

# TARGETED MICROVASCULAR DISRUPTION IN RAT CORTEX USING ULTRASHORT LASER PULSES

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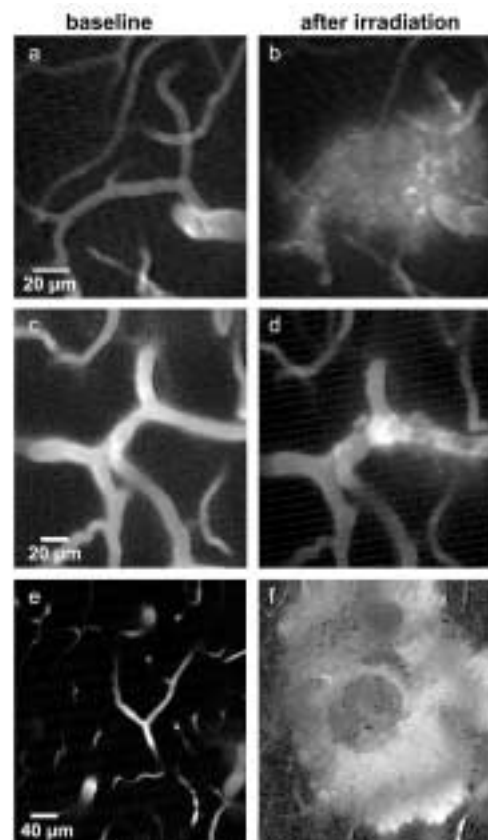
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We describe a model of microvascular injury in rat neocortex that enables the disruption of individual cortical vessels. Ultrashort laser-induced photodisruption is used to produce one of three types of vascular damage: blood brain barrier failure, ischemic clots, and hemorrhages. This method enables the three-dimensional targeting of damage to single microvessels that lie up to 500  $\mu\text{m}$  below the cortical surface. Our method leaves the surrounding tissue intact, allowing the progression of pathology in the local neuronal tissue to be studied. We monitor the formation and progression of these vascular insults, as well as the local blood flow in specific vessels, with *in vivo* two-photon laser scanning microscopy (TPLSM).

A craniotomy is performed over parietal cortex of urethane-anesthetized rats. To visualize the vasculature by TPLSM, the blood plasma is labeled with an intravenous injection of fluorescein-dextran. After mapping the vasculature and selecting the target vessel, a train of 1-kHz, 100-fs, 800-nm laser pulses is focused into the lumen of the vessel. One of three types of vascular injury is selected by varying the laser pulse energy (0.1 to 10  $\mu\text{J}$ ) and the number of pulses (2 to 10). The precise localization of the target enables us to study structural and biochemical effects on the immediately surrounding tissue by *in vivo* TPLSM and post-mortem histology.

Examples of the three types of vascular damage are shown by *in vivo* TPLSM images in Fig. 1. A blood brain barrier failure is seen as an extravasation of fluorescein-labeled blood plasma (Figs. 1a and b). This results in the exposure of parenchymal tissue to blood constituents without ischemia or the cessation of blood flow in the vessel. Figures 1c and d show an ischemic clot in which the target vessel is occluded. At higher laser pulse energies ( $\sim 5 \mu\text{J}$ ) the target vessel is ruptured and red blood cells invade the parenchyma, resulting in a hemorrhage (Figs. 1e and f).

The blood brain barrier failure and the ischemic clot are potential models of vascular pathologies related to clinically observed silent strokes, lacunar strokes, and cognitive deficits.



**Figure 1.** TPLSM images of cortical microvessels and vascular injury induced in the target vessel by ultrashort laser pulses. (a) Before and (b) after blood brain barrier failure. (c) Before and (d) after ischemic clot. (e) Before and (f) after hemorrhage.