

## Different strokes for rodent folks

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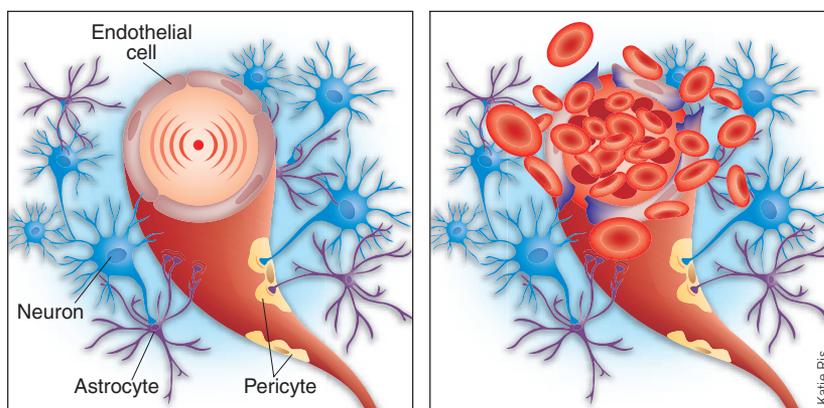
Understanding of the sequelae of cerebral microvascular injury has been hampered by a lack of animal models to enable precise localization of injury. In this issue, Nishimura *et al.* describe a stroke model that couples two-photon laser-scanning mapping of the cerebral cortex with femtosecond laser technology to produce three distinct microvascular injuries characterized by hemorrhage, vessel leakage or vessel occlusion.

The decade of the 1990s produced considerable advances in the molecular understanding of neuronal cell death after stroke<sup>1</sup>. Intricate intracellular pathways were delineated, involving overexcitation of neurons, oxidative stress and apoptosis<sup>2</sup>. However, so far, clinical trials targeting these specific neuronal pathways have failed.

A paradigm shift has taken place in the past 2–3 years. Instead of a pure focus on the neurobiology of stroke, an emerging consensus has postulated that an integrative approach to the entire neurovascular unit is required. Normal brain function is mediated by cell-cell and cell-matrix interactions between all components of the neurovascular unit, comprising

neurons, glia and the vasculature (**Fig. 1**). Consequently, brain disease is manifested as perturbations in homeostatic signaling in the neurovascular unit. The utility of the ‘neurovascular unit’ idea is now apparent. For example, caveats regarding the use of the thrombolytic agent tissue plasminogen activator can now be understood, because tissue plasminogen activator acts not only in blood as a clot-lysis agent but also as a neurovascular unit matrix protease and signaling agent in neurons and microglia<sup>3</sup>. Similarly, the putative efficacy of activated protein C in stroke may also be explained in part by its pleiotropic actions at multiple levels of the neurovascular unit<sup>4,5</sup>. The emphasis on the neurovascular unit places a premium on techniques for ‘dissecting’ its function in live animals. New tools capable of simultaneously imaging and perturbing the neurovascular interface are required.

In this issue of *Nature Methods*, a considerable new advance in the study of cerebral microvascular injury is described by Kleinfeld and colleagues<sup>6</sup>. The authors have used femtosecond laser pulses to generate precise cerebral vascular injuries. Tissue injury resulting from femtosecond pulses of laser light differs from injuries caused by constant-wave or long-pulsed lasers in two fundamental ways. First, femtosecond laser pulses do not result in a thermal effect, thus the injury is not secondary to the heating of tissue. Second, the participation of nonlinear processes enabled by the localization of photons in time and space in tightly focused pulses results in high peak intensities that reduce the energy threshold for tissue injury and minimize direct damage to adjacent tissue<sup>7</sup>. These characteristics make precise localization of the injury readily achievable<sup>8</sup>. A second technical development on which these investigators capitalize is the use of *in vivo* two-photon laser-scanning microscopy<sup>9</sup>. This approach allows the investigators to construct detailed three-dimensional maps of the cerebral microvasculature by stacking multiple optical slices. By coupling a sophisticated mapping strategy with precise photodisruption of vessels, the investigators control



**Figure 1** | Femtosecond laser pulses induce precisely localized cerebral microvascular injury. The neurovascular unit includes neurons (blue), glial cells such as astrocytes (purple), endothelial cells (gray) and pericytes (yellow). A train of femtosecond laser pulses is directed at the center of the blood vessel and is thought to induce injury by generating a cavitation bubble and ‘shock wave’ (left). Depending on the intensity and sequence in which the pulses are delivered, it is possible to cause a vessel hemorrhage, extravasation or occlusion. Right, a hemorrhagic lesion with blood cells released from a vessel.

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both the location and the nature of lesions generated by laser ablation.

Not all strokes are the same. Blood vessels can be occluded (ischemia) or ruptured (hemorrhage) or they may be leaky (edema). A principal challenge in research is to create reproducible models of the different types of strokes. Here the authors have observed three different types of microvessel perturbations depending on how they apply the femtosecond laser pulses. The delivery of a train of pulses with an average energy per pulse of 0.8  $\mu\text{J}$  uniformly results in vessel rupture with hemorrhage (Fig. 1). In contrast, when they deliver pulses of lower intensity (0.1–0.5  $\mu\text{J}$ ), there is extravasation of a fluorescein isothiocyanate–dextran tracer in the absence of erythrocyte loss approximately 80% of the time. A third type of injury is made by delivery of a single train of laser pulses capable of inducing extravasation, followed by the delivery of additional laser pulses of increased energy until blood flow stops. Using this method they are able to elicit occlusion 60% of the time. In addition, blood flow is monitored by line scans along single vessels. This approach provides mapping of blood flow dynamics in microvascular networks.

The main promise of this new technique is that individual components of the neurovascular unit can potentially be targeted. What happens to neuronal function when the microvessel is lesioned? What happens to microvessel dynamics when astrocytes are knocked out? The imprecision of older cerebral vascular injury models precluded the possibility of such discrete ‘dissection’ of the neurovascular unit. Of course, the use of femtosecond laser pulses to induce injury is not without limitations. The insult is nonphysiological and the mechanism of injury is not well understood or characterized at the cellular level. For example, the occlusive lesion generated by this technique contains fibrin but is not sensitive to tissue plasminogen activator. Thus, its nature is not apparent. Nonetheless, the precision of the injury coupled with the resolution of the two-photon laser-scanning microscopy enables unprecedented control in the perturbation and monitoring of the neurovascular unit *in vivo*.

Another advantage of this technique is afforded by the ability to map blood flow relatively deep in the brain. The cerebral microvascular is inherently three-dimensional and demonstrates high

connectivity. It has been difficult to predict the quantity and spatial relationship of microvascular lesions that will result in ischemia. The ability to monitor flow dynamics as described in the article by Nishimura *et al.* will allow investigators to quantitatively assess the effects of specific patterns of vascular occlusion on blood flow and ischemia. The susceptibility of different microvascular beds to ischemia may differ by location. Indeed, the authors suggest that occlusion of deep microvessels results in a substantial reduction in flow in ‘downstream’ vessels, whereas occlusion of superficial vessels results in only a minor compromise of ‘downstream’ flow. The methodology described in this article will allow for systematic evaluation to determine how the anatomical locale and depth of a microvascular bed

influence its response to occlusive insults. Such analysis could identify areas that are at high risk for ischemia. These issues and others, previously refractory to investigation, can now be addressed using the versatile stroke model described by Kleinfeld and coworkers.

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## NMR mapping of protein interactions in living cells

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Intracellular protein–protein interactions form the basis of most biological processes. Structural aspects of these reactions can now be analyzed in living prokaryotic cells and in atomic detail by nuclear magnetic resonance spectroscopy.

Almost all biology has evolved within the boundaries of cellular structures, and every intracellular space represents a highly crowded, viscous solute that harbors an intricate network of biological activities simultaneously exerted by a vast number of macromolecules. Yet biochemical and structural investigations of biomolecules are typically confined to artificial, dilute, isolated *in vitro* experimental setups.

Departing from these limitations, in this issue of *Nature Methods*, Burz *et al.*<sup>1</sup> report a new application of in-cell NMR spectroscopy, ‘STINT-NMR’, which provides a means of analyzing the structural changes that accompany protein–protein interactions *in vivo* and at atomic resolution.

To allow the study of biomolecules in their natural environment (that is, in cells), recent attempts have aimed to develop new *in vivo* techniques for structural biology

and cellular imaging<sup>2</sup>. X-ray crystallography and high-resolution electron microscopy are intrinsically excluded from *in vivo* approaches because of their requirement for pure samples and crystalline or vitrified specimen. Nuclear magnetic resonance (NMR) spectroscopy, as the only other method for the structural investigation of biomolecules at the atomic level, allows for the direct observation of ‘NMR-active’ atomic nuclei in any NMR-distinguishable environment and can thus be used to investigate labeled proteins structurally *in vivo* and inside cells<sup>3</sup>.

High-resolution NMR measurements in living prokaryotic cells have gained popularity because they allow the collection of both qualitative structural<sup>4</sup> and quantitative dynamic<sup>5</sup> information on proteins in the context of intact living cells. All applications of in-cell NMR spectroscopy so far

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