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INSIGHTS

Oligodendroglia-to-neuron material transfer lights up the mouse CNS

Stefano Pluchino[1] and Pranathi Prasad[1]

Intercellular material transfer in the central nervous system (CNS) supports neuronal survival and activity. Mayrhofer et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20221632) characterize extensive regionally coordinated transfer of oligodendroglial ribosomal and nuclear material toward neurons, linked to satellite oligodendrocyte–neuron pairs in the mouse CNS.

As myelinating cells of the central nervous system (CNS), oligodendrocytes support the survival and function of the neurons whose axons they sheath. Some of the oligodendrocyte support of neuronal integrity occurs via the intercellular transfer of extracellular vesicles (including exosomes) carrying specific cytoplasmic protein and RNA cargoes (Frühbeis et al., 2013; Mukherjee et al., 2020). In pathological settings associated with neuronal injury, the transfer of ribosome-containing extracellular vesicles from myelinating cells plays a major role in supporting injured axons in the peripheral nervous system (Court et al., 2008; Lopez-Verrilli et al., 2013; Müller et al., 2018; Rostami et al., 2017). Conversely, the transfer of material from other glial cells to neurons through extracellular vesicles, exosomes, and tunneling nanotubes contributes to the spread of pathogenic proteins including tau and alpha-synuclein in mouse models of neurodegeneration (Asai et al., 2015; Rostami et al., 2017). However, the extent to which material transfer to neurons takes place in the healthy CNS has yet to be fully established.

Using Cre-Lox tools for the expression of fluorescent ribosomal Rlp10a and inner nuclear membrane protein Sun1 specifically in the oligodendroglial-lineage cell *Sox10*-Cre mouse line, Mayrhofer et al. (2023) describe abundant presence of oligodendrocyte lineage Rpl10a-EGFP or nuclear Sun1-sfGFP proteins in neuronal cell bodies throughout the entire CNS. Considering regional differences in distribution, a striking 25–60% of all neurons in the cortex, thalamus, and striatum containing oligodendroglial-lineage cell–transferred ribosomal and nuclear material is described. To control for the presence of reporter protein in neurons due to unintended transient expression (or transfer) of Cre, the authors also make use of *Sox10*-Cre mice crossed with Sun1-sfGFP nuclear reporter mice carrying the inducible diphtheria toxin receptor transgene. In the offspring, the stereotaxic injection of diphtheria toxin specifically ablates the oligodendrocyte-lineage cells at the injection site, but not the neurons, implying that that neurons in Sox10-Cre mice do not express reporter protein. Applying these controls as well as another oligodendrocyte-lineage cell reporter mouse line (*Pdgfra*-Cre:Rpl10a-EGFP) with 60–100% of neurons containing transferred material, this work convincingly demonstrates extensive material transfer from oligodendrocyte-lineage cells to neurons in the healthy mouse brain.

To further investigate the dynamics of the transfer in the adult mouse brain, an inducible CreER^{T2}/LoxP system where tamoxifen injection allows expression of reporters Rpl10a-EGFP or Sun1-sfGFP in a *Sox10*-iCreER^{T2} mouse line is employed. At days 4–30 following injection, both reporter-positive oligodendrocytes and neurons are described. Intriguingly, frequent reporter-positive satellite (not myelinating) oligodendrocyte-lineage cell–neuron pairs, with their nuclei located in proximity, are found.

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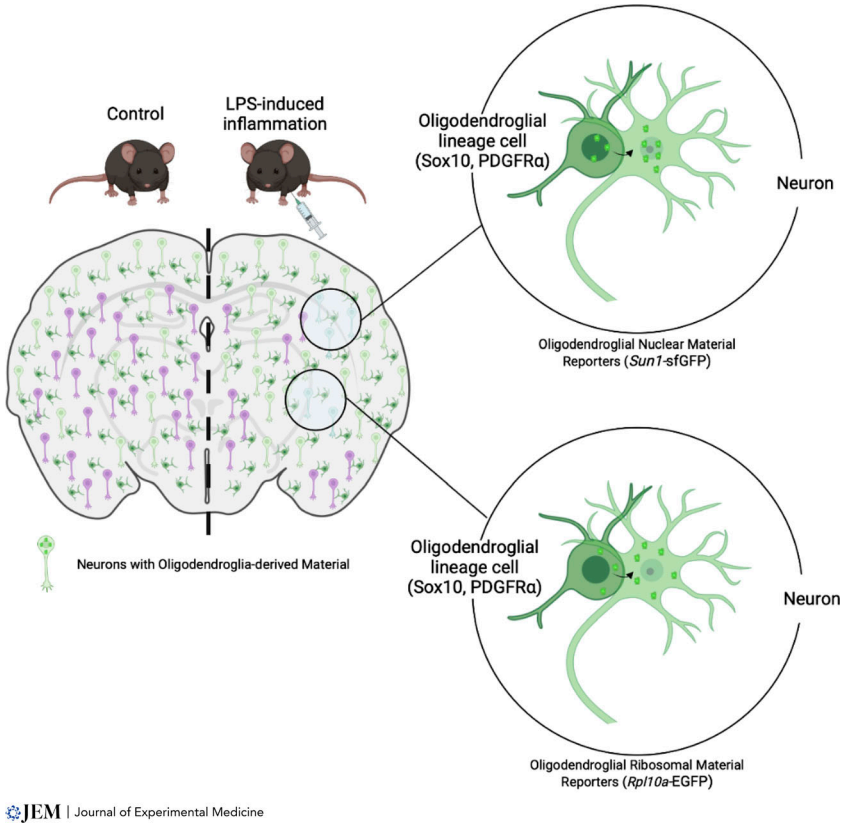
The appearance and frequency of these nuclear pairs responds dynamically to systemic inflammation, as the intraperitoneal injection of the bacterial endotoxin LPS leads to a first slight decrease in the number of nuclear reporter-positive neurons and nuclear pairs in the cortex 24 h after injection, followed by an increase in their number at day 5, which they found to coincide with chronic neuroinflammation, astrocyte hypertrophy, and astrogliosis.

Further examination of satellite oligodendrocyte-lineage cell–neuron pairs in the cortex through super-resolution confocal imaging combined with transmission electron microscopy (TEM) confirmed

[1]Department of Clinical Neurosciences and NIHR Biomedical Research Centre, University of Cambridge, Cambridge, UK.

Stefano Pluchino: spp24@cam.ac.uk.



Oligodendroglia-to-neuron nuclear and ribosomal material transfer in the adult mouse CNS. Using Cre-Lox tools for the expression of fluorescent ribosomal Rlp10a and inner nuclear membrane protein Sun1 specifically in the oligodendroglial-lineage cell *Sox10*-Cre mouse line, Mayrhofer et al. (2023) show presence of oligodendrocyte lineage Rpl10a-EGFP or nuclear Sun1-sfGFP proteins in neuronal cell bodies throughout the entire adult CNS. An increase in reporter-positive neurons, as well as oligodendroglial–neuronal nuclear pairs (i.e., oligodendroglia and neurons with nuclei in proximity) is detected 5 d after LPS injection. Created with Biorender.com.

close positioning of nuclei and visualized plasma membrane contact sites. Strikingly, TEM revealed that in addition to more numerous nuclear pairs separated by plasma membranes, lower frequency nuclear pairs with loss of plasma membrane integrity between nuclei were also imaged. In these latter pairs, nuclei were positioned 250–1,000 nm apart, with some organelles shifted away from the contact site, and mitochondria, recycling vesicles, and free ribosomes in the intranuclear space. Overall, cell type–specific proteins, such as Cre and Olig2, were confined to the oligodendrocyte-lineage cell, therefore arguing against complete cell fusion, and formation of multinucleate cells (heterokaryons). As such, the work by Mayrhofer et al. establishes that the healthy mouse CNS is home to extensive and highly dynamic material transfer between oligodendroglial-lineage cells and neurons, occurring occasionally through direct cell–cell transfer in satellite non-myelinating oligodendroglial–neuronal nuclear pairs. That material can be transferred between cells (and to neurons too) via exosomes, extracellular vesicles, and tunneling nanotubes, across synapses or gap junctions (Frühbeis et al., 2013; Lopez-Verrilli et al., 2013; Rostami et al., 2017; Shakhbazau et al., 2016) is an established concept in cellular biology. The novelty here is that intercellular communication does not necessarily cross a plasma membrane.

Further, Mayrhofer et al. also challenge the preconception that material transfer from glia to neurons is a feature predominantly associated to pathological or injury-related settings (Court et al., 2008; Lopez-Verrilli et al., 2013; Müller et al., 2018; Rostami et al., 2017). In fact, they show that the transfer of nuclear and ribosomal material is widespread across the healthy mouse CNS, observing certain areas of the brain with 100% of neurons containing ribosomal material derived from oligodendrocyte-lineage cells. While others have previously demonstrated proof-of-concept material transfer from glia to neurons (Chamberlain et al., 2021; Court et al., 2008; Frühbeis et al., 2013; Lopez-Verrilli et al., 2013; Müller et al., 2018; Shakhbazau et al., 2016), the extent to which material derived from satellite oligodendrocyte-lineage cells accumulates in neurons is novel. It also supports the notion that specific oligodendroglial-lineage cell states provide support to neurons beyond myelination, which includes transfer of material contributing to axonal metabolic support, as well as of ribosomes after nerve injury (Chamberlain et al., 2021; Müller et al., 2018; Shakhbazau et al., 2016).

While providing an extremely elegant and detailed characterization of potentially novel means of glia–neuron intercellular and internuclear interaction, this paper falls short in terms of the mechanism by which this interaction is possible. Hence, several key questions arise, the first being why such material transfer is necessary and why this is so extensive in the healthy CNS, seeing as neurons have the ability to express nuclear and ribosomal proteins on their own. Second is inevitably whether the activity (and the type of activity) of the recipient cells of the material transfer plays any role, and whether material transfer to neurons is an activity-dependent event, akin to activity-dependent myelination (de Faria et al., 2019). Third, the mechanism of nuclear interaction and cellular machinery involved in selective material transfer, and how specific it is to this type of glia and neurons or even the CNS, also remains to be elucidated. Previous work has demonstrated the potential formation of non-oligodendroglial–neuronal pairs, including (grafted) neural stem cell–neuronal pairs, grafted neural progenitor cell–endogenous macrophage pairs, and even cell fusion between transplanted bone-marrow-derived cells and adult Purkinje neurons (Johansson et al., 2008; Weimann et al., 2003). As such, it is conceivable that similar nuclear pairs may form between neurons and other cell types in the healthy mouse brain and remains to be investigated. Fourth, a further characterization of nuclear pairs without separation by a plasma membrane is needed to ensure the TEM finding is not

artefactual. Studies into the involvement of junctional coupling through connexins, as described previously (Cusimano et al., 2012; Jäderstad et al., 2010; Pluchino and Cossetti, 2013), may help shed some light on the mechanism of the widespread material transfer reported by Mayrhofer et al. (2023). Finally, whether material transfer is limited to proteins or may also extend to cytosolic and nuclear content such as metabolites and genetic material is likewise of great interest.

Another limitation of the study is that the visualization of material transfer to neurons depends on expression of *Sox10* in oligodendrocyte-lineage cells, which establishes postnatally. As such, the true dynamics of this material transfer and how/when it establishes and influences early CNS development remains to be elucidated. How these dynamics correspond with potential material transfer in the human CNS is likewise a major question that merits investigation.

Mayrhofer et al.'s findings also raise pertinent questions regarding the distribution and dynamics of transfer of pathogenic proteins in neuroinflammatory and neurodegenerative disorders across the CNS.

In accordance with previous studies reporting an increase in cell fusion between transplanted bone-marrow-derived cells and adult Purkinje neurons in response to chronic inflammation, Mayrhofer et al. (2023) demonstrate that material transfer may respond dynamically to neuroinflammation following LPS injection (Johansson et al., 2008). Whether this is a response to the inflammation elicited by LPS injection or the subsequent tissue and neuronal damage remains to be seen. These findings also raise important questions regarding the potential involvement of inflammation-associated or -dependent mechanisms of material trafficking and sorting, including the potential involvement of cytokine signaling. In fact, inflammatory cytokine-dependent modulation of material trafficking and transfer through extracellular vesicles is described in neural stem/progenitor cells (Cossetti et al., 2014). As such, it is plausible that inflammatory conditions may both increase material transfer, as well as modulate the cellular contents involved in the transfer, potentially contributing to the transcellular spread of cytokine signaling and inflammatory responses.

In addition to this, evidence exists for the transfer of pathogenic and non-pathogenic cytosolic proteins between spinal cord motor neurons and from motor neurons to neighboring oligodendrocytes, implicating oligodendrocytes as mediators of protein transfer (Thomas et al., 2017). Interestingly, such material transfer happens to take place in nerve motor nuclei known to be affected in amiotrophic lateral sclerosis (ALS), but not in those known to be spared in ALS. Hence, the differences in the distribution and dynamics of material transfer reported by Mayrhofer et al. might work as potential variables affecting the spread of pathogenic proteins across the CNS.

Again, whether areas in the CNS with increased material transfer are more susceptible to the spread of pathogenic proteins, and/or whether the speed at which the spread of pathogenic proteins occurs is enhanced in these areas, remains to be clarified. Further characterization of material transfer in different areas of the CNS in disease conditions, in comparison to healthy aging, may aid in our understanding of the spread of pathogenic proteins in neurodegenerative diseases, and aid in the identification of neuroprotective interventions.

In summary, Mayrhofer et al. (2023) describe widespread transfer of nuclear and ribosomal material from oligodendrocyte lineage cells to neurons throughout the healthy mouse CNS, previously described predominantly in disease or injury-related settings. They show this material transfer to occur extensively across the CNS, sometimes occurring through direct transfer between oligodendrocyte-lineage cell–neuronal pairs, not separated by a plasma membrane. Greater characterization of nuclear interaction and selective material transfer between cells is needed.

Further work aimed at investigating the biological role of material transfer to neurons across early development and aging is necessary to gain a greater understanding of intercellular communication in the CNS. Such investigation in the context of neuroinflammatory and neurodegenerative diseases may unveil new opportunities for neuroprotective interventions through material transfer.

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