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Going with the **Flow:**

New **insights** into the **plumbing** of the ***retina*** and ***optic nerve***

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Abstract

Fluid and solute movement in the posterior segment of the eye is not fully understood. Much like the brain, the retina has high fluidic demands, and yet, is completely devoid of conventional lymphatics. In a recently published study, we present evidence of a novel fluid clearance pathway through the retina and optic nerve that involves perivascular spaces and lymphatics, much like the glymphatic system of the brain. These discoveries bring new insights into diseases like glaucoma with new opportunities for treatment.

Water is the universal solvent of life but does not flow completely freely through our body. Ever since the Nobel Prize-winning discovery of aquaporins by Peter Agre, our eyes have been opened to the molecular machinery governing where and how water and solutes move at the microscopic level - our subcellular plumbing.[1] Directional fluid and solute movement driven by hydrostatic and electrochemical gradients, known as advection, is notoriously difficult to study. This is because advection is easily drowned out by the much larger amounts of random fluid movements, or diffusion. However, on a tissue or organ level, advective preferential pathways for fluid movement reveal themselves.[2]

The importance of this dichotomy between the macro- and microscopic functions of our subcellular plumbing was recently highlighted by the discovery of a novel "lymphatic-like" system for fluid and waste clearance in the brain. Although aquaporin water channels were known to be densely expressed by supporting astroglial cells plastering the cerebrovasculature, their exact role was not understood. That is, until imaging of solute transport in the brain was done on a larger organ-wide scale.[3]

In 2012, Iliff et al. showed that in our crowded brains, a quasi-lymphatic system facilitated by water channels in glial-cells allowed for rapid movement of solutes and fluid through doughnut shaped paravascular

spaces surrounding the blood vessels.[4] This fluid moves through the brain tissues, is collected in the peri-venous spaces, and is dumped in the meningeal lymphatics (**Figure 1**). The system was described as the *glymphatic system* (combining the words glia- and lymphatics), sometimes also termed the *brainphatic system.* These findings sparked a revolution in research and understanding of glial-cells and their function. Further research has shown that the brain's glymphatic system is critical for the elimination of amyloid-beta plaques linked to Alzheimer's disease, and seems to hold clues to the importance of sleep for "washing" our brains.[5] The drive and mechanism underlying this flow is not quite understood, though the pulsatility of the brain and cerebral vessels is postulated to serve a role in the movement of this fluid.[6]

How is this relevant to ophthalmologists?

The neural tissue of the eye is developmentally a direct protrusion of the forebrain. For this reason, the optic nerve is physiologically like a white matter tract, and therefore vulnerable to diseases like multiple sclerosis. The optic nerve is ensheathed by the meninges, which contains lymphatic vessels in the dura mater layer.[7]

By some estimates, the eye contains more than 98% water. In the anterior part of the eye, production and drainage routes for water and solutes via trabecular and uveoscleral pathways are well described (**Figure 2**).[8,9] However, the posterior neural tissues of the eye have some of the highest metabolic rates and fluidic demands per gram of any tissues in the body. And yet, similar to the brain parenchyma, the eye is devoid of lymphatics to facilitate fluid and solute transport.[10]

In 2017, Mathieu et al. demonstrated the movement of small cerebrospinal fluid tracers

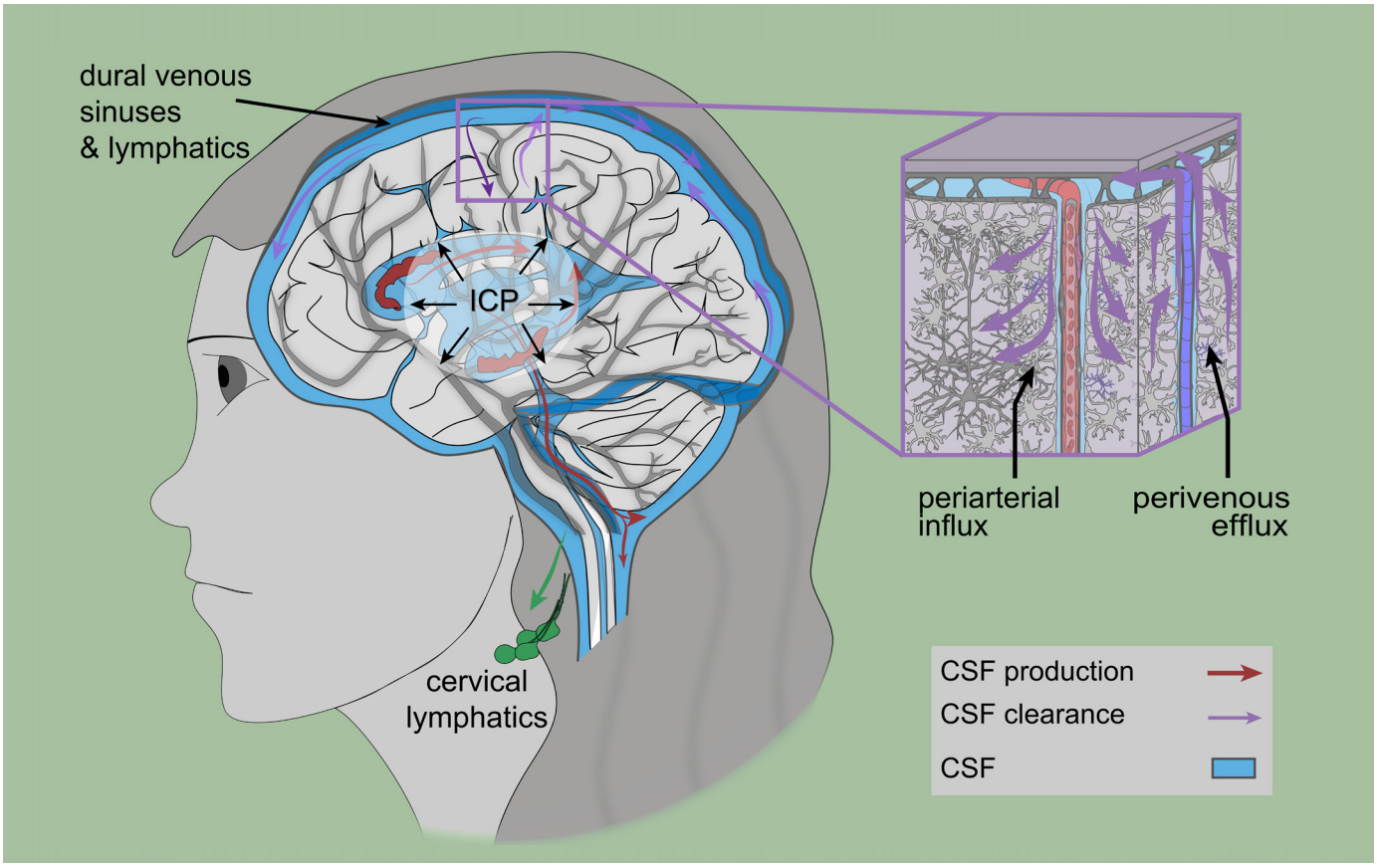

Figure 1. Proposed function of the brain glymphatic system. Periarterial fluid flows through interstitial brain tissue, to the perivenous-spaces, and is cleared by dural lymphatics. Adapted from Thrane et al. Trends Neurosci 2014 (https://pubmed.ncbi.nlm.nih.gov/25236348/, Fig 1)

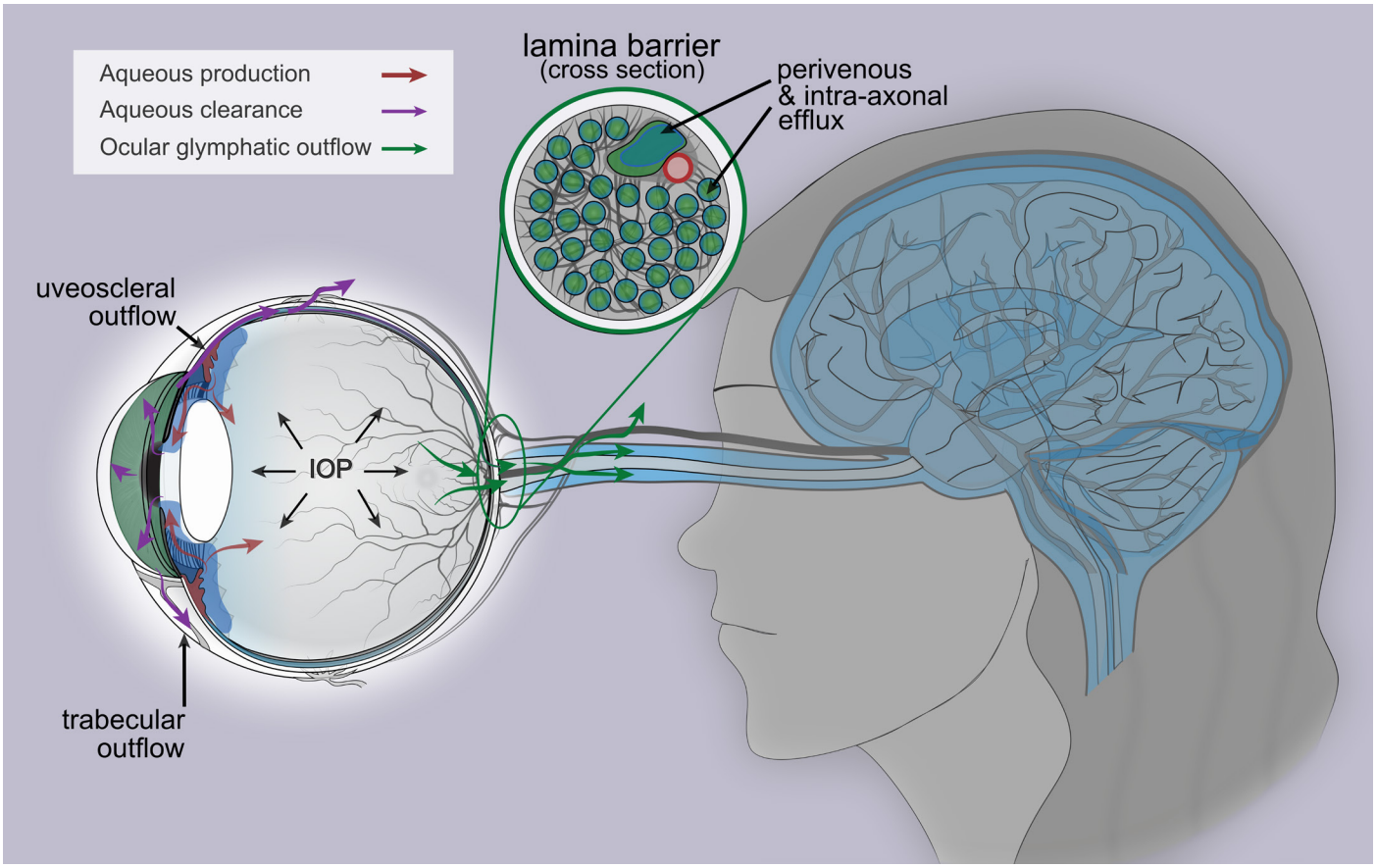

Figure 2. Known fluid transport systems of the eye. Aqueous fluid produced in the ciliary body (red arrows). Aqueous drained by the trabecular meshwork in the anterior chamber angle and through the uveoscleral outflow pathway (purple arrows). The newly discovered ocular glymphatic flow moving from the intravitreal and suprachoroidal spaces, though the lamina cribrosa, to the perivenous spaces, to the dural lymphatics surrounding the optic nerve (green arrows). Adapted from Rangroo Thrane et al. Acta Ophthalmol 2021 (https://pubmed.ncbi.nlm.nih.gov/32706172/, Fig 1)

from the brain into the optic nerve, consistent with glymphatic transport in the *retrograde direction*, from the brain towards the eye.[11] In collaboration with several laboratories, we recently published a study outlining the existence and basic function of a two-way ocular glymphatic system (**Figures 3 & 4**). This system shares similarities with the brain glymphatic system, with some local adaptations.[12]

Following various tracer infusions into the vitreous, suprachoroidal space, intravascularly and intra-cisternally, imaging of transparent rodent heads showed a bidirectional glymphatic transport between the eye and the optic nerve (**Figure 4**).[13] This fluid system was directly dependent on the high-to-low pressure gradient between the intraocular- and intracranial pressures (**Figure 5**). The system was also affected by the status of the lamina cribrosa, a fibrous connective tissue structure perforated by axon bundles and vessels. It acts as a supporting structure and barrier between the eye and the optic nerve and is essentially a gateway between the eye and the brain. In our study, when intracranial pressure was raised, the glymphatic drainage through the lamina was completely halted. Conversely, lowering the intracranial pressure *increased* the glymphatic flow (**Figure 5**).

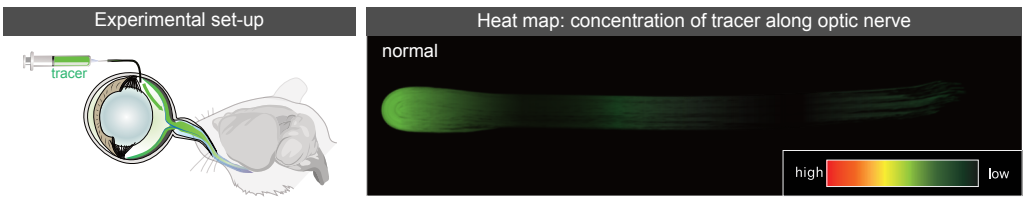

Figure 3. Heat-map of intravitreal tracer (green) found in optic nerve showing baseline glymphatic flow. Tracer has moved from the vitreous, through the neuro-retina and lamina cribrosa, to the optic nerve. Adapted from Wang et al. Sci Transl Med 2020 (https://pubmed.ncbi.nlm.nih.gov/32213628/, Fig 4A)

Small tracer molecules like amyloid-beta and radio-labelled potassium enter retinal axons and the perivenous spaces of both the retina and optic nerve, before being cleared by dural lymphatics around the optic nerve, and eventually, cervical lymph nodes.[12] Interestingly, larger molecules like dextrans were blocked by the lamina in rats and mice. This indicates that under physiological conditions, the glympathic outflow pathway exhibits selectivity, allowing some, but not all, molecules to pass. The lamina has the anatomical function of not only supporting axon bundles but also acts as a hydrostatic barrier by redirecting fluid and solute movement into axons and the perivenous spaces at the optic nerve head.[14]

Simultaneous injections of different tracers into the vitreous of the eye and the cisterna magna of the brain demonstrated that the forward and backward glymphatic flow are spatially separated (**Figure 4**). Cisterna magna tracers were transported along periarterial and pericapillary spaces in the optic nerve, stopping several hundred micrometres from the globe itself. Meanwhile, the intravitreal tracers were transported along peri*venous* spaces in the optic nerve (**Figure 4**).

Müller glial cells of the retina express large amounts of the water channel aquaporin-4 (AQP4).[15,16] In the brain, AQP4 is heavily expressed by astroglia end-feet, and line virtually all of the central nervous system vasculature.[17] Comparing control mice with knock-out mice, in which the AQP4 gene was deleted, we found a significant reduction in amyloid-beta clearance. This supports existing evidence that AQP4 facilitates water movement into and out of paravascular spaces in the brain. AQP4 thus seems to act in the eye and brain much like catch basins and grates of city streets that direct water into larger sewage pipes.

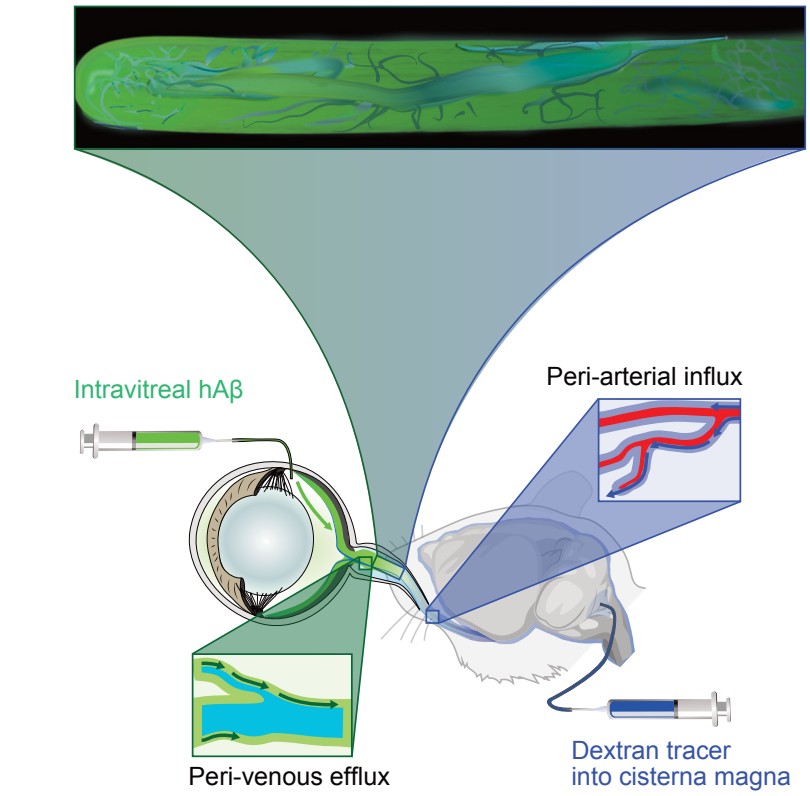

Figure 4. Illustration of mouse with dextran tracer (blue) injected into the CSF of the cisterna magna, which moves into peri-arterial spaces, while intravitreal human amyloid-beta (green) tracer moves through to the optic nerve and is found peri-venously. Proof of spatially and functionally separated bi-directional glymphatic flow. Adapted from Wang et al. Sci Transl Med 2020 (https://pubmed.ncbi.nlm.nih.gov/32213628/, Fig 1H)

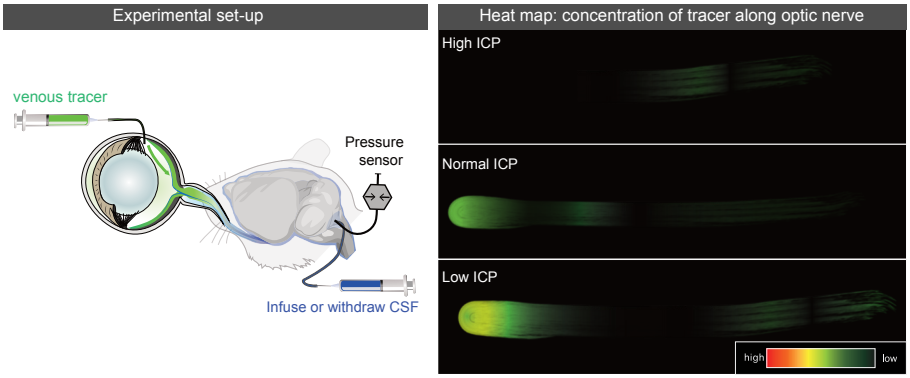

Figure 5. Changing glymphatic flow by manipulating intracranial pressure (ICP). Increased ICP reduced flow, while lowering ICP encouraged flow. Adapted from Wang et al. Sci Transl Med 2020 (https://pubmed.ncbi.nlm.nih.gov/32213628/, Fig 3A)

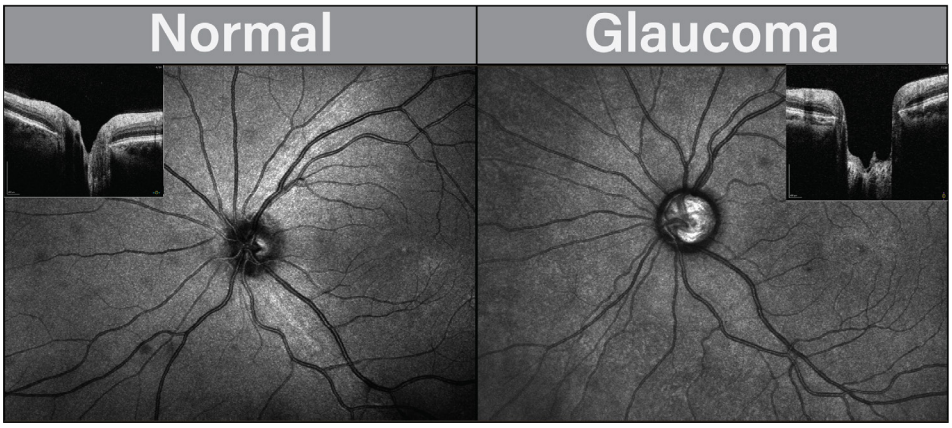

Figure 6. Confocal scanning laser ophthalmoscopy (C-SLO) and spectral domain optical coherence tomography (OCT, inset) images of normal and glaucomatous optic nerve head from Canon HS100. Glaucoma is associated with thinning of the retinal nerve fiber layer (RNFL) and optic disc rim tissues increasing the cup/disc ratio and causing a loss of the striated RNFL fundus reflex. With advancing glaucoma, we also see a posterior deflection and damage to the lamina cribrosa with increased pore size and sometimes acquired optic pits, resulting in pathological glymphatic flow.

Glymphatics & Glaucoma

Glaucoma is a neurodegenerative eye disease characterized by a recognized pattern of retinal ganglion cell death, optic disc excavation, and visual field loss. Increased intraocular pressure is one of the main risk factors of glaucoma.[18] Amyloid-beta has been shown to accumulate in the retina of glaucomatous eyes and to induce apoptosis of retinal ganglion cells.[19] Alzheimer's disease, the most common cause of dementia, is a neurodegenerative disease hallmarked by the accumulation of amyloid-beta in extracellular brain tissue. The brain glymphatic system has been tightly implicated in the pathophysiology of dementia.

Our initial hypothesis was that a reduction in glymphatic flow might contribute to glaucoma. However, using two distinct animal models of glaucoma, we found an *increase* in glymphatic tracer clearance, even *after* normalization of the intraocular pressure. Excessive outflow during high intraocular pressure could be explained by an increase in the translaminar pressure gradient. However, the increased flow after normalization of intraocular pressure, suggested that damage to the lamina cribrosa had occurred.

Destruction and outward deflection of the lamina cribrosa is a well-known feature of glaucoma (**Figure 6**), but its role in the disease development is not established.[14]

By examining the microscopic distribution of tracer clearance in glaucomatous and age-matched healthy controls, we found that clearance no longer was intra-axonal and perivascular, but primarily *extracellular*. When comparing the lamina barrier of glaucomatous and healthy mice by electron microscopy, we found obvious structural defects in the lamina. Functionally, these defects allowed passage of large dextrans out of the eye. The lamina no longer re-directed fluid into axons, and instead allowed free passage via large defects.

We hypothesize that excessive and unfiltered glymphatic outflow across the eye-brain hydrostatic gradient may cause wash-out of important metabolites and nutrients. Slowing of intra-axonal tracer clearance might lead to build-up of metabolic waste products, like amyloid-beta, leading to neurotoxicity, dysfunction, inflammation, and cell death.[20]

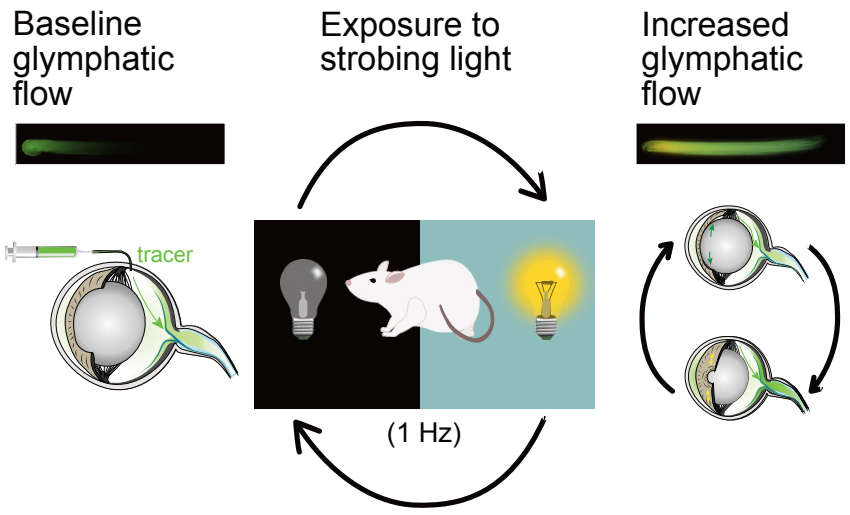

Figure 7. Significantly increased glymphatic flow through the optic nerve compared to baseline after flickering-light exposure. Adapted from Wang et al. Sci Transl Med 2020 (https://pubmed.ncbi.nlm.nih.gov/32213628/, Fig 4)

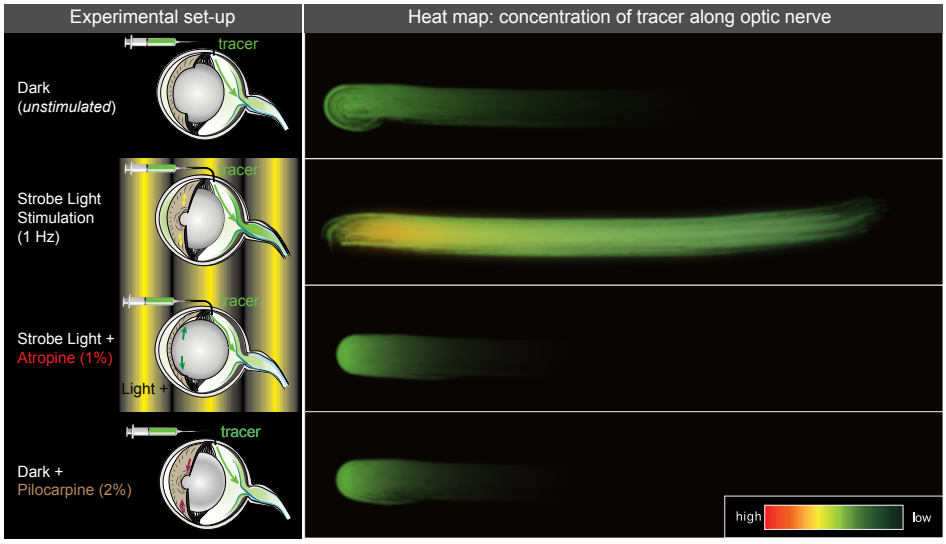

Figure 8. Heat-map showing basal glymphatic flow through the optic nerve and a significant increase in flow after unhindered repeated pupillary constriction/dilation induced by flickering light. In mice after first administering atropine or pilocarpine eye drops (which paralyzed pupillary constriction/dilation) flow did not increase from baseline, suggesting a link between pupillary movement and glymphatic flow. Adapted from Wang et al. Sci Transl Med 2020 (https://pubmed.ncbi.nlm.nih.gov/32213628/, Fig 4)

Another intriguing and puzzling finding in our study was that tracer outflow via the glymphatic pathway could be accelerated by the pupillary light response using flickering light (**Figure 7**). Rodents that were exposed to a flickering light once per second had a marked increase in glymphatic flow. This enhancement was blocked by topical atropine and pilocarpine, indicating that the movement of the pupil itself contributed to the increased glymphatic flow (**Figure 8**). Rodents have a rather rudimentary ciliary muscle compared to humans, so an even stronger functional link between glymphatic drainage, pupillary movement, and accommodation may exist in humans. When we injected tracers into the suprachoroidal space of rodents, a portion could be found in the optic nerve. Is it possible that an explanation could be found in an interplay between the glymphatic and uveoscleral outflow systems?

The net amount of fluid that exits through this novel glymphatic clearance route at the posterior pole of the eye is unknown and difficult to quantify. Large fluid and solute movement across the optic nerve head is physically impossible due to space constraints. It is likely to be orders of magnitude lower than intraocular fluid clearance from the anterior segment. Our findings indicate that the net amount of fluid clearance is not as important as the delicate balance of fluid and solute management.

REMAINING QUESTIONS:

- What other molecules are transported by the ocular glymphatic clearance pathway?
- What other eye diseases could be linked to glymphatic impairment?
- Can we develop diagnostic tracers to study this pathway in a clinical setting?
- Can we develop treatments that modulate transport or prevent damage to the glymphatic system?

Conclusion

To summarize, we have described a novel ocular glymphatic system characterized by selective fluid and solute transport from the retina along axons, across the lamina cribrosa, and via perivenous spaces to “classical” lymphatics. This system is dependent on the pressure gradient between the eye and the brain, and the clearance is stimulated by pupil movement. Glaucomatous damage to the lamina barrier greatly disturbs glymphatic function which impairs intra-axonal clearance of potentially neurotoxic solutes. Several important questions remain. Further characterization and knowledge of the ocular glymphatic system could be vital in the understanding of various eye diseases, and in the development of new diagnostics and treatments.

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NEW DISCOVERIES

KEY POINTS:

- ***We discovered a novel ocular glymphatic system***, which selectively transports solutes and fluids from the retina, along axons, across the lamina cribrosa, to the lymphatic system.
- ***The pressure gradient between the eyes and the brain is key*** to this transport and clearance is stimulated by pupil movement.
- ***Damage caused by glaucoma can impact this system***, potentially preventing proper clearance of neurotoxic solutes.

REFERENCES

1. Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S. Aquaporin CHIP: the archetypal molecular water channel. Am J Physiol. 1993 Oct;265(4 Pt 2):F463-76. doi: 10.1152/ajprenal.1993.265.4.F463. PMID: 7694481.
2. A. S. Thrane, V. Rangroo Thrane, M. Nedergaard, Drowning stars: reassessing the role of astrocytes in brain edema. Trends in neurosciences 37, 620-628 (2014).
3. M. Amiry-Moghaddam, O. P. Ottersen, The molecular basis of water transport in the brain. Nature reviews. Neuroscience 4, 991-1001 (2003).
4. J. J. Iliff et al., A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Science translational medicine 4, 147ra111 (201).
5. L. Xie et al., Sleep drives metabolite clearance from the adult brain. Science (New York, N.Y.) 342, 373-377 (2013).
6. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The Glymphatic System: A Beginner's Guide. Neurochem Res. 2015;40(12):2583-2599. doi:10.1007/s11064-015-1581-6
7. A. Louveau et al., Structural and functional features of central nervous system lymphatic vessels. Nature 523, 337-341 (2015).
8. A. Alm, S. F. Nilsson, Uveoscleral outflow--a review. Exp Eye Res 88, 760-768 (2009).
9. A. S. Huang, A. Camp, B. Y. Xu, R. C. Penteado, R. N. Weinreb, Aqueous Angiography: Aqueous Humor Outflow Imaging in Live Human Subjects. Ophthalmology 124, 1249-1251 (2017).
10. Y. H. Yucel et al., Identification of lymphatics in the ciliary body of the human eye: a novel "uveolymphatic" outflow pathway. Exp Eye Res 89, 810-819 (2009).
11. E. Mathieu et al., Evidence for Cerebrospinal Fluid Entry Into the Optic Nerve via a Glymphatic Pathway. Invest Ophthalmol Vis Sci 58, 4784-4791 (2017).
12. X. Wang et al., An ocular glymphatic clearance system removes beta-amyloid from the rodent eye. Science translational medicine 12 (2020).
13. C. Pan et al., Shrinkage-mediated imaging of entire organs and organisms using uDISCO. Nat Methods 13, 859-867 (2016).
14. M. D. Roberts et al., Remodeling of the connective tissue microarchitecture of the lamina cribrosa in early experimental glaucoma. Invest Ophthalmol Vis Sci 50, 681-690 (2009).
15. A. S. Verkman, J. Ruiz-Ederra, M. H. Levin, Functions of aquaporins in the eye. Progress in retinal and eye research 27, 420-433 (2008).
16. E. A. Nagelhus et al., Aquaporin-4 water channel protein in the rat retina and optic nerve: polarized expression in Muller cells and fibrous astrocytes. The Journal of neuroscience : the official journal of the Society for Neuroscience 18, 2506-2519 (1998).
17. T. M. Mathiisen, K. P. Lehre, N. C. Danbolt, O. P. Ottersen, The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia 58, 1094-1103 (2010).
18. R. N. Weinreb, T. Aung, F. A. Medeiros, The pathophysiology and treatment of glaucoma: a review. Jama 311, 1901-1911 (2014).
19. L. Guo et al., Targeting amyloid-β in glaucoma treatment. Proceedings of the National Academy of Sciences 104, 13444-13449 (2007).
20. M. A. Meraz-Ríos, D. Toral-Rios, D. Franco-Bocanegra, J. Villeda-Hernández, V. Campos-Peña, Inflammatory process in Alzheimer's Disease. Front Integr Neurosci 7, 59-59 (2013).

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News from the **European Glaucoma Society Guidelines 2020** – *How, why, & what* to avoid?

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EGS guidelines: History

In December 2020, the 5th edition of the European Glaucoma Society (EGS) Guidelines became available. Since the first edition of the EGS guidelines was published in 1998, the EGS has been continuously working to update the evidence-based guidelines for dealing with the increasing number of glaucoma patients. The Danish Glaucoma Guidelines from 1997 greatly inspired the first edition, especially regarding classification and terminology.

5th edition EGS guidelines: How?

For the 5th edition, the process began by identifying