1609. [PubMed: 24360280]

Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, et al. Astrocytic purinergic signaling coordinates synaptic networks. Science. 2005; 310:113–116. [PubMed: 16210541]

Petrzilka S, Taraborrelli C, Cavadini G, Fontana A, Birchler T. Clock gene modulation by TNF-alpha depends on calcium and p38 MAP kinase signaling. Journal of Biological Rhythms. 2009; 24:283–294. [PubMed: 19625730]

Pitman JL, McGill JJ, Keegan KP, Allada R. A dynamic role for the mushroom bodies in promoting sleep in Drosophila. Nature. 2006; 441:753–756. [PubMed: 16760979]

Price JL, Blau J, Rothenfluh A, Abodeely M, Kloss B, Young MW. Double-time is a novel Drosophila clock gene that regulates PERIOD protein accumulation. Cell. 1998; 94:83–95. [PubMed: 9674430]

Prolo LM, Takahashi JS, Herzog ED. Circadian rhythm generation and entrainment in astrocytes. Journal of Neuroscience. 2005; 25:404–408. [PubMed: 15647483]

Renn SCP, Park JH, Rosbash M, Hall JC, Taghert PH. A pdf neu-ropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in Drosophila. Cell. 1999; 99:791–802. [PubMed: 10619432]

Richardt A, Kemme T, Wagner S, Schwarzer D, Marahiel MA, Hovemann BT. Ebony, a novel nonribosomal peptide synthetase for beta-alanine conjugation with biogenic amines in Drosophila. Journal of Biological Chemistry. 2003; 278:41160–41166. [PubMed: 12900414]

Rusan ZM, Kingsford OA, Tanouye MA. Modeling glial contributions to seizures and epileptogenesis: Cation-chloride cotransporters in Drosophila melanogaster. PLoS One. 2014; 9:e101117. [PubMed: 24971529]

Salter MW, Beggs S. Sublime microglia: Expanding roles for the guardians of the CNS. Cell. 2014; 158:15–24. [PubMed: 24995975]

Schmitt LI, Sims RE, Dale N, Haydon PG. Wakefulness affects synaptic and network activity by increasing extracellular astrocyte-derived adenosine. Journal of Neuroscience. 2012; 32:4417–4425. [PubMed: 22457491]

Seluzicki A, Flourakis M, Kula-Eversole E, Zhang L, Kilman V, Allada R. Dual PDF signaling pathways reset clocks via TIMELESS and acutely excite target neurons to control circadian behavior. PLoS Biology. 2014; 12:e1001810. [PubMed: 24643294]

Seugnet L, Suzuki Y, Merlin G, Gottschalk L, Duntley SP, Shaw PJ. Notch signaling modulates sleep homeostasis and learning after sleep deprivation in Drosophila. Current Biology. 2011; 21:835–840. [PubMed: 21549599]

Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. Correlates of sleep and waking in Drosophila melanogaster. Science. 2000; 287:1834–1837. [PubMed: 10710313]

Shimosako N, Hadjieconomou D, Salecker I. Flybow to dissect circuit assembly in the Drosophila brain. Methods in Molecular Biology. 2014; 1082:57–69. [PubMed: 24048926]

Silies M, Klambt C. Adhesion and signaling between neurons and glial cells in Drosophila. Current Opinion in Neurobiology. 2011; 21:11–16. [PubMed: 20832289]

Stacey SM, Muraro NI, Peco E, Labbe A, Thomas GB, Baines RA, et al. Drosophila glial glutamate transporter Eaat1 is regulated by fringe-mediated notch signaling and is essential for larval locomotion. Journal of Neuroscience. 2010; 30:14446–14457. [PubMed: 20980602]

Stork T, Bernardos R, Freeman MR. Analysis of glial cell development and function in Drosophila. Cold Spring Harbor Protocols. 2012; 2012:1–17. [PubMed: 22194269]

Stork T, Sheehan A, Tasdemir-Yilmaz OE, Freeman MR. Neuron-glia interactions through the heartless FGF receptor signaling pathway mediate morphogenesis of Drosophila astrocytes. Neuron. 2014; 83:388–403. [PubMed: 25033182]

Strausfeld NJ, Hirth F. Deep homology of arthropod central complex and vertebrate basal ganglia. Science. 2013; 340:157–161. [PubMed: 23580521]

Stuart AE, Borycz J, Meinertzhagen IA. The dynamics of signaling at the histaminergic photoreceptor synapse of arthropods. Progress in Neurobiology. 2007; 82:202–227. [PubMed: 17531368]

Suh J, Jackson FR. Drosophila ebony activity is required in glia for the circadian regulation of locomotor activity. Neuron. 2007; 55:435–447. [PubMed: 17678856]

Szuplewski S, Kugler JM, Lim SF, Verma P, Chen YW, Cohen SM. MicroRNA transgene overexpression complements deficiency-based modifier screens in Drosophila. Genetics. 2012; 190:617–626. [PubMed: 22095085]

Tanenhaus AK, Zhang J, Yin JC. In vivo circadian oscillation of dCREB2 and NF-kappaB activity in the Drosophila nervous system. PLoS One. 2012; 7:e45130. [PubMed: 23077489]

Thomas A, Lee PJ, Dalton JE, Nomie KJ, Stoica L, Costa-Mattioli M, et al. A versatile method for cell-specific profiling of translated mRNAs in Drosophila. PLoS One. 2012; 7:e40276. [PubMed: 22792260]

Turner JR, Ecke LE, Briand LA, Haydon PG, Blendy JA. Cocaine-related behaviors in mice with deficient gliotransmission. Psychopharmacology. 2013; 226:167–176. [PubMed: 23104263]

Ueno T, Tomita J, Tanimoto H, Endo K, Ito K, Kume S, et al. Identification of a dopamine pathway that regulates sleep and arousal in Drosophila. Nature Neuroscience. 2012; 15:1516–1523. [PubMed: 23064381]

Viktorin G. Using MARCM to study Drosophila brain development. Methods in Molecular Biology. 2014; 1082:79–96. [PubMed: 24048928]

Vodala S, Pescatore S, Rodriguez J, Buescher M, Chen YW, Weng R, et al. The oscillating miRNA 959–964 cluster impacts Drosophila feeding time and other circadian outputs. Cell Metabolism. 2012; 16:601–612. [PubMed: 23122660]

Womac AD, Burkeen JF, Neuendorff N, Earnest DJ, Zoran MJ. Cir-cadian rhythms of extracellular ATP accumulation in suprachiasmatic nucleus cells and cultured astrocytes. European Journal of Neuroscience. 2009; 30:869–876. [PubMed: 19712092]

Wu H, Friedman WJ, Dreyfus CF. Differential regulation of neurotrophin expression in basal forebrain astrocytes by neuronal signals. Journal of Neuroscience Research. 2004; 76:76–85. [PubMed: 15048931]

Wu MN, Ho K, Crocker A, Yue Z, Koh K, Sehgal A. The effects of caffeine on sleep in Drosophila require PKA activity, but not the adenosine receptor. Journal of Neuroscience. 2009; 29:11029–11037. [PubMed: 19726661]

Yang M, Lee JE, Padgett RW, Edery I. Circadian regulation of a limited set of conserved microRNAs in Drosophila. BMC Genomics. 2008; 9:83. [PubMed: 18284684]

Yuan Q, Joiner WJ, Sehgal A. A sleep-promoting role for the Drosophila serotonin receptor 1A. Current Biology. 2006; 16:1051–1062. [PubMed: 16753559]

Zerr DM, Hall JC, Rosbash M, Siwicki KK. Circadian fluctuations of period protein immunoreactivity in the CNS and the visual system of Drosophila. Journal of Neuroscience. 1990; 10:2749–2762. [PubMed: 2117644]

Zhang Y, Ling J, Yuan C, Dubruille R, Emery P. A role for Drosophila ATX2 in activation of PER translation and circadian behavior. Science. 2013; 340:879–882. [PubMed: 23687048]

Zwarts L, Van Eijs F, Callaerts P. Glia in *Drosophila* behavior. Journal of Comparative Physiology A. 2014 http://dx.doi.org/10.1007/s00359-014-0952-9.

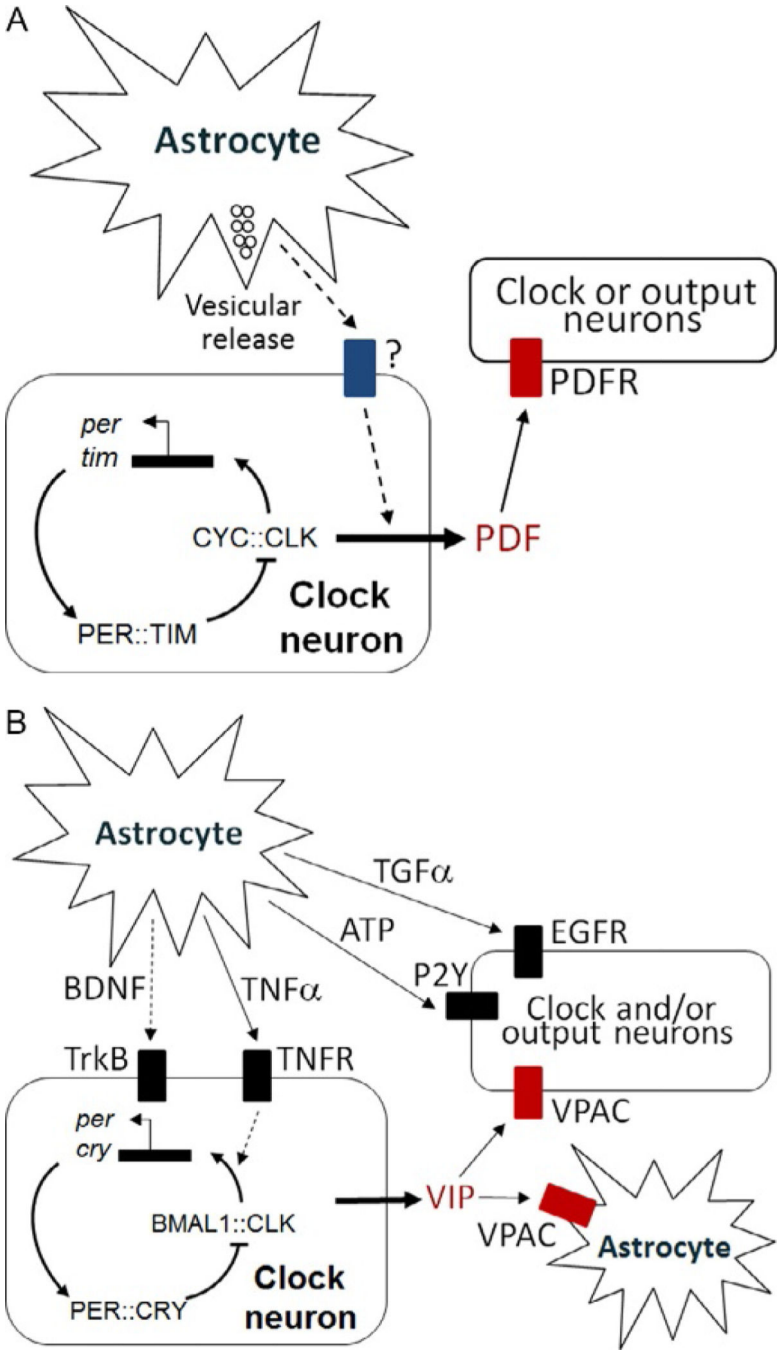

Figure 1.

Schematic models illustrating glia–neuron interactions within the fly and mammalian pacemaker systems. In flies and mammals, PDF and VIP, respectively, act on G-protein-coupled receptors of the same class (Class II, family B). Both neuropeptides act on cognate receptors present on pacemaker neurons to coordinate neuronal clocks. PDF also mediates clock output. (A) Working model for astrocyte regulation of fly pacemaker neuron physiology. The neuronal PER:TIM-based molecular oscillator is represented by an abbreviated transcriptional/translational loop. Blue (dark gray in the print version) rectangle

represents a hypothetical receptor or transporter that mediates effect of a secreted glial factor. Dotted lines indicate undefined elements or pathways involved in glia–neuron communication that regulates PDF downstream of the molecular clock. (B) Model illustrating known and hypothesize interactions between neurons and glia of the mammalian SCN. The neuronal PER:CRY-based molecular oscillator is represented by an abbreviated transcriptional/translational loop. Dotted lines indicate uncertainty regarding secretion of BDNF from SCN astrocytes or the nature of the pathway through which TNFR activation affects the molecular oscillator. Note that ATP may act on glial cells as well as neurons. BDNF is also expressed in SCN neurons as are many neuropeptides in addition to VIP (not shown in this figure). VPAC, VIP/PACAP receptor; P2Y, ATP receptor. Other abbreviations are defined in the text.