PNAS COMMENTARY OPEN ACCESS

Bilateral astrocyte reaction to unilateral insult in the optic projection to the brain

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Image information from the two eyes integrates in the striate cortex of the brain's occipital lobe (also known as area V1) to create a seamless representation of the visual world. The binocularity of V1 neurons distinguishes them from neurons at earlier stages, especially in subcortical structures that include the lateral geniculate nucleus (LGN) of the thalamus and the superior colliculus (SC) of the midbrain (1). The responses of neurons in the LGN and SC and in other subcortical structures are similar in quality to those providing their direct ocular input: the retinal ganglion cells (RGCs), whose axons comprise the optic nerve and its projection into the brain (2). The tacit assumption from decades of physiological studies is that information from the two eyes remains separate until cortical integration and up to that point "*never the twain shall meet*" or so the Kipling poem goes (3). Now, in light of the findings by Cooper et al. (4), once again this tenet must be reconsidered, or at least that part which defines visual information and how it is conveyed. This elegantly conducted study leverages the utility of the visual pathways for studying not only interactions between the two optic projections, but also how stress-related signals travel in both anterograde (toward the brain) and retrograde (toward the eye) directions. In doing so, the work adds substantially to growing evidence that ocular stress is its own form of shareable information, well before image-related information is combined in the cortex. The refinement of what comprises "information" in the visual system is a subtext to the main finding that astrocyte glia on one side of the optic projection can represent stress induced in the other. Since astrocytes interact with and support RGC axons, this crosstalk is a surrogate for the ocular response to stress more broadly.

One well-known stress to the eye involves intraocular pressure (IOP), which depends critically upon balanced fluid flow into and drainage from the anterior portion of the eye. Many factors contribute to IOP homeostasis, and its management is an important clinical problem (5). Sensitivity to IOP is the hallmark characteristic of glaucoma. Glaucoma causes vision loss through degeneration of RGC axons in the optic projection caused by IOP-dependent stress conveyed at the optic nerve head (6). This important structure contains a meshwork of astrocyte processes through which RGC axons pass unmyelinated from the retina into the optic nerve proper, where they become myelinated by oligodendrocytes. Studies of neurodegeneration in glaucoma model sensitivity to IOP by inducing its elevation, through techniques such as microbead occlusion of the anterior eye, which is precisely what Cooper et al. did in their investigation.

Building upon earlier work (7), the team elevated IOP unilaterally in mice for a week. They then used light sheet microscopy to trace transport of fluorescently labeled cholera toxin B (CTB) to subcortical retinal projection sites in whole brains

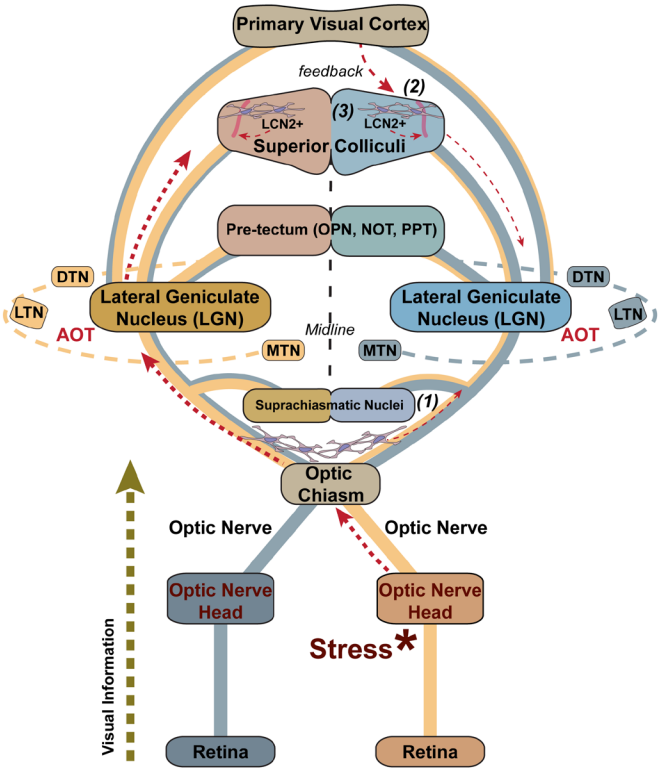

Fig. 1. Schematic of visual projections. Main central targets for RGC axons in the rodent, with dominance of the contralateral projection indicated (thicker solid lines). The AOT (accessory optic tract) is also contralateral and includes the dorsal terminal nucleus (DTN), the lateral terminal nucleus (LTN), and medial terminal nucleus (MTN). There are nearly 50 central targets for RGC axons (8), including the suprachiasmatic nucleus (SCN) and the LGN, which is the primary RGC target in primates and provides the direct retinal input to the primary visual cortex. The pretectum includes the olivary pretectal nucleus (OPN), nucleus of the optic tract (NOT), and posterior pretectal (PPT) nucleus. In rodents, all or nearly all RGCs project to the SC, while extending axon collaterals to other nuclei. Cooper et al. (4) show that unilateral IOP stress causes not only the expected contralateral pathophysiology that travels anterograde from the chiasm (thick red dashed line) but also an unexpected ipsilateral effect. Three possible mechanisms include (1) stress permeating anterograde through astrocyte-coupled networks that reflect cross-talk between the nerves, (2) feedback of stress signals from the visual cortex to the ipsilateral colliculus that travels retrograde to other structures, and (3) systemic circulation of inflammatory signals from LCN2+ astrocytes in the two colliculi.

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Author contributions: J.M.H. and D.J.C. wrote the paper.

The authors declare no competing interest.

See companion article, "Astrocytes in the mouse brain respond bilaterally to unilateral retinal neurodegeneration," 10.1073/pnas.2418249122.

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Published March 31, 2025.

cleared of lipids (delipidation), which allowed imaging in three dimensions. The transport of different CTB fluorophores from the two eyes was compared to markers of astrocyte reactivity including glial fibrillary acidic protein (GFAP) and lipocalin 2 (LCN2), the latter being a marker for rapid reactivity and a better surrogate than GFAP. The power of the experiment lies not only with the wonderful resolution brain clearing affords but also in the fact that in rodents, the optic projection is primarily contralateral (Fig. 1; 8), with only a small fraction (< 5%) projecting ipsilaterally. This feature stands in contrast to primates (like us) in which the projection from each eye is split about equally between ipsilateral and contralateral. This anatomical feature in rodents allows one to probe how unilateral ocular stress influences progression of degeneration not only in the projection contralateral to the stressed eye, which is expected, but in the ipsilateral as well.

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The quantification of RGC axonal transport to subcortical regions aligned with previous work showing decreased transport to the projection contralateral to the stressed eye (e.g., refs. 9 and 10). They report increased astrocyte GFAP, some with LCN2 fluorescence, in retinotopic regions with degraded anterograde CTB transport. A similar phenomenon occurs in the DBA2J mouse model of heritable glaucoma, in which SC regions of depleted transport demonstrate increased brain-derived neurotrophic factor coincident with elevated astrocyte GFAP (11). The surprising finding is that Cooper et al. also observed increased astrocyte hypertrophy ipsilateral to the IOP-stressed eye in regions receiving the corresponding retinal projection from the unaffected eye. Thus, astrocyte reactivity increased both in regions receiving innervation from transport-impaired axons and in mirrored regions in the opposite hemisphere, along with LCN2 reactivity in GFAP-positive astrocytes in both. That the retinotopic pattern of astrocyte GFAP reactivity was mirrored in the opposing hemisphere in the absence of abject transport deficits argues against the possibility that the few ipsilaterally projecting axons from the IOP-stressed optic projection could directly initiate astrocyte reactivity. Interestingly, the team also reported dramatic loss of transport to and volumetric shrinkage of the accessory optic tract (AOT). Although this projection is much less robust than to the SC or LGN, this finding could help explain early deficits in the optokinetic reflex, as the AOT is critical for this process (12). Moreover, as the authors discuss, since a subset of directional sensitive RGCs project primarily into the AOT, its early susceptibility may reflect their heightened sensitivity to IOP.

Bilateral effects from unilateral activity are not without precedent. For example, for some patients with age-related macular degeneration or macular edema, unilateral injection of agents that neutralize vascular endothelial growth factor causes clinical improvement in the contralateral eye (13–14). Most notably, unilateral gene therapy using enadogene nolparvovec (GS010) to treat Leber hereditary optic neuropathy has beneficial effects on vision preservation in both eyes (15), with transfer of the viral transcript between the eyes occurring via gap-junction coupled astrocyte networks within the optic nerves (16). These same astrocyte networks subserve the transfer of metabolic resources to ocular tissues stressed by unilateral IOP elevation from the contralateral unstressed projection (7). Since gap–junction coupling is bidirectional (or rather, directional agnostic), it is possible that astrocytes in subcortical structures challenged by degraded transport from the retina convey this signal to their counterparts across the optic chiasm, allowing for the communication of unilateral stress. Alternatively, LCN2+ astrocytes on the descending vasculature of the SC could circulate potential inflammatory mediators systemically. The authors suggest a neural-centric mechanism, in which higher-order bilateral visual centers (e.g., striate cortex) sense a change in net retinal signaling in one projection and feedback to the other via corticocollicular projections to initiate focal astrocyte reactivity (17). Astrocyte reactivity in the unstressed hemisphere could represent a compensatory mechanism to prime and preserve function against the possibility of additional stress.

1. T. R. Candy, L. K. Cormack, Recent understanding of binocular vision in the natural environment with clinical implications. *Prog. Retin. Eye Res.* **88**, 101014 (2022).
2. X. Shi *et al.*, Retinal origin of direction selectivity in the superior colliculus. *Nat. Neurosci.* **20**, 550–558 (2017).
3. T. S. Eliot, *A Choice of Kipling's Verse Made by TS Eliot* (Faber and Faber, London, 1963).
4. M. L. Cooper *et al.*, Astrocytes in the mouse brain respond bilaterally to unilateral retinal neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2418249122 (2025).
5. K. Mansouri, A. P. Tanna, C. G. De Moraes, A. S. Camp, R. N. Weinreb, Review of the measurement and management of 24-hour intraocular pressure in patients with glaucoma. *Surv. Ophthalmol.* **65**, 171–186 (2020).
6. D. J. Calkins, Critical pathogenic events underlying progression of neurodegeneration in glaucoma. *Prog. Retin. Eye Res.* **31**, 702–719 (2012).
7. M. L. Cooper *et al.*, Redistribution of metabolic resources through astrocyte networks mitigates neurodegenerative stress. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 18810–18821 (2020).
8. L. P. Morin, K. M. Studholme, Retinofugal projections in the mouse. *J. Comp. Neurol.* **522**, 3733–3753 (2014).
9. S. D. Crish, R. M. Sappington, D. M. Inman, P. J. Horner, D. J. Calkins, Distal axonopathy with structural persistence in glaucomatous neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 5196–5201 (2010).
10. N. J. Ward, K. W. Ho, W. S. Lambert, C. Weitlauf, D. J. Calkins, Absence of transient receptor potential vanilloid-1 accelerates stress-induced axonopathy in the optic projection. *J. Neurosci.* **34**, 3161–3170 (2014).
11. S. D. Crish *et al.*, Failure of axonal transport induces a spatially coincident increase in astrocyte BDNF prior to synapse loss in a central target. *Neuroscience* **229**, 55–70 (2013).
12. O. Dhande *et al.*, Genetic dissection of retinal inputs to brainstem nuclei controlling image stabilization. *J. Neurosci.* **33**, 17797 (2013).
13. J. Hanhart *et al.*, Fellow eye effect of unilateral intravitreal bevacizumab injection in eyes with diabetic macular edema. *Eye (Lond)* **28**, 646–653 (2014).
14. H. Isildak, S. G. Schwartz, H. W. Flynn Jr., Therapeutic effect of anti-VEGF for age-related macular degeneration in the untreated fellow eye. *Case Rep. Ophthalmol. Med.* **2018**, 8561895 (2018).
15. P. Yu-Wai-Man *et al.*, Bilateral visual improvement with unilateral gene therapy injection for Leber hereditary optic neuropathy. *Sci. Transl. Med.* **12**, eaaz7423 (2020).
16. N. R. McGrady *et al.*, Ocular stress enhances contralateral transfer of lenadogene nolparvovec gene therapy through astrocyte networks. *Mol. Ther.* **31**, 2005–2013 (2023).
17. B. Zingg *et al.*, AAV-mediated anterograde transsynaptic tagging: Mapping corticocollicular input-defined neural pathways for defense behaviors. *Neuron* **93**, 33–47 (2017).