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Training stay from 21st of July 2014 to the 20th of September 2014

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Metabolic interactions between neurons and astrocytes: role in neurodegenerative diseases
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REPORT TRAINING STAY

Both abnormal brain glucose utilization and hypoperfusion have been described in Alzheimer's disease (AD) patients using Positron Emission Tomography (PET). Besides being a biomarker of AD, energy metabolism and blood flow are essential to maintain brain homeostasis, tissue integrity and synaptic activity. Although early metabolic/vascular alterations in AD are likely to initiate, or at least could contribute later neurodegenerative processes, the mechanisms responsible for such deficits are not yet characterized.

We use 3xTg-AD mice, an animal model of Alzheimer's disease (AD) mimicking numerous hallmarks of the disease, to elucidate the cellular/molecular origins of those metabolic/vascular alterations using multi-scaled imaging tools.

The purpose of my internship in Zurich was to monitor blood flow in those 3xTg mice using two different methods that are not available in my laboratory:

- laser speckle imaging (LSI) and multiwavelength spectroscopy to measure blood flow changes during whisker stimulation in the barrel cortex area
- 2-photon (2P) imaging to quantitatively measure basal blood flow in multiple blood vessels

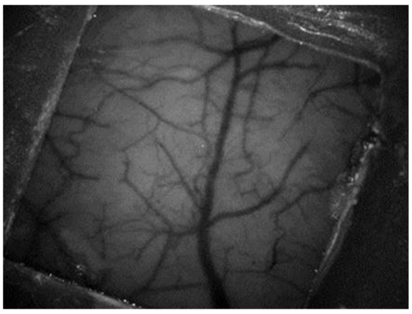
Preparation of the mice

Under anesthesia, a headpost is placed to allow for the fixation of the mouse in a frame compatible with the use of the microscopes. Then, a thinned-skull preparation (adapted from the protocol described by Shih *et al*[1]) is performed to allow imaging of the barrel cortex vasculature.

Laser speckle and multiwavelength spectroscopy imaging

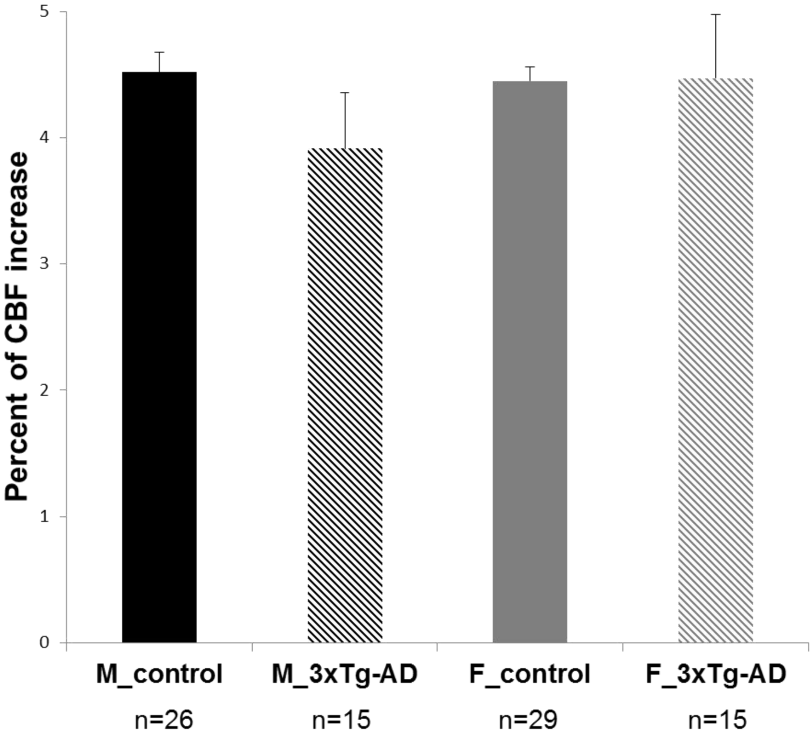
The mice were allowed to recover for a week before starting the imaging. We measured cerebral blood flow (CBF), oxygenated-hemoglobin and total-hemoglobin fluctuations during electrical whisker pad stimulation. We determined the protocol of stimulation based on the vascular response to the stimulus (intensity, duration, frequency). Thanks to the thinned-skull preparation, mice can be imaged several times. Four groups of 6-month old mice were imaged (male control, male 3xTg-AD, female control and female 3xTg-AD).

Results showed that there is no significant difference among the groups at this age for increased CBF, oxygenated-hemoglobin or total-hemoglobin.



Chronic window 2 weeks post-surgery

Mouse prepared for LSI imaging

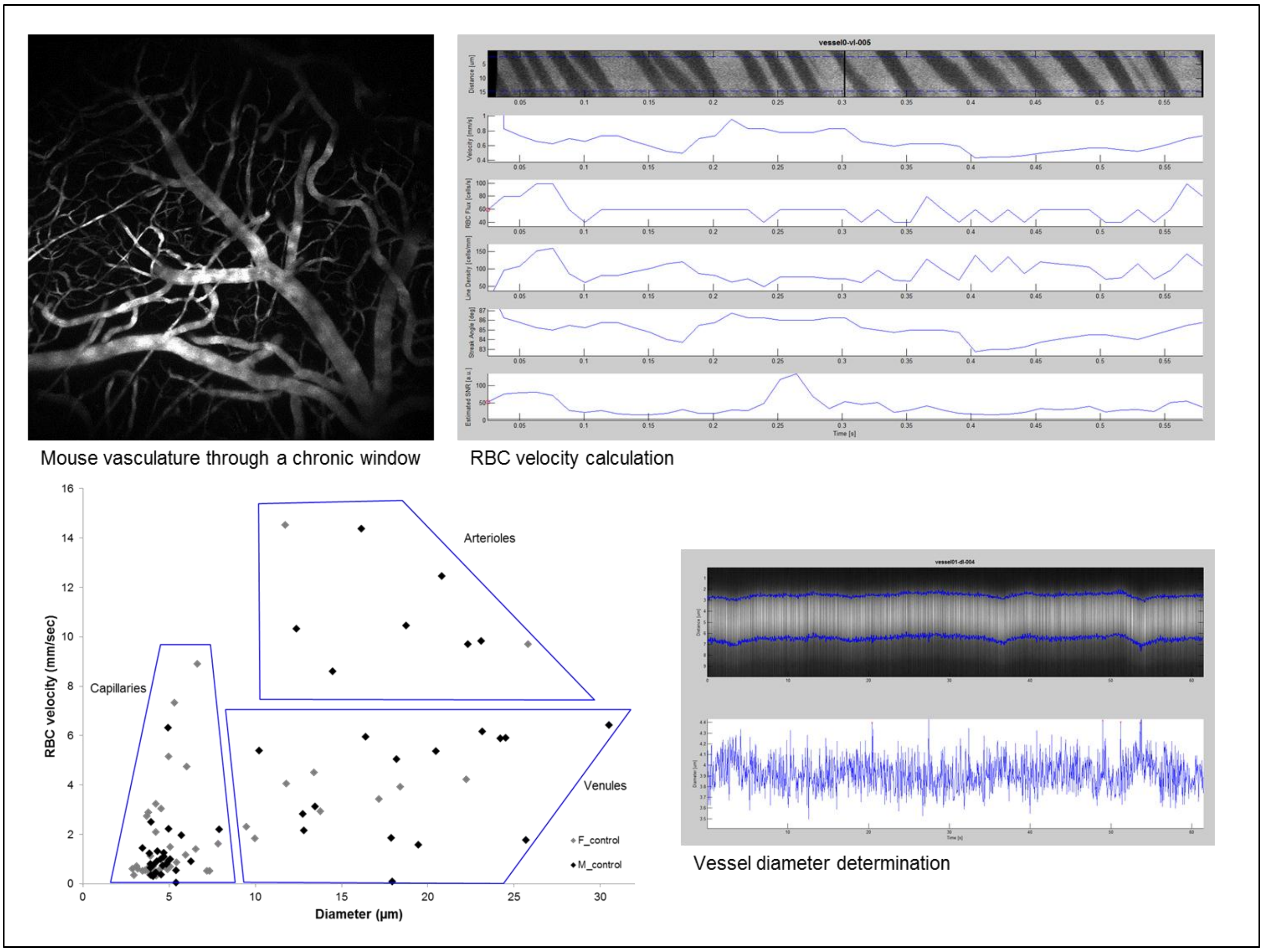


Two-photon imaging

A catheter was inserted in the tail vein and a fluorescent dye (Texas Red Dextran) was injected before starting 2P-imaging. The diameter of the vessels and the red blood cells (RBC) velocity was determined using a Radon transform-based algorithm similar that the one used by Drew *et al*[2]. This method allows us to determine the RBC velocity according of the diameter of different vessel populations (capillaries, venules and arterioles). Up to now, only the control mice were imaged but I will go back to Bruno Weber's lab for a couple of days in November to image 3xTg-AD mice.

Conclusion

I am very satisfied with my training since I have learned how to perform the surgical preparation of the mouse to allow access of the cortex for in vivo imaging and also because I was trained on the use of two different techniques that are complementary and very useful for recording blood flow. My internship also strengthened the collaboration between the two labs.



Bibliography

1 Shih, A. Y., Mateo, C., Drew, P. J., Tsai, P. S. & Kleinfeld, D. A polished and reinforced thinned-skull window for long-term imaging of the mouse brain. *Journal of visualized experiments : JoVE*, doi:10.3791/3742 (2012).

2 Drew, P. J., Blinder, P., Cauwenberghs, G., Shih, A. Y. & Kleinfeld, D. Rapid determination of particle velocity from space-time images using the Radon transform. *Journal of computational neuroscience* **29**, 5-11, doi:10.1007/s10827-009-0159-1 (2010).