**Description of the experimental data**

**Data collection.**

We collected four different types of data:

*1) Volume scan.*

Fluorescence from a volume of brain tissue, containing several vessels of interest, in time. We selected a brain volume of interest (*xyz*) and recorded 2D images (spanning *xy* range of the volume) at different *z*-coordinates (depth) within the *z*-span of the volume. Typically, individual images in the recorded stack are ~1-2 µm away from each other along the *z*-axis. Each time point (whole volume recorded one time) takes ~1-2 sec to collect. This type of data, therefore, can only be used to track a relatively slow changes of a vessel diameter (e.g. too slow to resolve 0.2-sec long single heartbeats). On the other hand, the volume scan allows to follow diameters of multiple vessels simultaneously even if their position changes substantially (e.g. by several µm, e.g. when inducing hypertension), because they will still be contained in the imaging volume (selected large enough to account for possible vessel drift).

We recorded two types of volume scans: a “hypertension” volume scan during injection of ANG II (red in Figure 1) and a “whisker stimulation” volume scan, during which we stimulated whiskers of the mouse (green in Figure 1). The purpose of the first is to study how blood vessels react to a large increase in blood pressure. The purpose of the second is to determine how different treatments affect neurovascular coupling in the brain (vessels dilate when neuronal activity increases).

“Hypertension” volume scan is recorded as follows: Start imaging a volume of the brain in time, containing penetrating arteriole, precapillary sphincter, 1st-order and 2nd-order capillaries from the selected vessel tree. Inject ANG II into the blood, which increases the blood pressure. Stop injection of ANG II. Record “hypertension” volume scan until the blood pressure returns to the pre-injection baseline. Estimates below are calculated for the four vessel types listed above. The five estimated quantities below correspond to different time points of the above protocol.

Estimated quantities:

(i) Mean vessel diameter before inducing hypertension (injection of ANG II) (diam\_mean\_before\_hypertension) [µm];

(ii) Mean vessel diameter 20-sec after the blood pressure starts to increase (diam\_mean\_start\_hypertension) [µm];

(iii) Mean vessel diameter during last 20-sec of the volume scan recording (diam\_min\_hypertension) [µm]. It is called “min” because vessels diameters typically reach their minimal values in these period.

(iv) Mean vessel diameter before stopping injection of ANG II (diam\_mean\_before\_stop\_hypertension) [µm].

(v) Mean vessel diameter when the blood pressure returned to the baseline, i.e. before injection of ANG II (diam\_mean\_after\_hypertension) [µm].

“Whisker stimulation” volume scan is recorded as follows: Start imaging a volume of the brain in time, containing penetrating arteriole, precapillary sphincter, 1st-order and 2nd-order capillaries from the selected vessel tree. Stimulate infraorbital (whisker) region of the mouse. Estimates below are calculated for the four vessel types listed above.

Estimated quantities:

(i) Mean vessel diameter before stimulation (diam\_mean\_before\_stim) [µm];

(ii) Maximum vessel diameter after the stimulation (diam\_max\_after\_stim) [µm];

(iii) Minimum vessel diameter after the stimulation (diam\_min\_after\_stim) [µm].

*2) Line scan (diameter).*

Fluorescence along a line that goes perpendicular a vessel’s axis (vessel’s axis lies in the focal plane) in time. Compared to the volume scan, a line scan allows only to record one vessel at a time, but much faster ­­– with ~10 ms time resolution compared with ~1-2 sec for a volume scan. Line scan data allows to track diameter of a blood vessel during a single heartbeat (~200 ms long).

The Iine scan is recorded as follows: For each vessel in the vascular tree (including structures like precapillary sphincter and bulb, located between a penetrating arteriole and a 1st-order capillary), record a time series of line profiles of fluorescence intensity (*xt*-imaging = kymogram) perpendicular to the vessels axes.

Estimated quantities: (i) mean vessel diameter (diam\_mean) [µm]; (ii) heartbeat frequency (pulse\_freq) [Hz]; (iii) Power of the vessel diameter oscillation at up to three harmonics, h1-3 (power\_diam\_h1,2,3) [µm2]; (iv) Power of the vessel center position oscillation at up to three harmonics, h1-3 (power\_center\_h1,2,3) [µm2].

*3) Line scan (RBC speed).*

Fluorescence along a line (1D) that goes along a single vessel’s axis (vessel’s axis lies in the focal plane) in time.

The Iine scan is recorded as follows: For each vessel in the vascular tree (including structures like precapillary sphincter and bulb, located between a penetrating arteriole and a 1st-order capillary), record a time series of line profiles of fluorescence intensity (xt-imaging = kymogram) along the vessels axes.

Estimated quantities: (i) RBCs speed (speed) [µm/sec]; (ii) RBCs flux (flux) [RBCs/sec].

*4) Blood pressure.*

Blood pressure measured from a femoral artery as a function of time.

Estimated quantities:

(i) mean arterial blood pressure (pressure\_d) [mmHg] recorded simultaneously with *Line scan (diameter)*;

(ii) mean arterial blood pressure (pressure\_s) [mmHg] recorded simultaneously with *Line scan (speed).*

(iii-viii) pressure\_before\_hypertension, pressure\_start\_hypertension, pressure\_min\_hypertension, pressure\_before\_stop\_hypertension, pressure\_after\_hypertension – all five are mean arterial blood pressures, estimated from time points of the corresponding estimated quantities in the “hypertension” volume scan, explained above. E.g. pressure\_start\_hypertension was estimated from the same data, from which we estimated diam\_mean\_start\_hypertension. All are measured in [mmHg].

**Experimental protocol.**

We describe the experimental protocol (Figure 1) starting from placing an operated mouse under the objective of a two-photon microscope.

A diagram of different types of ablation

Description automatically generated

Figure : The experimental protocol consisting of five groups of measurements, which we denoted as "treatments" (black boxes). During each treatment, we recorded volume scans (green and red) and line scans (blue). The scheme doesn’t show the vessel selection part, which precedes the treatments and is done as follows: select a penetrating arteriole, from which one can easily trace branching capillaries down to the fifth order. This penetrating arteriole and five capillaries branching from it we denote as a vessel tree. We recorded only one vessel tree from a mouse. “Baseline” treatment is a control, to which the following treatments can be compared to. During the 1st and 2nd “hypertension” treatment, blood pressure of the mouse is increased by injection of angiotensin II (ANG II). The “after hypertension” treatment corresponds to the time when the blood pressure of the mouse returned back to values similar to “Baseline” (the blood pressure return back to “baseline” because we stop injecting ANG II). The “after hypertension” treatment serves as a control for the “after ablation” treatment, in which we ablated (destroyed) the precapillary sphincter (a structure located where a penetrating arteriole branches into a first order capillary) to test how it affects the vascular tree (“after ablation”). Finally, “2nd hypertension” treatment is done to test if ablation of the precapillary sphincter affects vascular response to an increasing blood pressure.

**How to read** *data.csv*

*mouse*: the date of the experiment (format: date month year)

*treatment*: baseline, hyper (during first hypertension), after\_hyper (after first hypertension), after\_ablation (after sphincter ablation), hyper2 (during second hypertension).

*vessel*: pen\_art (penetrating arteriole), sphincter, bulb, cap1–5 (capillaries from first to fifth order).

*age*: adult or old mouse

*diam\_mean*: mean vessel diameter, measured in µm; see *Line scan (diameter) in* **Data collection**.

f*\_heart*: heartbeat frequency estimated from the power spectra measured in Hz; see *Line scan (diameter) in* **Data collection**.

*power\_diam\_h1(h2, h3):* Power of the vessel’s diameter fluctuations at the heartbeat frequency (h1), its second (h2) and third harmonic (h3), measured in µm2/Hz; see *Line scan (diameter) in* **Data collection**. Each individual power value is exponentially-distributed.

*power\_center\_h1(h2, h3):* Power of the vessel’s center fluctuations at the heartbeat frequency (h1), its second (h2) and third harmonic (h3), measured in µm2/Hz; see *Line scan (diameter) in* **Data collection**. Each individual power value is exponentially-distributed.

speed: RBCs speed measured in µm/sec; see *Line scan (speed) in* **Data collection**.

flux: RBCs flux measured in RBCs/sec; see *Line scan (speed) in* **Data collection**.

speed: RBCs speed measured in µm/sec; see *Line scan (speed) in* **Data collection**.

Pressure\_d(s, \_before\_hypertension, \_start\_hypertension, \_min\_hypertension, \_before\_stop\_hypertension, \_after\_hypertension): blood pressure measured in mmHg; see *blood pressure in* **Data collection**.

diam\_mean\_before\_stim: Mean diameter of a vessel before whisker stimulation, measured in µm; see *Volume scan in* **Data collection**.

diam\_max\_after\_stim: Maximal diameter of a vessel after whisker stimulation, measured in µm; see *Volume scan in* **Data collection**.

diam\_min\_after\_stim: Minimal diameter of a vessel after whisker stimulation, measured in µm; see *Volume scan in* **Data collection**.

diam\_mean\_before\_hypertension: Mean vessel diameter before inducing hypertension (injection of ANG II) measured in µm; see *Volume scan in* **Data collection**.

diam\_mean\_start\_hypertension: Mean vessel diameter 20-sec after the blood pressure starts to increase measured in µm; see *Volume scan in* **Data collection**.

diam\_min\_hypertension: Mean vessel diameter during last 20-sec of the volume scan recording measured in µm; see *Volume scan in* **Data collection**.

diam\_mean\_before\_stop\_hypertension: Mean vessel diameter before stopping injection of ANG II measured in µm; see *Volume scan in* **Data collection**.

diam\_mean\_after\_hypertension: Mean vessel diameter when the blood pressure returned to the baseline, i.e. before injection of ANG II measured in µm; see *Volume scan in* **Data collection**.

An empty cell means either no experimental data was recorded (e.g. no data for 5th order capillaries or during hypertension) or because the data was discarded due to low quality (read below for the exclusion criteria). If there is an empty cell in column Pc\_h2(h3), while there is a value in column Pc\_h1, it means that the second (third) harmonic was essentially zero. See section “Methods” for how the power spectra were estimated.

**Question #1:** How do ageing and sphincter ablation affect responses of different blood vessels to whisker stimulation?

a) Is there an overall change in the relative diameter increase for different vessels between adult and old mice?

b) Does sphincter ablation increase diameter to a different extent for adult and old mice?

Notes for the modelling: diameter values are likely to be normally distributed.

**A diagram of different numbers

Description automatically generated with medium confidence**

Figure 2: Comparison of average diameters before whisker stimulation (diam\_mean\_before\_stim in Oleg’s data; top row),maximum diameter after whisker stimulation (diam\_max\_after\_stim in Oleg’s data; second from the top row), diameter increase after whisker stimulation (diam\_max\_after\_stim - diam\_mean\_before\_stim in Oleg’s data; third from the top row), and relative diameter increase ((diam\_max\_after\_stim - diam\_mean\_before\_stim)/ diam\_mean\_before\_stim in Oleg’s data; bottom row), for different vessels (x-axis) and treatments (four treatments in columns) for adult (black) and old (red) mice. X-axis in panels contains penetrating arterioles (pa), sphincters (s), bulbs (b), and capillaries from 1st to 2th orders (c1-c2). Points with error bars show means ± s.e.m. (averaging over mice, minimum 5 points per average).

**Question #2:** How do ageing, hypertension, and sphincter ablation affect pulsatility of the diameter and center position of blood vessels?

a) Is there an overall change in Pd and Pc the for different vessels between adult and old mice? If yes, can this change be explained by the higher mean blood pressure in adult mice compared to old mice?

b) Does sphincter ablation affect Pd and Pc differently in adult and old mice?

c) Do Pd and Pc change with changing blood pressure?

d) D hypertension and sphincter ablation affect diameters, Pd, and Pc differently for different vessels?

Notes for the modelling: diameter values and blood pressure are likely to be normally distributed. Power spectral values are exponentially distributed.

A chart of different types of blood pressure

Description automatically generated

Figure 3: Comparison of average diameters (diam\_mean in Oleg’s data; top row), mean blood pressure (pressure\_d in Oleg’s data; second from the top row), power of diameter pulsations (Pd; power\_diam\_h1 in Oleg’s data; third from the top row), and power of center pulsations (Pc; power\_center\_h1 in Oleg’s data; bottom row) for different vessels (x-axis) and treatments (four treatments in columns) for adult (black) and old (red) mice. X-axis in panels contains penetrating arterioles (pa), bulbs (b), and capillaries from 1st to 5th orders (c1-c5). Points with error bars show means ± s.e.m. (averaging over mice, minimum 5 points per average). Dashed lines in panels of the bottom row show grand averages of Pc for all vessels for adult (black) and old (red) mice. Note that individual values of Pd andPd are exponentially-distributed.