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Myelin: more than just an insulator

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ABSTRACT

Oligodendrocytes build up the myelin sheath that insulates axons and speeds up action potential propagation. Moreover, oligodendroglia also regulates the ionic environment and fuels axons with metabolites to meet their energy demands. In this review, I discuss the mechanisms by which oligodendrocytes and myelin are metabolically coupled to axons, and how they adjust metabolic support to spiking activity. In sum, the major goal of this review is to have a better insight into how axons rely on their myelinating partners for fulfilling energy demands. I propose that this axon-to-myelin interaction can be critical to understand axonal and neuronal damage in neurodegenerative and demyelinating diseases, as well as connectivity alterations in neuropsychiatric disorders.

1. INTRODUCTION

Oligodendrocytes (OLGs), the myelin producing cells of the Central Nervous System (CNS), have been defined throughout decades by their axon insulating properties. Myelin is one of the most complex biological structures and is considered essential to enhance the speed of nerve impulses that allow neuronal communication in the human brain.

This introduction will provide a brief insight on how oligodendrocytes and their properties have been classically described, according to their morphology, physiological functions and myelinating properties.

1.1. HISTOLOGY OF OLIGODENDROCYTES

Pío del Río Hortega (1882-1945), mentored by Nicolás Achúcarro, rendered the first systematic description of oligodendrocytes in 1920. Using the silver carbonate impregnation method developed by him, he discovered microglia - also named as the "third element" -, and also noticed the existence of a new cell type of neuroglia with very fine processes that gathered around axonal tracts. Besides, years later del Río Hortega proposed the relationship between oligodendroglia and myelination, being the CNS counterparts of Schwann cells in the Peripheral Nervous Systems. Notably, he also suggested the trophic role of OLGs in supporting neuronal activity, a feature that has been finally confirmed a century later. Nevertheless, the demonstration of these myelinating functions had to wait until the 1960s, when the introduction of the Electron Microscopy (EM) allowed a more accurate description. Unfortunately, this time gap, together with staining difficulties and the fact that his results were published in Spanish, unfairly made his findings not recognized internationally (1).

In a review of his discoveries on the morphology and function of oligodendroglia published in 1928, del Río Hortega tried to classify OLGs based on their morphological characteristics according to the number and orientation of their cellular processes, shape and size of their somata, calibre of the axons they associate with, and their distribution within the CNS (1). This analysis resulted in four different subtypes (I to IV), which suggested a phenotypic diversity that, despite being initially neglected, has been later demonstrated by EM,

immunohistochemistry, intracellular dye injection and gene expression. Accordingly, we now group OLGs into two different phenotypes depending on the calibre of the axons they myelinate, below and above a diameter of 2 to 4 μm, which corresponds to del Río Hortega's types I/II and III/IV, respectively (2). As a whole, OLG phenotypes differ in the number of axons they myelinate, the diameter of the axon and the internodal length of the myelin sheath. Hence, while type I OLGs support a large number of small calibre axons with short internodal length, type IV cells myelinate a single large calibre fiber with a long internodal length. However, these limits appear to be theorical, being the four main types variants of a morphological continuum. All in all, these phenotypic differences are functionally relevant, as fiber diameter and internodal length determine axonal speed conduction (2).

Although originally described as morphologically heterogeneous, it is not yet clear whether OLGs become diversified during maturation through interaction with the environment or there is intrinsic heterogeneity (3). Classically, myelin sheath and axon growth have been considered developmentally interdependent, being OLG phenotypic divergence regulated by axon-derived factors and interactions (2).

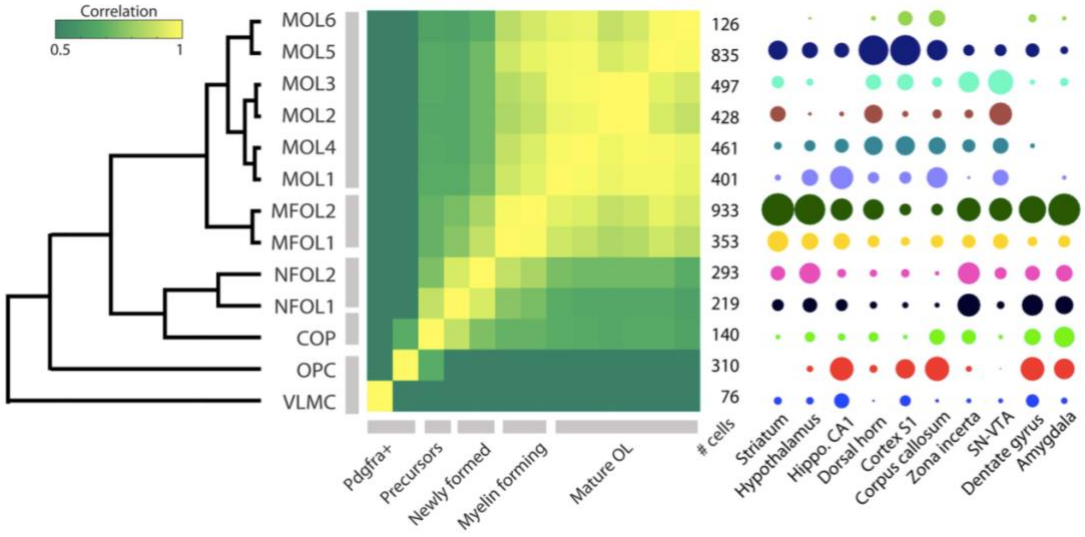

Figure 1. Single cell RNA-Seq analysis of 5072 cells expressing markers of the oligodendrocyte lineage in ten regions of the mouse CNS. Hierarchical clustering (left), correlation matrix (middle) and subclass abundances by region (right). VLMC, vascular and leptomeningeal cells; OPC, oligodendrocyte precursor cells; COP, differentiation-committed oligodendrocyte precursors; NFOL, newly formed oligodendrocytes; MFOL, myelin-forming oligodendrocytes; MOL, mature oligodendrocytes. *Taken from Marques et al (2016).*

Recently, however, transcriptional analysis revealed an unexpected heterogeneity along OLG differentiation. Thus, single-cell RNA sequencing of cells from the OLG lineage of mouse juvenile and adult CNS led to the identification of 13 different populations (3). This analysis revealed a differentiation path resulting in 12 different cell states from *Pdgfra*$^{+}$ oligodendrocyte precursor cells (OPCs) and myelin-forming oligodendrocytes to mature OLGs **(Figure 1)**, each population expressing different genes according to their level of differentiation. Thus, while precursors were enriched in cell fate commitment and adhesion genes, more mature populations expressed genes involved in ensheathment of axons and steroid biosynthesis (3). Subtype specification also occurred in an age- and region-specific manner, suggesting not only a transcriptional diversity in terms of maturation, but also a functional divergence.

Single cell transcriptomic analysis of OLG linage has recently been applied for the neuropathological analysis of diseases such as multiple sclerosis (MS), revealing an under-representation of some sub-clusters of mature OLGs in human MS tissue (4) and disease-specific OLG populations selectively expressing immunoprotective, innate and adaptative immunity genes (5). These findings suggest that OLG lineage and oligodendroglial heterogeneity might be important to understand disease origin, progression and developing therapeutic approaches.

1.2. PHYSIOLOGY OF OLIGODENDROCYTES

In accordance with the aforementioned oligodendroglial variety, OLGs show a heterogeneous receptor and channel expression. This subpopulation-specific expression of different subtypes of receptors and ion channels strongly suggests the possibility that distinct OLG subpopulations have diverse physiological properties. An in depth knowledge of the functional significance of this remarkable molecular heterogeneity will surely help in understanding the role of myelinating OLGs in cognition and behaviour, as well as their disorders (6).

In the following sections, I will describe the major ligand- and voltage-gated ion channels, as well as G-protein coupled neurotransmitter receptors, present in OLGs.

1.2.1. Voltage-dependent ion channels

Oligodendroglia expresses K^+, Na^+ and Ca^{2+} voltage dependent ion channels. In particular, K^+ channels – the largest and most diverse class – play an important role in maintaining the resting membrane potential. In contrast, Na^+ channels are less well characterized, being only expressed during early stages of the OLG lineage. Finally, voltage-operated Ca^{2+} channels may be involved in initiating and maintaining myelination via axoglial interactions mediated by K+ accumulation after axonal activity (6).

1.2.2. Ligand-gated ion channels

Ligand-gated ion channels represent a highly important family of proteins, as OLG lineage cells express functional ionotropic receptors for almost all neurotransmitters (6). I will mainly focus on ionotropic glutamate receptors (iGluR), which are not only fundamental to excitatory neurotransmission between neurons, but are also present in OLGs as key modulators of both physiological and pathological responses.

AMPA- and Kainate-sensitive iGluR (respectively, AMPARs and KARs) are heterotetrameric, cationic, receptor channels that can be composed by four different subunits for AMPAR (GluA1-GluA4) and five subunits for KAR (GluK1-GluK5) (6). The consequences of AMPA/KAR activation in OLGs are not yet clearly defined. Na^+ entry upon activation of these receptors depolarizes OLGs and may secondarily activate Na^+ and voltage-operated Ca^{2+} channels (6). AMPAR activation could also regulate OPC differentiation into myelinating OLGs by the blockade of K^+ channels mediated by Na^+ entry. In this way, as it will be discussed later, axons can regulate their own myelination, as axonal glutamate release allows surrounding OPCs and myelinating OLGs to be constantly informed about their activity, and thus regulate myelination (6). By contrast, in mature OLGs, the importance of AMPARs is less certain, but they might cause physiological cell death, as overactivation of AMPAR mediate OLG death in pathological conditions such as ischemia (6).

NMDARs are heterotetramers formed through combinations of NR1 (binding site for glycine/D-serine) with NR2 or NR3 subunits. These receptors are expressed in both OPCs and mature OLGs, inducing Ca^{2+} influx when activated. Unlike AMPARs,

NMDARs appear not to be critical in neuron-to-OPC synapses (6). Interestingly, however, NMDARs in OLGs have proved to be a key modulator of metabolic coupling to axonal activity in what it has recently been described as axo-myelinic synapse (AMS; see chapter 3). Similarly to AMPAR/KAR, glutamate excitotoxicity in OLGs during ischemia seemed to be also mediated by NMDAR activation. Yet, NMDAR specific knockout or blockade can worsen the outcome of white matter ischemia (6).

In addition, expression of Gamma-aminobutyric acid type A receptors ($GABA_AR$) and adenosine triphosphate (ATP) sensitive purinergic receptors (P2X), among others, has also been demonstrated in OLGs (6). However, the functional significance and relevance to pathology are not well known.

1.2.3. Metabotropic receptors

Metabotropic neurotransmitter receptors are G-protein coupled proteins that modulate the function of ligand- and voltage-gated ion channels or influence intracellular processes via second messengers such as Ca^{2+} and cAMP. Among others, OLGs and OPCs express glutamate (mGluR), GABA ($GABA_BR$) and purinergic metabotropic receptors (P2Y) (6).

1.3. MYELIN DYNAMICS

Myelin is known to insulate axons and to speed up action potential propagation. Although classically considered as a static interaction, recent studies have described novel functions for OLGs, proposing that neurons rely on these myelinating cells in a more dynamic manner.

1.3.1. Myelination and its regulating factors

OPCs proliferate through the CNS and eventually differentiate into OLGs. The process of differentiation and subsequent myelination of axons is driven by different regulatory factors. In what it could be considered as a “default program”, cultured OLGs develop the intrinsic capacity to expand their plasma membrane and even enwrap artificial nanofibers (7). In addition, dynamic interactions between neurons and OLGs also promote myelination driven by signals and molecules that

collectively determine when and how much to wrap based on "functional demand". Adjustments of actin filaments, for example, regulated by cytoplasmic levels of gelsolin, pushes the inner tongue forward and contributes to myelin plasticity by the adaptation of myelin sheath thickness or internodal length (8). At the same time, proper adjustment of myelinic channels appears critical for myelin dynamics and, hence, axonal function. Non-compacted myelinic channels – routes for metabolites towards the inner tongue – close with myelin compaction. However, $PI(3,4,5)P_3$-mediated signalling can restore channel opening, thereby increasing metabolite flow during myelin growth (7). Similarly, OLG-specific protein 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) acts together with F-actin to counteract myelin compaction mediated by myelin basic protein (MBP) (9) (**Figure 2**). All in all, the balance between these permissive and non-permissive factors might be critical for axonal remyelination in demyelinating diseases.

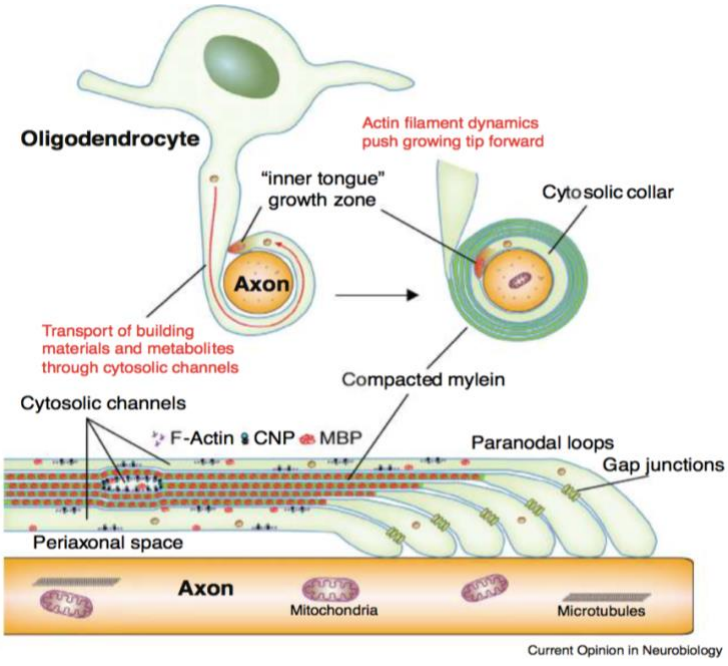

Figure 2. Model of myelin growth dynamics. Building materials and metabolites for membrane production and myelin growth are transported through a cytosolic channel system towards the growing tip. The force that pushes the inner tongue forward relies on actin filament assembly-disassembly cycles. Stacked layers of plasma membranes are subsequently compacted and myelin basic protein (MBP) is essential for membrane compaction. The membrane-associated 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) interacts with the actin cytoskeleton and counteracts the compacting force of MBP. This helps maintaining non-compacted cytosolic channels in mature sheaths, which are critical for metabolite diffusion. *Taken from Saab and Nave (2017).*

1.3.2. Influence of neuronal activity on OPC differentiation

The functional impact of neuronal activity on OPC differentiation is still uncertain. As previously mentioned, unlike NMDA receptors, AMPAR signalling could be the driver for OPC differentiation (6). Axonal spiking and vesicular release of glutamate promote *in vitro* myelination by stimulating formation of cholesterol-rich signalling domains between OLGs and axons, and increasing local synthesis of the major protein in the myelin sheath, MBP. However, this does not need synapse formation, suggesting that non-synaptic axon-glial junctions could mediate glutamate delivery to OPCs (10, 11). Accordingly, time-lapse imaging of OLG myelination in zebrafish confirmed that axonal vesicular release stimulated myelin sheath formation, showing that myelinating processes were stabilized preferentially around electrically active axons (12).

Although the cellular basis underlying learning are not well known, this form of activity-dependent regulation could be important in modifying development of brain circuits according to environmental experience. Thus, regulation of myelination by impulse activity in individual axons could regulate neurodevelopment and thus influence information transmission in the brain (10).

On the other hand, as OPCs are functionally heterogeneous among regions and with age (13), in accordance to already described OLG heterogeneity (3), the impact of neuronal activity on OPC differentiation and myelination probably depends on neuronal subtype, brain region and neurotransmitters released from different cellular sources (7).

1.3.3. Myelin plasticity

Importantly, neuronal activity regulates not only myelinization during development, but also during plasticity in the adult brain. Thus, myelin sheath thickness, internodal length and perinodal organization can change following sensory stimuli and motor performance (7). This plasticity can be essential for synchronizing complex neuronal networks, as demonstrated in recent studies. For example, learning a motor skill on a “complex” running wheel caused a rapid production of new OLGs and changes in myelin structure (14). Conversely, social isolation provoked thinner and fewer

myelin sheaths, along with impaired cognitive and social behaviours (15). Strikingly, social reintegration restored myelin appearance and normal behaviour. All these data suggest that experience-mediated and activity-driven neuronal activity promotes changes in myelination that have an impact on brain function (7).

In addition to speed up action potential propagation, proper myelination is crucial to fine-tune the brain's computational power throughout life. In the results section, I will describe a recently highlighted novel feature, in which the myelinated axons receive metabolic support from OLGs in an activity-dependant manner. Thus, it appears that myelin is more than just an insulator.

2. METHODS

The bibliography search was conducted in PubMed database using the terms *myelin*, *oligodendrocytes*, *oligodendroglia* and *axo-myelinic interaction* together with each of the terms *metabolism* and *energy*. The search was limited to articles written in English and published in the last 10 years. All references were assessed and, mainly, seminal papers and most recent reviews were read in detail. Citations in these articles were noted as well, and all relevant citations not appearing in the original search were also included.

2.1. STUDY SELECTION

Due to the unmanageable number of articles that resulted from the search, publications were included only if they referred to axo-glial metabolic coupling. Studies referring to OLG metabolism itself were excluded, as were out-dated reviews that did not include facts described in the following years.

Ultimately, among the articles retrieved from the initial search, only 41 met the criteria for review. However, the impact factor of each journal was also checked on Thomson ISI "Journal Citation Report 2017", discarding those publications with lower citation indexes. Therefore, the bibliography on which I will base the main ideas of this review was finally comprised of 30 publications, including three seminal articles (Fünfschilling et al, 2012; Lee et al, 2012; Saab et al, 2016) and two highly relevant reviews (Saab and Nave, 2017; Micu et al, 2017).

3. RESULTS: AXO-MYELINIC METABOLIC COUPLING

Glucose is the primary energy source for the brain and is ultimately oxidized to CO_2 by mitochondrial respiration. Neurons depend on mitochondria for ATP homeostasis and long-term integrity, whereas glial cells can survive with ATP generated only by anaerobic glycolysis (16).

The interactions between different cell types and the mechanisms by which they exchange metabolites are essential to understand brain energy homeostasis. Hereafter, I will give a deep insight into how neurons rely on their myelinating partners for fulfilling their energy demands, which in the end could be critical to understand cognition, as well as the cognitive decline associated to neuropsychiatric diseases characterized by white matter pathology.

3.1. OLIGODENDROCYTES SUPPORT NEURONAL FUNCTION

In 2012, Fünfschilling et al discovered axon-glia metabolic coupling (16). By using conditional *Cox10* mutant mice, that is, a model for mitochondrial disease where the assembly of the terminal complex cytochrome *c* oxidase (COX) of the electron transport chain was blocked, they assessed the effect of glial cell metabolism inhibition. They hypothesized that in the absence of functional COX, OLGs would fail to fully metabolize glucose, and would instead generate ATP mostly by glycolysis and produce lactate.

Surprisingly, myelinated tracts were normally developed in the CNS, with no signs of demyelination or white matter pathology. Genomic recombination in OLGs of newborn mutant mice did not interfere with postnatal myelination, as mutant OLGs myelinated using pre-existing mitochondria that will subsequently decline in respiratory function (16). Even at 9 months of age, histological analysis failed to show any signs of demyelination or neurodegeneration. Thus, once myelination had occurred, reduced mitochondrial functions in OLGs do not perturb their survival, myelin maintenance or axonal integrity (16) **(Figure 3)**.

In view of these results, Fünfschilling et al proposed that OLGs survived by enhanced glycolysis (16). By localized proton magnetic resonance spectroscopy (MRS), mutants showed increased lactate levels only under isoflurane anaesthesia, a

finding compatible with a model in which oligodendroglial release of lactate – the ultimate by-product of anaerobic glycolysis – is followed by its rapid use in other cellular compartments **(Figure 4)**. In addition, it was demonstrated in vitro that glial lactate is efficiently metabolized by myelinated axons.

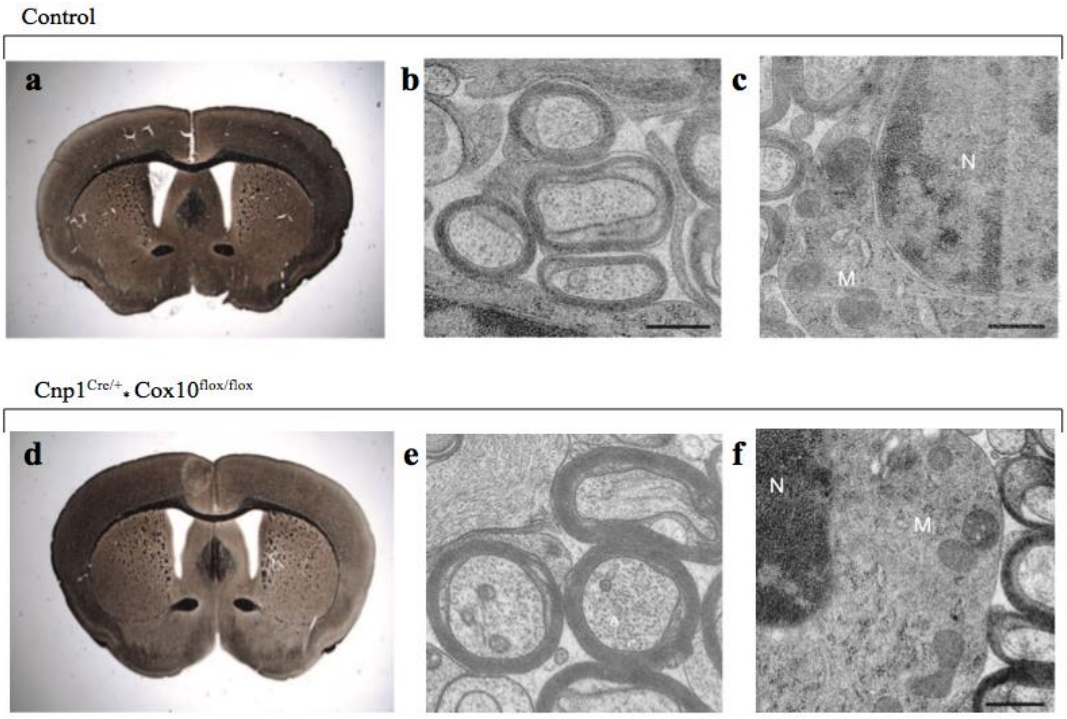

Figure 3. Oligodendroglial survival, myelin preservation and white matter integrity in Cnp1$^{Cre/+}$* Cox10$^{flox/flox}$ mice. a, **d**, By Gallyas' silver impregnation of myelin at 9 months of age, the corpus callosum and other white matter tracts appear normally developed and mature in mutant mice. **b**, **c**, **e**, **f**, Electron microscopy of high-pressure frozen optic nerve shows intact myelination of CNS axons (**b**, **e**), and healthy oligodendroglia nuclei (**c**, **f**). A, axon; N, nucleus; M, mitochondria. Scale bars, 500 nm. *Modified from Fünfschilling et al (2012).*

All in all, for the first time, this study suggested that the increased rate of OLG glycolysis could supply metabolic products to support axonal energy needs.

Almost simultaneously, Lee et al (2012) further clarified the mechanisms by which OLGs support neurons and axons. They mainly focused on the contribution of monocarboxylate transporter 1 (MCT1) to this lactate shuttle. This carrier, along with MCT2 and MCT4, transports monocarboxylic acids (such as lactate, pyruvate and ketone bodies) in the CNS. By using transgenic mice with a fluorescent reporter, they demonstrated in vivo what it had inconsistently been suggested by in vitro studies,

namely, that MCT1 is almost exclusively expressed by myelinating OLGs and, in addition, its expression is closely apposed to the axolemma (17).

Mouse models of OLG injury demonstrate axon loss without considerable demyelination, suggesting that OLGs support axon survival through a myelin-independent mechanism possibly, as previously remarked, as a result of insufficient axonal energy support (17). Therefore, Lee et al investigated the effect of MCT1 inhibition on neuronal death **(Figure 5a-d)**, showing that neurons in vitro were vulnerable to genetic or pharmacological reduction of MCT1 (17). Not only that, this toxicity was enhanced by removing glucose **(Figure 5d-f)** or increasing the metabolic activity of neurons (not shown) and, in contrast, prevented by supplying exogenous lactate **(Figure 5f)**. These results confirmed that when neuronal energy-requirements increase, either because of energy-deprivation or enhanced activity, reduced lactate release from OLGs leads to neuronal loss. Accordingly, focal MCT1 downregulation in vivo in the spinal cord also produced motoneuron death (17).

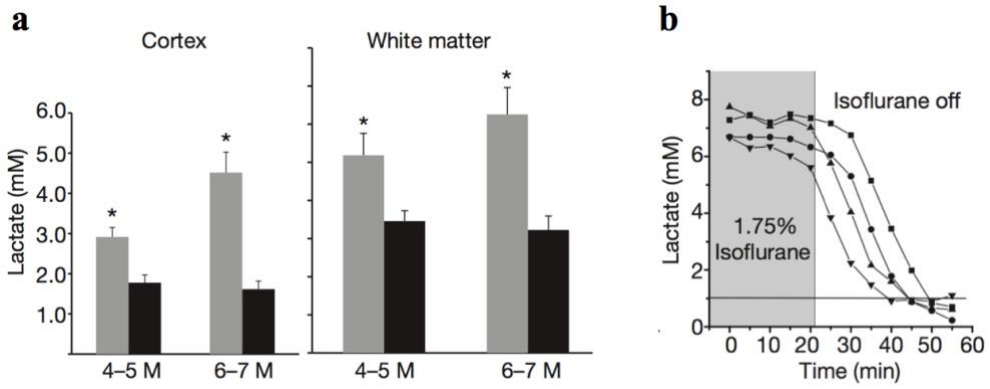

Figure 4. Rapid use of lactate shown by proton magnetic resonance spectroscopy (MRS). **a**, Lactate levels in the cortex (left) and white matter (right) are increased in mutant mice (grey bars) compared with controls (black bars) under isoflurane anaesthesia. Data are mean ± s.e.m. *P<0.05. Note that under isoflurane anaesthesia the control mice have higher lactate levels in white matter than in grey matter. M, months. **b**, Increased brain lactate drops to undetectable levels in less than 60 min at the end of anaesthesia. This suggests that lactate (produced by oligodendrocytes) is rapidly metabolized by other cellular compartments in the white matter tracts of awake mutant mice. *Taken from Fünfschilling et al (2012).*

Similarly, heterogeneous MCT1-null mice eventually developed axonopathy, but notably, OLG morphology and number were not changed and myelination was preserved, again suggesting that axonal degeneration was not due to OLG damage,

but to a reduction of MCT1, crucial for the normal function of CNS axons through a myelin dependent-mechanism (17). Indeed, Lee et al proposed that reduced expression of MCT1 is one of the mechanism by which oligodendroglia produce neurotoxicity in amyotrophic lateral sclerosis (ALS; see section 3.3.1) (17).

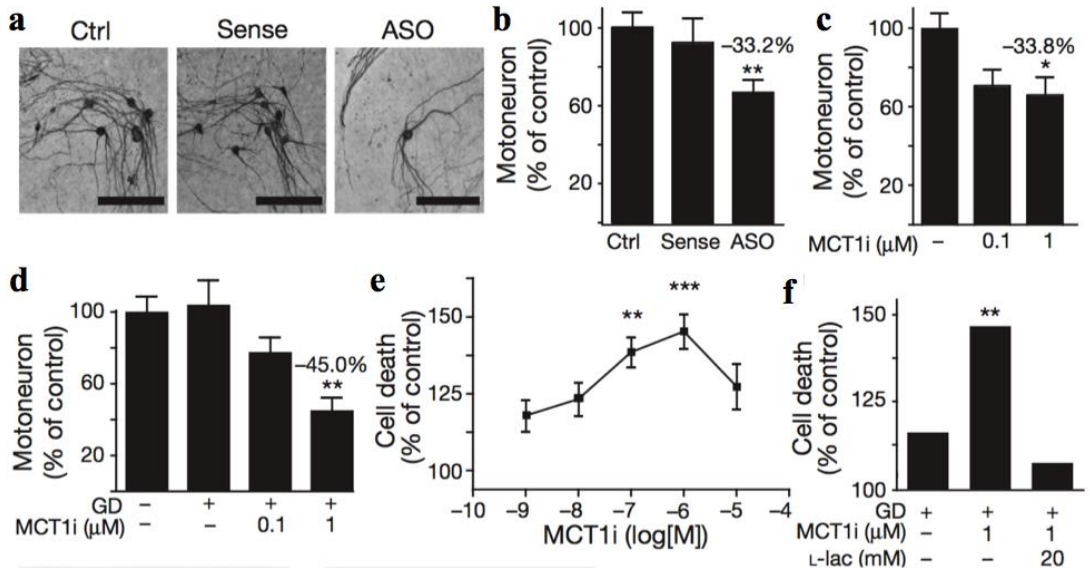

Figure 5. MCT1 is required for neuronal survival *in vitro*. a–d, Photomicrographs (**a**) and quantification (**b–d**) of motoneurons in spinal cord slice cultures treated with media only (ctrl), MCT1 sense or antisense (ASO) oligonucleotides for 3 weeks (**b**), after 3 weeks of treatment with MCT1 inhibitor (MCT1i) (**c**), or 2 h of glucose deprivation (GD) with or without MCT1i (**d**). **e**, **f**, Propidium iodide uptake in slice cultures with 2 h glucose deprivation plus MCT1i (**e**) or 2 h glucose deprivation with or without MCT1i and 20 mM lactate (**f**). Error bars denote s.e.m. *P<0.05; **P<0.01; ***P<0.001. *Taken from Lee et al (2012).*

Taken together, these results confirmed what Fünfschilling et al suggested: lactate export from glycolytic OLGs, regulated by MCT1, is a crucial component of the local energy supply to axons, and the disruption of this transport leads to axonal dysfunction and ultimately to neuronal degeneration **(summary in Figure 6)**. Thus, these data expand the known roles of myelin sheaths and reveals how the interruption of metabolic support through myelin might cause disease.

On the other hand, astrocytes constitute a glycogen store (18), possibly being an important glucose source for glycolytic OLGs. Presumably, astroglia can also provide lactate to neurons (19) and even OLGs could use MCT1 to take up lactate, which helps them to survive and produce myelin particularly in low-glucose

conditions (19). In addition, the demonstration that exogenous lactate can overcome the neuronal dysfunction caused by deficient lactate supply from OLGs is intriguing, as it could have a therapeutic potential and be one of the ways in which physical exercise benefits the brain (19).

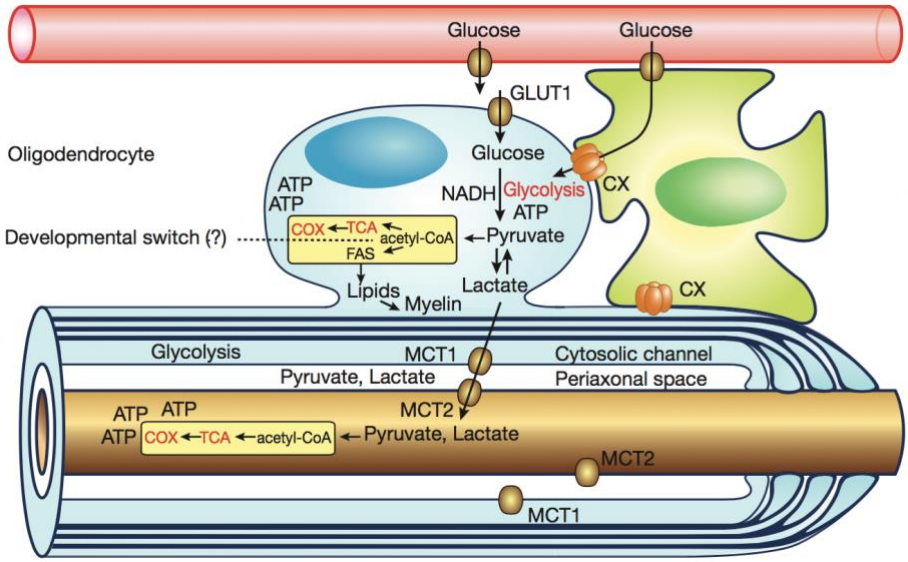

Figure 6. Model of metabolic coupling between oligodendrocytes and myelinated axons. Oligodendrocytes (OLGs) import glucose for glycolysis through glucose transporter GLUT1 and possibly via astrocytes and gap junctions (CX, connexin), which produce metabolic precursors from glycogen and their contacts with the vasculature. Pyruvate is metabolised in mitochondria (yellow) for ATP generation (TCA, tricarboxylic acid cycle). With the onset of myelination, glucose also serves the synthesis of fatty acid (FAS) and myelin lipids from acetyl-CoA. In post-myelination OLGs, glycolysis can yield sufficient ATP to support OLG survival. Glycolysis products are used by myelinated axons when energy levels are low. Lactate can be directly transferred via monocarboxylate transporter 1 (MCT1), which resides in internodal myelin near MCT2 present in the axolemma, in a way in which lactate ultimately reaches the axon. Lactate can also be produced in astrocytes and then transferred to axons by means of gap junctions. *Adapted from Fünfschilling et al (2012) and Lee et al (2012).*

3.2. METABOLIC SUPPORT IS DRIVEN BY NEURONAL ACTIVITY

This new paradigm of metabolic interaction between axons and OLGs raised the following question: could OLGs glucose utilization be quantitatively regulated to match neuronal energy needs? That is, as ATP consumption differs greatly depending on the spiking activity of myelinated axons, could OLGs "know" their association with fast spiking axons to adapt their own metabolism and, thus, their lactate supply?

In 2016, Saab et al tested this hypothesis. The function of NMDARs (see chapter 1) in OLGs was relatively unknown by that time, but it had been described that glutamate release upon axonal spiking induced calcium elevations in myelin (20). Consequently, Saab et al proposed NMDAR signalling as a link for axonal ATP consumption and oligodendroglial lactate supply.

To that end, they showed that similar to neurons, treatment with NMDA in cultured OLGs triggered glucose transporter GLUT1 surface expression, which in turn was efficiently blocked by NMDAR inhibitors (21). This NMDAR activation was followed by an increase on glucose uptake and the release of lactate was simultaneously enhanced, suggesting that as expected, lactate release relied on glucose availability. In contrast, MCT1 expression was unaffected by NMDA **(Figure 7)**.

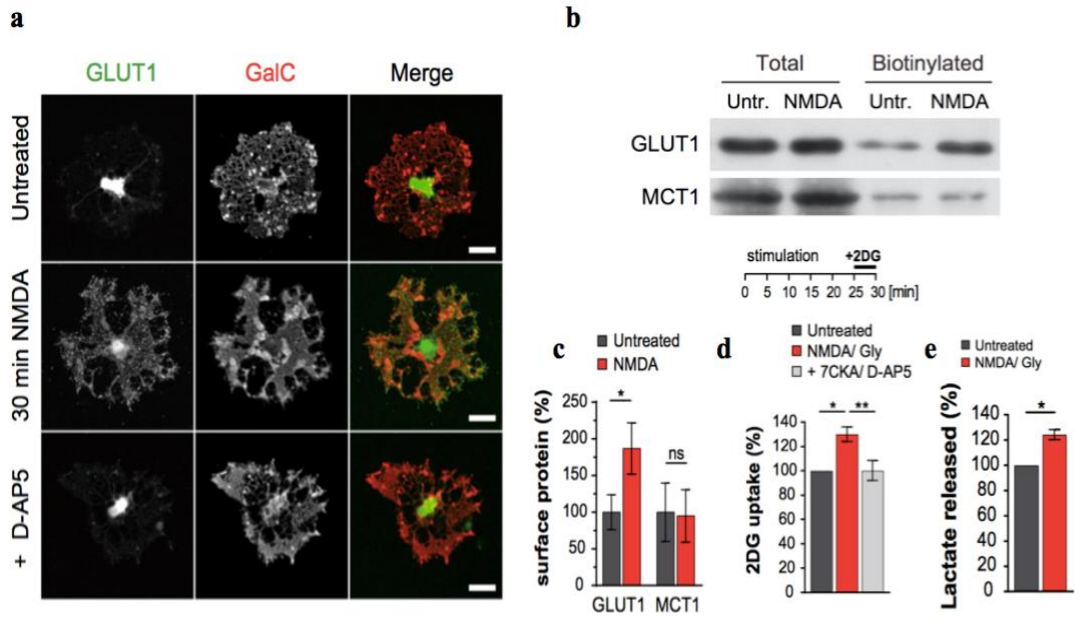

Figure 7. NMDA stimulates GLUT1 surface expression and glucose uptake by cultured oligodendrocytes. **a,** OLG immunostained for GLUT1 and GalC. NMDA receptor stimulation mobilizes GLUT1 and increases its cell surface expression, which is blocked by D-AP5. Scale bar, 20 μm. **b**, GLUT1 and MCT1 immunoblots following cell surface biotinylation of immunopanned NMDA-treated OLGs and controls. **c**, Quantification of the experiments in (**b**), normalizing biotinylated to total protein. MCT1 surface expression is unchanged. **d**, Immunopanned OLGs stimulated with NMDA/Gly (25 min) before switching to 2-deoxyglucose (2DG, 10 mM, 5 min). Increased 2DG uptake was blocked by 7CKA and D-AP5. **e**, Lactate release after NMDA receptor stimulation increased to 124% ± 4%. *P<0.05; **P<0.01. *Modified from Saab et al (2016).*

Considering glucose as a source for lipid precursor metabolites essential for myelination (see Figure 6), Saab et al hypothesized if reduced glutamate signalling could affect myelination during development. However, they showed that NMDARs were not essential for myelination *per se*, although the decrease of GLUT1 in OLGs could affect the rate of myelin growth "metabolically controlled" at highest myelination rates (third postnatal week) **(Figure 8)**. Nevertheless, differences were transient, as myelin thickness caught up with time (21).

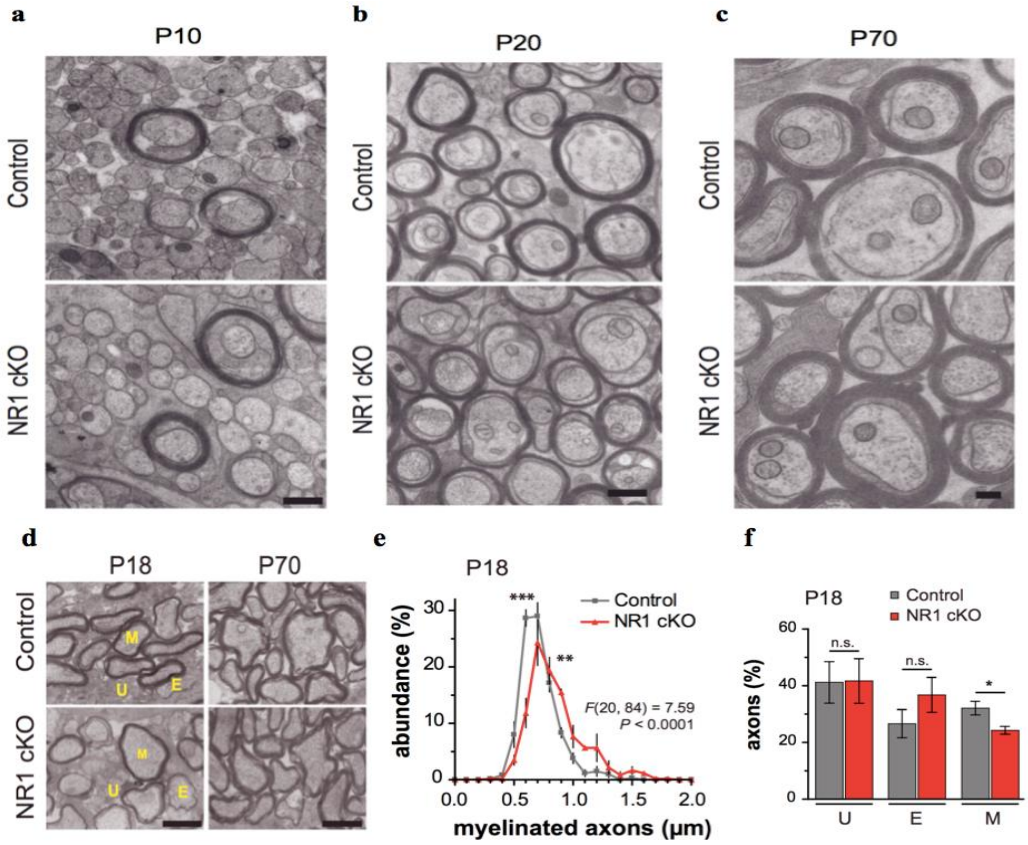

Figure 8. Myelination in the absence of oligodendroglial NMDA receptors in vivo is transiently delayed. a-c, High-pressure freezing electron microscopy of the developing optic nerve. Overview of optic nerve cross sections from control and NR1 knockout (NR1 cKO) mice at P10 (**a**), P20 (**b**), and P70 (**c**). At early and late stages, NR1 mutant nerves are indistinguishable from controls. A minor hypomyelination is apparent around P20. Scale bars, 0.5 µm (**a** and **b**) and 0.2 µm (**c**). **d**, Electron microscopy of conventionally fixed optic nerves from mutant and controls, with unmyelinated (U), ensheathed (E), and myelinated (M) axons. Scale bar, 1 µm. **e-f**, Axon size distribution and myelin sheath thickness (g-ratio) at P18. **e**, Diameter profile of myelinated axons with relatively more myelinated small caliber axons in control nerves than in NR1 cKO at P18. **f**, At P18, myelinated (M) axons are reduced in mutant optic nerves, while the number of unmyelinated (U) and merely ensheathed (E) axons remain similar. *P<0.05; **P<0.01; ***P<0.001. *Modified from Saab et al (2016).*

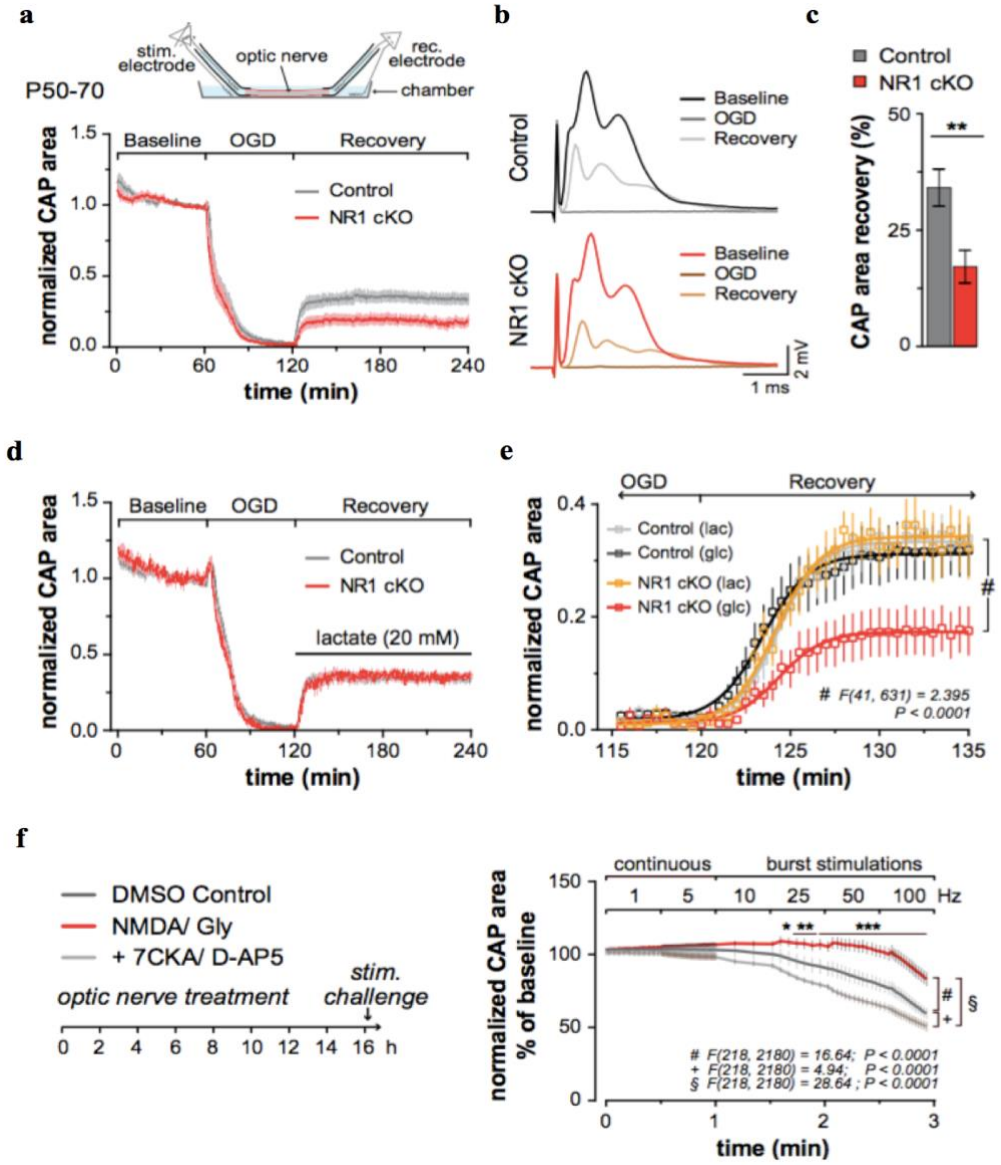

Figure 9. Axonal energy metabolism regulated by NMDAR and GLUT1-dependent lactate export from myelinating oligodendrocytes. a, Optic nerve compound action potential (CAP) areas normalized to baseline. After 1 hr, nerves were subjected to 60 min oxygen glucose deprivation (OGD) followed by reperfusion with artificial cerebrospinal fluid (ACSF) containing 10 mM glucose. Note the rapid decline of nerve conduction and the incomplete recovery after reperfusion, which is more pronounced in NR1 knockout mutants (NR1 cKO) (red) compared to controls (black). **b**, Average optic nerve CAPs during baseline, OGD, and recovery phase in control (top) and NR1 cKO (bottom). **c**, Quantification of data in (A) with reduced functional recovery after OGD in mutants versus controls (**P<0.01). **d**, With 20 mM lactate, the functional recovery after OGD was the same in NR1 mutants and controls. **e**, Axonal recovery at higher temporal resolution, comparing 10 mM glucose (glc) and 20 mM lactate (lac). **f**, Wild-type optic nerves, maintained functional ex vivo for 16 hr in the presence of NMDA/Gly (100 µM), or NMDA/Gly plus 7CKA/D-AP5 (100 µM), or only DMSO (control), were subsequently challenged with increasing stimulation frequencies. Note that nerves treated with NMDA/Gly show less decline of CAP area at higher frequency (***P<0.001). *Modified from Saab et al (2016).*

More interestingly, Saab et al assessed myelinated axon conduction to study functional differences between NMDAR mutants and controls. By recording compound action potentials (CAPs) ex vivo under basal conditions, they revealed a transiently reduced conduction velocity only at the peak of myelination (again at around the third postnatal week) that could be best explained due to the previously mentioned delay of CNS myelination (data not shown) (21).

However, when myelinated nerves were assessed under metabolic stress to test their ability to recover from transient oxygen-glucose deprivation (OGD), differences arose. In those experiments, they induced nerve conduction blockade by OGD for 60 min, and then re-perfused for an additional hour using oxygenated artificial cerebrospinal fluid (ACSF) to determine the recovery of axonal function. Surprisingly, the recovery of axonal conduction in wild-type nerves was better than in NMDAR mutant nerves **(Figure 9)**. As myelin injury was the same in both mutants and controls, they concluded that the loss of axonal conductivity in mutant nerves was caused by less-efficient axonal recovery from metabolic stress (21). Interestingly, axonal recovery after OGD was normal prior to myelination (data not shown), suggesting that axons require OLG support mainly after the formation of the myelin sheath, which limits rapid axonal access to extracellular metabolites (21).

Thereafter, according to the model proposed by Fünfschilling et al and Lee et al (2012) **(Figure 6)**, they tested whether lactate could restore axonal conduction independent of prior NMDAR signalling and replace glucose as an energy source through MCT1 transporters. As expected, by using lactate-containing ACSF for reperfusion, nerves from NMDAR mutants recovered as well as control nerves and even better than in the presence of glucose **(Figure 9)**.

Accordingly, nerves that had been isolated and treated for 16 hours with NMDA showed a much better maintained conductivity under increasing stimulation frequencies in comparison with those nerves without NMDA exposure. Consistently, the decline of axonal conduction was even more aggravated when nerves had been treated with NMDAR inhibitors **(Figure 9)**. These results were confirmed as well by in vivo studies, where after high-frequency stimulation, CAPs dropped fast in mutants and recovered more slowly (21).

Taken together, Saab et al demonstrated that OLGs regulate glucose utilization by using NMDA receptor signals as a switch for axonal spiking activity **(Summary in Figure 10)**.

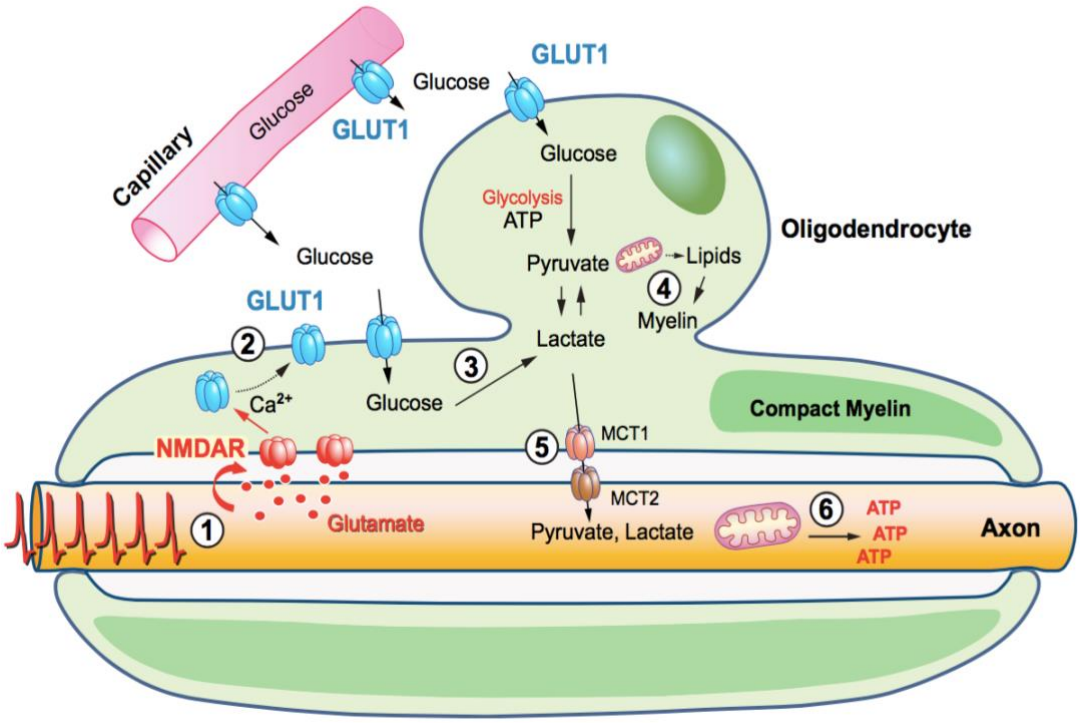

Figure 10. Schematic depiction of oligodendroglial NMDA receptor signalling. Working model in which axonal electrical activity in developing white matter tracts constitutes a glutamatergic signal for the surrounding OPC/oligodendrocytes/myelin compartments (**1**). After myelination, NMDA receptors associated with the internodal/ paranodal membrane respond to axonal glutamate release as a surrogate marker for increased axonal electrical activity and energy needs, causing (**2**) the incorporation of additional glucose transporters (GLUT1) into OLGs and myelin membrane and the adaptation of glucose uptake (feed-forward regulation). Glycolysis products (**3**) are initially used for ATP and lipid synthesis (**4**). Later, mature oligodendrocytes release lactate (or pyruvate) to fuel the axonal compartment (**5**) for mitochondrial ATP production (**6**). Regulation of oligodendroglial glucose uptake by axonal energy needs could help prevent abnormal accumulation of lactate. *Taken from Saab et al (2016).*

3.3. CLINICAL IMPLICATIONS TO DISEASE OF IMPAIRED MYELIN METABOLIC SUPPORT

Axons comprise a vulnerable neuronal compartment that serves connectivity in the CNS. Metabolic support from myelinating OLGs appears critical to maintain long-term axonal integrity, so hereafter I will describe how impaired metabolic coupling in white matter tracts could perturb axonal viability and thereby contribute to neurodegenerative diseases.

3.3.1. MCT1 expression is reduced in ALS

Lee et al (2012) proposed that the reduced ability of grey and white matter OLGs to support motoneurons caused by altered MCT1 expression, might contribute to ALS pathogenesis (17). They investigated the expression levels of MCT proteins in patients with ALS, showing a greater than 50% decline in MCT1 and MCT4 expression compared with gender- and age-matched control patients **(Figure 11)**. OLGs were preserved (although it is possible the oligodendroglia were immature) thus suggesting alterations in OLG MCT1 as a possible contributor to motoneuron degeneration in ALS (17).

Interestingly, it is well known that a mutation in the gene encoding superoxide dismutase 1 (SOD1), an enzyme involved in the removal of potentially harmful reactive oxygen species (ROS), leads to mitochondrial dysfunction and causes a hereditary form of ALS – it is also a commonly used transgenic mice model for this disease **(Figure 11)** – (17,19). Hence, the reduction of MCT1 expression could be linked to mitochondrial dysfunction, since the lack of metabolic substrates (such as lactate) could damage the mitochondria (19).

Taken together, this metabolic coupling is presumably not as critical for all myelinated axons, because they vary significantly in length, diameter, and firing frequencies, suggesting that their long-term energy demands differ greatly as well. This could explain why neurons of the cortico-spinal tracts, for example, are more vulnerable to neurodegeneration in MCT1 knock out mice (19). Besides their higher energy demands, the supply of lactate along the axon could be less efficient in motoneurons than in neurons with shorter axons (19). That is, assuming that energy

substrates enter into the axons only at the nodes of Ranvier, diffusion times from the node to the internode could take long for larger-calibre axons with long internodes. These distances are critical, as ATP-consuming pumps and axonal mitochondria are largely localized in internodal regions (22). Thus, additional energy supplies delivered from OLGs or astrocytes are likely vital to support the dynamic range of firing frequencies of myelinated fibre tracts (22).

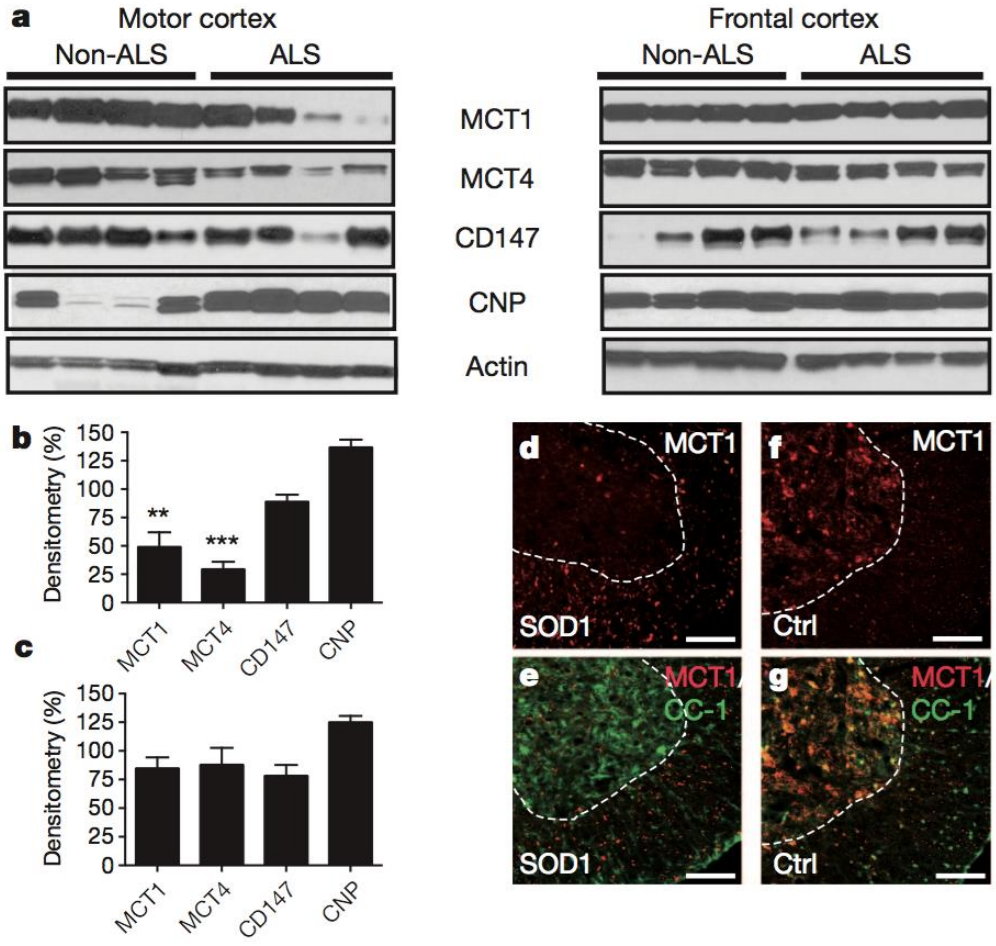

Figure 11. MCT1 is reduced in ALS patients and SOD1 (G93A) mice. a, Immunoblots of MCT1 and myelin-related proteins from patients with sporadic ALS and non-ALS patients. **b**, **c**, Relative densitometry of proteins from the motor (**b**) and frontal (**c**) cortex of patients with ALS compared with control patients. Error bars denote s.e.m. **P<0.01; ***P<0.001. **d–g**, Immunofluorescence of the MCT1 reporter alone (**d**, **f**; red) and double-labelling with CC-1 (**e**, **g**; green), in end-stage SOD1(G93A) transgenic mice (**d**, **e**) and littermate controls (**f**, **g**). Scale bars, 100 μm. Dashed lines delineate the boundary of ventral horn grey matter. *Taken from Lee et al (2012).*

3.3.2. Neuroinflammation and axonopathy in NMDAR mutant mice

Saab et al (2012) predicted that NMDAR mutant mice, with a developmentally reduced presence of GLUT1 in OLGs, would develop at least some signs of axonal pathology (21). As expected, mutant mice kept in standard housing – without physical challenges – showed signs of neuroinflammation and axonopathy beginning in the medulla and spinal cord at 10 months of age and later progressing to all CNS white matter tracts (21). Moreover, 1 year old NMDAR mutants revealed a significant deficit in performance comparing to age-matched controls when they performed a motor-behavioural analysis by a simple rotarod test, a feature that can be explained considering that myelinated axons in the cortico-spinal tracts fire at high frequency and are energy-demanding (21). Later, at the age of 19 months, NMDAR mutants showed a severe neurological phenotype caused by the on-going neurodegeneration that was already visible at 10 months **(Figure 12)**.

As pointed out by Saab et al, it is tempting to compare NMDA receptor-dependent regulation of GLUT1 with insulin-dependent GLUT4 trafficking in other cell types such adipocytes (7,21). However, in their study, they were unable to mobilize GLUT1 with insulin in OLGs. Despite analogies, like calcium-dependent GLUT trafficking or the need for a stable microtubule network in both cell types, in OLGs glucose transporters are more likely to serve long-term functions rather than fast (“insulin-like”) adaptations to changing energy needs (7,21). In fact, their in vivo experiment showed that it takes up to 16 hours for OLGs to metabolically respond to the loss of NMDAR signalling **(Figure 9)**. Acute changes in axonal activity might require thereby additional mechanisms to regulate lactate supply (7,21).

All in all, activity-dependent regulation of axonal energy metabolism suggests that axon-glial signalling is critical to adjust the metabolic machinery for long-term integrity, also suggesting why neural functions must be practiced or they will deteriorate (“use it or lose it”) (7,12). Thus, better understanding of these mechanisms might be clinically relevant to comprehend, for example, the aging brain or neurodegenerative diseases and help, at the same time, in the design of new therapeutic strategies.

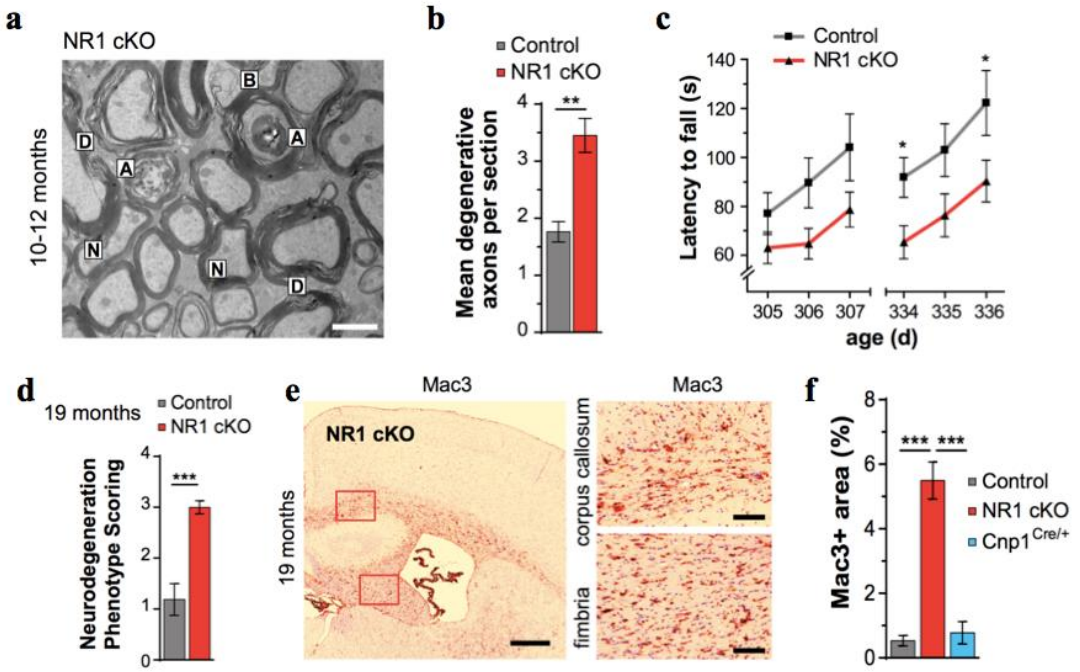

Figure 12. Late-onset neuroinflammation and axonopathy in NMDAR mutant (NR1 cKO) mice. **a**, **b**, By electron microscopy of ventral cervical spinal cord cross sections (**a**), ultrastructural features of axonal pathology and degeneration were more frequent in NR1 cKO mice compared to controls (**b**) Scale bar, 2 μm. In (**a**): A, axonal degeneration; B, blebbing membranes; D, delamination; N, normal myelin. **c**, Motor deficits of NR1 cKO mutants at 10-11 months of age, demonstrated by Rotarod testing on three consecutive days (repeated the following month). The latency to fall is decreased in NR1 mutant mice (red line) compared to littermate controls. **d**, At age 19 months, NR1 cKO mice display significant neurological deficits compared to controls. **e**, Brain sections of 19-month-old NR1 cKO mice immunostained for Mac3+ show widespread signs of neuroinflammation in white matter tracts; corpus callosum and fimbria are magnified (right panel). **f**, Quantification of Mac3+ immunostained area in NR1 mutants compared to littermate controls and age-matched Cnp1Cre/+ mice. *P<0.05; **P<0.01; ***P<0.001. *Taken from Saab et al (2016).*

4. DISCUSSION

The data reported above indicates that oligodendrocyte-to-axon metabolic support is important for normal physiology. Therefore, disruption of this coupling may be responsible for a host of diseases displaying axonopathy and degeneration. Hereafter I would like to propose a hypothesis as to how dysfunction of this metabolic interaction may contribute to various CNS disorders with white matter involvement, especially in the earliest stages of pathology.

4.1. AXO-MYELINIC "SYNAPSE" DYSFUNCTION

The interactions between myelin and axons resemble in many regards those occurring in classical synapses; in particular, in the way these two compartments use neurotransmitter signalling. Thus, neurotransmitters including glutamate are released from axons ("the presynaptic site") and activate receptors mostly located in myelin and OLGs ("the postsynaptic site").

Myelinated axons release neurotransmitters in an activity-dependent manner, thereby stimulating receptors on the inner myelin surface, and thus forming an axo-myelinic synapse (AMS). As I explained, this form of communication is vital to both myelin and axon, as one likely function of the AMS is to couple electrical activity to the metabolic output from the OLG (22). Thus, dysregulation of the AMS may lead to deleterious effects that could hypothetically be involved in the pathogenesis of diseases including multiple sclerosis (MS) and Alzheimer's disease (AD).

4.1.1. Multiple Sclerosis

MS is a chronic progressive disease of unknown aetiology, characterized by multifocal lesions of inflammatory demyelination exhibiting perivascular inflammation and complement deposition (22). However, the absence of substantial inflammation in a subgroup of very early lesions has raised the following question: could degenerative CNS pathology precede inflammation? (23).

Particularly, pathology at the inner tongue of myelin suggests that a perturbation of the AMS – with subsequent biochemical alterations of myelin components, possibly impaired transfer of metabolites and other deleterious effects – plays a primary role in the onset of MS lesions (22). Indeed, the inner tongue pathology in some cases of MS includes the loss of myelin-associated glycoprotein, suggesting that structural perturbation of the interface between axon and myelin is one possible mechanism by which an oligodendropathy could lead to disease (22).

In this sense, Micu et al (2017) proposed that aberrant glutamatergic transmission represent a potential mechanism by which the AMS might contribute to MS pathogenesis. Noteworthy, MS genome-wide association studies have identified disease-associated genes involved in glutamate homeostasis whose alteration may

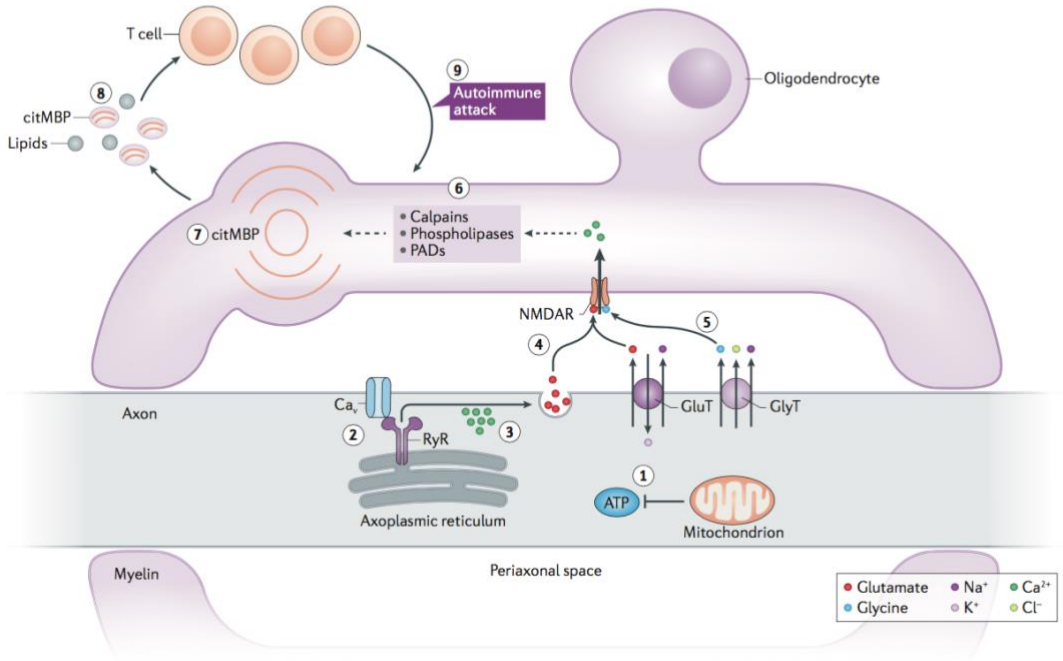

Figure 13. Proposed pathological consequences of overactivation of the axo-myelinic synapse. White-matter 'energy failure' would impair the ability of the OLG to generate lactate for export to the axon, also impeding the ability of axonal mitochondria to synthesize ATP (**step 1**). The reduction in axonal ATP results in failure of ion transporters, which in turn causes axons depolarization, activation of voltage-gated Ca2+ channels (Cav) (**step 2**) and excessive release of Ca2+ from stores (**step 3**). This Ca2+ release not only directly injures the axon by overactivation of Ca2+-dependent axonal enzymes but also stimulates excessive vesicular glutamate release into the periaxonal space (**step 4**). In addition, pathological entry of Na+ into the axon together with loss of K+ promotes reversal of Na+-dependent glutamate transporters (GluTs) and glycine transporters (GlyTs) (**step 5**), further exacerbating the increase of agonists at myelinic receptors. Excessive Ca2+ entry through these receptors over time, together with the inability of the myelin and/or OLG to buffer these Ca2+ loads because of energy deprivation, could overactivate several key enzymatic pathways, leading to aberrant biochemical modification of myelin components (**step 6**). Ca2+-activated calpains and phospholipases will degrade myelin proteins and lipids, respectively, and if persistent and exceeding the capacity for repair, this will eventually lead to demyelination of the axon. Peptidylarginine deiminases (PADs) are Ca2+- dependent enzymes that convert positively charged arginine residues on myelin basic protein (MBP) to citrulline. The deficit of positive charge on citrullinated MBP (citMBP) could focally disrupt the compacted myelin sheath (**step 7**), promoting the release of antigenic citMBP and lipid debris (**step 8**). In a host predisposed to immune-system overactivation, a T cell-driven adaptive immune response could result in a secondary wave of inflammatory pathology and autoimmune attack, causing further damage to myelin and the axon (**step 9**). RyR, ryanodine receptor. *Taken from Micu et al (2017).*

lead to glutamate excitotoxicity in relapsing-remitting MS (24). Thereby, it is tempting to suggest that glutamatergic dysregulation could establish an environment of "chronic excitotoxicity" where overstimulation of the AMS might have adverse effects on myelin structure and, consequently, on the axon by impairing the ability of OLGs to provide metabolic support **(Figure 13).** At the same time, AMS overstimulation could activate enzymatic pathways resulting in demyelination and release of antigenic debris that could lead to secondary inflammation with further damage to myelin and the axon (22). Accordingly, the axo-myelinic signalling machinery would be a potential key target, as therapies controlling chronic axo-myelinic excitotoxicity could provide cytoprotection for both the axon and myelin, and thus preserve the functional integrity of myelinated tracts. Considering that effective therapies for MS are very limited, especially for later-stage progressive MS, AMS would represent a novel target for drug development to treat this disease (22).

4.1.2. Neurodegenerative disorders

AD is caused by the neurotoxic accumulation of β-amyloid (Aβ) and hyperphosphorylated tau proteins. In addition, evidence increasingly points to a role for white matter abnormalities in disease pathogenesis, as defective axonal transport precedes amyloid accumulation and subsequent grey matter pathology (25). Indeed, imaging of patients with mild cognitive impairment suggests that white matter damage could even precede grey matter atrophy (26).

Regarding AMS, AD-related Aβ peptides increase the activity of NMDARs (27), which could as well promote chronic glutamatergic overstimulation of the oligodendroglial compartment (22). Considering that Aβ binds copper-bound major prion protein (PRP) with high affinity – a known inhibitor of NMDARs –, this could lead to chronic excitotoxicity at the AMS with consequent white matter pathology (22), with similar consequences for axonal integrity as described for MS.

Taken together, impairment of this new mode of communication between axons and OLGs may underlay the pathogenesis of chronic CNS diseases such as AD or MS, which highlights the need of research on the molecular architecture of the AMS to allow potential pharmacological strategies.

4.2. CONSEQUENCES OF MYELIN METABOLIC SUPPORT DYSFUNCTION TO BRAIN CONECTIVITY: PSYCHIATRIC DISEASES

Human brain white matter tracts constitute an enormous cellular network that connects the cortex with subcortical structures and, importantly, interconnect cortical areas with each other. Lesions affecting white matter disconnect brain areas causing motor and sensory deficits, as well as cognitive dysfunction (28). Besides, abnormal metabolic support can slow down or interrupt signal propagation, which may result in neurodegeneration (e.g. motoneuron cell death in ALS; 17) and contribute to psychiatric diseases (28).

Several clinical studies using imaging and neuropathology techniques have reported white matter changes and oligodendroglia alterations in depression, schizophrenia and autism, among others. These changes may stress axons metabolically and compromise axon potential propagation (28).

Table 1. Hypothetical mechanisms underlying primary and secondary involvement of myelin-forming oligodendrocytes in the course of psychiatric diseases. *Taken from Nave and Ehrenreich (2014).*

Oligodendrocyte Defect	Physiological Effect	Effect on Higher Brain Functions
Hypomyelination	Reduced nerve conduction velocity	Overall slowing of mental processes
	Reduced range of cortical synchrony	Partial loss of connectivity
	Axonal loss of millisecond precision	Loss of spike-timing-dependent plasticity
Loss of axonal metabolic support	Transport slowing, axonal swellings	Altered synaptic properties
	Transient conduction blocks	Transient loss of connectivity
	Axonal degeneration	Persistent loss of connectivity
Cortical hypermyelination	Reduced axonal sprouting	Loss of neuroplasticity and consolidation

Indeed, mutant mice with genetically defined defects in OLGs reveal unusual phenotype consisting of catatonia and depression-like symptoms (29) and interestingly, post-mortem brain of patients with schizophrenia shows specifically

reduced OLG gene transcripts (30). Consequently, primary defects of OLGs and myelin could well affect higher brain functions in different ways **(Table 1)**.

The continuous motor-driven transport of organelles and vesicles containing signalling proteins for synaptic transmission is an energy-consuming process. In heterozygous mice for OLG-specific genes (*Cnp1*+/-), for example, the first signs of pathology are axonal swellings, which indicate transport problems evocative of those seen in axonopathies caused by mitochondrial disease (28). This is well matched with the role of OLGs in supporting axonal energy metabolism, as reduced axonal ATP levels could lead to a slowing down of transport along the axon and eventually cause its arrest, abnormal calcium entry and caspase-mediated degeneration. Thus, decreased fuelling by myelin of axons could damage the synaptic machinery and cause behavioural disease symptoms (28).

Myelin thickness and internodal length in myelinated axons are designed to synchronize impulse transmission, specifically when parallel input differs in axon length. Transcallosal and interhemispheric connections, for example, are extraordinarily stable over time, and this may be critical for coupling between cortical regions, spike-timing-dependent plasticity or attention, processes that need a high temporal precision (28).

Whether these ideas are amenable to clinical applications remains to be seen, nonetheless it is certain that metabolic interactions between axons and OLGs are key for proper white matter function and axon survival, as well as to prevent neurodegenerative and psychiatric diseases. Thereby, such a complex model of metabolic interaction highlights the need to broaden the focus of research from neurons to other brain cells.

5. CONCLUSION

Oligodendroglia represents a much more diverse class of CNS cells than previously thought. Functionally, they subserve roles including not only insulation of axons, but also a source of energy substrates for proper propagation of action potentials. Notably, the supply of fuel to axons is controlled on demand according to neuronal activity via NMDA receptors localized in the myelin sheath, which are activated by

glutamate release from axons. Conceptually, the relationship between the axon and the enwrapping myelin constitutes a structure resembling the classical neuronal synapse. Disruption of this communication can lead to demyelination or axonopathy, and contribute to the physiopathology of diseases including multiple sclerosis, amyotrophic lateral sclerosis and Alzheimer's disease.

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