Analyzing the Morphology of the Neurovascular Network through Two-Photon Imaging Over time

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Introduction: Neurovascular dysfunction, caused by e.g. stroke, Alzheimer's, neuropathy, is a major cause of death and disability worldwide, however researchers have not yet well-characterized the plasticity of the neurovascular system. Therefore, there is a need for observing the morphology of the cortex microvasculature in a living animal model over time. Multiphoton Microscopy has enabled images up to one millimeter in depth into the mouse brain. Prior studies have shown that the morphology of the healthy adult mouse microvasculature in the motor cortex remained stable over months even in response to voluntary exercise (Cudmore, 2017)[1]. We have continued this investigation into neurovascular plasticity with the help of semi-automated vessel vectorization software.

Materials and Methods: We utilized two-photon microscopy (2PM), in vivo, three-dimensional images of adult mice microvasculature. To vectorize the vascular network (capillaries, descending arterioles/venules) depicted in the image, we used the Segmentation-Less, Automated, Vascular Vectorization (SLAVV) software. SLAVV simplifies vascular analysis by obtaining the centerline vector of every single vessel segment as well as all the branch points of these vascular networks. Vectorization also enables the reconstruction of the blood vessels in a three-dimensional model. Morphological characteristics of interest are extracted from the vectorized vessel network.

Results and Discussion: We captured 18 two-photon images (512x512x100) from three imaging sessions, each taken 2 weeks apart, to compare their morphological properties longitudinally. All imaging sessions observed the same 2x3 tiled field of view (~1 mm^3). Vessel strands were defined as non-bifurcating vessel segments beginning and ending at bifurcations or end-points, and Figure 1 shows distributions of strand statistics for the three-imaging sessions. The average radius and length statistics had stable distributions over the time period observed.

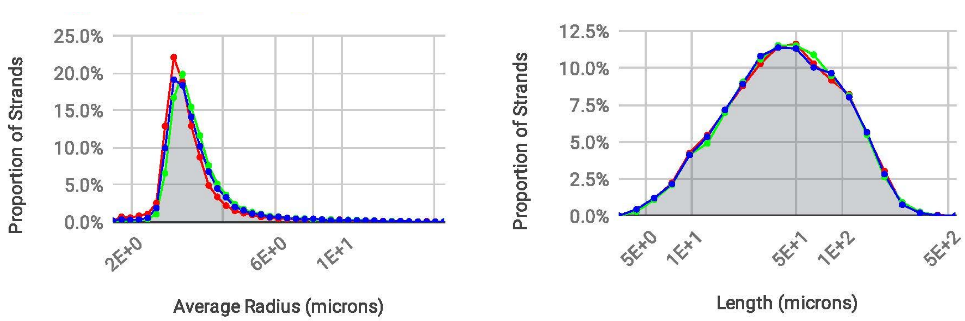

Figure 1: Red is week 1, green week 3, and blue week 5. **Left.** The average radius measured in microns for each strand vessel was consistent throughout the time period. **Right.** The length for each vessel strand was consistent throughout the time period

Conclusion: Our network statistical analysis agrees with the current hypothesis that vasculature changes do not happen frequently in the adult mouse brain. This establishes a baseline to compare to neurovascular networks affected by neurological diseases. Future studies include individual capillary strand-by-strand comparison and extending this study to stroke and diabetes disease models. We hope to elucidate the effects of common neurovascular diseases on the microvasculature in the living brain.

References:

[1]Cudmore RH. J. Cereb. Blood Flow Metlab. *Cerebral vascular structure in the motor cortex of adult mice is stable and is not altered by voluntary exercise*. 2017. *37*(12), 3725–3743.