

## Laser lesions simulate microvessel stroke

Researchers may soon be looking at stroke in a new light: ultrashort laser pulses targeted on tiny, subsurface, cortical blood vessels have successfully been used in rats to induce precise lesions that simulate three types of microvascular accident (*Nat Methods* 2006; **3**: 99–108).

The microvessels of the cerebral cortex are a common site of silent strokes—events that can contribute to cognitive dysfunction, dementia, and gait problems, etc. However, understanding their pathology and the testing of potential treatments have long been hampered by a lack of suitable models.

“Those [models] available relied on surgical interventions that could never really localise an induced stroke very well”, explains David Kleinfeld (University of California, San Diego,

CA, USA), leader of the team that developed the new technique. “With our method, we can not only induce a lesion in a microvessel within the cortex with pinpoint accuracy, we can also produce different lesions mimicking different types of stroke.”

The new method relies on the delivery of pulses of laser light to a target microvessel whose position has been precisely mapped by two-photon laser scanning microscopy techniques (TPLSM). “TPLSM allows you to take ‘slice images’ of the brain and build up a 3D map of the microvessels”, explains Kleinfeld. “After opening a viewing window in the animal’s skull, a fluorescent dye is injected into the bloodstream and planar images taken at different depths in the exposed cortex. Gradually you build up a stack of images of fluorescing microvessels, giving you a very accurate 3D map of their trajectories.” This map then helps pinpoint a short burst of laser pulses each lasting just 100 fs. The accuracy with which the beam can be delivered into the cortex means the target vessel is selectively damaged.

However, the researchers found they could do more than just select what they hit; by varying the energy and number of pulses they also found they could induce different types of lesions. “High intensity pulses rupture vessels causing the dye to visibly spill out in

TPLSM images”, explains Kleinfeld. “Using a lower energy can cause extravasation of blood components, and when you irradiate for longer at low energy levels you can induce a clot to form. Basically the technique allows you to decide what kind of stroke you want to [simulate] at exactly the point where you want it.”

After inducing a clot, the team used TPLSM to confirm that blood flow downstream was affected and that neither alteplase nor heparin had any effect. Haemodilution with saline, however, did increase blood flow. “This mimics the clinical results commonly seen”, explains coauthor Patrick Lyden, “suggesting this model is valid.”

“The precision of the induced lesion site and the variety of lesions that can be induced is impressive”, remarked Richard Frackowiak (University College, London). “One question that remains is whether rodents [can] provide information of relevance to humans. The history of this subject is that it is unlikely. Nevertheless much may be understood about the local biochemical, immunological, and other inflammatory responses of the brain to ischaemia in vivo. It is also true that this is one of the first small vessel models that is easily controlled.”

Adrian Burton

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Microvascular lesions mimicked with mouse and laser technique

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## From stem-cell to dopamine neuron: the molecular make-up

A newly discovered molecular programme that controls the generation of dopamine neurons during development might enable the engineering of cells for replacement therapy to treat Parkinson’s disease (PD).

The lead investigators, Thomas Perlmann and Johan Ericson (Karolinska Institute, Stockholm, Sweden) explained to *The Lancet Neurology* “we have identified two transcription

factors, *Lmx1a* and *Msx1*, that function very early in the normal development of the CNS in the specification of midbrain dopamine neurons. This basic developmental process is generally highly conserved between species. Thus, we are convinced that the human versions of these transcription factors will also be intrinsic determinants during the development of this neural population in humans”.

In *Msx1* knock-out mice, dopamine neurons do not develop properly. However, when artificially expressed in the midbrain of chick embryos, only *Lmx1*, but not *Msx1*, induced the extensive generation of extra dopamine neurons. Furthermore, the elimination of *Lmx1* resulted in notable reductions in both *Msx1* concentrations and the number of neurons (*Cell* 2006; **124**: 393–405).