Ultrafast ultrasound Doppler and confocal microscopy correlative approach: blood flow and vascular structure in adult wild type mice

Background, Motivation and Objective

An important goal in biology is to be able to reveal the structural composition of systems at different scales. In this work two different techniques were used to compare the vasculature of mice brain hippocampus. Ultrafast ultrasound Doppler (μD) reveals large scale vasculature by measuring the blood flow, while confocal microscopy (CM) reveals vessel structures.

Statement of Contribution/Methods

Experiments were conducted in 3 wild type (C57BL/6), 5-months-old, male mice. After craniotomy, each mouse was placed in a stereotaxic system. For μD, a 128 element, 15 MHz probe, driven by Verasonics Vantage System, was aligned to the coronal plane. Figure 1A shows a μD image obtained after SVD clutter filtering and averaging 350 compound images acquired at 1 kHz rate. For CM, the brain was fixed, cut into coronal vibratome sections and incubated with Isolectin GS-IB4 for vascular endothelial recognition. Figure 1C shows a CM image obtained in a tile scan modality (Zeiss 800). To quantify blood flow, each μD hippocampal image was segmented using the quartile cut-off values (Q1 to Q4) of their intensity distribution. To characterize the corresponding vascular structure, each hippocampus was analyzed using ImageJ software (3D counter) to extract the Vessel Volume Fraction (VVF). In Fig. 1E μD and CM images were superposed to visualize the structural coincidences.

Results/Discussion

Significant differences were found between all quartiles of the μD images (Fig.1B), and between the distribution of VVF ranges for CM (Fig. 1D). We found that high flow-rates (Q1 and Q2, Fig. 1B) and large VVF (>1 range, Fig. 1D), corresponds to the great ventral artery and the sulcal vein pathways. We conclude that blood flow measured by μD correlates to the vascular network and vessel distribution measured by CM. In addition, the overlap images (Fig.1E) showed visual coincidence between groups of small vessels seen in MC images, and big structures of blood flow seen in μD images, suggesting a functional organization of small vessels. Future work will focus on using this correlative approach to study changes due to physiology and unhealthiness. Moreover, functional ultrasound studies are being developed to establish ground for future experiments.

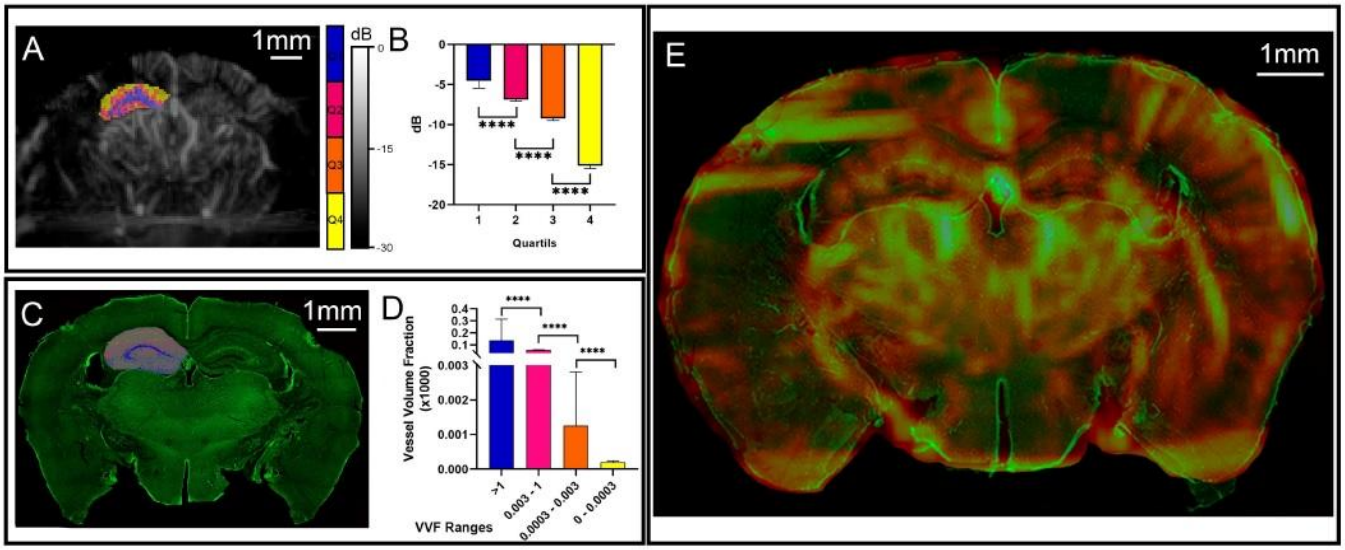

Figure 1. Flow analysis and vascular structure. A. μD image (gray scale) and hippocampus after quartile segmentation (color). **B.** Cut off value distribution for different quartiles of μD. [Q1 vs Q2]: Wilcoxon test, p<0. 0001, W=-465; [Q2 vs Q3]: Student-t test, p<0.0001, t=37.86, df=29; [Q3 vs Q4]: Student-t test, p<0.0001, t=40.85, df=29. **C.** CM image with the hippocampal mask obtained by 3D counter ImageJ-plugin. **D.** Distribution of VVF (x1000) for different VVF ranges. Man-Whitney U test: [>1] vs [1-0.003]: p<0,0001, U=0; [1-0.003] vs [0.003-0.0003], p<0,0001, U=0; [0.003-0.0003] vs [0.0003-0]: p<0,0001, U=0. **E.** Superposition of μD (hot colormap) image and CM (green colormap) image corresponding to the same coronal plane. Normality was evaluated by Shapiro-Wilk test. **** p< 0.0001.

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