chronic and acute inhibition experiments agree, these complementary strategies may help to confirm that the observed effect is not caused by artifacts such as light-induced heating.

Overall, Owen et al. demonstrate that light-induced tissue heating can be a confounding factor that should be controlled for in optogenetic silencing experiments. Together with other recent reports about germline recombination and non-cell-type-specific expression in Cre-driver mouse lines[13,14], paradoxical excitation effects of inhibitory opsins[11] and off-target effects of metabolites from the CNO ligand used in DREADD experiments[15], the work by Owen and colleagues is an important reminder that as our technology grows increasingly complex to enable ever defter control over neural activity, our application of these resources must also evolve to be more sophisticated so that we may account for the inevitable caveats and limitations that will always be associated with our tools. ❐

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Published online: 17 June 2019
https://doi.org/10.1038/s41593-019-0426-z

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Acknowledgements

D.C.P. is supported by a Graduate Research Fellowship from the National Science Foundation. S.L. is supported by a John P. Stock Faculty Fellowship and Rita Allen Scholarship. This work was supported by grants from NIDA (R01-DA042889), NIMH (R01MH112721), TRDRP (26IP-0035), the Rita Allen Foundation, the Wayne and Gladys Valley Foundation and the Shurl and Kay Curci Foundation.

Competing interests

The authors declare no competing interests.

CEREBROVASCULATURE

Sharpening the tools for pericyte research

The creation of a murine Cre driver specific to CNS capillary pericytes has opened a major bottleneck in brain microvascular research. Using this tool, pericyte loss in the adult brain is shown to induce neuronal loss due to concurrent microcirculatory failure and depletion of the protective trophic factor pleiotrophin.

Andrée-Anne Berthiaume and Andy Y. Shih

A remarkable 400 miles (~650 km) of vasculature is packed into our brains to provide an 'on-tap' supply of oxygen and nutrients to all brain cells. This is largely composed of a dense labyrinth of capillaries[1], formed by thin endothelial tubes lined with specialized cells called pericytes. Numerous functions have been ascribed to pericytes in the adult brain. These range from canonical roles in maintaining blood–brain barrier integrity and regulating capillary flow to exciting, albeit less understood, roles in immune cell trafficking, scar formation and stem cell activity after brain injury[2]. If pericytes are indeed modulators of these diverse cerebrovascular functions, it will be critical to learn how to preserve or even harness their abilities to improve vascular health during disease. Underscoring this need, mounting evidence points to an accelerated rate of pericyte loss in Alzheimer's disease and related dementias, a factor thought to exacerbate disease progression[3–7]. However, progress in this area has been hampered by the lack of genetic models to conditionally, and specifically, target pericytes in vivo. The crux of the problem is that singular pericyte markers are not exclusive to pericytes. For example, widely used markers such as PDGFRβ, NG2 and CD13 are also expressed in functionally distinct arteriolar smooth muscle cells just upstream in the vascular network[8].

In this issue of *Nature Neuroscience*, Nikolakopoulou and colleagues[9] report the first Cre driver with specificity for capillary pericytes, providing an opportunity to explore pericyte physiology and pathophysiology in detail. This was achieved using an intersectional strategy to gate the expression of CreER under both *Pdgfrb* and *Cspg4* gene promoters, which encode the PDGFRβ and NG2 proteins, respectively. These promoters are also active in smooth muscle cells, but to a lesser extent than in capillary pericytes[8]. The authors predicted that the combined requirement for both promoters would further reduce the odds of targeting non-pericyte cell types. This notion proved correct, as crossing the new pericyte-CreER line with a tdTomato reporter mouse led to inducible fluorescent protein expression limited to pericytes, excluding even mural cells in upstream pre-capillary arterioles (Fig. 1). A detailed appraisal of these mice revealed the further advantage that pericytes of several peripheral organs were only sparsely targeted, due to their limited expression of NG2 compared to in the CNS.

In their first application of this tool, the authors crossed pericyte-CreER mice with *loxP*-flanked reporter mice to selectively express the gene encoding diphtheria toxin receptor (DTR) in pericytes following tamoxifen induction. Using a daily regimen of systemic diphtheria toxin (DTX), which

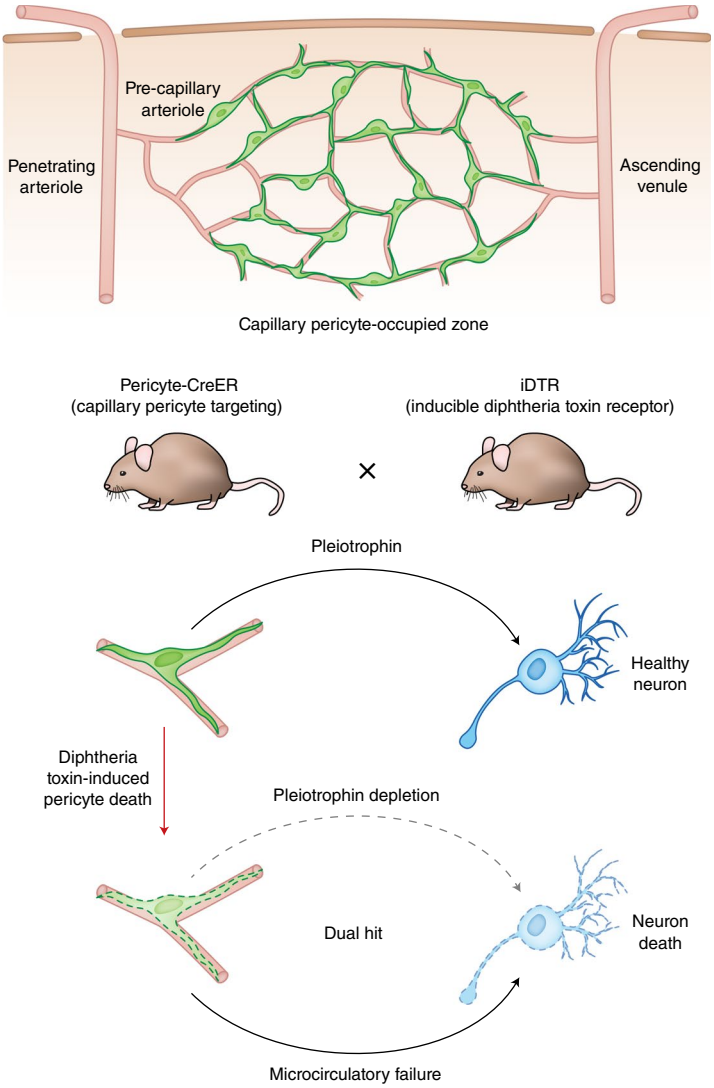

Fig. 1 | Selective ablation of capillary pericytes in the adult brain leads to concurrent microcirculatory failure and depletion of pleiotrophin levels. Pericyte-CreER mice are generated using an intersectional genetic strategy to selectively target brain capillary pericytes, which are located in the capillary bed, downstream of Acta2-expressing mural cells of penetrating and pre-capillary arterioles. This conditional Cre driver was crossed with *loxP*-flanked *DTR* mice to enable cell-specific ablation of capillary pericytes. Pericyte ablation resulted in impairment of microcirculatory flow and integrity, as well as concurrent depletion of the neuroprotective factor pleiotrophin. The authors suggest this 'dual hit' is a basis for neuronal loss caused when pericytes are lost globally in the adult brain.

causes cell death only in DTR-expressing cells, pericytes could be conditionally ablated in the adult brain over 6–9 days. This led to progressive pericyte loss until only about 40% of the original pericytes remained. This dropout of pericytes caused breakdown of the blood–brain barrier, reductions in cerebral blood flow, and rapid neuronal loss.

These findings are consistent with earlier reports from the same group[10], in which they showed that PDGFRβ$^{+/-}$ mice exhibiting progressive, age-dependent loss of pericyte coverage experienced cerebrovascular dysfunction followed by neurodegeneration. However, in this earlier work, a caveat remained that the phenotype involved developmental defects in capillary and/or neuronal function due to congenital impairment of PDGFRβ signaling. The current study sets this concern to rest, showing clearly that brain-wide loss of pericytes in adulthood is sufficient to cause severe microvascular pathology and neuronal death. An attractive advantage of the new model is that dose-dependency of pericyte loss can be achieved by titrating both tamoxifen and DTX dose. This opens the possibility of tuning the extent of pericyte loss over different time scales and with graded severities to mimic pericyte loss seen in a variety of disease states.

The authors then asked whether microcirculatory failure during pericyte ablation was the primary reason for the observed neuron loss, or whether pericytes have direct supportive roles in maintaining neuronal health. To explore this possibility, they turned to the neurotrophic factor pleiotrophin (PTN), whose elevated expression following ischemia promotes neuronal survival and recovery[11]. Recent single-cell transcriptomic analysis of the adult mouse brain has revealed that *Ptn* gene expression is enriched in capillary pericytes[8,12]. For this reason, Nikolakopoulou and colleagues[9] hypothesized that widespread pericyte ablation may be eliminating a major source of PTN from the brain, thereby leaving neurons vulnerable when challenged by a second hit, such as ischemia.

In line with their hypothesis, the authors showed that DTX-induced pericyte ablation reduced overall PTN protein levels in purified brain capillaries and in cerebrospinal fluid. This provides evidence that capillary pericytes are a key source of PTN in the adult mouse brain, suggesting a previously unrecognized neuroprotective role for these cells. They further found that brain infusion of exogenous PTN in mice with ablated pericytes was sufficient to counteract neuron loss despite the persistence of cerebrovascular dysfunction. Interestingly, small interfering RNA-mediated knockdown of PTN alone was not sufficient to cause neuronal loss without concurrent microcirculatory dysfunction. PTN knockdown in the context of focal stroke or NMDA-induced excitotoxicity, however, resulted in worsened brain injury. Together, these findings suggest that capillary pericytes are a major producer of PTN and that pericyte loss in the adult brain produces a dual hit of microcirculatory failure and PTN loss that culminates in neurodegeneration.

With exciting results come a host of new questions and directions. Does the extent of pericyte loss induced in this study mimic the nature of pericyte loss in Alzheimer's disease? The pathology described here seems too rapid for chronic small-vessel disease. Yet one can also argue that the time course of pericyte loss in humans is unknown and could very well involve waves of rapid cell death initiated by vascular disease. A related question is whether a slower-paced pericyte loss can be achieved using the DTX approach, allowing time for mechanisms of pericyte repair and recovery to factor in[13], as they likely do in pathology. Further, can PTN expression be augmented

in pericytes for vascular protection? Could PTN in the cerebrospinal fluid serve as a useful biomarker of pericyte loss in humans? Finally, are pericytes the predominant source of PTN in the healthy brain, and if so, is this still the case following brain injury? Indeed, other cells also express PTN basally, and there is evidence that microglial cells and astrocytes augment PTN expression during ischemia, likely in direct response to oxygen and/or glucose deprivation[11,14].

Regardless of these open questions, Nikolakopoulou and colleagues[9] have provided cerebrovascular researchers with a potentially game-changing tool to dissect mechanisms of pericyte function in adulthood following normal neurovascular development. They have also reminded us not to develop tunnel vision when studying blood vessels in the brain. The microvasculature is not simply a conduit to supply blood, but possibly also an elaborate distribution network for protective factors ❐ that support neuronal health.

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Published online: 24 June 2019
https://doi.org/10.1038/s41593-019-0437-9

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Competing interests
The authors declare no competing interests.

NEURODEGENERATION

A traffic jam leads to Lewy bodies

Shahmoradian and colleagues report that the structure of Lewy bodies in Parkinson's disease consists of α-synuclein and lipid vesicle clusters instead of the long-assumed amyloid fibril core. This finding has implications for our understanding of the underlying pathogenesis of synucleinopathies.

Tim Bartels

What goes awry when we develop brain diseases? Research into the pathogenesis of neurodegenerative disorders typically focuses on protein misfolding and aggregation. The pathological hallmark of these disorders is focal accumulation of a specific protein—amyloid-β in Alzheimer's or α-synuclein (α-Syn) aggregation in Parkinson's disease (PD)—that has a propensity to aggregate and deposit in the brain. For most neurodegenerative diseases, these protein inclusions are thought to consist of amyloid fibrils, rod-like protein structures with a predominantly β-sheet fold. Assembly of these proteins into amyloid fibrils is thought of as the toxic event driving cell death. For PD, structural analyses of its pathological hallmarks, the Lewy body in the soma of a cell and the Lewy neurite along its processes, have revealed a dense accumulation of α-Syn (ref. [1]) consisting of amyloid fibrils[2]. These findings have driven therapeutic and diagnostic research over the last two decades toward studying the α-Syn amyloid formation.

In their structural analysis of Lewy bodies in this issue of *Nature Neuroscience*, Shahmoradian et al.[3] now take a fresh look at brain tissue of patients with PD and find an unexpected answer to the question of what makes up a Lewy body. Using correlative light and electron microscopy (CLEM), they show that the vast majority of Lewy bodies actually consist of clusters of various membranous compartments, instead of amyloid fibrils as previously assumed. The indication of vesicle trafficking defects and the absence of fibril formation in the pathology of PD has several important implications for future research of disease mechanisms.

Traditional structural analysis of Lewy bodies relied on light microscopy of α-Syn accumulation via immunohistochemical staining[4]. These techniques lack the resolution to provide structural information on the basis of focal accumulation. By contrast, ultrastructural techniques like electron microscopy (EM) are mainly guided by visual inspection of anticipated structures. Given the absence of protein specific markers that would indicate all points of interest, only spots that showed characteristic rod-like structures were analyzed in the past, assuming that both microscopy and EM were looking at all present α-Syn inclusions. The important new findings of Shahmoradian et al.[3] stem from a barrage of cutting-edge imaging techniques that the authors employed to look in detail at the Lewy bodies and Lewy neurites in the brains of patients with PD.

The authors identified α-Syn aggregates by immunohistological staining and, using the CLEM technique, directly conducted EM ultrastructural analysis of the same inclusion. This allowed them to now probe what an average, classically defined Lewy body or neurite looks like at the EM level. Via this approach, and extending it using serial block-face scanning EM, the authors identified various vesicle clusters coated with high local concentrations of non-fibrillar α-Syn molecules in the core of a Lewy body, explaining the punctate appearance of α-Syn staining in past neuropathological analysis. Unexpectedly, only about 20% of all Lewy bodies and neurites appeared to have large amyloid fibrils associated with them, indicating that α-Syn amyloid formation is not as integral to PD pathology as formerly believed.

Interestingly, morphological analysis led to the identification of various lysosomal vesicles associated with the core and further