



The *Drosophila* blood-brain barrier: development and function of a glial endothelium

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The efficacy of neuronal function requires a well-balanced extracellular ion homeostasis and a steady supply with nutrients and metabolites. Therefore, all organisms equipped with a complex nervous system developed a so-called blood-brain barrier, protecting it from an uncontrolled entry of solutes, metabolites or pathogens. In higher vertebrates, this diffusion barrier is established by polarized endothelial cells that form extensive tight junctions, whereas in lower vertebrates and invertebrates the blood-brain barrier is exclusively formed by glial cells. Here, we review the development and function of the glial blood-brain barrier of *Drosophila melanogaster*. In the *Drosophila* nervous system, at least seven morphologically distinct glial cell classes can be distinguished. Two of these glial classes form the blood-brain barrier. Perineurial glial cells participate in nutrient uptake and establish a first diffusion barrier. The subperineurial glial (SPG) cells form septate junctions, which block paracellular diffusion and thus seal the nervous system from the hemolymph. We summarize the molecular basis of septate junction formation and address the different transport systems expressed by the blood-brain barrier forming glial cells.

Keywords: *Drosophila*, glia, blood-brain barrier, septate junction formation, transmembrane transporter, astrocyte-neuron lactate shuttle hypothesis

INTRODUCTION

In all animals, an efficient separation of metabolic and ionic balance between nervous system and circulation is necessary. This in consequence led to the evolution of the so-called blood-brain barrier (Abbott et al., 2006). Vertebrates are characterized by a highly vascularized nervous system, while the insect nervous system floats in the hemolymph, which circulates through the body by the action of a primitive heart (Figures 1A–C). In the mammalian nervous system, the blood-brain barrier is established by an interplay of polarized endothelial cells and pericytes that leads to the formation of endothelial tight junctions (Armulik et al., 2010, 2011; Daneman et al., 2010b). These tight junctions prevent uncontrolled paracellular leakage of solutes into the brain. In more primitive vertebrates such as in elasmobranch fish (sharks, skates, and rays), but also in some bony fish (sturgeon), the blood-brain barrier is formed by perivascular astrocytes. These glial cells form interdigitating lamellae but do not establish tight junctions (Bundgaard and Abbott, 2008). A morphologically similar blood-brain barrier is found in insects. Here, only the outer surface of the nervous system, which is formed exclusively by glial cells, contacts the hemolymph. Although this glial barrier appears to be related to the evolutionary ancestral form of the blood-brain barrier, *Drosophila* has only recently emerged as a genetic model to study blood-brain barrier biology (Carlson et al., 2000; Abbott et al., 2006). Here, we summarize what is currently known on the

organization and the physiological properties of the *Drosophila* blood-brain barrier.

Why is *Drosophila* a good system to study blood-brain barrier properties? One of the advantages is that the nervous system is small and almost all cells are known. In addition, geneticists established rich resource and tool kits, which allow the easy manipulation of individual cells at any time of development (Dietzl et al., 2007; Venken et al., 2011; Jenett et al., 2012; Li et al., 2014). The basis of neuronal transmission is identical in flies and man and, moreover, even complex behavioral aspects appear to be controlled by similar mechanisms (Davis, 2011; Anholt and Mackay, 2012). Thus, based on the overwhelming wealth of data documenting the evolutionary conservation of central biological processes, one can expect that work on the *Drosophila* blood-brain barrier, which correctly should be termed hemolymph-brain barrier, might provide further insights into the general biology of this essential boundary.

THE NERVOUS SYSTEM OF DROSOPHILA

Drosophila is a holometabolic insect. Following 1 day of embryogenesis, three larval stages spread over the next 4 days. During the subsequent pupal stage, which covers another 5 days, metamorphosis takes place and the imago, the adult fly, emerges. Accordingly, the nervous system of *Drosophila* develops in two phases.

The larval nervous system originates from stem cells called neuroblasts that delaminate in five waves shortly after gastrulation into the interior of the embryo (Campos-Ortega and Hartenstein, 1997). Analysis of the nervous system is simplified by the fact that the thoracic and abdominal segments are mostly alike and

Abbreviations: ABC, ATP binding cassette; ATP, Adenosine triphosphate; FACS, fluorescence-activated cell sorting; GCM, Glial Cells Missing; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; IPC, Insulin-Producing Cell; RNA, Ribonucleic acid; SPG, subperineurial glia; TCA cycle, tricarboxylic acid cycle; UDP, Uridine diphosphate.

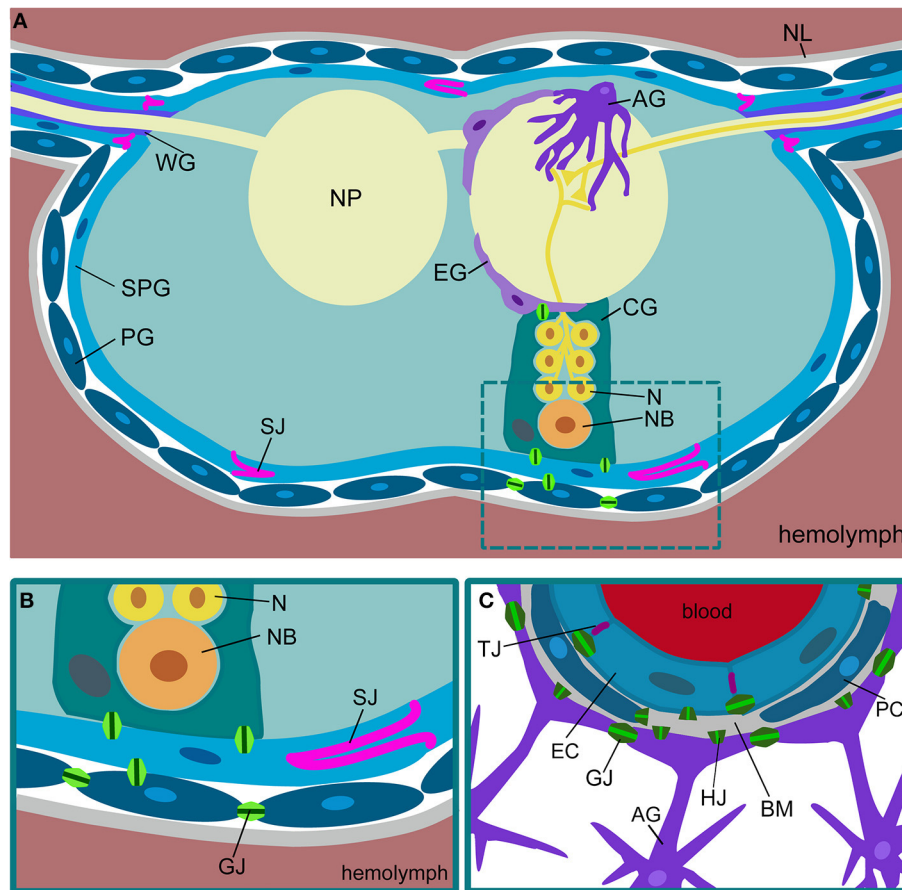


FIGURE 1 | Comparison of *Drosophila* and mammalian blood-brain barriers. (A) Schematic view of a cross-section of a *Drosophila* ventral nerve cord. The nervous system is covered by a sheath of extracellular matrix, called neural lamella (NL). The outermost glial layer consists of perineurial glial cells (PG). The subperineurial glia (SPG) forms pleated septe junctions (SJ) and blocks paracellular transport. Neurons (N) project into the neuropil (NP). Neuronal cell bodies and neuroblasts (NB) are surrounded by cortex glia (CG). The neuropil is covered by ensheathing glia (EG). Astrocytes (AG) invade the neuropil. In the peripheral nerves, wrapping glia (WG) ensheath axons.

(B) In *Drosophila*, the blood-brain barrier is built by perineurial and subperineurial glia. The latter form septe junctions (SJ) to prevent paracellular diffusion. The different glial cells are connected via gap junctions (GJ). **(C)** The mammalian blood-brain barrier is built by endothelial cells (EC) that form tight junctions (TJ) to prevent paracellular diffusion. The endothelium is in close contact with pericytes (PC). Both are surrounded by the basal membrane (BM). Gap junctions (GJ) can be found between the endothelial cells and between the astrocytes (AG). Gap junction hemichannels (HJ) can be found in all the cell types.

thus, a large part of the nervous system called ventral nerve cord represents an array of repeated and almost identical neuromeric units. Currently, the identity and the lineage of all neuroblasts are known and by the end of embryogenesis about 650 neurons and 65 glial cells are found in each neuromer of the ventral nerve cord (Broadus et al., 1995; Landgraf et al., 1997; Schmid et al., 1999; Wheeler et al., 2006; Beckervordersandforth et al., 2008; Rickert et al., 2011). The brain lobes of the *Drosophila* larvae originate from somewhat less well-defined head neuroblasts (Urbach and Technau, 2003). The neuronal circuits established by the many neurons in the larval brain are currently being deciphered by analyzing serial TEM sections of a larval brain (Cardona et al., 2010). Thus, it can be anticipated that within the next years the complete anatomical building plan of the larval nervous system is known.

The adult nervous system, including the elaborate compound eyes, develops during early pupal stages. Neuroblast proliferation

is reactivated at the end of the larval stage to generate a large number of neurons, particularly in the two brain lobes. When fully developed, the fly central nervous system harbors about 30,000 neurons (Lovick et al., 2013). Upon GFP labeling of neurons or glial cells and subsequent FACS sorting we counted about 25,000 neuronal and 10,000 glial cells per adult brain (Limmer et al., unpublished).

DROSOPHILA GLIAL CELLS

As in primitive vertebrates, the *Drosophila* blood-brain barrier is formed by glial cells (Stork et al., 2008). The fly nervous system harbors seven morphologically and molecularly distinct glial subtypes, namely midline glia, perineurial glia, subperineurial glia (SPG), cortex glia, ensheathing glia, astrocytes, and wrapping glia (Figures 1A,B) (Ito et al., 1995; Peraanu et al., 2005; Silies et al., 2007; Awasaki et al., 2008; Stork et al., 2008, 2012, 2014; Hartenstein, 2011). The entire nervous system is covered

by a layer of perineurial glial cells. These cells participate in blood-brain barrier function although their exact contribution is currently unknown. The subjacent glial cell layer is represented by the SPG cells. These flat and interdigitating cells form elaborate septate junctions, which prevent paracellular diffusion (Bainton et al., 2005; Stork et al., 2008; Mayer et al., 2009). The cortex glial cells engulf neuronal stem cells and their progeny and most likely exert some nutritional functions. The ensheathing glial cells form a sheath around the neuropil area, which lacks cell bodies and harbors only axons and dendrites. Astrocytes invade the neuropil to modulate synaptic transmission as it is known from their vertebrate homologs (Awasaki et al., 2008; Stork et al., 2014). The wrapping glia is mostly found in the peripheral nervous system where these cells engulf individual axons (Pereanu et al., 2005; Awasaki et al., 2008; Franzdóttir et al., 2009).

All glial subtypes are generated in the Drosophila embryo and emerge from stem cells programmed by the expression of the gene *glial cells missing* (*gcm*) (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). The presence of the Gcm transcription factor specifies glial identity except for the midline glia, which requires the activity of the master regulator gene *single minded* (Crews et al., 1988). Gcm subsequently activates a cascade of transcription factors. The transcription factor Pointed promotes glial differentiation and the Zn-finger protein Tramtrack inhibits neuronal differentiation in glial cells (Klaes et al., 1994; Giesen et al., 1997). Together with other transcriptional regulators such as Prospero, Distal-less and Deadringer, these factors most likely are involved in specifying the glial subtype identity (Shandala et al., 2003; Thomas and van Meyel, 2007; Schmidt et al., 2011). The relevant transcriptional regulators that specify blood-brain barrier identity are currently unknown.

THE DROSOPHILA BLOOD-BRAIN BARRIER

The establishment of the Drosophila blood-brain barrier occurs at the end of embryogenesis and requires the SPG cells. Only 16 of these cells are formed in every neuromere and four additional SPG cells are generated along every segmental nerve (Beckervordersandforth et al., 2008; von Hilchen et al., 2013). During larval stages, when the animal grows in size by a factor of 100, and likewise during metamorphosis, no additional SPG cells are formed but the blood-brain barrier remains intact (Awasaki et al., 2008; Stork et al., 2008; Unhavaithaya and Orr-Weaver, 2012). Thus, the few SPG cells generated during embryonic stages must grow enormously in size during development, and at the same time they have to maintain their elaborate junctional contacts that prevent paracellular diffusion (see below).

Once the SPG cells are born at about mid-embryogenesis, they form numerous filopodia-like processes and eventually spread to touch their neighbors at the end of embryogenesis (Schwabe et al., 2005). The SPG cells now establish a contiguous, very flat, endothelial-like sheet that covers the entire nervous system and the cell contact zones interdigitate extensively. Interestingly, in cuttlefish as well as in sturgeon, the glial blood-brain barrier also involves highly overlapping glial lamellae (Lane and Abbott, 1992; Bundgaard and Abbott, 2008). In Drosophila, glial cells of the blood-brain barrier in addition form extensive septate junctions that further restrict the paracellular diffusion between different

glial cells (Carlson et al., 2000; Schwabe et al., 2005; Stork et al., 2008). The first experimental confirmation of the physiological relevance of septate junctions for blood-brain barrier integrity was provided by the genetic analysis of the septate junction component NeurexinIV. In the absence of this protein, which is homologous to the Caspr protein found in septate-like junctions in vertebrate paranodes, the blood-brain barrier is permissive to even large molecules like dextran and in consequence, the high potassium content of the hemolymph can spread into the nervous system where it blocks any neuronal activity (Baumgartner et al., 1996).

The SPG cells are very large: a single SPG can cover the size of one half of the eye imaginal disc thus covering an area equivalent to about 10,000 epithelial cells (Silies et al., 2007). In order to achieve this enormous cell growth, the SPG cells undergo polyploidization (Unhavaithaya and Orr-Weaver, 2012). All SPG cells form a polarized endothelium that in most areas does not even reach a thickness of 1 μm . The thin nature of these barrier-forming cells has hindered detailed electron microscopic studies for a long time. Only the ability to label individual cells with GFP using subperineurial specific Gal4 driver strains such as *gliotactin-Gal4* or *moodyGal4* has allowed the description of the intricate morphology of these cells (Schwabe et al., 2005; Silies et al., 2007; Stork et al., 2008; Hatan et al., 2011; Unhavaithaya and Orr-Weaver, 2012). Currently, only few molecular markers for the blood-brain barrier are available. The multidrug resistance protein Mdr65 was shown to reside in the apical domain of the SPG cell, whereas the GPCR Moody is found at the basal portion of the cell (DeSalvo et al., 2011).

FORMATION OF SEPTATE JUNCTIONS

The most characteristic feature of the SPG is the formation of extensive septate junctions. This type of cell-cell junction has been particularly well studied in ectodermal cells, such as tracheal cells (Tepass and Hartenstein, 1994). Septate junctions are a complex crystalline array of comb-like structures built by a bewildering number of different proteins that connect individual cells, as revealed by freeze-fracture studies (Figure 2) (Lane and Swales, 1979; Lane, 1991). The cell-cell distance in these junctions is about 20 nm and thus a bit larger than in tight junctions that seal the brain endothelial cells in mammals (Farquhar and Palade, 1963, 1965). Many membrane-associated proteins are known to be involved in septate junction formation (Table 1). The core group of septate junction proteins contains the cation pump ATPalpha, the claudin family members Megatrachea and Sinous, the Ig-domain protein Neuroglian, the potassium pump subunit Nervana2, the Caspr homolog NeurexinIV and the two cytoplasmic proteins Coracle and Varicose (Oshima and Fehon, 2011, see also references in Table 1). These proteins recruit a large number of additional membrane proteins that together build the septate junctions (Table 1). Loss of most of these proteins results in the disruption of septate junctions.

Septate junction structure becomes even more elaborate at tricellular junctions. Here, septate junction strands of three cells meet and appear to be linked to a central core in the extracellular space between three neighboring cells (Figure 2). The tricellular junctions are among the first junctional complexes

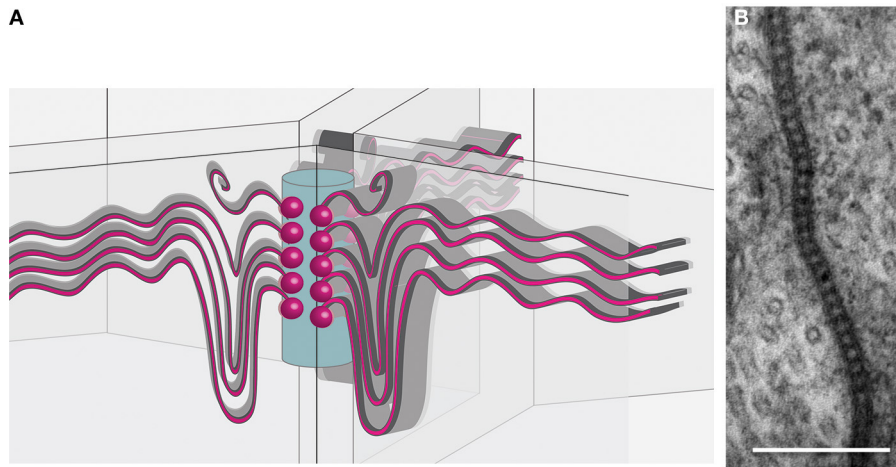


FIGURE 2 | Organization of tricellular junctions. (A) Schematic view of septate junctions at tricellular contacts according to the tricellular plug model (Graf et al., 1982; Noirot-Timothee et al., 1982; Schulte et al., 2003). Septate junctions (red sinuous lines) span the membranes of

two adjacent cells. At a central core (blue cylinder), emanating from transmembrane proteins (red balls), the septate junctions extend. **(B)** Transmission electron microscopic image of pleated septate junctions between two SPG cells.

to differentiate and are characterized by several specific proteins such as Gliotactin or Macroglobin (Auld et al., 1995; Genova and Fehon, 2003; Schulte et al., 2003; Padash-Barmchi et al., 2010; Bätz et al., 2014; Furuse et al., 2014; Oda et al., 2014). Possibly septate junction formation initiates from these positions to match the stretch growth of the SPG cells during larval development (Figure 2). The extent of septate junction formation is in part controlled by the G protein-coupled receptor Moody (Bainton et al., 2005; Schwabe et al., 2005). The Moody protein is continuously required for septate junction formation and a temporal lack of *moody* function, evoked by conditional RNA interference, results in a transient opening of the blood-brain barrier (Bainton et al., 2005). It is important to note that Moody, like possibly other developmentally required proteins, may affect both, development and the physiology of the blood-brain barrier. Indeed some effects of Moody on barrier function, such as increased cocaine sensitivity, can be seen under experimental conditions (Bainton et al., 2005). However, in the normal life of a fly no serious defects are observed when *moody* function is lacking. It was noted by Schwabe et al. (2005) that *moody* mutations are lethal, but it was also mentioned that few homozygous females and hemizygous males survive to adulthood. Interestingly, homozygous *moody* flies can be easily kept as a living stock, suggesting that blood-brain barrier physiology can be efficiently regulated by additional pathways.

How Moody signaling regulates septate junction dynamics remains unclear. *moody* null mutants show normal septate junction morphology but a somewhat reduced junctional length (Schwabe et al., 2005). In addition, Moody was shown to affect the formation of actin-rich structures along the lateral borders of the SPG cells, which also contributes to the effects on blood-brain barrier integrity (Hatan et al., 2011).

In conclusion, SPG cells are intimately interconnected by septate junctions. They set up a tight seal around the nervous system,

such that metabolite import into the brain, and the exit of waste products and xenobiotics out of the nervous system can be tightly controlled.

TRANSPORT OF METABOLITES ACROSS THE BLOOD-BRAIN BARRIER

A classical function of the blood-brain barrier is to control transport of ions and metabolites. Once the septate junctions are formed between individual SPG cells, paracellular diffusion is blocked. This, in consequence, calls for efficient and highly active transport mechanisms that shuttle all required metabolites into the brain. The relevant transporters are mostly defined by bioinformatic criteria and their expression has been globally addressed by tissue specific transcriptomics and is summarized in FlyAtlas (Chintapalli et al., 2013).

Metabolite transport across the SPG cell layer not only requires an efficient uptake mechanism into the SPG cells, but also an efficient secretion of metabolites into the nervous system. This aspect may not be confined to the blood-brain barrier, since glial cells in general and in particular SPG cells establish extensive gap junctions, which is similarly observed in endothelial cells (Figure 1) (Lane and Swales, 1979; Lane, 1991; Holcroft et al., 2013; Gaete et al., 2014). Thus, once metabolites have entered the blood-brain barrier forming cells, they might be easily distributed throughout the whole nervous system via these intercellular connections. Gap junctions are a hallmark of invertebrate glia and most glial cells express several *Drosophila* innexins, which constitute the functional connexin homologs of invertebrates (Holcroft et al., 2013). The direct coupling of glial cells by gap junctions is also reflected by glial Ca^{2+} waves, which can be seen in the blood-brain barrier, the cortex and the astrocyte glial population (Melom and Littleton, 2013; Spéder and Brand, 2014; Stork et al., 2014).

In addition to the transport of metabolites into the brain, waste products and xenobiotics need to be shuttled out of the brain. In vertebrates, chemoprotection is mediated by ATP-binding

Table 1 | Septate junction proteins.

Gene	Vertebrate homolog	Structural domains	Function	References
<i>ATPα</i>	NA ⁺ /K ⁺ ATPase α-subunit		Ion pump ATPase	Genova and Fehon, 2003
<i>nervana 2</i>	NA ⁺ /K ⁺ ATPase β-subunit		Ion pump ATPase	Genova and Fehon, 2003
<i>boudin</i>		Ly-6, GPI	Secreted, non-autonomous	Hijazi et al., 2009
<i>coiled</i>		Ly-6, GPI	Homophilic adhesion, symmetrical expression of adjacent cells necessary	Nilton et al., 2010; Syed et al., 2011
<i>crimped</i>		Ly-6, GPI		Nilton et al., 2010
<i>crooked</i>		Ly-6, GPI		Nilton et al., 2010
<i>retroactive</i>		Ly-6	Chitin cable formation	Moussian et al., 2006
<i>Contactin</i>	Contactin	Ig, FnIII, GPI	Cell-adhesion	Faivre-Sarrahil et al., 2004
<i>Fasciclin III</i>		Ig	Cell-adhesion	Narasimha et al., 2008
<i>Lachesin</i>		Ig, GPI	Cell-adhesion	Llimargas et al., 2004
<i>Neuroglian</i>	Neurofascin 155	Ig, FnIII	Cell-adhesion	Genova and Fehon, 2003
<i>Neurexin IV</i>	Caspr/Paranodin	Laminin G and EGF domains	Cell-adhesion	Baumgartner et al., 1996
<i>coracle</i>	Protein 4.1	FERM	Linker protein	Fehon et al., 1994
<i>kune-kune</i>	Claudin	PDZ-domain	Cell-adhesion	Nelson et al., 2010
<i>megatrachea</i>	Claudin	PDZ-domain	Cell-adhesion	Jaspers et al., 2012
<i>sinuous</i>	Claudin	PDZ-domain	Cell-adhesion	Wu and Beitel, 2004
<i>discs large</i>	hDLG/Sap97	MAGUK, SH3, PDZ, guanylate kinase domain	Linker protein	Woods et al., 1996
<i>scribble</i>	hscrib1	PDZ-Domain, LRR	Linker protein	Bilder and Perrimon, 2000
<i>Glilotactin</i>	Neuroigin3	Noncatalytic cholinesterase like molecule	Cell-adhesion	Auld et al., 1995
<i>Macroglobulin complement-related</i>	α2M	Thioester protein (TEP) family		Bätz et al., 2014; Hall et al., 2014
<i>Melanotransferrin</i>	MTF	Iron binding, GPI	Iron-binding, endocytosis	Tiklová et al., 2010
<i>varicose</i>	Pals2	MAGUK	Linker protein	Wu et al., 2007
<i>wunen</i>		Lipid phosphatase		Ile et al., 2012
<i>yurt</i>				Laprise et al., 2009

The known *Drosophila* septate junction proteins are listed. Structural domains and the predicted molecular functions are indicated. Ly-6, lymphocyte antigen-6; GPI, glycosyl phosphatidylinositol; Ig, immunoglobuline; FnIII, fibronectin type III; EGF, epidermal growth factor; MAGUK, membrane-associated guanylate kinases; SH3, SRC homology 3; LRR, leucine-rich repeat.

cassette (ABC) transporters such as the Multidrug-resistance protein 1 (Mdr1). Quite similar, *mdr65* performs related functions in the Drosophila nervous system (Mayer et al., 2009). In line with this, Mdr65 levels are elevated upon exposure to insecticides (Dermauw and Van Leeuwen, 2014). Mdr65 belongs to a large class of transporters with 56 members in Drosophila of which several are expressed in the nervous system (Dermauw and Van Leeuwen, 2014). Their function, however, is largely unknown and will not be further discussed here.

Neural cells need to take up specific lipids from the hemolymph, which feeds into specific anabolic pathways (Palm et al., 2012) or is directly used as energy source during β -oxidation (Palanker et al., 2009). In the following we will focus on metabolite import across the blood-brain barrier and review the main classes of the relevant transport systems. Due to space constraints, however, we will neglect lipid metabolism. We anticipate that the wealth of tools available in Drosophila will promote a deeper understanding of the physiological roles of this important barrier.

WATER HOMEOSTASIS

Mechanisms for controlling influx and efflux of water are essential for osmotic control of fluids in the nervous system. Water can enter the nervous system through aquaporins, small membrane-spanning proteins that form channels to facilitate water flow along the osmotic gradient. 14 aquaporins are known in humans and in particular aquaporin-4 (AQP4) has been linked to blood-brain barrier function. AQP4 localizes to astrocyte endfeet at the CSF-CNS and blood-CNS barriers and is crucially required for the regulation of water homeostasis and the definition of the extracellular space in the CNS (Nagelhus and Ottersen, 2013; Papadopoulos and Verkman, 2013).

The Drosophila genome harbors eight aquaporin-encoding genes (FlyBase, CV: water transmembrane transporter activity, **Table 2**). The DRIP protein has the highest sequence similarity to vertebrate AQP4 but shows no prominent CNS expression (Chintapalli et al., 2013). *big brain* (*bib*) was initially identified as a neurogenic gene and encodes a protein related to aquaporins (Rao et al., 1990). Although *Bib* has water transport properties, this function is still controversial (Tatsumi et al., 2009). The Drosophila aquaporin most prominently expressed in the CNS is CG7777 (Chintapalli et al., 2013), but currently no functional analysis has been performed.

ION HOMEOSTASIS

The hemolymph is an ion rich fluid (**Table 3**). In particular the high potassium concentration, which is characteristic for the invertebrate hemolymph, would be problematic for normal neuronal function. In the mammalian blood, potassium levels are only around 1–5 mM and thus almost a factor of 10 lower compared to the Drosophila hemolymph. The blood-brain barrier prevents any uncontrolled influx of ions into the nervous system. In consequence, the potassium concentration in the Drosophila brain fluid is about 5 mM or less (Armstrong et al., 2012).

The Drosophila genome harbors a large number of proteins linked to potassium influx or export (Chintapalli et al., 2013). However, only in few cases, their relevance for regulated ion

transport across the blood-brain barrier has been demonstrated. One example is the Fray kinase and its target Ncc69, the homolog of the human SLC12 $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter (Leiserson et al., 2000, 2011). Ncc69 is expressed in SPG cells and *Ncc69* mutant larvae develop a peripheral neuropathy with fluid accumulations between glia and axons (Leiserson et al., 2011). The effect of Ncc69 on CNS physiology is not reported. Several other ion transport proteins of the SLC12 family are also expressed in the Drosophila brain (Sun et al., 2010) but their possible glial functions are unexplored.

In a genetic screen for temperature-sensitive conditional seizure mutants the glial-specific $\text{Na}^+/\text{Ca}^{2+}$, K^+ exchanger Zydeco was identified. Zydeco is mostly expressed by the cortex glia and not by the blood-brain barrier glia (Guan et al., 2005; Melom and Littleton, 2013). In addition to channels, active ion pumps such as the Na^+/K^+ ATPase are involved in regulating ion homeostasis in the brain. The two Drosophila *nervana* genes (*nrv1* and *nrv2*) encode beta subunits of the Na^+/K^+ ATPase. *Nrv2* and the ATPase alpha are expressed in the SPG, where they are localized to septate junctions. Hypomorphic mutants for the ATPase alpha subunit show a bang and ouabain sensitivity (Schubiger et al., 1994). Septate junction formation, however, does not depend on ATPase function (Genova and Fehon, 2003; Paul et al., 2007).

UPTAKE OF AMINO ACIDS INTO THE NERVOUS SYSTEM

Amino acids are not only required for protein synthesis, but particular amino acids or their direct derivatives are also used as neurotransmitters. Cells are able to generate most amino acids from intermediates of the citric acid cycle. However, some amino acids cannot be produced and thus their uptake and transport is essential. In Drosophila, 10 proteinogenic L-amino acids are essential: tryptophan, phenylalanine, leucine, histidine, valine, isoleucine, lysine, methionine, arginine, and threonine (Boudko, 2012). Therefore, specific transport systems for these amino acids must be expressed by the SPG cells.

In vertebrates, nine distinct amino acid transport systems have been reported to be present at the brain capillary endothelium (Smith, 2000): the X-system is a high affinity, sodium-independent transport system for anionic amino acids, the L-system transports large neutral amino acids, the A- and ASC-systems transport small neutral amino acids, the Y^+ -system is a sodium-independent cationic amino acid transport system, the B^{O^+} -system represents a high affinity transport system and transports both neutral and basic amino acids and the β -system is a low capacity, sodium-dependent transporter for taurine and β -alanine. The N-system mediates the sodium-dependent transport of L-glutamine, L-histidine and L-asparagine and the T-system transports thyroid hormones.

Two well-known X-system transporters are EAAT1 and EAAT2, which in mammals are expressed by astrocytes (Rothstein et al., 1994). In Drosophila, both proteins have been described, but are expressed in glial cells other than the blood-brain barrier cells (Soustelle et al., 2002; Freeman et al., 2003; Stacey et al., 2010). In total, 51 Drosophila genes are annotated to have an “organic acid transmembrane transporter activity” or as “amino acid transporter” (**Table 4**). For a few of these proteins

Table 2 | *Drosophila* Aquaporins.

Gene	CG number	Human homolog	CNS expression			References
			FlyAtlas	BDGP	Literature	
<i>drip</i>	CG9023	AQP1, AQP4	–	–	No	Kaufmann et al., 2005
<i>big brain</i>	CG4722	AQP4	Yes	Yes	Yes	Rao et al., 1990
<i>aquaporin</i>	CG12251	AQP12	No	No	–	
CG7777	CG7777	AQP1	Yes	No	Yes	Kaufmann et al., 2005
CG17664	CG17664	MIWC, AQP4	No	–	No	Kaufmann et al., 2005
CG17662	CG17662	MIWC, AQP4	No	–	–	
CG4019	CG4019	MIWC, AQP4	Low	No	No	Kaufmann et al., 2005
CG5398	CG5398	AQP2	No	–	No	Kaufmann et al., 2005

All predicted *Drosophila* aquaporins (FlyBase, CV: water transmembrane transporter activity) are listed. Information about the CNS expression of each gene were obtained from FlyAtlas, the Berkely *Drosophila* Genome Project (BDGP), and the literature shown. AQP, aquaporin; MIWC, mercurial-insensitive water channel.

Table 3 | Ion concentration in the hemolymph.

[Na ⁺] [mM]	[K ⁺] [mM]	[Cl ⁻] [mM]	[Mg ²⁺] [mM]	[Ca ²⁺] [mM]	Remark/method	References
THIRD INSTAR LARVAL						
52 ± 1	36 ± 1	30 ± 1	–	–	Photometry and chemical methods	Croghan and Lockwood, 1960
56.5	40.2	42.2	41.6	15.9	Physical and chemical methods	Begg and Cruickshank, 1962
~45	~23	–	–	–	Ion-selective microelectrodes	Naikhwah and O'Donnell, 2011
ADULT						
70 – 110	26				Ion-selective microelectrodes	Naikhwah and O'Donnell, 2011
123.7 ± 9.2	28.4 ± 2.4				Ion-selective microelectrodes	MacMillan and Hughson, 2014

Drosophila third instar larval and adult hemolymph concentrations of selected ions are listed. The method of concentration determination is indicated.

expression in CNS glial cells has been noted (Soustelle et al., 2002; Besson et al., 2005; Augustin et al., 2007; Grosjean et al., 2008; Featherstone, 2011). In some cases, expression in the blood-brain barrier was found (e.g., CG15088, Thimman et al., 2006) but functional analysis is lacking so far.

ENERGY SUPPLY OF THE BRAIN

The energy expenditure for normal neuronal function is enormous. The human brain requires about 20% of the total resting oxygen consumption of the entire body although it comprises only about 2% of the body mass. Likewise in flies, just the photoreceptor cells in the retina consume about 10% of the total ATP production (Laughlin et al., 1998). Therefore, an efficient transport of energy-rich nutrients such as sugars needs to be established at the blood-brain barrier.

THE ASTROCYTE-NEURON LACTATE SHUTTLE HYPOTHESIS

The intense metabolic interactions between glial cells and neurons led to the astrocyte-neuron lactate shuttle (ANLS) hypothesis (Pellerin and Magistretti, 1994, 2012; Allaman et al., 2011). In the mammalian brain, the main energy source is glucose, which is shuttled into the nervous system via the Glut1 transporter. Glut1 is asymmetrically expressed in endothelial cells and is also found in astrocytes surrounding the endothelium (Leybaert, 2005). Glucose that is taken up by astrocytes is then metabolized through glycolysis to lactate or pyruvate. These small C₃ metabolites are then released into the extracellular space to be utilized by

neurons, which is supported by experimental and as well as theoretical considerations (Rouach et al., 2008; Jolivet et al., 2009; Harris et al., 2012). Enhanced neuronal activity might be linked to an increase in Glut1 expression, which would account for increased energy supply (Leybaert, 2005). This energetic coupling may be of more general relevance, since also the survival of myelinated axons depends on metabolic support by the corresponding glial cells (Fünfschilling et al., 2012; Lee et al., 2012). In invertebrates, a similar compartmentalization of energy metabolism is likely to be established as well. In the honeybee retina, glucose is exclusively taken up by glial cells and alanine, which is generated from pyruvate through transamination, seems to be shuttled to neurons to fuel the TCA cycle (Tsacopoulos et al., 1994).

TREHALOSE TRANSPORTERS IN THE DROSOPHILA BLOOD-BRAIN BARRIER

Several different sugars are present in the *Drosophila* hemolymph (Table 5). The main carbohydrate found in the insect hemolymph is trehalose, a non-reducing disaccharide with two D-glucose units linked by an α , α -1,1-glycosidic bond. Smaller amounts of glucose and fructose are also found, but their respective concentrations appear to vary depending on the metabolic state of the animal (Blatt and Roces, 2001). Upon feeding, carbohydrates are taken up by the intestinal epithelium and glucose is secreted into the hemolymph. The glucose is then shuttled to the fat body, where trehalose is synthesized from glucose-6-phosphate and UDP-glucose by the enzyme Trehalose phosphate synthase,

Table 4 | Drosophila amino acid transporters.

Gene	CG	Human homolog	CNS expression			Function	References
			FlyAtlas	BDGP	Literature		
	CG13248	SLC7	Yes	Yes	Yes	Cationic AA transporter; putative arginine transporter	Romero-Calderón and Krantz, 2006; Park et al., 2011
	CG5535	SLC7	Yes		No	Cationic AA transporter	Romero-Calderón and Krantz, 2006
	CG12531	SLC7	Yes			Cationic AA transporter	Romero-Calderón and Krantz, 2006
	CG7255	SLC7	No		No	Cationic AA transporter	Romero-Calderón and Krantz, 2006
<i>slimfast</i>	CG11128	SLC7	No			Cationic AA transporter	
<i>Jhl-21</i>	CG12317	SLC7	Yes			L-AA transporter; lysine and other neutral AA transport light chain of heterodimeric transporter	Freeman et al., 2003; Reynolds et al., 2009
<i>pathetic</i>	CG3424	SLC36	Yes	Yes	Yes	AA transporter; alanine, glycine transporter	Goberdhan et al., 2005
<i>minidiscs</i>	CG3297	SLC7	Yes	Yes		AA transporter	Reynolds et al., 2009
<i>Nutrient Amino Acid transporter 1</i>	CG3252	SLC6	No		No/yes	K ⁺ /AA symporter; L/D-amino acid transporter Neutral AA transporter	Thimgan et al., 2006; Miller et al., 2008; Romero-Calderón et al., 2008
	CG17119	CTNS	Yes			AA transporter; lysosomal L-cysteine transporter	Romero-Calderón and Krantz, 2006
	CG1139	SLC36	No	No		AA transporter; cysteine, alanine, glycine transport	Goberdhan et al., 2005; Romero-Calderón and Krantz, 2006
	CG1607	SLC7	Yes			AA transporter	Romero-Calderón and Krantz, 2006
	CG13384	SLC36	Yes			AA transporter	Romero-Calderón and Krantz, 2006
	CG13743	SLC38	Yes			AA transporter	Romero-Calderón and Krantz, 2006
	CG7708	SLC5	Yes	Yes		AA transporter; Proline/Na ⁺ symporter; Choline transporter	Romero-Calderón and Krantz, 2006
	CG7888	SLC36	Yes			AA transporter	Romero-Calderón and Krantz, 2006
	CG9413	SLC7	Yes			AA transporter	Romero-Calderón and Krantz, 2006
<i>List</i>	CG15088	SLC6	Yes		Yes	K ⁺ /AA symporter; neuro-transmitter/Na ⁺ symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006; Kasuya et al., 2009
	CG30394	SLC38	Yes			AA transporter	Romero-Calderón and Krantz, 2006

(Continued)

Table 4 | Continued

Gene	CG	Human homolog	CNS expression			Function	References
			FlyAtlas	BDGP	Literature		
<i>tadr</i>	CG9264	SLC7	Yes			AA transporter	
	CG4991	SLC36	Yes			AA transporter	Romero-Calderón and Krantz, 2006
	CG1628	SLC25	Yes			AA transporter; L-ornithine transporter	Romero-Calderón and Krantz, 2006
	CG8785	SLC36	No			AA transporter	Romero-Calderón and Krantz, 2006
	CG12943	SLC36	No			AA transporter	Romero-Calderón and Krantz, 2006
	CG13646	no	No			AA transporter	Romero-Calderón and Krantz, 2006
	CG32079	SLC36	No		No	AA transporter	Romero-Calderón and Krantz, 2006
	CG32081	SLC36	No			AA transporter	
<i>hoepel1</i>	CG12787	OCA2	Yes			L-tyrosine transporter	
<i>hoepel2</i>	CG15624	OCA2	Yes			L-tyrosine transporter	
<i>kazachoc</i>	CG5594	SLC12	Yes	Yes	Yes	K ⁺ /Cl ⁻ symporter; AA transporter	Hekmat-Scafe et al., 2006; Sun et al., 2010 Filippov et al., 2003
	CG31547	SLC12	Yes		Yes	AA transporter; Na ⁺ /K ⁺ /Cl ⁻ symporter	Filippov et al., 2003; Romero-Calderón and Krantz, 2006; Sun et al., 2010
<i>Ncc69</i>	CG4357	SLC12	Yes		No/yes	Na ⁺ /K ⁺ /Cl ⁻ cotransporter; AA transporter	Filippov et al., 2003; Romero-Calderón and Krantz, 2006; Sun et al., 2010; Leiserson et al., 2011
	CG12773	SLC12	Yes		No/yes	AA transporter; Na ⁺ /K ⁺ /Cl ⁻ symporter	Filippov et al., 2003; Romero-Calderón and Krantz, 2006; Sun et al., 2010
	CG1698	SLC6	No		No	K ⁺ /Cl ⁻ symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006
<i>karmoisin</i>	CG12286	SLC16	Yes			Monocarboxylic acid transporter	
<i>Silnoon</i>	CG8271	SLC16	Yes			Secondary active monocarboxylate transporter; Butyrate, lactate transport	Jang et al., 2008
<i>outsiders</i>	CG8062	SLC16	No			Monocarboxylic acid transporter	
<i>Dietary and metabolic glutamate transporter</i>	CG5304	SLC17	Yes		No	High affinity inorganic phosphate/Na ⁺ symporter; Glutamate transporter; Na ⁺ -independent glutamate transporter	Laridon et al., 2008; Shim et al., 2011

(Continued)

Table 4 | Continued

Gene	CG	Human homolog	CNS expression			Function	References
			FlyAtlas	BDGP	Literature		
<i>Eaat 1</i>	CG3747	SLC1	Yes		Yes	Glutamate/Na ⁺ symporter; L-aspartate transporter; Na ⁺ /Dicarboxylate symporter; L-Glutamate transporter	Besson et al., 2000; Freeman et al., 2003; Rival et al., 2004
<i>Eaat 2</i>	CG3159	SLC1	Yes		Yes	Glutamate/Na ⁺ transporter; L-aspartate Taurine transporter; Na ⁺ /Dicarboxylate symporter	Besson et al., 2000, 2005, 2011; Soustelle et al., 2002; Freeman et al., 2003
<i>genderblind</i>	CG6070	SLC7	Yes	Yes	Yes	AA transporter Glutamate transporter	Augustin et al., 2007, Grosjean et al., 2008
<i>Ntl</i>	CG7075	SLC6	No		No	Neurotransmitter/Na ⁺ symporter; Glycine transporter	Thimgan et al., 2006; Romero-Calderón et al., 2008
<i>Vesicular GABA transporter</i>	CG8394	SLC3	Yes		Yes	GABA/H ⁺ symporter; AA transporter	Romero-Calderón et al., 2008; Fei et al., 2010
<i>Vesicular glutamate transporter</i>	CG9887	SLC17	Yes		Yes	High affinity inorganic phosphate/Na ⁺ symporter; L-glutamate transporter	Daniels et al., 2004
	CG1732	SLC6	Yes	Yes	Yes	GABA/Na ⁺ symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006
	CG4476	SLC6	Yes		No	K ⁺ /AA symporter; Neurotransmitter/Na ⁺ symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006
	CG16700	SLC36	Yes			AA transporter; GABA/H ⁺ symporter	Romero-Calderón and Krantz, 2006
	CG5549	SLC6	No		Yes	Glycine/Na ⁺ symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006
	CG8850	SLC6	No		No	K ⁺ /AA symporter; Na ⁺ /AA symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006
	CG13796	No	No			Glycine/Na ⁺ symporter	Romero-Calderón and Krantz, 2006
	CG15279	SLC5	No		No	Cation/Na ⁺ symporter	Romero-Calderón and Krantz, 2006; Thimgan et al., 2006

Amino acid transporters annotated in FlyBase as "organic acid transmembrane transporter activity" or as "amino acid transporter" are listed. Information about the CNS expression of each gene was obtained from FlyAtlas, the Berkely *Drosophila* Genome Project (BDGP), or literature. The predicted function of each protein is indicated. SLC, solute carrier; AA, amino acid; OCA2, oculocutaneous albinism II; GABA, γ -Aminobutyric acid.

which is encoded by an essential gene in *Drosophila* (*Tps1*, CG4104, Chen and Haddad, 2004). Among others, trehalose is used for the maintenance of energy metabolism during fasting and non-feeding periods (Friedman, 1978; Arrese and Soulagés, 2010; Chen et al., 2010).

These observations suggest that specific sugar transporters exist, which import either trehalose or glucose into the brain. Two dedicated trehalose transporters, *Tret1-1* and *Tret1-2*, have been described in *Drosophila melanogaster* (Kikawada et al., 2007). A comparison with other insect species shows that only one trehalose transporter gene is conserved in insects whereas

the second trehalose transporter gene found in *Drosophila melanogaster* (*Tret1-2*) arose from a recent gene duplication event. *Tret1-1* but not *Tret1-2* is able to transport trehalose when expressed in *Xenopus* oocytes suggesting that the two trehalose transporters exert non-redundant functions (Kanamori et al., 2010). Trehalose transporters belong to the solute carrier 2 (SLC2) facilitated glucose transporter family with strongest homology to the human SLC2A8 protein. According to microarray data, *Tret1-1* is strongly expressed in the brain. *Tret1-2* is expressed only at very low levels. Its function is currently unknown (Kanamori et al., 2010). In conclusion, the Trehalose transporter is in a prime

Table 5 | Sugar concentration in the insect hemolymph.

Organism	Trehalose	Glucose	Fructose	Remark	References
<i>A. mori</i> , <i>G. mellonella</i> , <i>T. polyphemus</i> , <i>P. cecropia</i>	2–13 mg/ml	0–2.8 mg/ml	Not tested	Based on chromatography and chemical methods	Wyatt and Kalf, 1957
Several insects	2–50 mg/ml	Generally low amounts, but <i>Apis mellifica</i> 6–32 mg/ml, <i>Phormia regina</i> 7–12.5 mg/ml	Generally low amounts, but <i>Apis mellifica</i> 2–16 mg/ml	Review on hemolymph composition in insects	Jeuniaux, 1971
<i>Apis mellifica</i>	40 mg/ml	10 mg/ml	10 mg/ml	HPLC, trehalose concentration changes depending on metabolic rate	Blatt and Roces, 2001
Drosophila larvae	60 mg/ml	50 mg/ml	Not tested	Commercial kit	Lee and Park, 2004
Drosophila larvae	13.7–17.2 mg/ml	5.4–7.2 mg/ml	Not tested	Commercial kit	Broughton et al., 2008
Drosophila larvae	6 mg/ml	1 mg/ml	Not tested	Commercial kit	Pasco and Léopold, 2012
Drosophila adults	17.2 mg/ml	1.8 mg/ml	Not tested	Commercial kit	Broughton et al., 2008

Trehalose, glucose, and fructose concentrations in the hemolymph of different organisms and developmental stages are listed. Methods for concentration measurements are indicated.

position to control the import of high-energy carbohydrates at the blood-brain barrier.

CARBOHYDRATE TRANSPORTERS IN THE DROSOPHILA BLOOD-BRAIN BARRIER

In total, 78 genes of *Drosophila* harbor a sugar transporter motif (Interpro domain search; IPR005829, **Table 6**). Most of the encoded proteins, however, probably will not transport sugar across the plasma membrane but, as the SLC35 member Meigo, organize intracellular trafficking of different sugars to ensure glycosylation (Sekine et al., 2013). As an alternative to trehalose, other sugars such as glucose or fructose could be taken up by the nervous system to meet the high neuronal energy demand. In vertebrates, the SLC2 family includes Glut2 and Glut5 transporters, which have been characterized as glucose and fructose transporters (Douard and Ferraris, 2008; Kellett et al., 2008; Mueckler and Thorens, 2013). No clear homolog of Glut2 and Glut5 can be identified in *Drosophila*, so it remains uncertain whether fructose can be imported into the nervous system. Interestingly, however, a fructose receptor (Gr43a) has been shown to be expressed on several CNS neurons (Miyamoto et al., 2012). Thus, a possible explanation for the apparent lack of Glut2 or Glut5 homologs is that fructose might be either generated in the brain or that the fructose sensing CNS neurons form dendrites that leave the CNS to detect fructose in the hemolymph.

Unlike in the *Drosophila* hemolymph, glucose is the main energy supply in the mammalian blood. Glucose is transported by Glut1 and Glut3, which are also members of the SLC2 family. For

both of these facilitated glucose transporters, glucose, galactose, and mannose transport activities have been found (Uldry and Thorens, 2004). Glut1 functions in the mammalian endothelial blood-brain barrier and in astrocytes, whereas Glut3 is expressed by neurons (Leino et al., 1997). In *Drosophila*, Glut1 is specifically expressed in the embryonic nervous system and microarray data indicate continued expression in brain tissue (FlyBase). Future work needs to discriminate whether Glut1 is expressed in neurons or glial cells or both. In contrast, Glut3 is only expressed in imaginal discs of late larval stages and in adult testis (FlyBase). Thus, Glut1 might be responsible for glucose uptake into the nervous system. In support of this notion, loss of *glut1* function is lethal (Saito et al., 2002) and expression of *glut1* is able to improve locomotor behavior and survival of flies when mitochondrial activity is reduced in glial cells (Besson et al., 2010).

In addition, members of the SLC5A family (SLC5A1 and SLC5A2) have been shown to mediate sodium-dependent glucose uptake (Featherstone, 2011). One *Drosophila* SLC5A family member is CG9657, which is also expressed in glial cells (Freeman et al., 2003).

BLOOD-BRAIN BARRIER AND HORMONAL FUNCTION

In addition to the control of metabolism, the blood-brain barrier must also permit the entry and exit of hormones into or out of the nervous system. This is especially true for the *Drosophila* neuroendocrine system, which consists of neurosecretory cells (NSCs) in the brain. The *Drosophila* genome encodes eight insulin-like peptides (Dilps), which are the functional homologs

Table 6 | Drosophila sugar transporters.

Gene	CG	Human homolog	CNS expression		Function	Reference
			FlyAtlas	BDGP		
<i>Tret1-1</i>	<i>CG10960</i>	SLC2A8 (Glut8)	Yes	Yes	glucose transporter	Kikawada et al., 2007; Kanamori et al., 2010
	<i>CG30035</i>	SLC2A8 (Glut8)	Yes		glucose transporter; trehalose transport	
<i>Glut1</i>	<i>CG1086</i>	SLC2	Yes	Yes	glucose transporter	
	<i>CG1213</i>	SLC2	Yes		glucose transporter	
<i>sut1</i>	<i>CG8714</i>	SLC2	Yes		sugar/H ⁺ symporter; glucose transporter	
<i>Glut3</i>	<i>CG3853</i>	SLC2	No		sugar transporter; glucose transporter	
<i>Tret1-2</i>	<i>CG8234</i>	SLC2A8 (Glut8)	No		fructose transporter; glucose transporter;	Kanamori et al., 2010
<i>sut2</i>	<i>CG17975</i>	SLC2	No		sugar/H ⁺ symporter; glucose transporter	
<i>sut3</i>	<i>CG17976</i>	SLC2	No		sugar/H ⁺ symporter; glucose transporter	
<i>sut4</i>	<i>CG1380</i>	SLC2	No		sugar/H ⁺ symporter; glucose transporter	
	<i>CG1208</i>	SLC2	Yes		glucose transporter	
	<i>CG7882</i>	SLC2	No		glucose transporter	
	<i>CG8249</i>	SLC2	No		glucose transporter	
	<i>CG4797</i>	SLC2	Yes		glucose transporter	
	<i>CG8837</i>	SLC2	Yes		transporter activity	
	<i>CG15406</i>	SLC2	No		fructose transporter	
	<i>CG4607</i>	SLC2	Yes		substrate-specific transporter	
	<i>CG3285</i>	SLC2	No		transporter activity	
	<i>CG33281</i>	SLC2	No		monosaccharide transporter	
	<i>CG6484</i>	SLC2	No		glucose transporter	
	<i>CG33282</i>	SLC2	No		monosaccharide transporter	
	<i>CG15408</i>	SLC2	No		fructose transporter	
	<i>CG14605</i>	SLC2	No		transmembrane transporter activity	
	<i>CG14606</i>	SLC2	No		hexose transporter	
	<i>CG11976</i>	SLC2	Yes		monosaccharide transporter	
<i>Orct</i>	<i>CG3168</i>	SLC22/SV2	Yes	Yes	transporter activity	Altenhein et al., 2006
	<i>CG6126</i>	SLC22	Yes	Yes	organic cation transporter	
	<i>CG6331</i>	SLC22	Yes	Yes	organic cation transporter	
	<i>CG3790</i>	SLC22	Yes		carnitine transporter	
	<i>CG6356</i>	SLC22	Yes		secondary active organic cation transporter	
	<i>CG7084</i>	SLC22	Yes		secondary active organic cation transporter	
	<i>CG7442</i>	SLC22	Yes		secondary active organic cation transporter	
<i>Orct2</i>	<i>CG13610</i>	SLC22	Yes	Yes	organic cation transporter	
	<i>CG8654</i>	SLC22	Yes	Yes	secondary active organic cation transporter	
	<i>CG10486</i>	SLC22	No		secondary active organic cation transporter	
	<i>CG16727</i>	SLC22	No		organic cation transporter	
	<i>CG17751</i>	SLC22	No		secondary active organic cation transporter	
	<i>CG17752</i>	SLC22	No		secondary active organic cation transporter	

(Continued)

Table 6 | Continued

Gene	CG	Human homolog	CNS expression		Function	Reference
			FlyAtlas	BDGP		
	CG14855	SLC22	Yes		secondary active organic cation transporter	
	CG14856	SLC22	No		secondary active organic cation transporter	
	CG6006	SLC22	Yes		carnitine transporter	
	CG6231	SLC22	Yes		secondary active organic cation transporter	
	CG7333	SLC22	No		secondary active organic cation transporter	
	CG5592	SLC22	No		secondary active organic cation transporter	
	CG7342	SLC22	No		secondary active organic cation transporter	
	CG42269	SLC22	No		secondary active organic cation transporter	
	CG7458	SLC22	No		secondary active organic cation transporter	
	CG8925	SLC22	No		carnitine transporter	
	CG9317	SLC22	Yes		secondary active organic cation transporter	
<i>Csat</i>	CG2675	SLC35	Yes		UDP-galactose transporter; sugar/H ⁺ symporter	Segawa et al., 2002
<i>Efr</i>	CG3774	SLC35	Yes		nucleotide-sugar transporter; GDP-fucose transporter	
<i>frc</i>	CG3874	SLC35	Yes		triose-phosphate transporter; pyrimidine nucleotide-sugar transporter	
<i>meigo</i>	CG5802	SLC35	Yes		UDP-N-acetylglucosamine transporter	
<i>sll</i>	CG7623	SLC35	Yes		UDP-N-acetylglucosamine transporter; 3'-phosphoadenosine 5'-phosphosulfate transporter	
<i>Papst2</i>	CG7853	SLC35	Yes		UDP-N-acetylglucosamine transporter; 3'-phosphoadenosine 5'-phosphosulfate transporter	
<i>Gfr</i>	CG9620	SLC35	Yes		GDP-fucose transporter	
	CG33181	SLC41	Yes		cation transporter	
	CG11537	HIAT1	Yes		carbohydrate transporter	
<i>Prp38</i>	CG30342	PRPF38	Yes		pre-mRNA-splicing factor	
	CG7009	FTSJ1	Yes		tRNA methyltransferase activity	
<i>slv</i>	CG8717	SLC50	Yes		sweet sugar transporter	
<i>rtet</i>	CG5760	MFSD	Yes		sugar transporter	
	CG15096	SLC17	Yes		high affinity inorganic phosphate/Na ⁺ symporter	
	CG30344 (CG8054)	SLC46	Yes		transporter activity	Freeman et al., 2003
	CG6901	SVOP	No		transporter activity	
	CG12783	SV2	No		transporter activity	
	CG30345	SLC46	No		transporter activity	
	CG15553	SLC46	No		transporter activity	

(Continued)

Table 6 | Continued

Gene	CG	Human homolog	CNS expression		Function	Reference
			FlyAtlas	BDGP		
	CG14160	–	No		transporter activity	
	CG17929	–	No		transporter activity	
	CG17930	–	No		transporter activity	
	CG32053	–	No		transporter activity	
	CG32054	–	No		transporter activity	
	CG42825	–	No		transporter activity	
	CG31103	SV2	No		transporter activity	
	CG33233	SV2	No		transporter activity	
	CG4324	SVOPL	No		organic cation transporter	
	CG5078	HIAT1	No		carbohydrate transporter	

Carbohydrate transporters harboring a sugar transporter motif (Interpro domain search, IPR005829) are listed. Information about the CNS expression of each gene was obtained as indicated. The predicted function of each protein is noted. SLC, solute carrier; HIAT, hippocampus abundant transcript 1; PRPF, pre-mRNA processing factor; FTSJ1, FtsJ RNA methyltransferase homolog 1; MFSD10, major facilitator superfamily domain containing 10; SVOP, SV2 related protein homolog; SV2, synaptic vesicle glycoprotein 2; SVOPL, SVOP-like.

of vertebrate insulin and insulin-like growth factors (IGFs) that affect a wide range of processes (Erion and Sehgal, 2013; Shim et al., 2013). Dilp2, 3 and 5 are expressed by 14 insulin-producing cells (IPCs) and released into the hemolymph to regulate growth, metabolism, reproduction, and life span (Nässel et al., 2013). A key trigger of Dilp release from IPCs is food intake. The associated increase in hemolymph sugar and amino acid levels is sensed by the fat body and causes release of the leptin-like peptide Unpaired 2 (Upd2), which acts on IPCs via GABAergic neurons (Rajan and Perrimon, 2012). However, how this fat body-derived signal passes the blood-brain barrier or how it is sensed by the surface glia and then further transmitted into the nervous system is not known.

An additional fat body derived signal coordinates the second wave of neurogenesis at the end of larval stages, which must be matched to the nutritional status of the animal. This still elusive signal triggers expression of Dilp6 in the SPG, which in turn activates the proliferation of larval neuroblasts (Chell and Brand, 2010; Sousa-Nunes et al., 2011). Interestingly, both, the expression and the secretion of Dilp6 from the SPG cells depend on the presence of gap junctions in these cells (Spéder and Brand, 2014).

Moreover, the blood-brain barrier influences the physiology of the animal. It is known that some hemolymph proteins modulate the mating behavior of *Drosophila* (Lazareva et al., 2007). Interestingly, the sex of the blood-brain barrier matters and male-specific factors of the blood-brain barrier are required for normal male courtship behavior (Hoxha et al., 2013). These aspects of blood-brain barrier function are currently not extensively studied but it appears likely, that in the near future more surprising findings will be made.

CONCLUSIONS

Nervous system function strongly depends on a well-balanced ion and metabolite milieu. To ensure this homeostasis in the brain, the blood-brain barrier fulfills a variety of functions. It seals the

brain from circulation and, in consequence, active transport systems are required for all solutes that have to be shuttled into or out of the brain. Therefore, a number of specific transporters must be expressed in the barrier forming cells. In *Drosophila*, as in primitive vertebrates, the blood-brain barrier is formed by glia. To date, our knowledge about essential transporters expressed in the glia is very limited. However, genomic information and transcriptomic data as already available for the mouse (Daneman et al., 2010a) will soon enable us to identify many relevant genes in *Drosophila* and the wealth of genetic tools will ease their analysis. One of the future challenges will be to decipher how the metabolic supply through the blood-brain barrier is matched to neuronal activity. In light of the apparently well-conserved blood-brain barrier, functional studies using *Drosophila* are expected to deepen our understanding of how the blood-brain barrier keeps our nervous system functional.

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