Targeted single blood vessel occlusion in rodent pial arteries to study astrocytic-vascular dysfunction in a mini stroke model

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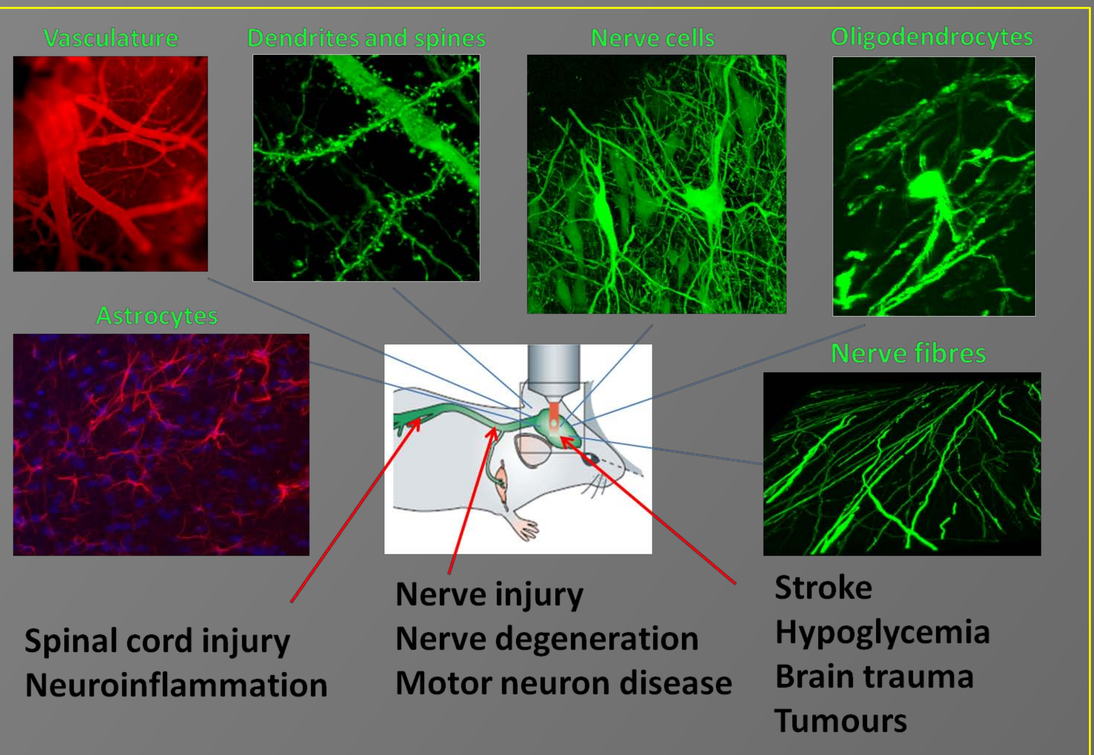
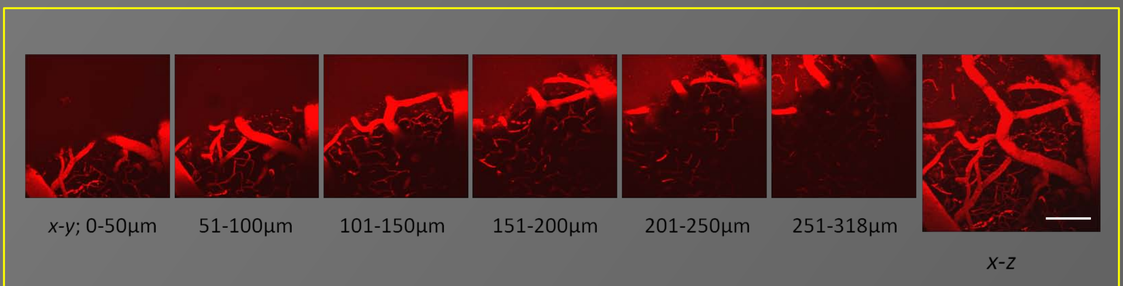
INTRODUCTION

The ability to form targeted vascular occlusions in small vessels of the brain is an important technique to study the microscopic basis of cerebral ischemia. We utilize a method that enables the targeted occlusion of any single blood vessel within the upper 350 µm of mouse neocortex to generate highly localized regions of ischemia by blocking capillary or surface arteries. This method makes use of linear optical absorption by a photosensitizer, transiently circulated in the bloodstream, to induce a clot in a surface or near-surface segment of a vessel after activation with a green 532nm continuous laser. Using two-photon microscopy of green fluorescent protein-labeled astrocytes (GFEC) and 70-kDa Texas Red dextran-labeled blood flow, we explore the alteration of the spatial relationship between cortical microcirculation and astrocytic endfeet structure in vivo during ischemia. This two-photon imaging method allows extremely high spatial and temporal resolution for studying pathological mechanisms that underlie ischemic injury, including abnormal changes in cell signaling and structure, vascular dysfunction, and inflammation. We aim to identify the cellular basis of cerebrovascular dysregulation in transgenic animal models of neurological disease and identify molecular targets for the pharmacotherapy of these pathological pathways.

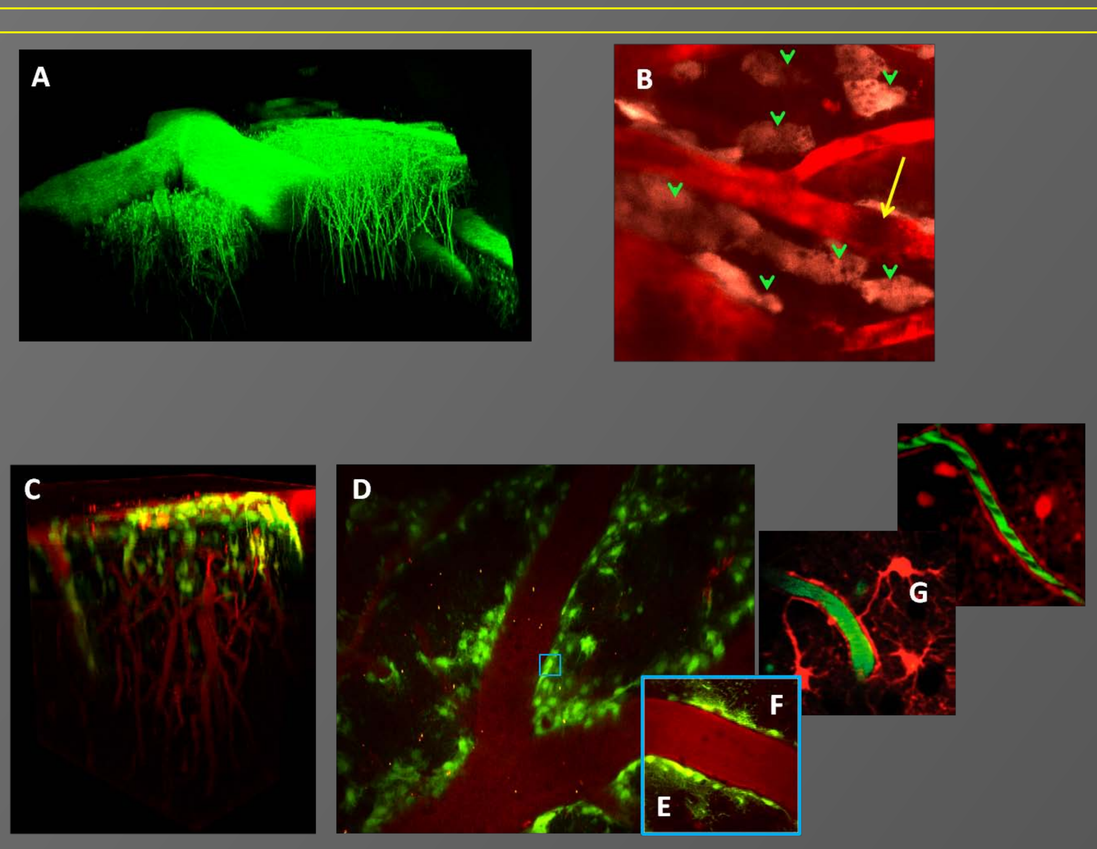


Studying the cellular architecture of the brain *in vivo*

Numerous parts of the nervous system can be visualized *in vivo*, including the cortex, cerebellum, olfactory bulb, retina, spinal cord, peripheral nerves and autonomic ganglia. There are transgenic labels for tracing many disease-relevant structures in the nervous system,including neurons,axons,glia,oligodendrocytes, blood vessels and immune cells. Intravital two-photon imaging in transgenic mice with cell-specific expression of fluorescent labels is an important tool to study the ionic, cellular and vascular dynamics in disease states as outlined above.

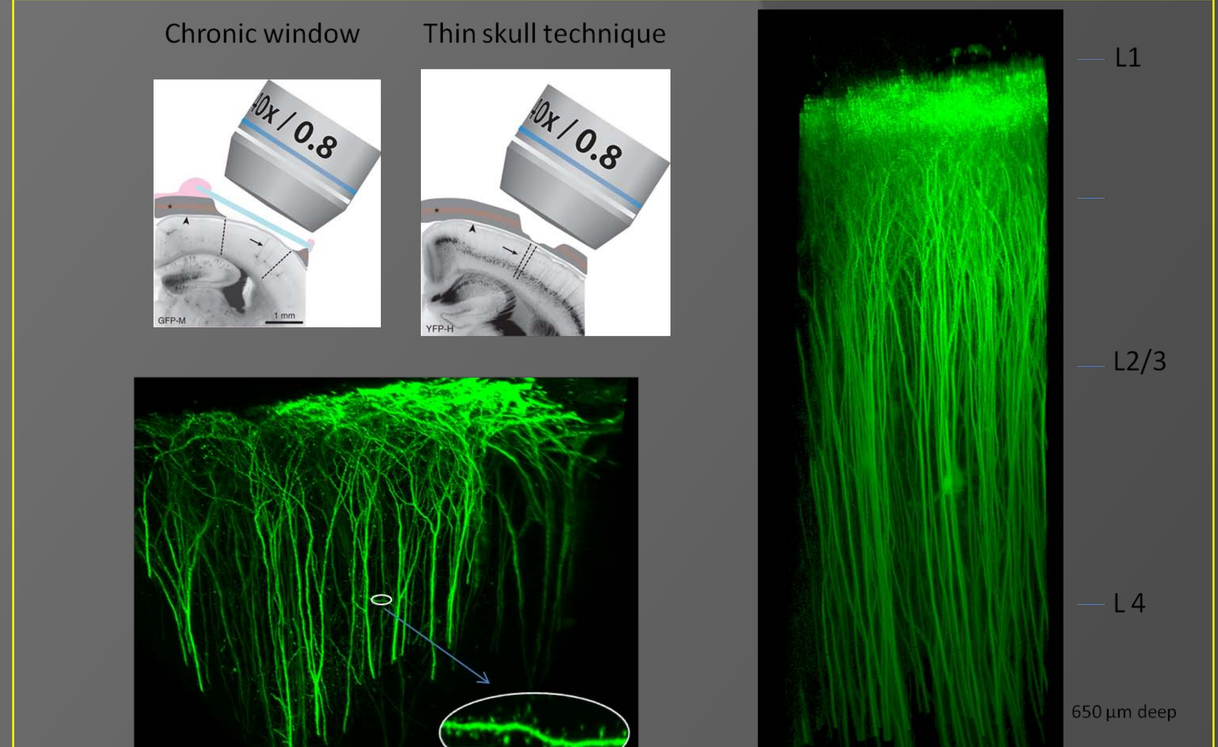


Maximum z-axis projections below the thin-skull preparation across 20-250µm of Texas red -conjugated dextran-filled vasculature, 60 d after surgery (average of 5 frames; dwell time, 6µs pixel^{-1}; and average power, 30-45mW). The last frame shows the projected z-stack in 3-D of all the collected images.Scale bars, 100µm.



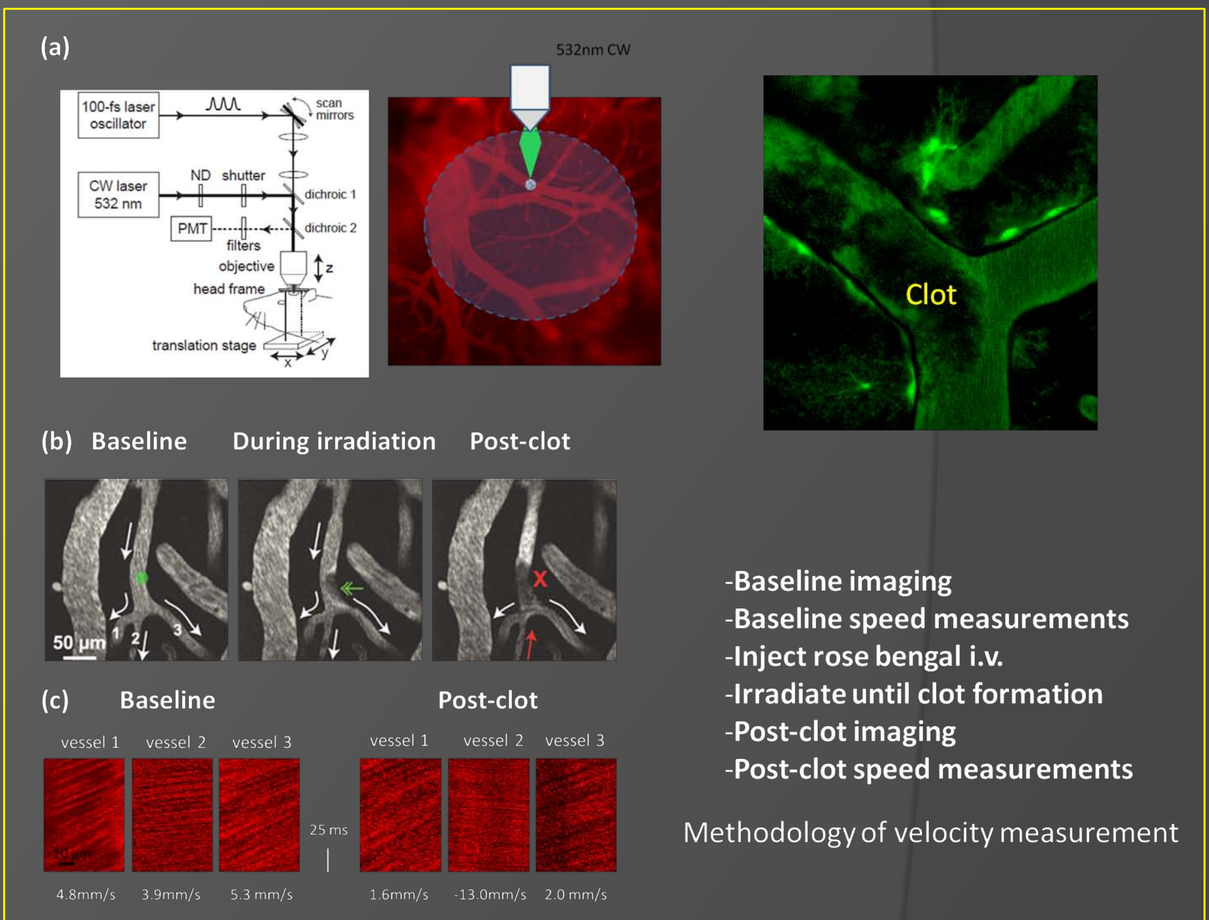
Examples of *in vivo* fluorescence imaging of the neurovascular unit

(A) A 3-D reconstructed image showing the entire vasculature and neurons through a 1mm^2 cranial window from a YFP-H transgenic mouse brain. The vasculature was labeled with FITC-dextran dye that labels the plasma and outlines the pial vasculature. imaged under two-photon microscopy. **(B)** 30 minutes of ischemia from a thrombosed artery (yellow arrow) shows evident signs of astrocyte injury in the vicinity of the injured blood vessel. Extensive cytoplasmic vacuoles and irregularity of structure (arrowheads) is marked in the labelled (arrowheads) astrocytes. **(C)** Intravital staining of microvessels and astrocytes by i.v of Texas red dextran (red) into the blood plasma from GFEC astrocyte-specific transgenic mice showing high resolution imaging up to a depth of 320µm. **(D)** Astrocyte-vasculature interaction as visualized on the brain surface from EGFP-labelled astrocytes using the above procedure. **(E-F)** High resolution images showing enwrapped astrocytes on a blood vessel after topical application of SR101 on the dura **(G)** Fast image acquisition showing red blood cells moving inside an artery.



In vivo two-photon imaging in the intact neocortex

Different types of brain access. Open cranial window with the *dura mater* removed so that micropipettes for cell labeling and electrophysiological recordings can be inserted (left). Pulsation of the exposed brain is reduced by covering the craniotomy with agar and a coverglass. Thinned-skull (20–40µm thickness) preparation. Cellular structures are either prelabeled (for example, with fluorescent proteins in transgenic mice) or stained through a tiny hole lateral to the thinned area. Chronically implanted glass window replacing the skull (bottom). Agar is used underneath the window for stabilization. Images show examples of deep two-photon imaging in mouse neocortex. Maximum-intensity side projection of a fluorescence image stack, obtained in a transgenic mouse expressing YFP. Data were taken with a 10 W pumped Ti:sapphire oscillator using a 25X, NA 1.2 water-immersion lens (Olympus). Note that nearly the entire depth of the neocortex can be imaged.



-Baseline imaging
-Baseline speed measurements
-Inject rose bengal i.v.
-Irradiate until clot formation
-Post-clot imaging
-Post-clot speed measurements

Methodology of velocity measurement

Photothrombotic clotting of targeted surface blood vessels mantains a level of blood supply by reversal of flow at junctional branches

a) Schematic illustration[1] of the targeted photothrombotic occlusion of a vessel and experiment timeline. After baseline imaging and blood flow measurements, rose bengal is intravenously injected into the animal. Green laser light is focused onto the wall of the target vessel, which excites the rose bengal and ultimately triggers the natural clotting cascade.

b) Planar TPLSM images of photothrombotic clotting of a surface arteriole. The green circle indicates the region of the targeted arteriole that was irradiated, whereas the white arrows indicate the blood flow direction, as determined from line-scan measurements in the targeted vessel and in the vessels downstream from the target. The numbers over the downstream vessels correspond to the numbered line-scan data shown. The streaked appearance of the vessels is due to the motion of RBCs during the acquisition of the image[2]. The center frame is taken after an intravenous injection of rose bengal and 2-min irradiation with 0.5 mW of 532-nm laser light. The vessel is partially occluded (indicated by green double arrow). The right frame is taken after one more minute of irradiation. The target vessel is completely clotted (indicated by red X) whereas surrounding vessels are unaffected. Stalled blood flow is seen by the dark mass of clotted cells in the target region and the brightly fluorescent region of stagnant blood plasma upstream from the target region.

c) Blood flow is maintained in the branches downstream from the occluded vessel by a reversal in the direction of blood flow in the center branch, as determined from the line-scan data. Baseline and post-clot line-scan data for the numbered vessels is indicated. The average RBC speed determined from the line-scan data is indicated for each case, with a positive speed taken to be along the baseline direction of flow.

CONCLUSIONS

- **2-PTM enables analysis of the structure and dynamic activity of different cell types in the brains of living animals at high temporal and spatial resolution.**
- **We show that *in vivo* fluorescence microscopy can be utilized to videomonitor local cerebral microcirculation under control and ischemic conditions, and that focal ischemia through a cranial window leads to reproducible injury, which can be explored in longitudinal studies.**
- **This model is relevant to thromboembolic stroke in humans, also because spontaneous reperfusion of arteries occurred in most experiments.**

References

[1] Journal of Neuroscience Methods,170 (2008); 35-44.
[2] Imaging in Neuroscience, Rafael Yuste, editor. 2011.

Acknowledgements

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