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# Brain Energy Metabolism: Conserved Functions of Glycolytic Glial Cells

Andrea Trevisiol[1] and Klaus-Armin Nave[1,*]
[1]Max Planck Institute of Experimental Medicine, Department of Neurogenetics, 37075 Göttingen, Germany
*Correspondence: nave@em.mpg.de
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**The discovery in mammals that axons are metabolically supported by myelinating glial cells explains why neurons can extend meters in length. In this issue, Volkenhoff et al. (2015) show that, in *Drosophila*, the transfer of lactate from the glial to the neuronal compartment is conserved in evolution, independent of body size.**

The brain is a complex network of electrically excitable neurons that communicate with each other through long axonal projections, antenna-like dendritic processes, and an astronomic number of synaptic contact points. It is less well known that in virtually all nervous systems, from invertebrate ganglia to the human brain, neurons are associated with glia (γλια, Greek for "glue"). By definition, glial cells are unable to generate action potentials, but they can respond to neuronal activity. Originally a cellular minority, the glia-to-neuron ratio has significantly increased in evolution: in the mammalian brain the glial and neuronal cells are at least equal in number.

Why does the brain need neurons and glial cells? In the vertebrate central nervous system (CNS), astrocytes contribute to the blood-brain barrier (BBB) and take up neurotransmitters at glutamatergic synapses. Oligodendrocytes ensheath axons with myelin, which enables rapid "saltory" conduction. Microglia are phagocytic cells of the innate immune system that respond to injury. In the peripheral nervous system (PNS), Schwann cells either sort out single axons for myelination or they tightly associate with and engulf several small caliber axons in so-called Remak bundles. Specifically, the function of these non-myelinating Schwann cells, which are reminiscent of "wrapping glia" in the PNS of *Drosophila* (Figure 1), has remained obscure. In invertebrates, glial cells have been mostly implicated in BBB formation, in neurotransmitter transport, and in providing phagocytic functions (Edwards and Meinertzhagen, 2010; Limmer et al., 2014). Compared to the advanced knowledge of neuronal networks, however, our understanding of most neuron-glia interactions is still at its infancy, awaiting fundamental discoveries still to be made. The paper by Volkenhoff et al. (2015) is a remarkable example.

A key structural feature that sets a neuron apart from all other cell types is the length of its axonal process. In all animals, axons exceed the length of the neuronal somata severalfold: in the largest vertebrates up to 100,000-fold. This poses a logistic problem not least for the energy-consuming transport processes essential for maintaining presynaptic compartments, in addition to the metabolic costs of impulse conduction and axonal membrane repolarization (Nave, 2010). In mammals, genetic evidence suggested the importance of glial cells in maintaining the functional integrity of these long axons. Here, oligodendrocyte-specific genes were studied in mouse mutants that appeared initially well myelinated, but later developed axonal swellings and Wallerian degeneration throughout the CNS, causing premature death (Griffiths et al., 1998). Such mouse models are also of clinical interest, because human myelin diseases often lead to axonal degeneration, which is blamed on demyelination rather than the loss of specific axon-supportive mechanisms. Recently, mechanistic insight has emerged with the discovery that oligodendrocytes are metabolically coupled to the axonal compartment and provide lactate to myelinated axons (Fünfschilling et al., 2012; Lee et al., 2012). It is thought that lactate shuttles through glial and axonal monocarboxylate transporters (MCTs) to be metabolized in neuronal mitochondria for ATP generation. Indirect evidence suggests that also Schwann cells provide peripheral axons with energy-rich metabolites, independent of the presence of myelin (Beirowski et al., 2014). This concept of axonal metabolic support differs in many ways from the astrocyte-to-neuron lactate shuttle (ANLS) thought to reenergize cortical glutamatergic synapses (Magistretti and Allaman, 2015). Local glycolytic support solves the unique problem arising from axon length and myelin itself, which may physically prevent rapid axonal uptake of glucose. However, neither concept assumes there is a principle division of labor between neuronal and glial energy metabolism. This is what the findings of Volkenhoff et al. (2015) suggest and which may be evolutionarily conserved.

Volkenhoff et al. (2015) systematically studied the energy metabolism of the neuronal and glial compartments in the *Drosophila* CNS. Here, neuronal cell bodies and axonal projections are shielded by a simple BBB, made up of perineurial and subperineurial glial (SPG) cells, the latter physically separating neurons from the surrounding hemolymph. "Wrapping" glial cells engulf bundles of axons similar to non-myelinating Schwann cells in vertebrates. The authors report a surprising division of labor between neurons and associated glial cells that closely matches the findings in mice and suggest a new principle of neuron-glia interaction that appears conserved in neural evolution.

In *Drosophila*, the main energy source circulating in the hemolymph is trehalose, a disaccharid hydrolyzed by trehalase to generate two molecules of glucose. A CRISPR/Cas-induced null mutation of the Tret1-1 gene, encoding a trehalose transporter, is expectedly lethal. Surprisingly, rescue experiments indicate that Tret1-1 is essential in glia but dispensible

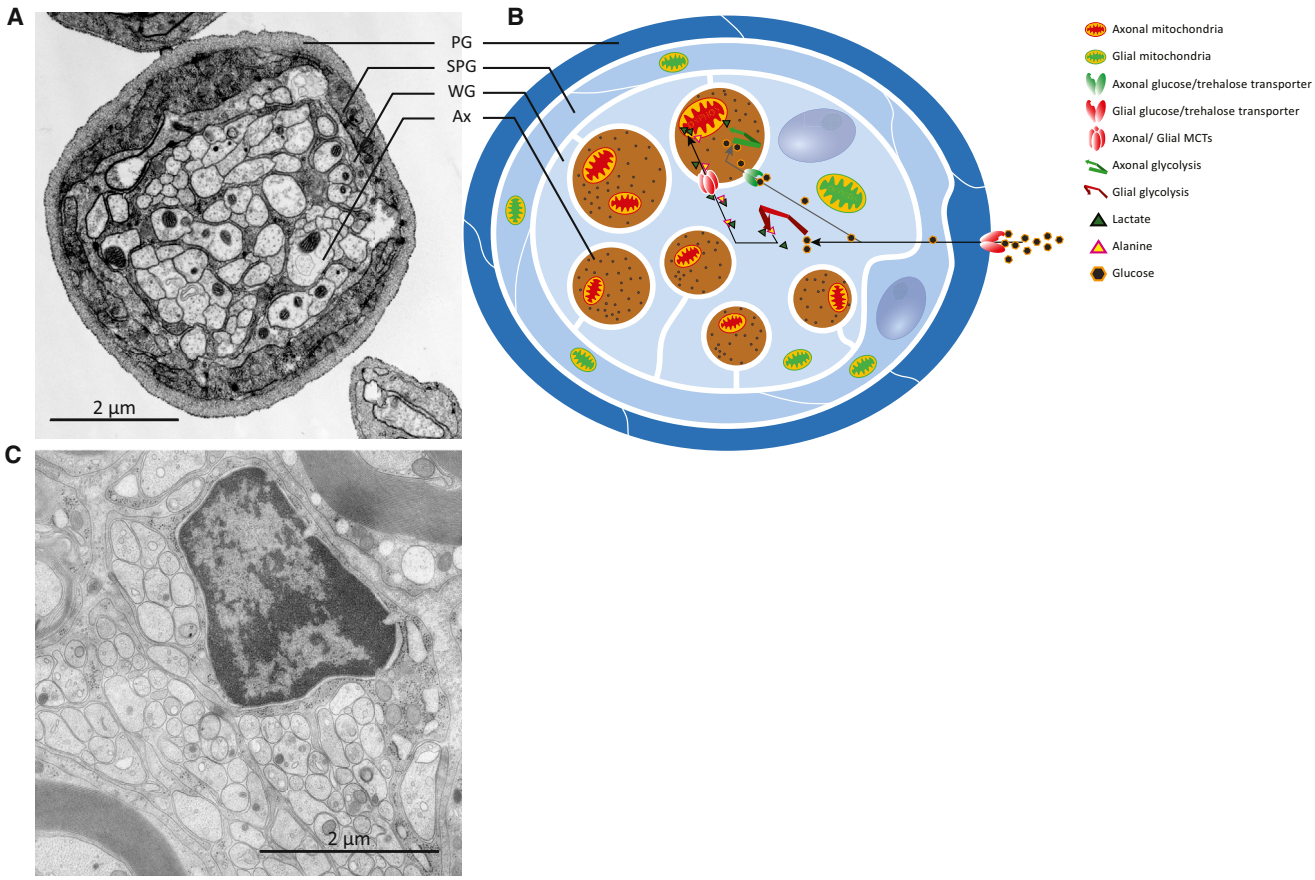


**Figure 1. Glycolytic Glial Cells in Invertebrates and Vertebrates**

(A) Electron microscopic cross sections of neuronal compartments (axons) and their ensheathing glial cells in the PNS of *Drosophila*.

(B) Schematic depiction of neuronal and glial compartments and metabolite fluxes in *Drosophila*. Red color marks essential metabolic pathways and metabolite transporters in the genetic experiments of Volkenhoff et al. (2015), whereas those in green are dispensable. Note that glial cells import glucose (trehalose in *Drosophila*) and provide glycolysis products (lactate) to the neuronal compartment, which fuels mitochondrial oxidative phosphorylation and the generation of ATP.

(C) Neuronal and glial compartments in the PNS of mice appear morphologically similar (Remak bundle). Small axons are engulfed by a single Schwann cell, which provides metabolic support (Beirowski et al., 2014). Abbreviations: PG, perineurial glia; SPG, subperineurial glia; WG, wrapping glia; Ax, Axon; MCT, monocarboxylate transporter. Images courtesy of C. Klämbt (Münster) and W. Möbius (Göttingen).

in neurons. This, and the specific expression of this transporter, shows that trehalose enters the nervous system through perineural glial cells. RNAi-mediated silencing of trehalase or genes for glycolytic enzymes (aldolase and pyruvate kinase) reveals their vital function in glial cells, but glycolysis is quite unexpectedly dispensable in neurons. Indeed, these glycolytic enzymes are more prominently expressed in BBB-forming glia than neurons. In contrast, silencing the nuclear genes for mitochondrial TCA cycle functions in neurons causes strong neurodegeneration, whereas the same genes appear dispensable when inactivated in glial cells. This strongly suggests that glial glycolysis products are metabolized in the neuronal compartment, in analogy to the ANLS and the recent findings in vertebrates. Indeed, when purified by fluorescence-activated cell sorting and cultured in vitro, feeding *Drosophila* cells with $^{13}$C-labeled trehalose revealed that glia (unlike neurons) release lactate and alanine. This function involves a family of MCTs, and silencing these genes in glia causes lethality, reminiscent of Mct1 mutant mice.

What was considered an odd specialization of photoreceptor and pigment cells in the retina of honey bees (Tsacopoulos et al., 1988) emerges in *Drosophila* as a widespread partitioning of energy metabolism between neurons and glia and more than a developmental phenomenon. When glycolytic genes were downregulated in adult flies, using a cell-type-specific and temperature-controlled (Gal80$^{ts}$-mediated) gene silencing strategy, impaired glycolysis in glia resulted in neurodegeneration and reduced lifespan but was remarkably tolerated in neurons. These genetic data, when combined, are surprising and provide a new view on neuron-glia interactions. They should not be taken as evidence that under normal conditions glial cells are 100% glycolytic and that neurons metabolize only glial lactate or exclusively rely on mitochondrial respiration. However, they clearly suggest that metabolic fluxes exist between these major cell types and that the underlying division of labor is likely an ancestral feature of nervous system design, remarkably independent of axon length.

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# Redox Modulation by Reversal of the Mitochondrial Nicotinamide Nucleotide Transhydrogenase

**Michael P. Murphy**[1,*]
[1]MRC Mitochondrial Biology Unit, Hills Road, Cambridge CB2 0XY, UK
*Correspondence: mpm@mrc-mbu.cam.ac.uk
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**The mitochondrial nicotinamide nucleotide transhydrogenase (NNT) uses the protonmotive force to transfer electrons from NADH to NADP, thereby maintaining the NADP/NADPH pool reduced to protect mitochondria from oxidative damage. In this issue, Nickel et al. (2015) show that NNT also operates in reverse, oxidizing the NADP/NADPH pool, thereby disrupting antioxidant defense.**

The way we have been taught inevitably influences how we think of metabolic processes—I always remember the Krebs cycle as a complete circle that only goes clockwise! Of course, the textbook presentation is just a way of compiling information concisely, and in vivo metabolic networks can be quite different. Similar simplifications affect our view of mitochondrial proton translocating complexes. A good example is the $F_oF_1$-ATP synthase that utilizes the protonmotive force across the inner membrane to drive ATP synthesis during oxidative phosphorylation. However, if the protonmotive force is too low then the protein can turn into an ATPase, hydrolysing ATP and pumping protons out of the mitochondria. This occurs in vivo, for example during ischemia when respiration is prevented, thereby draining away the ATP synthesized by glycolysis (St-Pierre et al., 2000). Mitochondrial respiratory complexes can also be driven "backwards," compared to their conventional direction, depending on the balance of thermodynamic forces. For example, a recent study to which I contributed suggested that complexes I and II may operate in reverse and contribute to ischemia-reperfusion injury (Chouchani et al., 2014). During ischemia, complex II utilizes the reduced Coenzyme Q pool to convert fumarate to succinate, then upon reperfusion the accumulated succinate drives reversal of complex I, thereby producing damaging reactive oxygen species (ROS) (Chouchani et al., 2014).

More interesting than the reversal of respiratory complexes in pathology is the possibility that such unconventional operation may occur in normal physiology. For example, the reversal of complex II during anoxia may generate succinate as a hypoxic signal, complex I going backward could be a physiological ROS signal, and ATP hydrolysis by the $F_oF_1$-ATP synthase might enable maintenance of a protonmotive force independently of the respiratory chain (Brown et al., 2006; Chouchani et al., 2014).

In this issue, Nickel et al. show that the latest proton pumping mitochondrial complex that may operate in two directions is the nicotinamide nucleotide transhydrogenase (NNT) (Nickel et al., 2015). The NNT is a mitochondrial inner membrane protein that takes electrons from NADH to reduce NADP to NADPH, utilizing the protonmotive force across the inner membrane to drive the process (Figure 1A). This coupling of electron transfer to the protonmotive force is essential to maintain the difference in redox state between the mitochondrial NADP/NADPH and NAD/NADH pools, despite their identical midpoint potentials (Rydström, 2006). This redox difference is essential for the quite different biological roles of the two nicotinamide nucleotides. The NAD/NADH pool mainly acts a conduit for electrons from substrates to the respiratory chain and is thus only partially reduced ($E_h \sim -300$ mV). In contrast, the mitochondrial NADP/NADPH pool supplies electrons to glutathione reductase, to maintain the mitochondrial glutathione/glutathione disulfide ratio high, and to thioredoxin reductase 2, which keeps the matrix thioredoxin 2 pool reduced (Murphy, 2012).