Relationship between blood flow and vascular structure at hippocampal level is revealed by correlating ultrafast ultrasound Doppler and confocal microscopy

Maximiliano Anzíbar[1*], Mariana Martínez[2*], Lucía Vázquez[2*], Miguel Calero[3], Mickael Tanter[4], Carlos Negreira[1], Nicolás Rubido[1,5], Alejandra Kun[2+], Javier Brum[1+], [1]Instituto de Física, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay, [2]Lab Biología Celular del Sistema Nervioso Periférico, IIBCE-Facultad de Ciencias-UdelaR, Montevideo, Uruguay, [3]Chronic Disease Programme (UFIEC), Instituto de Salud Carlos III, CIBERNED, Madrid, Spain, [4]Physics for Medicine Paris, Inserm, CNRS, ESPCI, Paris, France, [5]University of Aberdeen, Aberdeen Biomedical Imaging Centre, Aberdeen, United Kingdom. *equal contributions, [+]corresponding authors

Background, Motivation and Objective

The hippocampus plays an important role in learning and memory, which requires high neuronal oxygenation, making its vascular network and physiology of utmost importance. Ultrafast ultrasound Doppler (µD) and confocal microscopy (CM) are powerful imaging modalities of the brain's blood flow and vascular structure, respectively. In this work, we apply both imaging modalities to study the relationship between blood flow and structure in mouse brain hippocampi.

Statement of Contribution/Methods

Experiments are conducted in 3 wild type (C57B/6), 5 months old, male mice. After craniotomy, each mouse is placed in a stereotaxic system. For µD, a 128 element, 15 MHz probe (Vermon) driven by Verasonics Vantage System is aligned to the coronal plane and moved along the anteroposterior axis. 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 is fixed, cut into coronal vibratome sections and incubated with Isolectin GS-IB4 (ThermoFisher) for vascular endothelial recognition. Figure 1B shows a CM image obtained in a tile scan modality (Zeiss 800). To quantify blood flow, each µD image of the hippocampus is segmented using the quartile cut-off values (Q_1 to Q_4) of their intensity distribution (Fig. 1A). To characterize vascular network, each tile stack is analyzed using ImageJ software (3D counter plug-in) (Fig. 1C) to extract the number and Vessel Volume Fraction (VVF) distribution (normalized by the hippocampal volume) as a function of the VVF.

Results/Discussion

We find significant differences between all quartiles of the µD images (Fig. 1D), and between the vessel number and VVF for CM images (Figs. 1E & 1F). Comparing these results, high flow-rates (i.e., Q_1 and Q_2) located in the hippocampus' center (Fig. 1A) relate to fewer vessels (Fig. 1E) but with large VVF (>1 range in Fig. 1F), corresponding to the great ventral artery and the sulcal vein pathways (Fig. 1C). We conclude that blood flow measured by µD correlates to the vascular network and vessel distribution measured by CM. Future work can focus on using this correlative approach to study changes in flow and vascular structure due to physiology (e.g. ageing) or unhealthiness (e.g. neurodegeneration).

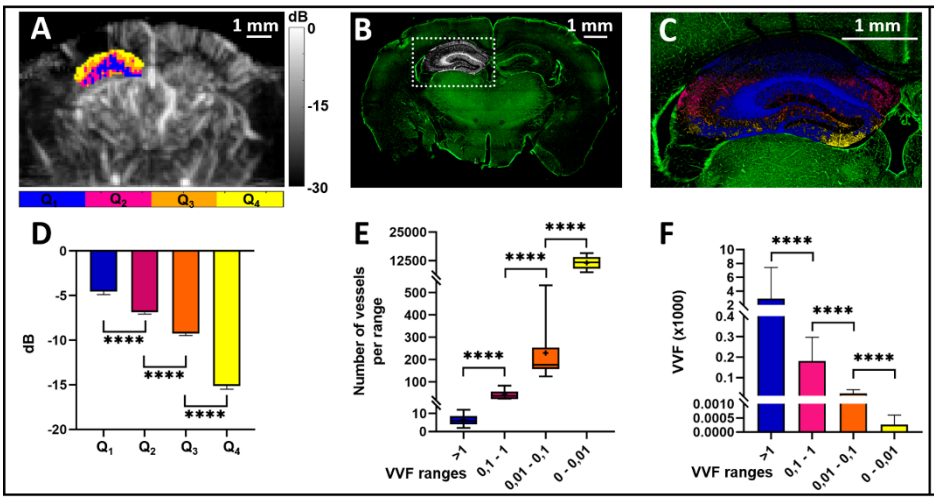

Fig. 1 A. µD image (gray scale) and hippocampus after quartile segmentation (color). **B.** CM image correlative to panel A with hipocampus highlighted in gray. **C.** Zoom of the white dashed reactangle in panel B. The vessels after ImageJ analysis are highlighted by different colors corresponding to each VVF range. **D.** Cut-off value distribution for different quartiles of µD. Normality was evaluated by Shapiro-Wilk test. Q_1 vs Q_2: MW-test, U=86; Q_2 vs Q_3: t-test, t=7.5, df=58; Q_3 vs Q_4: t-test, t=13.26, df=58. **E.** Number of vessels for each VVF range. [>1] vs [0.1-1]: t-test, t=5.934, df=22. MW-test [0.1-1] vs [0.01-0.1]: U=0; [0.01-0.1] vs [0-0.01]: U=0. **F.** Distribution of VVF (x1000) for different VVF ranges. MW-test [>1] vs [0.1-1]: U=0; [0.1-1] vs [0.01-0.1]: U=0; [0.01-0.1] vs [0-0.01]: U=0. ****p<0.0001

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