NMDA receptors: power switches for oligodendrocytes

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The role of NMDA receptors in oligodendrocytes has been controversial. A new paper suggests that they play a key role in regulating glucose uptake in response to axonal glutamate release, thus regulating bi-directional metabolic cooperation between oligodendrocytes and axons.

Myelinated axons face unique energetic challenges. During development, oligodendrocytes require a large amount of energy and carbon skeletons to construct myelin sheaths (Harris & Attwell, 2012). Furthermore, once a sheath is formed, it isolates most of the underlying axon from the energetic substrate glucose present in the extracellular fluid, and some axons are so long that the supply of new mitochondria and glycolytic enzymes from the soma may take weeks to years (Nave, 2010). This raises the question of where axons get their energy from, and how the energy supply can adapt to immediate energy needs. A paper in this issue of Neuron (Saab et al., 2016) reveals a novel NMDA receptor dependent mechanism that coordinates the transfer of energetic substrates between oligodendrocytes and axons, to aid the initial development of the myelin sheath and to ensure the health of the axon once it is myelinated.

The role of NMDA receptors (NMDARs) in oligodendrocyte linage cells has been controversial. Originally they were thought to be absent, but three separate studies (Káradóttir et al. 2005; Salter & Fern 2005; Micu et al. 2006) suggested that glutamate could activate an NMDAR-mediated current and calcium influx into oligodendrocyte precursors and myelinating oligodendrocytes, and that the Ca^{2+} influx damages myelin in ischemia. The receptors were reported to contain weakly Mg^{2+}-blocked subunits, allowing them to function even at the normal resting potential of oligodendrocytes. However, NMDAR expression is downregulated after the development of oligodendrocytes, and two previous studies reported that knock-out of the NR1 subunit did not produce a major effect on myelination.

Saab et al. (2016) found that applying NMDA to cultured oligodendrocytes increased trafficking of the glucose transporter GLUT1 to the surface membrane, while conditional knock-out of the obligate NMDAR subunit NR1 in oligodendrocytes (driven by the CNP promoter which turns on during oligodendrocyte differentiation, between P5 and P10) led to a

fall in the amount of GLUT1 in myelin. This is consistent with exocytosis of glutamate from axons acting on NMDARs to maintain the level of GLUT1 in ensheathing oligodendrocytes (Fig. 1). Although how NMDAR activation increases GLUT1 level in the surface membrane was not investigated in depth, Saab et al. (2016) attributed this to an NMDAR-evoked $[Ca^{2+}]_i$ rise, as has been reported to occur in the myelin sheath when axons are stimulated (Micu et al., 2016). It will be of interest to determine whether prevention of $[Ca^{2+}]_i$ transients in oligodendrocytes affects their sheath formation. Knock-out of oligodendrocyte NMDARs starting after postnatal day 5 did not prevent a normal myelin sheath thickness being attained, but at postnatal day 20 the myelination of small optic nerve axons was delayed (conceivably, use of a Cre line that turns on earlier in oligodendrocyte development to delete NMDAR signalling might result in a larger delay). This delay is consistent with the finding, from a cell culture model of myelination, that upregulation of oligodendrocyte NMDARs by neuregulin or BDNF leads to myelination becoming faster and dependent on neuronal activity (Lundgaard et al., 2013). Presumably, therefore, release of glutamate by axonal action potentials activates NMDARs in oligodendrocyte lineage cells and increases their glucose uptake, as a result of which myelination is preferentially focused on active cells (Lundgaard et al., 2013; Mensch et al. 2015), rather than on inactive axons that are undergoing developmental pruning or belong to neurons undergoing apoptosis.

Once inside the developing oligodendrocyte, glucose can be converted to ATP and acetyl-CoA, and used to synthesise lipids for myelination. However, acetyl-CoA can also be formed from lactate or from N-acetyl-aspartate (NAA, Fig. 1). NAA is expressed at a high level in axons and exported to oligodendrocytes, where a loss of the aspartoacylase enzyme that converts it to acetate and thus to acetyl CoA leads to a failure of myelination in Canavan disease (it will be of interest to determine whether NMDAR activation also increases NAA uptake into oligodendrocytes). Thus, during developmental myelination there is a two-fold metabolic support of oligodendrocytes by axons: provision of NAA, and upregulation of glucose uptake by activation of NMDARs in oligodendrocytes near active axons.

Once the myelin sheath is formed, the direction of this metabolic cooperation may be reversed. Most of the axon is isolated by the myelin from glucose in the extracellular space, and it has been proposed that axons are at least partly powered by a sequence of processes in which glycolysis in oligodendrocytes generates lactate (or possibly pyruvate), which is passed by monocarboxylate transporters (Fig. 1) to the ensheathed axon, where it can be used as a substrate in mitochondria (Fünfschilling et al. 2012; Lee et al. 2012). This is similar to the proposed astrocyte-neuron lactate shuttle, in which ATP use on glutamate uptake and recycling in astrocytes promotes astrocyte glycolysis and export of lactate to neurons (Fig. 1). Saab et al. (2016) extend this concept by suggesting that maintenance of oligodendrocyte glucose transport, through activation of NMDARs, is crucial for the function and long-term health of myelinated axons. Transmission of high frequency trains of action potentials failed more rapidly if NMDARs were blocked, or knocked out in oligodendrocytes, and ran down less rapidly if NMDARs were activated prior to the trains being stimulated. More dramatically, in the medulla and spinal cord of 1 year old mice with oligodendrocyte NMDARs deleted, descending white matter tracts exhibited astro- and microgliosis, myelin delamination and degenerating axons, and these were associated with deficits on a rotarod test, while by 19 months of age a more severe neurological phenotype was apparent with generalised neuroinflammation and axonopathy.

Activation of oligodendrocyte NMDARs may also be important in the more immediate response of the white matter to ischemia. Activation of oligodendrocyte NMDARs by glutamate released in ischemia was reported to raise oligodendrocyte $[Ca^{2+}]_i$ and thus damage myelin sheaths (Káradóttir et al. 2005; Salter & Fern 2005; Micu et al. 2006). This view has been challenged by recent work (Hamilton et al. 2016) demonstrating that much of the NMDAR-mediated current in oligodendrocytes in brain slices is generated indirectly by a $[K^+]_o$ rise in the tissue, and that the ischemic $[Ca^{2+}]_i$ rise is mediated by TRPA1 channels rather than by oligodendrocyte NMDARs. Nevertheless, it is surprising that Saab et al. (2016) found deletion of NMDARs to decrease action potential recovery after oxygen-glucose deprivation of the optic nerve - a phenotype rescued by superfusion with lactate. This was attributed to NMDAR

activation being needed to maintain glucose import into oligodendrocytes and downstream transfer of lactate to axons (although it is not clear whether the superfused lactate would be provided as rapidly to the ensheathed axon as lactate generated within oligodendrocytes). Interestingly, unlike NMDAR deletion, block of NMDARs with MK-801 during oxygen-glucose deprivation did not affect recovery of the action potential. This suggests that turnover of GLUT1 transporters in the oligodendrocyte membrane is slow, so that a brief removal of NMDAR activation does not lead to much decrease of glucose transport.

The work of Saab et al. (2016) demonstrates that, in a sense, oligodendrocyte NMDARs act as "power switches", controlling the flow of energetic substrates both to the oligodendrocytes themselves and to their ensheathed axons. This raises a number of exciting questions relating to the energetic design of the white matter and how it can be exploited therapeutically in diseases of great personal and economic impact, such as stroke, spinal cord injury, multiple sclerosis and the genetic leukodystrophies. First, how local is the trigger for the power switch: the model of Saab et al. envisages an axon signalling solely to its own sheath, but immunogold images indicate NMDAR expression in both the outer and inner margins of the sheath (Káradóttir et al. 2008; Micu et al. 2006), suggesting that oligodendrocytes could also respond to glutamate release from other cells. This might imply tract-specific in addition to axon-specific control of energy supply. Second, how is glial energy supply regulated by myelinated GABAergic axons (such as Purkinje cells): does GABA do the same job as glutamate (e.g. via $GABA_B$ receptors), or could glutamate be released by the GABAergic axon within the sheath? Third, what is the precise mechanism by which the GLUT1 density in the oligodendrocyte membrane is controlled, and on what time scale is the GLUT1 level increased or decreased when the axon firing frequency is altered? If a $[Ca^{2+}]_i$ rise is needed to trigger GLUT1 trafficking to the surface membrane, could TRPA1 channels (which raise oligodendrocyte $[Ca^{2+}]_i$ in ischemia: Hamilton et al., 2016) or Ca^{2+}-permeable AMPA receptors also be involved in this control?

Turning to therapy, could the long-term health of white matter tracts be promoted by activation of oligodendrocyte NMDARs (a strategy that inverts previous conceptual

approaches) or by increasing GLUT1 trafficking to the oligodendrocyte surface membrane? Could pharmacologically potentiating the expression or function of the monocarboxylate transporters that allow lactate to move from oligodendrocytes to axons be used to increase the energy supply to axons? How does expression of GLUT1 and the monocarboxylate transporters change with age, diabetes, Alzheimer's disease or when new oligodendrocytes are formed after demyelination? At present, all we can say is that the metabolic coordination characterised by Saab et al. (2016) is likely to play a role in many neurological conditions.

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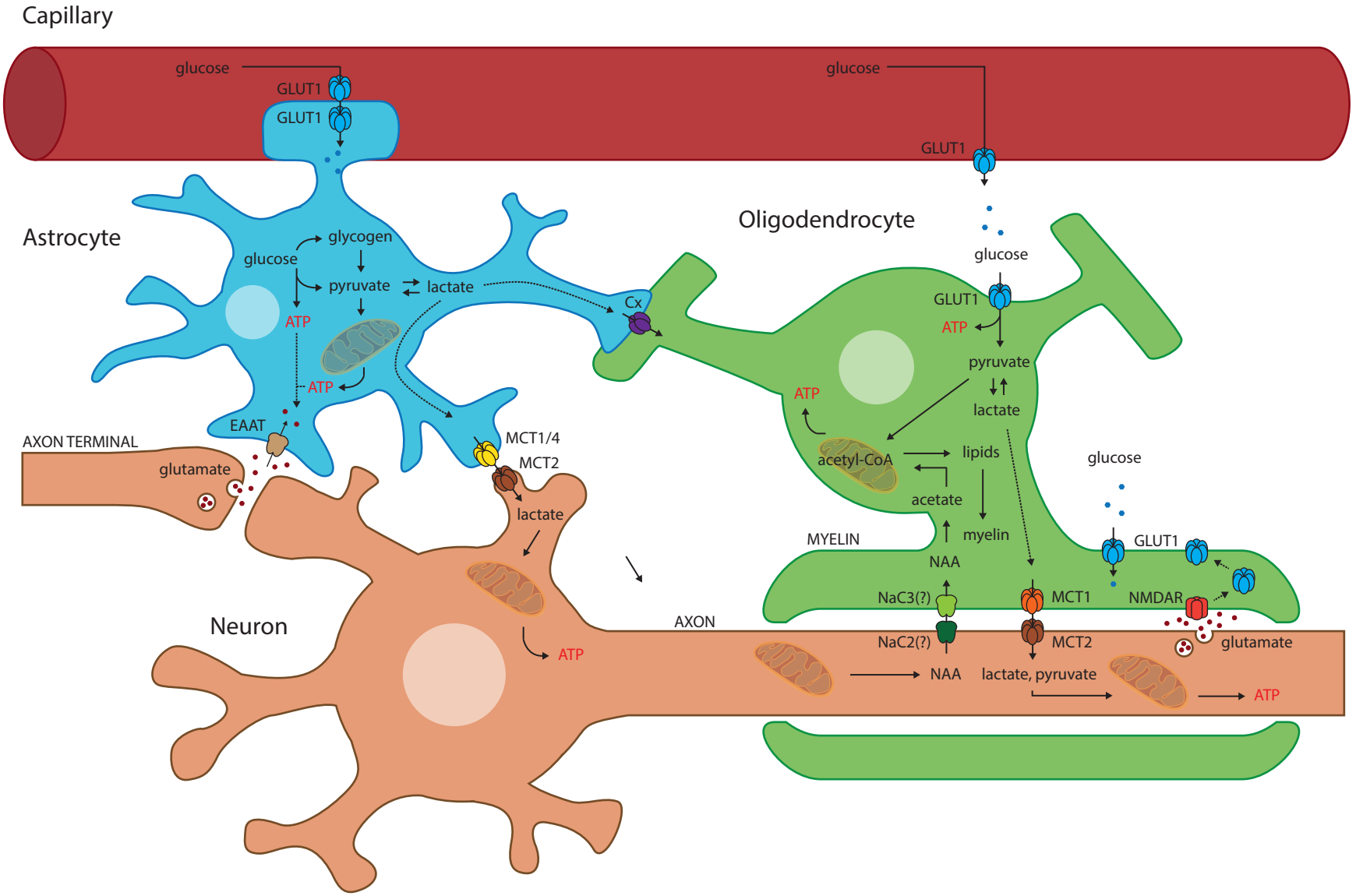
Figure Legend

Fig. 1. Metabolic cooperation in the CNS

Glucose provided by the blood is imported into the extracellular fluid by glucose transporters (largely GLUT-1), and then taken up into astrocytes, oligodendrocytes and neurons. In astrocytes glucose is used to make ATP via glycolysis, and some of the pyruvate thus formed generates more ATP via oxidative phosphorylation in mitochondria, while some is converted to lactate that can be exported to neuronal dendrites and somata to generate ATP in their mitochondria. ATP expenditure on glutamate uptake (EAAT) and recycling is proposed to stimulate glycolysis and lactate export (the astrocyte-neuron lactate shuttle, the quantitative significance of which is controversial). In developing oligodendrocytes, glucose is similarly used to form ATP, but also donates carbon skeletons for the formation of acetyl CoA and thus myelin lipids. Axonal N-acetyl-aspartate (NAA) is also transferred to oligodendrocytes, possibly via the Na^+-dependent dicarboxylate transporters NaC2 and NaC3 (also known as NADC3) and used to make acetyl CoA for myelination. In mature oligodendrocytes, lactate (or pyruvate) formed from glucose can be exported to neuronal axons via monocarboxylate transporters (MCT1 and 2), providing them with a substrate for oxidative phosphorylation. Glutamate release from active axons activates NMDARs in oligodendrocyte lineage cells which, probably via a $[Ca^{2+}]_i$ rise, triggers an increase in the level of glucose transporters in the cell membrane. This provides oligodendrocytes with more glucose to make myelin, and to export as lactate to axons.


Capillary
glucose
GLUT1
GLUT1
glucose
GLUT1
Astrocyte
glucose
glycogen
pyruvate
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ATP
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Oligodendrocyte
glucose
GLUT1
ATP
pyruvate
lactate
ATP
acetyl-CoA
lipids
acetate
myelin
MYELIN
NAA
glucose
GLUT1
AXON TERMINAL
glutamate
MCT1/4
MCT2
lactate
Neuron
ATP
AXON
NaC3(?)
NaC2(?)
MCT1
MCT2
NMDAR
glutamate
NAA
lactate, pyruvate
ATP