Vascular changes due to ageing using Ultrafast Ultrasound Doppler combined with Scanning Laser Confocal Microscopy

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Background, Motivation and Objective

Ultrafast ultrasound Doppler (μD) is a novel in vivo imaging modality that measures cerebral blood volume (CBV) at microscopic scale. Conversely, Scanning Laser Confocal Microscopy (SLCM) is an elegant optical microscopy modality that reveals vascular structure with nanometer resolution. In this work, both imaging modalities were used to study vascular aging in mice hippocampi, a region of utmost importance associated with learning and memory processes which is affected by several neurodegenerative diseases.

Statement of Contribution/Methods

μD experiments were conducted in 12 wild type (C57BL/6) male mice of 5 months old (n=6) and 21 months old (n=6), from which ten were used in SLCM experiments. Animals were anesthetized for craniotomy and then placed in a stereotaxic system. For μD, a 128 element, 15 MHz probe, driven by Verasonics Vantage System, was aligned to the coronal plane. Each μD image was built from averaging 350 frames using a four angle compound sequence and SVD clutter filter. For SLCM, the brain was fixed, cut into coronal vibratome sections and incubated with Isolectin GS-IB4 for vascular endothelial recognition. To quantify blood flow, each hippocampal (left and right) μD image was segmented using the quartile cut-off values (Q1 to Q4) of the intensity distribution (Fig 1A). To characterize vascular structure with SLCM, each hippocampus was analyzed using ImageJ software (3D counter) to extract the Vessel Volume Fraction (VVF), i.e. the vessel volume normalized by the hippocampus volume. The VVF distribution was segmented into ranges aligned with the reserve capacity of veins-arteries, venules, arterioles and capillary vessels (Fig 1B). To assess differences between age, normally distributed parameters (i.e. ranges and quartiles) were compared using the unpaired Student's t-test, while non-normal distributions were compared using the Mann-Whitney U test.

Results/Discussion

Figure 1C shows that the mean quartile cut-off values are significantly higher in younger mice as compared to older ones. Significant differences in VVF between young and old mice (Fig. 1D) demonstrate a detriment of the vascular network that can be clearly associated with the decrease in μD quartile cut-off values, both of which are related to the normal aging process. This result demonstrates the sensibility of this combined approach to establish a structural-functional correlation and pave the way for studying other progressive (e.g., neurodegeneration) or induced pathological changes.

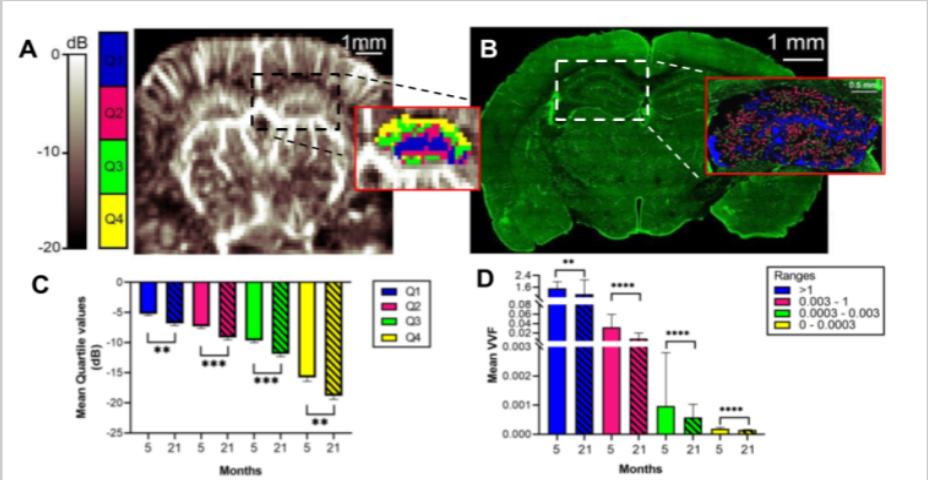

***Figure 1:** **(A)** μDoppler segmentation of the right hippocampus, overlaid with the quartile distribution of the CBV intensity. **(B)** Tile-scan image of a coronal section analyzed by SLCM; the vascular endothelium marked with GS-IB4 appears green. In color we show the distribution of the vessels identified by the 3D counter plugin. **(C)** Comparison with age of the quartile values obtained by μDoppler. 5-month-old vs 21-month-old: [Q1] p = 0.0019, t = 3.537, df = 22; [Q2] p = 0.0008, t = 3.872, df = 22; [Q3] p = 0.0005, t = 4.047, df = 22; [Q4] p = 0.0020, t = 3.504, df = 22). **(D)** Comparison with age of the mean VVF values obtained by SLCM. 5-month-old vs 21-month-old: [>1], p = 0.0023; U = 19535; [1-0.003], [0.003-0.0003], [0.0003-0], p < 0.0001, U = 2106914. ** p < 0.0021; *** p < 0.0002, **** p < 0.0001.*

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