Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

A role for glia in cellular and systemic metabolism: insights from the fly

Jean-François De Backer[1,2] and Ilona C Grunwald Kadow[1,2]

Excitability and synaptic transmission make neurons high-energy consumers. However, neurons do not store carbohydrates or lipids. Instead, they need support cells to fuel their metabolic demands. This role is assumed by glia, both in vertebrates and invertebrates. Many questions remain regarding the coupling between neuronal activity and energy demand on the one hand, and nutrient supply by glia on the other hand. Here, we review recent advances showing that fly glia, similar to their role in vertebrates, fuel neurons in times of high energetic demand, such as during memory formation and long-term storage. Vertebrate glia also play a role in the modulation of neurons, their communication, and behavior, including food search and feeding. We discuss recent literature pointing to similar roles of fly glia in behavior and metabolism.

Addresses
[1] Technical University of Munich, School of Life Sciences, Liesel-Beckmann-Str. 4, 85354 Freising, Germany
[2] University of Bonn, Faculty of Medicine, UKB, Institute of Physiology II, Nussallee 11, 53115 Bonn, Germany

Corresponding author: Ilona C Grunwald Kadow (ilona.grunwald@uni-bonn.de)

Current Opinion in Insect Science 2022, **53**:100947

This review comes from a themed issue on **Neuroscience**

Edited by **Jean-Christophe Sandoz** and **Julie Carcaud**

For complete overview about the section, refer "Neuroscience (December 2022)"

Available online 27th June 2022

https://doi.org/10.1016/j.cois.2022.100947

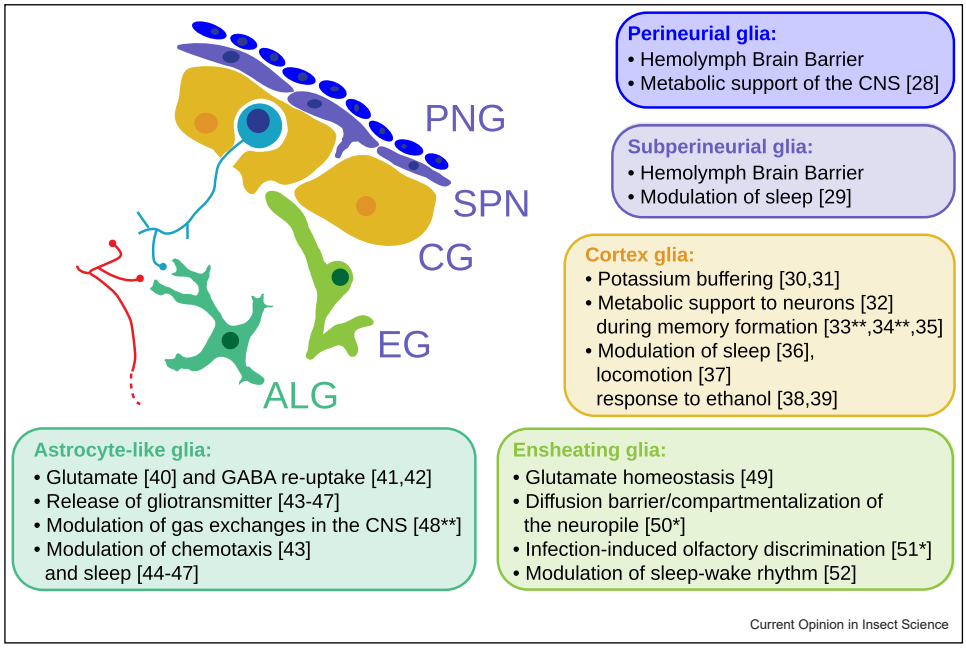
Introduction

The neuronal computation performed by the brain makes it an energy-expensive organ. In mammals, it consumes about 20% of the oxygen budget [1–3]. In insects, the main energy consumers are the flight muscles [4]. However, the insect nervous system ranks the second highest as, for instance, in flies, the retinal photoreceptors alone account for 10% of the adenosine triphosphate (ATP) consumed [5]. Among the main reasons for this high-energy demand, in mammals and insects, is the maintenance of transmembrane ion gradients and the process of synaptic transmission, as well as the maintenance of a pool of releasable synaptic vesicles [2,6].

Research using the genetic model insect, *Drosophila melanogaster*, has a long history in the study of metabolism [4] as well as in neurosciences [7]. The relatively small size and numerical simplicity of the anatomically well-mapped fly brain, combined with an extensive genetic toolkit and advances in light-microscopy technologies, have helped to discover fundamental principles of the interaction between an organism's nervous system and its metabolism [8–15]. Moreover, the emergence of in vivo neuronal population and whole-brain imaging in behaving flies further accelerates the study of the role of physiological state on nervous-system processes [16]. In a remarkable study, Mann et al. [17••] monitored in vivo how neuronal activity provokes changes in cell metabolism. Interestingly, neuronal activity drives an increase in cellular ATP concentration that significantly outlasts the recorded neuronal calcium signal, suggesting that the active brain might anticipate future energy consumption. Moreover, not only neuronal activity but also the metabolic flux represented by pyruvate and ATP concentrations, albeit not as precisely, predicted leg movements [17••]. These data suggest that, in the higher-activity state during behavior, neurons not only consume but also drive the production of ATP. This, in turn, triggers an increase in energy metabolism and subsequently in the consumption of nutrients.

Despite this high-energy demand, neurons produce ATP mainly through cellular respiration but do not store nutrients such as carbohydrates or lipids [3]. Therefore, in the insect and mammalian nervous systems, glial cells play the role of nutrient suppliers that support the high energetic demand of neurons. Albeit still largely underrepresented in neuroscience research, the study of glia in *Drosophila* has been growing in the last 10 years, largely thanks to an increased number of genetic tools and, specifically, to the availability of specific Gal4 driver lines that enable precise targeting of the different glia subpopulations in the fly central nervous system (CNS) [18,19]. The structure and function of fly glia, and particularly their relationship to neurons, have been extensively reviewed elsewhere [20–25] (Figure 1). In this review, we will first give an update of the current understanding of the role of glia as metabolic support in the fly brain. In addition to this classical role, it is now

Figure 1



Schematic representation of the different glia subtypes in the fly CNS and summary of their main functions. The perineurial (PNG, *blue*) and subperineurial glia (SPN, *purple*) form the hemolymph-brain barrier around the entire nervous system. The cortex glia (CG, *orange*) encapsulate the neuronal cell bodies. Astrocyte-like glia (ALG, *turquoise green*) interact with the synapse, while the ensheathing glia (ENG, *green*) form anatomical borders within the neuropile [30,31,37–42,46,50,51].

accepted that glia participate in the modulation of neurotransmission and therefore of behavior in a large variety of model organisms, including *Drosophila* [25]. As nutrient sensors, glial cells are in a good position to instruct neuronal circuits regulating systemic metabolism, as it has been shown in rodent models [26,27]. While such a role for fly glia remains to be demonstrated, recent advances in the study of the fly glia strongly point to a similar function in metabolism and behavior. We will discuss this possibility in the second part of this review.

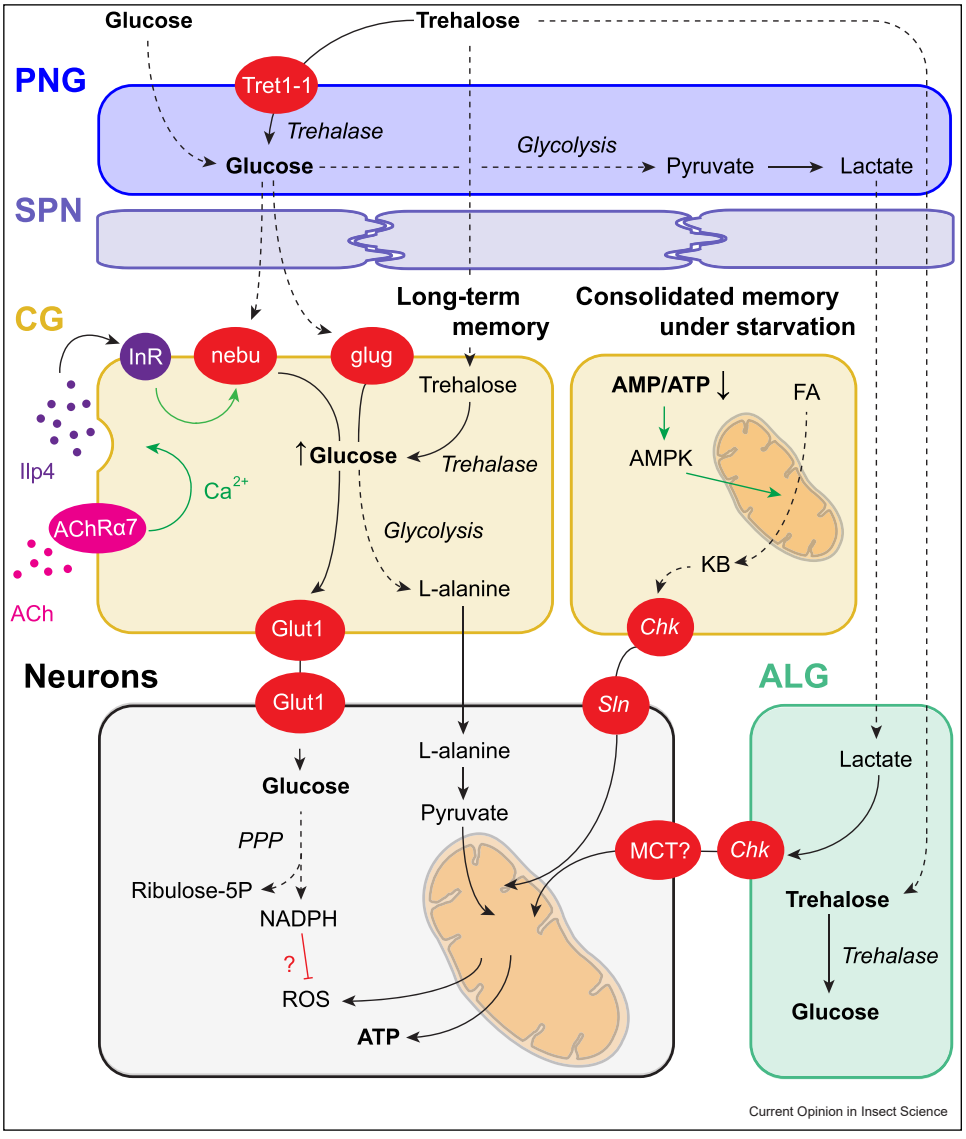
Nutrient supply of brain metabolism

A barrier preventing the uncontrolled exchange of the body's circulating fluids such as blood or hemolymph and the interstitial medium of the brain appeared early in the evolution of animals [53]. In flies, this separation is ensured by the surface glia — perineurial and subperineurial glia — that surround the entire peripheral and central nervous system and form the hemolymph-brain barrier (HBB) [54,55] (Figure 1). Septate junctions between the surface glia cells form the diffusion barrier. The integrity of the HBB is necessary to maintain proper neuronal function. A leak in the HBB would provoke an increase in potassium concentration in the interstitial fluid of the brain, and therefore, trigger neuronal hyperexcitability [56]. However, due to this diffusion barrier, the nutrients present in the hemolymph must pass through the HBB to reach the brain and satisfy its demand in energy. Interestingly, it has been recently shown that sugar transport at the HBB is upregulated in starved flies [57].

The main circulating sugar in insects is trehalose, a non-reducing disaccharide formed by two molecules of glucose in the fat body. [56] Trehalose can be taken up by the glia [28] (Figure 2). There, it is degraded and enters the glycolysis pathway to produce alanine and lactate. The latter are released by glia and used to fuel neuronal activity. Importantly, glycolysis in glia seems to be essential for brain homeostasis as knockdown of glycolytic enzymes specifically in glia impairs neuronal survival [28]. By contrast, neurons are insensitive to such a manipulation and rather rely on oxidative metabolism [28]. This study further suggested a metabolic compartmentalization of the fly brain similar to the astrocyte–neuron lactate-shuttle model (ANLS) proposed for mammals [58]. This is further supported by the presence of the lactate/pyruvate transporter *Chaski*, which has been found enriched in fly glia, and which is necessary for proper synaptic transmission and the increase in lactate in highly active neurons [32,59•].

In mammals, the ANLS model proposes a system that couples the energy supply by astrocytes to the energy demand of active neurons [58]. Here, a high rate of glutamatergic transmission triggers a

Figure 2



Simplified model of glia-to-neuron nutrient transfers. *Upper half:* Trehalose enters the brain through the perineurial glia (PNG) via the trehalose transporter *Tret1–1*. In PNG cells, trehalose is degraded into glucose that enters the glycolysis pathway to produce lactate, later excreted into the CNS [28]. Lactate can be transferred to neurons through monocarboxylate transporters (MCT) such as *Chaski* (*Chk*), predominantly expressed in glial cells such as astrocyte-like glia (ALG) [32,59•]. The enzyme responsible for the degradation of trehalose (*trehalase*) is also highly expressed in cortex glia (CG) and ALG [60]. *Lower half:* Glucose can be taken up by CG in response to high energetic demand, such as the formation of long-term memory [33••]. CG can sense neuronal activity through the activation of acetylcholine receptors (AChRα7) that trigger the release of the insulin-like peptide 4 (Ilp4) and the autocrine stimulation of the insulin receptor (InR). Activation of this pathway promotes glucose intake by the glucose transporter nebu [35] and transfer to neurons through the Glut1. Here, glucose is used by neurons to fuel the pentosephosphate pathway (PPP) that produces NADPH. NADPH provides reducing power that is potentially used by neurons to protect against the ROS resulting from mitochondrial respiration. In parallel, glucose can also enter CG cells via another transporter, 'glucose uptake by glia' (glug) [35]. Glucose imported by glug enters the glycolysis to produce L-alanine that is then transported to neurons to fuel oxidative metabolism in mitochondria. This mechanism does not seem to be specific to LTM as it also supports the formation of middle-term memory [35]. Starved flies do not form LTM [61,62]. However, during starvation, CG can switch from glucose to ketone bodies (KB) to support the consolidation of another form of memory [34••]. The KB production is triggered in CG by the intracellular energy sensor AMP-activated protein kinase (AMPK). KB are transferred to neurons through the MCTs *Chk* and *Silnoon* (*Sln*).

high rate of glutamate reuptake in astrocytes. As glutamate is cotransported along with sodium ions, the Na^+/K^+-ATPase is needed to restore the dissipated Na^+ gradient. The demand in ATP further promotes glucose uptake and upregulates glycolysis in astrocytes that in turn produce pyruvate subsequently transformed into lactate and eventually transferred to neurons. In neurons, lactate is transformed back into pyruvate to fuel the

tricarboxylic acid cycle (TCA) cycle in mitochondria. Although not yet fully demonstrated in flies, the metabolic flow described by Volkenhof et al. [28] suggests the existence of a similar mechanism in insects [22]. And although glutamatergic neurons are present in relatively small numbers as compared with cholinergic neurons in insects, fly glial cells express an excitatory amino-acid transporter named *Genderblind* that regulates glutamatergic transmission, and thereby, chemosensory processing and male courtship behavior [63]. Glutamatergic recycling in glia also contributes to long-term memory (LTM) formation and sleep regulation [36,64]. Moreover, the mitochondrial activity of ensheathing glia affects glutamate homeostasis [49]. Glutamatergic neuron activity is upregulated during active behavior such as walking [65]. Given these data and the high degree of glia interconnectivity, that is, through gap junctions [66], it is tempting to hypothesize the existence of a fly equivalent to the ANLS model with glutamatergic neurons as sensors of overall brain activity. In a recent preprint, Rabah et al. (2022) [35] propose an alternative to lactate in energy-demanding cholinergic neurons during LTM formation. In addition to lactate, L-alanine is produced during glycolysis in glia [28,35]. L-alanine can be then transferred to neurons where it is converted back into pyruvate to fuel mitochondrial oxidative metabolism [35] (Figure 2).

In addition to the surface glia, the ensheathing glia were recently shown to form another diffusion barrier around the neuropil [49•]. These cells are polarized, with an apical-like domain facing the neuropil and basolateral-like domain facing the cortex. Interestingly, the basolateral domain is enriched in the Na^+/K^+-ATPase *Nervana 2*, as discussed above, Na^+/K^+-ATPase is a key component of the ANLS model. However, there is still no evidence for an involvement of ensheathing glia in nutrient transfer for metabolic support in the fly brain.

Many fundamental questions still remain regarding the role of glia as nutrient supplier of the brain, including the important relationship between a high metabolic flow and high neuronal demand, for example, during metabolic state-dependent behavior. Exciting insights on that topic have been recently brought by the laboratory of Pierre-Yves Plaçais and Thomas Preat [33–35] (Figure 2). In addition to integrating sensory inputs and generating executive motor commands, an important property of the brain is the generation of memory. Memory formation is costly as it requires energy for neuronal firing and synaptic transmission but also an additional flow of nutrients to support gene expression and protein synthesis. Indeed, the formation of LTM is disabled in starved flies that cannot afford these additional costs [61,62]. In a follow-up paper, de Tredern et al. [33••] pinpoint the central role played by cortex glia in the transfer of glucose to neurons to sustain the formation of LTM. The authors showed that cortex glia respond to neuronal activity through the activation of acetylcholine receptors. This triggers an autocrine insulin-like signaling, which promotes glucose intake in cortex glia. Glucose is then transferred from cortex glia to neurons via the glucose transporter 1 (Glut1) transporter. Interestingly, this glucose transfer is necessary for the formation of LTM, but is not used to fuel glycolysis that could directly provide ATP. In this case, glucose is used in the pentosephosphate pathway that produces ribulose-5-phosphate and the reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$), NADPH. Ribulose-5-phosphate can be used in nucleotide synthesis or oxidized into pyruvate. NADPH could provide reducing power to protect cells from reactive oxygen species (ROS) produced by the increased mitochondrial oxidative metabolism that takes place during LTM formation [62].

Despite not being able to form LTM, starved flies can nonetheless still form a consolidated memory that does not require protein synthesis [67]. In this situation, and similar to what occurs in mammals when carbohydrates are scarce, the fly's metabolism switches to the use of lipids as an alternative source of energy [34,68]. This lipid source is present as ketone bodies and is again provided to neurons by the cortex glia in the context of starvation (Figure 2). Interestingly, this metabolic switch is orchestrated by the AMP-activated protein kinase, known to be a master energy sensor of the cell [69].

Oxygen supply of cerebral metabolism

Neurons mainly produce their ATP through oxidative metabolism [3]. Therefore, neurons must be supplied not only with nutrients but also with oxygen. In mammals, oxygen is delivered to metabolically active organs through the vascular system, along with nutrients. In the brain, it is thus essential to couple the blood flow to neuronal activity in order to ensure sufficient oxygen perfusion and prevent ischemia and subsequent neuronal cell injuries or death. Owing to their tight association with blood vessels, much of this key function is ensured by astrocytes in the mammalian brain [70,71]. Contrary to the mammalian vascular system, insects use their tracheal system to provide oxygenation of target organs by diffusion. In mammals, oxygen supply is mediated by the modulation of the diameter of blood vessels by smooth muscle cells [70]. In flies, it seems to occur through extension/retraction of the tracheal filipodia. Indeed, the terminal branches of the tracheae are highly dynamic structures that are regulated by oxygen availability [72,73]. Interestingly, Ma and Freeman [48••] recently showed that astrocyte-like glia is closely associated with tracheae and can modulate the dynamics of the filipodia that form their terminations. The interaction between tracheal terminals and astrocyte-like glia

is modulated by astrocytic spontaneous calcium activity located in microdomains close to the tracheae. This is one of the several forms of calcium activity that can take place in glia [74]. In the ventral nerve cord of the fly larva, as in the mouse brain, this microdomain activity is independent of neuronal firing but can be modulated by neurotransmitters [48••,75]. In both cases, microdomain calcium transients are modulated by the production of ROS from mitochondria.

The metabolic roles of glia and their relationship to behavior

Despite having been ignored for a long time, a recent accumulation of evidence from rodents highlights the role of glia in the regulation of information flux in the brain and thereby in the modulation of behavior (see, e.g. [76–80], reviewed in [25]). This is also the case in flies [23–25].

Similar to mammals [81,82], fly glia participate in the homeostatic regulation of sleep [36,44,45,83–85]. In particular, astrocyte-like glia have been recently proposed to be a sensor of sleep need [47•]. Interestingly, sleep has been associated with changes in metabolic rates [86,87], and several populations of fly glia might participate in the metabolic regulation of sleep. First, it has been observed that the permeability of the HBB changes according to the circadian rhythm of the fly [88]. In addition, endocytic trafficking in the HBB has been found to modulate sleep [29]. Second, the taurine transporter (excitatory amino acid transporter,EAAT2) is expressed in ensheathing glia and modulates metabolic rates during sleep [52].

The systemic metabolic state of the animal is another important driver for behavior. Hunger enhances the fly's arousal and affects its locomotion, sensory perception, and motivational state [89]. Hungry flies will start to forage for a food source and, once one is found start to feed. These behaviors and their sequence are tightly controlled by the internal physiological state of the animal [10]. Inside the fly brain, neurons can sense the circulating concentration of sugar such as fructose or glucose and promote or suppress feeding according to this metabolic state [8,11–13]. In addition, the energetic status is also monitored in the periphery at the level of the fat body, the *corpus cardiacum* and the gut. These organs modulate the flies' feeding behavior through endocrine signaling [10,14,15]. Flies, as mentioned above, possess an equivalent of the mammalian insulin/glucagon system [90]. Important data from rodent models have shown that glia participate in the control of food search and feeding behavior through the hypothalamic circuit [26,27]. In flies, cortex glia release and respond to insulin-like peptide 4 in an autocrine manner [33••]. In addition, during development, the subperineurial glia sense amino-acid availability and release insulin-like peptides to trigger neuroblast proliferation [91,92]. However, despite the recent evidence that points to a role for fly glia in metabolic support of neurons in high-energy demand [33–35], and in the control of behavior [25], there is still no report directly linking glial functions, and their modulation of the physiological state of the animal, to food seeking or consumption behaviors.

Concluding remarks

Despite noticeable structural differences between the vertebrate and invertebrate nervous system, insect glia seems to share most of the properties of their mammalian counterparts [25]. In particular, they provide metabolic support to the energy-demanding neurons that do not have direct access to the main form of circulating sugar, trehalose. This division of labor between glycolytic cellular metabolism in glia and oxidative metabolism in neurons may even be more important in flies than in mammals [22,28]. Contrary to the mammalian ANLS, there is still little evidence in flies that a metabolic shuttle between glia and neurons depends on neuronal activity. De Tredern et al. [33••] suggest that it might be the case by showing that acetylcholine-receptor activation promotes glucose intake in cortex glia. *Drosophila* astrocyte-like glia also respond to other neurotransmitters and signal back to neurons. This has been shown to modulate chemotaxis in larvae as well as sleep in adult flies [43,47•]. It is therefore tempting to speculate that similar mechanisms could participate in the regulation of systemic metabolism of insects.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

Research in the Grunwald Kadow lab received funding by the German research foundation (DFG, FOR2705, SFB870), the EU (ERCStG FlyContext), the state of Northrhine-Westphalia (NRW network iBehave), EMBO, and HFSP.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest.

1. Mink JW, Blumenschine RJ, Adams DB: **Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis**. *Am J Physiol Regul Integr Comp Physiol* 1981, **241**:R203-R212.

2. Harris JJ, Jolivet R, Attwell D: **Synaptic energy use and supply**. *Neuron* 2012, **75**:762-777.

3. Magistretti PJ, Allaman I: **A cellular perspective on brain energy metabolism and functional imaging**. *Neuron* 2015, **86**:883-901.

4. Chatterjee N, Perrimon N: **What fuels the fly: energy metabolism in *Drosophila* and its application to the study of obesity and diabetes**. *Sci Adv* 2021, **7**:eabg4336.

5. Laughlin SB, de Ruyter van Steveninck RR, Anderson JC: **The metabolic cost of neural information**. *Nat Neurosci* 1998, **1**:36-41.

6. Pulido C, Ryan TA: **Synaptic vesicle pools are a major hidden resting metabolic burden of nerve terminals**. *Sci Adv* 2021, **7**:eabi9027.

7. Owald D, Lin S, Waddell S: **Light, heat, action: neural control of fruit fly behaviour**. *Philos Trans R Soc B* 2015, **370**:20140211.

8. Miyamoto T, Amrein H: **Diverse roles for the Drosophila fructose sensor Gr43a**. *Fly* 2014, **8**:19-25.

9. Pool A-H, Kvello P, Mann K, Cheung SK, Gordon MD, Wang L, Scott K: **Four GABAergic interneurons impose feeding restraint in Drosophila**. *Neuron* 2014, **83**:164-177.

10. Pool A-H, Scott K: **Feeding regulation in Drosophila**. *Curr Opin Neurobiol* 2014, **29**:57-63.

11. Dus M, Lai JS-Y, Gunapala KM, Min S, Tayler TD, Hergarden AC, Geraud E, Joseph CM, Suh GSB: **Nutrient sensor in the brain directs the action of the brain-gut axis in Drosophila**. *Neuron* 2015, **87**:139-151.

12. Yapici N, Cohn R, Schusterreiter C, Ruta V, Vosshall LB, Taste A: **Circuit that regulates ingestion by integrating food and hunger signals**. *Cell* 2016, **165**:715-729.

13. Oh Y, Lai JS-Y, Mills HJ, Erdjument-Bromage H, Giammarinaro B, Saadipour K, Wang JG, Abu F, Neubert TA, Suh GSB: **A glucose-sensing neuron pair regulates insulin and glucagon in Drosophila**. *Nature* 2019, **574**:559-564.

14. Scopelliti A, Bauer C, Yu Y, Zhang T, Kruspig B, Murphy DJ, Vidal M, Maddocks ODK, Cordero JB: **A neuronal relay mediates a nutrient responsive gut/fat body axis regulating energy homeostasis in adult Drosophila**. *Cell Metab* 2019, **29**:269284.e10.

15. Nässel DR, Zandawala M: **Hormonal axes in Drosophila: regulation of hormone release and multiplicity of actions**. *Cell Tissue Res* 2020, **382**:233-266.

16. Aimon S, Grunwald, Kadow IC: **Studying complex brain dynamics using *Drosophila***. *J Neurogenet* 2020, **34**:171-177.

17. •• Mann K, Deny S, Ganguli S, Clandinin TR: **Coupling of activity, metabolism and behaviour across the Drosophila brain**. *Nature* 2021, **593**:244-248.

Using genetically encoded sensors for ATP and pyruvate, the authors provide the first *in vivo* wholebrain recording of neuronal metabolism in flies, in parallel to calcium signal. They show that neuronal activity provokes rapid changes in cytosolic ATP concentration that persist long after the termination of the calcium signal, suggesting that neurons may anticipate future energy expenses.

18. Li H-H, Kroll JR, Lennox SM, Ogundeyi O, Jeter J, Depasquale G, Truman JW: **A GAL4 driver resource for developmental and behavioral studies on the larval CNS of Drosophila**. *Cell Rep* 2014, **8**:897-908.

19. Kremer MC, Jung C, Batelli S, Rubin GM, Gaul U: **The glia of the adult *Drosophila* nervous system: glia anatomy in adult Drosophila nervous system**. *Glia* 2017, **65**:606-638.

20. Zwarts L, Van Eijs F, Callaerts P: **Glia in Drosophila behavior**. *J Comp Physiol A* 2015, **201**:879-893.

21. Freeman MR: ***Drosophila* central nervous system glia**. *Cold Spring Harb Perspect Biol* 2015, **7**:a020552.

22. Rittschof CC, Schirmeier S: **Insect models of central nervous system energy metabolism and its links to behavior**. *Glia* 2018, **66**:1160-1175.

23. Yildirim K, Petri J, Kottmeier R, Klämbt C: **Drosophila glia: few cell types and many conserved functions: YILDIRIM et al**. *Glia* 2019, **67**:5-26.

24. Bittern J, Pogodalla N, Ohm H, Brüser L, Kottmeier R, Schirmeier S, Klämbt C: **Neuron–glia interaction in the Drosophila nervous system**. *Dev Neurobiol* 2021, **81**:438-452.

25. Nagai J, Yu X, Papouin T, Cheong E, Freeman MR, Monk KR, Hastings MH, Haydon PG, Rowitch D, Shaham S, *et al*.: **Behaviorally consequential astrocytic regulation of neural circuits**. *Neuron* 2021, **109**:576-596.

26. García-Cáceres C, Balland E, Prevot V, Luquet S, Woods SC, Koch M, Horvath TL, Yi C-X, Chowen JA, Verkhratsky A, *et al*.: **Role of astrocytes, microglia, and tanycytes in brain control of systemic metabolism**. *Nat Neurosci* 2019, **22**:7-14.

27. Murat CDB, García-Cáceres C: **Astrocyte gliotransmission in the regulation of systemic metabolism**. *Metabolites* 2021, **11**:732.

28. Volkenhoff A, Weiler A, Letzel M, Stehling M, Klämbt C, Schirmeier S: **Glial glycolysis is essential for neuronal survival in Drosophila**. *Cell Metab* 2015, **22**:437-447.

29. Artiushin G, Zhang SL, Tricoire H, Sehgal A: **Endocytosis at the Drosophila blood–brain barrier as a function for sleep**. *eLife* 2018, **7**:e43326.

30. Melom JE, Littleton JT: **Mutation of a NCKX eliminates glial microdomain calcium oscillations and enhances seizure susceptibility**. *J Neurosci* 2013, **33**:1169-1178.

31. Weiss S, Melom JE, Ormerod KG, Zhang YV, Littleton JT: **Glial Ca2+ signaling links endocytosis to K+ buffering around neuronal somas to regulate excitability**. *eLife* 2019, **8**:e44186.

32. Delgado MG, Oliva C, López E, Ibacache A, Galaz A, Delgado R, Barros LF, Sierralta J: **Chaski, a novel Drosophila lactate/pyruvate transporter required in glia cells for survival under nutritional stress**. *Sci Rep* 2018, **8**:1186.

33. •• de Tredern E, Rabah Y, Pasquer L, Minatchy J, Plaçais P-Y, Preat T: **Glial glucose fuels the neuronal pentose phosphate pathway for long-term memory**. *Cell Rep* 2021, **36**:109620.

This study describes an elegant mechanism coupling the role of glucose supplier of glia to high demand from neurons during memory formation. Interestingly, glucose is not used to directly fuel energy metabolism but to provide building blocks and reductive power that could protect neurons against the ROS produced by mitochondria.

34. •• Silva B, Mantha OL, Schor J, Pascual A, Plaçais P-Y, Pavlowsky A, Preat T: **Glia fuel neurons with locally synthesized ketone bodies to sustain memory under starvation**. *Nat Metab* 2022, **4**:213-224.

The authors provide the first demonstration of a metabolic switch from carbohydrates to ketone bodies in fly glia. This metabolic adaptation enables starved flies to form memory when glucose is scarce.

35. Rabah Y, Frances R, Plaçais P-Y, Preat T: **Glycolysis-derived alanine from glia fuels neuronal mitochondria for memory in Drosophila**. *BioRxiv* 2022,, https://doi.org/10.1101/2022.05.30.493961

36. Farca Luna AJ, Perier M, Seugnet L: **Amyloid precursor protein in *Drosophila* glia regulates sleep and genes involved in glutamate recycling**. *J Neurosci* 2017, **37**:4289-4300.

37. Coutinho-Budd JC, Sheehan AE, Freeman MR: **The secreted neurotrophin Spätzle 3 promotes glial morphogenesis and supports neuronal survival and function**. *Genes Dev* 2017, **31**:2023-2038.

38. Lee KM, Mathies LD, Grotewiel M: **Alcohol sedation in adult Drosophila is regulated by Cysteine proteinase-1 in cortex glia**. *Commun Biol* 2019, **2**:252.

39. Lee KM, Talikoti A, Shelton K, Grotewiel M: **Tyramine synthesis, vesicular packaging, and the SNARE complex function coordinately in astrocytes to regulate *Drosophila* alcohol sedation**. *Addict Biol* 2021, **26**:e13019.

40. Rival T, Soustelle L, Strambi C, Besson M-T, Iché M, Birman S: **Decreasing glutamate buffering capacity triggers oxidative stress and neuropil degeneration in the Drosophila brain**. *Curr Biol* 2004, **14**:599-605.

41. Muthukumar AK, Stork T, Freeman MR: **Activity-dependent regulation of astrocyte GAT levels during synaptogenesis**. *Nat Neurosci* 2014, **17**:1340-1350.

42. Zhang YV, Ormerod KG, Littleton JT: **Astrocyte Ca^{2+} influx negatively regulates neuronal activity**. *eNeuro* (2) 2017, **4**:ENEURO.0340-16.2017, , https://doi.org/10.1523/ENEURO.0340-16.2017

43. Ma Z, Stork T, Bergles DE, Freeman MR: **Neuromodulators signal through astrocytes to alter neural circuit activity and behaviour**. *Nature* 2016, **539**:428-432.

44. Vanderheyden WM, Goodman AG, Taylor RH, Frank MG, Van Dongen HPA, Gerstner JR: **Astrocyte expression of the Drosophila TNF-alpha homologue, Eiger, regulates sleep in flies**. *PLoS Genet* 2018, **14**:e1007724.

45. Vanderheyden W, Van Dongen HP, Frank M, Gerstner J: **Sleep pressure regulates mushroom body neural-glial interactions in Drosophila**. *Matters Sel* 2019, https://doi.org/10.19185/matters.201903000008

46. Sengupta S, Crowe LB, You S, Roberts MA, Jackson FR: **A secreted Ig-domain protein required in both astrocytes and neurons for regulation of Drosophila night sleep**. *Curr Biol* 2019, **29**:2547-2554 e2..

47. • Blum ID, Keleş MF, Baz E-S, Han E, Park K, Luu S, Issa H, Brown M, Ho MCW, Tabuchi M, *et al.*: **Astroglial calcium signaling encodes sleep need in Drosophila**. *Curr Biol* 2021, **31**:150-162 e7.

In this study, the authors show that calcium dynamics in astrocyte-like glia follows circadian rhythm in flies. In addition, flies astrocyte-like provides homeostatic control of sleep in response to tyramine signaling and through the release of the IL-1 homolog *spätzle* in the sleep-driving circuit.

48. •• Ma Z, Freeman MR: **TrpML-mediated astrocyte microdomain Ca2+ transients regulate astrocyte–tracheal interactions**. *eLife* 2020, **9**:e58952.

This is the first study addressing the relationship between astrocyte-like glia, the tracheal system and the oxygenation of the flies nervous system. The authors show that astrocyte-like glia calcium microdomain activity is stimulated by ROS. In turn, astrocyte-like glia could promote tracheal filipodial retraction and therefore modulate gas exchanges in the nervous system.

49. Otto N, Marelja Z, Schoofs A, Kranenburg H, Bittern J, Yildirim K, Berh D, Bethke M, Thomas S, Rode S, *et al.*: **The sulfite oxidase Shopper controls neuronal activity by regulating glutamate homeostasis in Drosophila ensheathing glia**. *Nat Commun* 2018, **9**:3514.

50. • Pogodalla N, Kranenburg H, Rey S, Rodrigues S, Cardona A, Klämbt C: **Drosophila ßHeavySpectrin is required in polarized ensheathing glia that form a diffusion-barrier around the neuropil**. *Nat Commun* 2021, **12**:6357.

This study shows that ensheathing glia cells forming an additional diffusion barrier within the neuropile are polarized. Disturbance of this cellular polarization provokes abnormal locomotion in larvae, showing its requirement for proper nervous system functions.

51. • Cai XT, Li H, Borch Jensen M, Maksoud E, Borneo J, Liang Y, Quake SR, Luo L, Haghighi P, Jasper H: **Gut cytokines modulate olfaction through metabolic reprogramming of glia**. *Nature* 2021, **596**:97-102.

The authors demonstrate that gut cytokines produced in response to an enteric infection signal to ensheathing glial cells and modify their metabolism. The exact function of this metabolic switch is not yet understood but it influences flies olfactory response to bacteria-contaminated food. Furthermore, this response could contribute to the aging-related decline of olfactory signaling.

52. Stahl BA, Peco E, Davla S, Murakami K, Caicedo Moreno NA, van Meyel DJ, Keene AC: **The taurine transporter Eaat2 functions in ensheathing glia to modulate sleep and metabolic rate**. *Curr Biol* 2018, **28**:3700-3708 e4..

53. Verkhratsky A, Ho MS, Parpura V: **Evolution of neuroglia**. In *Neuroglia in Neurodegenerative Diseases*. Edited by Verkhratsky A, Ho MS, Zorec R, Parpura V. Springer; 2019:15-44.

54. Schirmeier S, Klämbt C: **The Drosophila blood-brain barrier as interface between neurons and hemolymph**. *Mech Dev* 2015, **138**:50-55.

55. Weiler A, Volkenhoff A, Hertenstein H, Schirmeier S: **Metabolite transport across the mammalian and insect brain diffusion barriers**. *Neurobiol Dis* 2017, **107**:15-31.

56. Schmidt I, Thomas S, Kain P, Risse B, Naffin E, Klambt C: **Kinesin heavy chain function in Drosophila glial cells controls neuronal activity**. *J Neurosci* 2012, **32**:7466-7476.

57. Hertenstein H, McMullen E, Weiler A, Volkenhoff A, Becker HM, Schirmeier S: **Starvationinduced regulation of carbohydrate transport at the blood–brain barrier is TGF-βsignaling dependent**. *eLife* 2021, **10**:e62503.

58. Pellerin L, Magistretti PJ: **Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization**. *Proc Natl Acad Sci USA* 1994, **91**:10625-10629.

59. • González-Gutiérrez A, Ibacache A, Esparza A, Barros LF, Sierralta J: **Neuronal lactate levels depend on glia-derived lactate during high brain activity in Drosophila**. *Glia* 2020, **68**:1213-1227.

This study shows that the increase of cytosolic lactate in highly active neurons depends on its transfer from glia. This paper supports the existence of a fly equivalent of the ANLS described in mammals.

60. Davie K, Janssens J, Koldere D, De Waegeneer M, Pech U, Kreft Ł, Aibar S, Makhzami S, Christiaens V, Bravo González-Blas C, *et al.*: **A single-cell transcriptome atlas of the aging Drosophila brain**. *Cell* 2018, **174**:982-998 e20..

61. Plaçais P-Y, Preat T: **To favor survival under food shortage, the brain disables costly memory**. *Science* 2013, **339**:440-442.

62. Plaçais P-Y, de Tredern É, Scheunemann L, Trannoy S, Goguel V, Han K-A, Isabel G, Preat T: **Upregulated energy metabolism in the Drosophila mushroom body is the trigger for long-term memory**. *Nat Commun* 2017, **8**:15510.

63. Grosjean Y, Grillet M, Augustin H, Ferveur J-F, Featherstone DE: **A glial amino-acid transporter controls synapse strength and courtship in Drosophila**. *Nat Neurosci* 2008, **11**:54-61.

64. Matsuno M, Horiuchi J, Ofusa K, Masuda T, Saitoe M: **Inhibiting glutamate activity during consolidation suppresses age-related long-term memory impairment in Drosophila**. *iScience* 2019, **15**:55-65.

65. Aimon S, Cheng KY, Gjorgjieva J, Grunwald, Kadow IC: **Walking elicits global brain activity in Drosophila**. *BioRxiv* 2022,.

66. Holcroft CE, Jackson WD, Lin W-H, Bassiri K, Baines RA, Phelan P: **Innexins Ogre and Inx2 are required in glial cells for normal postembryonic development of the *Drosophila* central nervous system**. *J Cell Sci* 2013, **126**:3823-3834, https://doi.org/10.1242/jcs.117994

67. Tully T, Preat T, Boynton SC, Del Vecchio M: **Genetic dissection of consolidated memory in Drosophila**. *Cell* 1994, **79**:35-47.

68. Chowdhury GM, Jiang L, Rothman DL, Behar KL: **The contribution of ketone bodies to basal and activity-dependent neuronal oxidation *in vivo***. *J Cereb Blood Flow Metab* 2014, **34**:1233-1242.

69. Mihaylova MM, Shaw RJ: **The AMPK signalling pathway coordinates cell growth, autophagy and metabolism**. *Nat Cell Biol* 2011, **13**:1016-1023.

70. Attwell D, Buchan AM, Charpak S, Lauritzen M, MacVicar BA, Newman EA: **Glial and neuronal control of brain blood flow**. *Nature* 2010, **468**:232-243.

71. Nortley R, Attwell D: **Control of brain energy supply by astrocytes**. *Curr Opin Neurobiol* 2017, **47**:80-85.

72. Ghabrial A, Luschnig S, Metzstein MM, Krasnow MA: **Branching morphogenesis of the *Drosophila* tracheal system**. *Annu Rev Cell Dev Biol* 2003, **19**:623-647.

73. Hayashi S, Kondo T: **Development and function of the *Drosophila* tracheal system**. *Genetics* 2018, **209**:367-380.

74. Bazargani N, Attwell D: **Astrocyte calcium signaling: the third wave**. *Nat Neurosci* 2016, **19**:182-189.

75. Agarwal A, Wu P-H, Hughes EG, Fukaya M, Tischfield MA, Langseth AJ, Wirtz D, Bergles DE: **Transient opening of the mitochondrial permeability transition pore induces microdomain calcium transients in astrocyte processes**. *Neuron* 2017, **93**:587-605 e7..

76. Adamsky A, Kol A, Kreisel T, Doron A, Ozeri-Engelhard N, Melcer T, Refaeli R, Horn H, Regev L, Groysman M, *et al.*: **Astrocytic activation generates De Novo neuronal potentiation and memory enhancement**. *Cell* 2018, **174**:59-71 e14..

77. Cui Y, Yang Y, Ni Z, Dong Y, Cai G, Foncelle A, Ma S, Sang K, Tang S, Li Y, *et al.*: **Astroglial Kir4.1 in the lateral habenula drives neuronal bursts in depression**. *Nature* 2018, **554**:323-327.

78. Yu X, Taylor AMW, Nagai J, Golshani P, Evans CJ, Coppola G, Khakh BS: **Reducing astrocyte calcium signaling in vivo alters striatal microcircuits and causes repetitive behavior**. *Neuron* 2018, **99**:1170-1187 e9..

79. Nagai J, Rajbhandari AK, Gangwani MR, Hachisuka A, Coppola G, Masmanidis SC, Fanselow MS, Khakh BS: **Hyperactivity with disrupted attention by activation of an astrocyte synaptogenic cue**. *Cell* 2019, **177**:1280-1292 e20..

80. Lim EY, Ye L, Paukert M: **Potential and realized impact of astroglia Ca2+ dynamics on circuit function and behavior**. *Front Cell Neurosci* 2021, **15**:682888.

81. Haydon PG: **Astrocytes and the modulation of sleep**. *Curr Opin Neurobiol* 2017, **44**:28-33.

82. Bojarskaite L, Bjørnstad DM, Pettersen KH, Cunen C, Hermansen GH, Åbjørsbråten KS, Chambers AR, Sprengel R, Vervaeke K, Tang W, *et al.*: **Astrocytic Ca2+ signaling is reduced during sleep and is involved in the regulation of slow wave sleep**. *Nat Commun* 2020, **11**:3240.

83. Seugnet L, Suzuki Y, Merlin G, Gottschalk L, Duntley SP, Shaw PJ: **Notch signaling modulates sleep homeostasis and learning after sleep deprivation in Drosophila**. *Curr Biol* 2011, **21**:835-840.

84. Ng FS, Tangredi MM, Jackson FR: **Glial cells physiologically modulate clock neurons and circadian behavior in a calcium-dependent manner**. *Curr Biol* 2011, **21**:625-634.

85. Ng FS, Sengupta S, Huang Y, Yu AM, You S, Roberts MA, Iyer LK, Yang Y, Jackson FR: **TRAP-seq profiling and RNAi-based genetic screens identify conserved glial genes required for adult Drosophila behavior**. *Front Mol Neurosci* 2016, **9**.

86. Petit J-M, Magistretti PJ: **Regulation of neuron–astrocyte metabolic coupling across the sleep–wake cycle**. *Neuroscience* 2016, **323**:135-156.

87. Yurgel ME, Kakad P, Zandawala M, Nässel DR, Godenschwege TA, Keene AC: **A single pair of leucokinin neurons are modulated by feeding state and regulate sleep–metabolism interactions**. *PLoS Biol* 2019, **17**:e2006409.

88. Zhang SL, Yue Z, Arnold DM, Artiushin G, Sehgal A: **A circadian clock in the blood-brain barrier regulates xenobiotic efflux**. *Cell* 2018, **173**:130-139 e10..

89. Sayin S, Boehm AC, Kobler JM, De Backer J-F, Grunwald Kadow IC: **Internal state dependent odor processing and perception—the role of neuromodulation in the fly olfactory system**. *Front Cell Neurosci* 2018, **12**:11.

90. Ahmad M, He L, Perrimon N: **Regulation of insulin and adipokinetic hormone/glucagon production in flies**. *WIREs Dev Biol* 2020, **9**:e360.

91. Chell JM, Brand AH: **Nutrition-responsive glia control exit of neural stem cells from quiescence**. *Cell* 2010, **143**:1161-1173.

92. Sousa-Nunes R, Yee LL, Gould AP: **Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in Drosophila**. *Nature* 2011, **471**:508-512.