The severity of microstrokes depends on local vascular topology and baseline perfusion.

Franca Schmid 1,2, Giulia Conti 2, Patrick Jenny 3, Bruno Weber 1

1 Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
2 Institute of Fluid Dynamics, ETH Zurich, Sonneggstrasse 3, CH-8092 Zurich, Switzerland

Corresponding authors: Franca Schmid (franca.schmid@uzh.ch) and Bruno Weber (bweber@pharma.uzh.ch)

Joint last author: Patrick Jenny and Bruno Weber

1 Abstract

Cortical microinfarcts are linked to pathologies like cerebral amyloid angiopathy and dementia. Despite their relevance for disease progression, microinfarcts often remain undetected and the smallest scale of blood flow disturbance has not yet been identified. We employed blood flow simulations in realistic microvascular networks from the mouse cortex to quantify the impact of single capillary occlusions. Our simulations reveal that the severity of a microstroke is strongly affected by the local vascular topology and the baseline flow rate in the occluded capillary. The largest changes in perfusion are observed in capillaries with two in- and two outflows. This specific topological configuration only occurs with a frequency of 8%. The majority of capillaries has one in- and one outflow and is likely designed to efficiently supply oxygen and nutrients. Taken together, microstrokes bear potential to induce a cascade of local disturbances in the surrounding tissue, which might accumulate and impair energy supply locally.

2 Introduction

As the brain’s energy storage is limited, a sustained supply of oxygen and nutrients is crucial to avoid local tissue damage. Accordingly, flow disturbances even at the level of individual vessels can result in cortical tissue lesions, so called microinfarcts [1-5]. In recent decades it has become evident that such microinfarcts are linked to various pathologies, e.g. cerebral amyloid angiopathy (CAA), Alzheimer’s disease (AD) and dementia [6-10]. Depending on the severity of flow disturbance the dimension of documented microinfarcts ranges between 50 µm to a few millimeters [2, 4, 5, 7, 9-11]. This small size renders the quantification of the brain’s microinfarct burden challenging and makes it difficult to gain insights on their role for local blood supply.

Animal models allow a more refined study of the etiology of microinfarcts [7]. By occluding vessels via photothrombosis [1-5, 12-16] or by injecting microemboli [17-22], microstrokes can be induced and their impact on blood flow and on the surrounding tissue can be studied. Here, most studies focus on the occlusion of penetrating vessels, which because of their one-dimensional topology [23-25] have been identified as the “bottleneck of perfusion” [1]. Less attention has been given to occlusions of descending arteriole (DA) offshoots and capillaries.

While the occlusion of DA offshoots causes a maximal infarct volume of 0.8 nl (275 times smaller than for DA occlusions), no tissue damage could be detected for the occlusion of capillaries >2 branches apart from the DA [2]. However, the effect of anesthesia on these results remains unknown. This is because anesthesia can act as a vasodilator and tends to increase red blood cell (RBC) flux and tissue oxygenation [26, 27]. Importantly, it has also been shown that single capillary occlusion causes flow reversals and RBC speed reductions of up to 90% in the vessels downstream of the occluded capillary [13]. Additionally, single capillary occlusion can induce the formation and alter the morphology of amyloid-beta (Aβ) plaques [14], which are related to AD and CAA. Taken together, even if single capillary occlusion might not directly cause local tissue damage, it disturbs blood flow locally and might impair tissue clearance. As such, single...
capillary occlusions could play an important role in the development and progression of larger disturbances and pathologies.

Further studies have investigated the effect of simultaneously occluding multiple capillaries or multiple microvessels of larger caliber [12, 22]. Underly et al. [12] showed that the occlusion of ~10 proximal capillaries via photothrombosis leads to deterioration of the blood brain barrier (BBB). The accumulation of occluded capillaries can also influence the total perfusion of the cortical vasculature. In a simulation study related to in vivo investigations in an AD mouse model, Cruz Hernandez et al. [28] showed that with 2% of capillaries stalled, cortical cerebral blood flow is reduced by ~5%. Capillary stalls have also been identified as an important factor for incomplete reperfusion after stroke [29, 30].

These aspects underline the need for an in-depth quantification of blood flow changes in response to single capillary occlusion, which will allow us to better understand the role of these small disturbances on local tissue perfusion. We will identify factors influencing the severity of micro-occlusions and quantify the area of impact. Additionally, by looking at the smallest possible scale of occlusion valuable insights on the robustness of perfusion within the capillary bed can be gained and our knowledge of topological characteristics of cortical capillary beds can be extended. In this context, we will also analyze the arrangement of arteriole- and venule sided capillaries. Moreover, identifying the smallest scale of disturbance is a prerequisite for the correct interpretation of disturbances and changes observed on a larger scale.

To address these questions, we employed blood flow simulations in realistic microvascular networks from the mouse cortex [25, 31, 32], in which individual capillaries have been occluded. Using an in silico approach comes with several advantages. First of all, it is challenging to monitor blood flow changes in vivo with single vessel resolution in multiple vessels or even entire vascular networks simultaneously. This problem is even more pronounced, if the focus is on blood flow changes in the capillary bed, which is highly interconnected [23, 25, 31, 33-35] and in which the flow field is highly heterogeneous and fluctuating [31, 32, 36-39]. Secondly, in silico studies allow us to investigate the impact of single capillary occlusions in an isolated manner. This is in contrast to in vivo analyses, where a capillary occlusion will always be accompanied by a response from directly neighboring cells (e.g., endothelial cells, mural cells, microglia).

By studying flow changes in response to the occlusion of 167 different capillaries, we reveal that the severity of a microstroke strongly depends on the local vascular topology and the baseline flow rate in the occluded capillary. More precisely, in the worst-case scenario the flow rate dropped by as much as 70% in the direct vicinity of the occluded capillary. As well as this, a microstroke locally reduces the number of available flow paths between DAs and ascending venules (AVs). This aspect might play an important role for the up-regulation of blood flow during neural activation, since the ability of the microvasculature to adapt to local changes in energy demand might be impaired in the disturbed flow field. The fact that the worst-case scenario only occurs with a frequency of 8% across all capillaries and the re-routing of blood to neighboring vessels suggests that the capillary bed offers an inherent robustness towards single capillary occlusions. These aspects as well as compensatory oxygen supply from neighboring capillaries likely help to avoid severe hypoxic conditions in response to single capillary occlusion. Our results further indicate that the different vascular topologies are not only relevant for the severity of the microstroke, but that they might in fact fulfill distinct functional tasks. We postulate that there is a topological difference between capillaries responsible for the distribution of blood and capillaries responsible for supplying oxygen and nutrients to the cortical tissue.

In summary our work provides an in-depth quantification of flow changes in response to single capillary occlusions and reveals novel topological characteristics of the cortical microvasculature. Our results give valuable insights into the role of microinfarcts, which are relevant
3 Results

The following results are based on time-averaged blood flow simulations in realistic microvascular networks (MVNs) embedded in a tissue volume of 1.6 mm$^3$ (MVN1) and 2.2 mm$^3$ (MVN2). The realistic MVNs and the simulation framework have been introduced in previous publications [25, 31, 40]. A brief description of both is provided in the Methods. In total we performed 167 single microstroke simulations to investigate the impact of a microstroke on local perfusion and to reveal key factors for the severity of the microstroke. To induce a microstroke we constricted the diameter of the microstroke capillary (MSC) to 0.01 μm, which reduced the flow rate in the MSC to $< 10^{-10}$ μm$^3$ms$^{-1}$. Details regarding the selection of MSCs and the computation of the relative flow changes $\Delta q_{ij}$ are provided in the Methods and Supplementary File 1a. Statistical validations are available in Supplementary File 1a-1e.

3.1 The severity of a microstroke is governed by the local vascular topology.

Microvascular bifurcations are either divergent or convergent. Thus, depending on the bifurcation types at the source and the target vertex of the MSC, four topological configurations are possible at the MSC (Figure 1a-d). To identify the MSC-type the flow directions in all five capillaries need to be known. An identification based purely on the topological arrangement and appearance of the vessel is not possible. To investigate the impact of the topological configuration on the severity of the microstroke we performed $>=20$ microstroke simulations per configuration. Based on the time-averaged flow field before and after stroke we computed the thresholded relative flow change $\Delta q_{ij}$ for each vessel (Methods). Note that, the response to long lasting stalls ($>20$ s) and permanent occlusions is equivalent for the observation period considered in this work.

In a first step, we analyzed the relative flow changes $\Delta q_{ij}$ in the vessels in up to five generations up- and downstream of the MSC. As more than 80% of all capillaries in the vicinity of the MSC experienced a decrease in flow (Figure 1 – figure supplement 1 e-h), the subsequent analyses focus on capillaries with a reduction in flow. Figure 1e-g shows that the relative changes are larger for MSCs fed by two upstream vessels and for MSCs feeding two downstream vessels. For the worst-case scenario, i.e. MSCs with a convergent bifurcation upstream and a divergent bifurcation downstream (2-in-2-out, Figure 1a and e), the median relative decrease is still $<25\%$ at generation ±2 from the site of occlusion. In contrast for the best-case scenario, i.e. MSCs with a divergent bifurcation upstream and convergent bifurcation downstream (1-in-1-out, Figure 1d and h), the median relative change is $\sim10\%$ at generation ±2 (p < 0.001 at generation ±2, Supplementary File 1c). The differences between 2-in-2-out and 1-in-1-out are even more pronounced for vessels of generation ±1. Here, the median relative drop in blood flow is as large as 70% for 2-in-2-out, while it is only 22% for 1-in-1-out (p < 0.001 at generation ±1, Supplementary File 1c). 2-in-1-out and 1-in-2-out are intermediate MSC-types (Figure 1 b-c and f-g). For example, on the upstream side 2-in-1-out experienced relative changes comparable to 2-in-2-out, while on the downstream side the trends correspond to the ones observed for 1-in-1-out (Supplementary File 1c).

![Figure 1](https://example.com/figure1.png)

**Figure 1** Impact of the local vascular topology on the severity of a microstroke. a)-d) Illustration of the four possible topological configurations at a microstroke capillary (MSC). For each topological configuration a schematic (upper left) and a realistic example (lower right) is provided: the MSC (dark) and its adjacent vessels (grey, generation -1 and 1) are depicted. The arrows show the flow direction. e)-f) Average relative change in flow rate $\Delta q_{ij}$ for capillaries up- and downstream of the MSC which experienced a flow decrease. For each topological configuration the flow field for $>=20$ microstrokes has been computed (n: number of microstroke simulations). The average relative decrease per generation for each simulation is depicted by the color- and marker-coded symbols. The boxplots are based on the data for each generation. Statistically significant two-way interaction between MSC-type and generation: Upstream: $F(7.67,217.33)=9.23$, p< 0.001, Downstream: $F(7.00,198.43)=2.71$, p=0.01 (two-way mixed ANOVA). For further...
3.2 Capillary occlusion reduces perfusion in the tissue around the MSC and causes a local redistribution of flow

To predict oxygen and nutrient supply in the tissue around the MSC it is important to account for changes in capillaries, which are in the direct vicinity of the MSC but not directly upstream or downstream of the MSC. Therefore, we defined an analysis box around the MSC and compute its total inflow before and after stroke (Figure 2a-e, Methods). The initial analysis box volume is set to 0.2 nl and was chosen such that each MSC fits into the initial box volume and that the box has at least five inflows. The box volume was increased progressively and the relative inflow difference has been recomputed (Figure 2a, Methods). This analysis allows us to comment on the reduction in perfusion of a tissue volume around the MSC capillary. Moreover, it provides an estimate of the tissue volume, which is affected by a reduced perfusion in response to the microstroke.

In line with the relative flow changes in the upstream and downstream vessels (Figure 1e-h) we observed the largest inflow reduction for 2-in-2-out (Figure 2b). For the initial box volume, i.e. a volume factor of 1.0, the median inflow reduction is as large as -14%. For a volume factor of 1.75 the median inflow reduction already drops to -5.1% (p=0.005, pairwise t-test with Bonferroni correction). However, it is not until a volume of 0.6 nl (volume factor: 3.0) that the median inflow reduction approaches 0%. For MSC-type 2-in-1-out the median inflow reduction for a volume factor of 1.0 is -13% (Figure 2c). The tissue around MSC-types 1-in-2-out and 1-in-1-out did not experience significant changes in total inflow. Here, for all volume factors the median inflow difference is smaller than 2.5%.

Importantly, the resulting inflow reduction in the analysis box is also affected by the topological connectivity around the MSC and the redistribution of flow in response to a microstroke. These aspects become apparent if we compare the relative flow changes in vessels with different topological positions with respect to the MSC. We discern three topological positions: 1) vessels that are directly upstream and downstream of the MSC, 2) vessels that run parallel to the MSC and 3) vessels that do not belong to the first two categories, i.e. distant vessels (Figure 2f, Figure 2 - figure supplement 1e, Methods).

Figure 2 - figure supplement 1a shows that for up to a volume factor of 2, more than 50% of the vessels in the analysis box are directly upstream or downstream of the MSC. In these vessels we had a significant flow reduction (Figure 2g). In contrast, in the vessels that run parallel to the MSC we observed an increase in flow (Figure 2h). This clearly indicates that during a microstroke the flow is redistributed to pathways parallel to the MSC. However, only ~15-20% of all capillaries in the analysis box are parallel (Figure 2 - figure supplement 1b) and consequently we still observed an overall flow reduction in the analysis box. In the third vessel
category, the distant vessels, the median relative flow difference is <2% for all volume factors (Figure 2i). This result confirms that the impact of a microstroke is most pronounced in vessels that are directly connected to the MSC.

[PLACEHOLDER FIGURE 2]

**Figure 2** Flow reduction in analysis boxes around the microstroke capillary (MSC). a) Schematic introducing how the analysis and the volume factor are defined (Methods). The MSC is highlighted in dark red. In- and outflow vessels of the analysis box are annotated with arrows. \( V_{\text{box}} \): initial analysis box volume. \( \Delta \text{incr} \): distance by which the analysis box has been increased. b) e) Relative inflow difference for an increasing box volume around the MSC for the four MSC types. b) 2-in-2-out, c) 2-in-1-out, d) 1-in-2-out and e) 1-in1-out. The initial box volume, i.e. volume factor = 1, is 0.2 nl. The relative inflow difference is computed by adding up the inflows across the borders of the box for the baseline and the stroke simulation (Methods). There is a statistically significant two-way interaction between MSC-type and volume factor: \( F(5.3,150.24)=5.23, p<0.001 \) (two-way mixed ANOVA). For further statistical details see Methods and Supplementary File 1a. f) Upper panel: Schematic to introduce the concept of vessels parallel to the MSC (Methods). Lower panel: Realistic example of the edges in a box volume of 1 nl, i.e. volume factor = 5. US: upstream. DS: downstream. g) i) Relative total flow difference for an increasing box volume around a 2-in-2-out MSC for upstream and downstream vessels (g), parallel vessels (h) and distant vessels (i). The relative total flow difference is calculated by comparing the length-weighted flow for the baseline and the stroke simulation (Methods). The \( \geq 2 \) microstrokes per topological configuration are depicted by the color- and marker coded symbols. The boxplots are based on the data for each volume factor.

Worthy of note is that for a tissue volume of 0.4 nl (i.e. volume factor = 2) ~50% of vessels in the analysis box are distant and parallel capillaries (Figure 2 - figure supplement 1 a-d). This topological configuration might be beneficial for the robustness of perfusion of the tissue volume around the MSC. Because even if the total inflow decreases in the tissue volume around the MSC, there is always a fraction of vessels within the analysis box that are not significantly affected by the microstroke (distant vessels) or that experience an increase in flow in response to the microstroke (parallel vessels). Therewith, an even larger drop in overall perfusion can be avoided and a minimum remaining perfusion can be ensured. This likely is beneficial to avoid a significant drop in oxygen partial pressure \( (pO_2) \) in the tissue around the MSC.

The most relevant haemodynamic quantity for local \( pO_2 \) is the RBC flux [41]. Thus, in addition to investigating changes in flow, we repeated our analyses for changes in RBC flux (Figure 2 - figure supplement 2). As RBC flux and flow rate are related the general trends are comparable for both quantities. Interestingly, the reduction in RBC flux in the analysis box around the MSC is larger than for the flow rate (Figure 2 - figure supplement 2 c-d). This indicates that a single capillary occlusion also affects the distribution of RBCs, which might further increase the risk of local tissue hypoxia. As our study is limited to changes in perfusion within the vasculature, further investigations resolving oxygen transport within the tissue are necessary to answer the question if a single capillary occlusion significantly affects local tissue \( pO_2 \).

**3.3 The baseline MSC flow rate increases the area of impact of a microstroke.**

Our results demonstrate that the local vascular topology plays a crucial role in the severity of a microstroke. To identify further structural and functional characteristics relevant to the level of flow change in response to microstroke, we repeated our analysis for eight additional cases. We looked at the impact of the baseline flow rate in the MSC (case 5), the cortical depth (case 8-12) and the distance to the penetrating vessels (case 6-7). An overview of the selection criteria for each vessel subset is provided in Supplementary File 1a. In this study we focused on 2-in-2-out MSCs because we expect the largest changes here.

For the case with a higher baseline flow rate we observed that the relative change tends to be larger at generations -3 and ±4 (Paired t-tests: -3: \( p=0.007 \), 3: \( p=0.014 \), 4: \( p=0.008 \), Figure 2 - figure supplement 3 a-b). However, no significant two-way interaction between baseline flow rate category and generation and no main effect of the baseline flow rate could be detected (likely because the relative changes do not differ at generations ±1, ±2 and ±5). The impact of the baseline flow rate on the changes in the vicinity of the MSC is further supported by the
analysis of the total inflow change into the analysis box around the MSC (Figure 2 - figure supplement 3 c-d), where we found a significant main effect of the baseline flow rate on the relative inflow change (F(1,45)=13.97, p<0.001, two-way mixed ANOVA). Here, the most relevant difference is that the occlusion of a capillary with a high baseline flow rate increases the volume in which a significant decrease in inflow can be observed.

No significant differences could be observed for the relative changes in up- and downstream capillaries for occlusions at different cortical depths or with varying distance to the penetrating vessels (Figure 2 - figure supplement 4 and Figure 2 - figure supplement 5 a-b). Regarding the analysis over cortical depth, it is important to note that the baseline flow rate of the chosen MSC has to be between 0.1-7.0 µm³/ms. This selection criterion might cancel out potential effects of the decrease in flow rate over depth [31, 38, 42, 43]. We observed a significant effect of the position of the MSC along the capillary path for inflow changes in the analysis box around the MSC (Figure 2 - figure supplement 5 c-d). We hypothesize that these differences are affected by the relative frequency of up- and downstream, parallel and distant capillaries in the analysis box (Figure 2 - figure supplement 1 g-h).

3.4 Multi-capillary occlusions

Our results show that single capillary occlusion affects the flow rate in multiple capillaries in the direct vicinity of the MSC. Moreover, in vivo observations suggest that capillary stalls are more likely in low flow capillaries [44, 45] and thus microstrokes might accumulate in the direct vicinity of the MSC. To investigate the impact of an accumulation of capillary occlusions around the MSC, we performed simulations in which three, five, seven and nine capillaries have been occluded in the analysis box around the MSC with a volume of 0.3 nl (volume factor = 1.5, Figure 3a). The simulations have been performed sequentially and in each step the two capillaries with the lowest time-averaged flow rate have been occluded for the subsequent simulation.

Figure 3b shows that the relative flow difference in the analysis box increases with the number of occluded capillaries. This is most apparent for the occlusion of nine capillaries, where we observed a flow decrease of ~20% in an analysis box 1.6 nl (volume factor = 8). To further analyse the perfusion changes within the analysis box we counted the number of vessels with a flow decrease within the analysis box (Figure 3c). The number of vessels with a flow decrease increased with the volume factor, which underlines that the capillary occlusions also affect the perfusion in neighbouring vessels. Interestingly, the number of vessels with flow decrease is smaller if more capillaries are occluded. This indicates that single capillary occlusion causes a small flow reduction in a larger number of capillaries. In contrast, if multiple proximal capillaries are occluded a re-routing of flow occurred and a smaller number of vessels experienced a flow decrease. This is consistent with the observations in Figure 2g-i, where we describe a flow increase in vessels parallel to the MSC and shows how the perfusion in the capillary bed adapts to local disturbances of increasing severity.

[PLACEHOLDER FIGURE 3]

Figure 3 Flow reduction in analysis boxes around the microstroke capillary (MSC) for multi-capillary occlusions. a) Capillaries in an analysis box of 1.6 nl (volume factor = 8). The occluded capillaries are highlighted in dark red (left: one occluded capillary, right: nine occluded capillaries). Two distinct examples are shown in the upper and lower row. b) Relative total flow difference for an increasing box volume around a 2-in-2-out MSC for an increasing number of occluded capillaries. The initial box volume, i.e. volume factor = 1, is 0.2 nl. The relative total flow difference is calculated by adding up the length-weighted flow for the baseline and the stroke simulation (Methods). While the two-way interaction between number of occluded capillaries and volume factor is not significant (F(4,92,30.7)=1.06, p=0.4), there is a statistical significant main effect of the number of occluded capillaries and the volume factor on the relative flow difference (Number of occluded capillaries: F(4,25)=3.52, p = 0.021, Volume factor: F(1,23,30.7)=100.3, p<0.001 , two-way mixed ANOVA). c) Number of vessels with a flow decrease in the analysis box around the MSC. Occluded capillaries are not counted. The six microstrokes per number of occlusions are depicted by the grey scatterplot. The boxplots are based on the data for each volume factor.
3.5 The minimum distance between an arteriole-sided and a venule-sided capillary point is on average 44 µm.

It is well established that pO2 in capillaries shortly downstream of DAs is higher than in capillaries just upstream of AVs [43, 46]. Moreover, it has been suggested that the tissue supplied by venule-sided capillaries might be more susceptible to hypoxia in the case of blood flow disturbances or during neural activation [46-48]. Consequently, the spatial arrangement of arteriole-sided and venule-sided capillaries with respect to each other might be an important topological feature for the robustness of oxygen and nutrient supply. A convenient way to avoid local hypoxia could be obtained by a topological structure where arteriole-sided and venule-sided capillaries are positioned in close proximity to each other.

To investigate the spatial arrangement of arteriole-sided and venule-sided capillaries with respect to each other we introduce the AV-factor. The AV-factor for each capillary has been computed by identifying all paths leading from the capillary to all possible DA-endpoints and to all possible AV-endpoints. The AV-factor has subsequently been calculated from the median distance to all DA/AV-endpoints (Figure 4a, Methods). The AV-factor is close to 0 if the capillary is close to a DA and is close to 1 if the capillary is adjacent to an AV. We define arteriole-sided capillaries as capillaries with an AV-factor < 0.5 and venule-sided capillaries with an AV-factor >= 0.5. The following investigations have been performed in MVN1 and MVN2. The precise analysis approaches are described in more details in the Methods.

In an initial analysis we computed the shortest distance between a discretization point along a venule-sided capillary and an arteriole-sided capillary. As a reference we additionally calculated the shortest distance to any vessel around a venule-sided capillary (Figure 4b) and the average distance between two capillaries. The median shortest distance from a venule-sided capillary to any vessel is 18 µm and 15 µm for MVN1 and MVN2, respectively. The average distance between two capillaries is 32 µm (MVN1) and 31 µm (MVN2). The shortest distance from a venule-sided to an arteriole-sided capillary is 2.73 (MVN1) and 2.78 (MVN2) larger than the shortest distance to any vessel (Figure 4c). This corresponds to a distance of 46 µm (MVN1) and 41 µm (MVN2) to the closest arteriole-sided-capillary, which are only factor 1.4 (MVN1) and 1.3 (MVN2) larger than the average distance between two capillaries.

Subsequently, we analyzed the average AV-factor in analysis spheres of 50 µm surrounding venule-sided capillary points (Figure 4d). For 68% (MVN1) and 60% (MVN2) of all venule-sided capillaries, the average AV-factor in the analysis sphere is smaller than the AV-factor of the venule-sided capillary under investigation (Figure 4e). This implies that these capillaries have multiple arteriole-sided points nearby, which potentially act as backup for oxygen and nutrient supply. The relative difference between the AV-factor of the venule-sided capillary and the mean of all points within the analysis sphere was -5.9% and -4.2% for MVN1 and MVN2, respectively.

In a further analysis we computed the average AV-factor for analysis cubes of different sizes (side length 30-120 µm, Figure 4 - figure supplement 1). The range of the average AV-factor per analysis cube goes from almost 0 to 1. Nonetheless, the median AV-factor across all analysis cubes is independent of the cube size and equal to 0.52 and 0.54 for MVN1 and MVN2, respectively.

Taken together, the shortest distance of 46 µm (MVN1) and 41 µm (MVN2) to an arterial-sided capillary and the frequent decrease of the average AV-factor in the 50 µm analysis spheres around venule-sided capillaries suggest that arteriole-sided capillaries are well distributed throughout the network. Nonetheless, further studies are necessary to estimate whether proximal arterial-sided capillaries help to avoid hypoxic tissue areas in the vicinity of venule-sided capillaries. Moreover, it has to be kept in mind that in the rodent cortical vasculature, AVs outnumber DAs [23]. This is in contrast to the primate vasculature where DAs are approximately twice as frequent as AVs [23, 38, 49, 50]. As such, these results might be species dependent.
3.6 MSC-type 1-in-1-out supplies the largest tissue volume and is the most frequent MSC-type.

The significant impact of the different topological configurations on the severity of the microstroke raises questions about the frequency of occurrence and the distribution of different MSC-types in realistic MVNs. The following investigations are based on the time-averaged flow field in two realistic MVNs from the mouse somatosensory cortex acquired by Blinder et al. [25], which jointly encompass a tissue volume of ~3.6 mm^3 and which contain 31,400 vessels (Methods).

Interestingly, the worst case scenario, i.e. MSC-type 2-in-2-out, only occurs with a frequency of 11% (MVN1) and 6% (MVN2), while the best-case scenario, i.e. MSC-type 1-in-1-out, represents 44% (MVN1) and 43% (MVN2) of all possible MSCs (Figure 5d-e). Moreover, the median supplied tissue volume of 1-in-1-out is 52% (MVN1) and 119% (MVN2) larger than the supplied tissue volume of 2-in-2-out (Figure 5f-g, Methods). Consequently, a total of 51% (MVN1) and 55% (MVN2) of the tissue is supplied by 1-in-1-out capillaries and only 8% (MVN1) and 4% (MVN2) is supplied by 2-in-2-out capillaries. This also becomes apparent in Figure 5b where the tissue volume of realistic MVN1 is color-coded based on the MSC-type by which it is supplied.

The differences in the median supplied tissue volume are partly caused by the larger median vessel length of 1-in-1-out capillaries (Figure 5 - figure supplement 1 b-c).

The small number of 2-in-2-out capillaries and the small flow reduction for the frequent MSC-type 1-in-1-out suggest that the cortical microvasculature is inherently robust to the occlusion of a single capillary. The significant differences in the supplied tissue volume further underline this aspect.

Figure 5h-i shows that the median flow rate in a 2-in-2-out capillary is 2.6 (MVN1) and 2.2 (MVN2) times larger than in a 1-in-1-out. As a higher baseline flow rate increases the area of impact of a microstroke, we conclude that these differences further contribute to the severity of a microstroke in a 2-in-2-out configuration. We hypothesize that different local topological configurations might fulfill different tasks in microvascular blood supply. While 2-in-2-out capillaries might be more relevant for distributing blood in the cortical microvasculature, 1-in-1-out capillaries are likely designed to robustly deliver oxygen and nutrients to the cortical tissue. This hypothesis is strengthened by the number of unique paths going from DA to AV through the different MSC-types (Figure 5j-k, Methods). While for 1-in-1-out we only have 37 (MVN1) and 15 (MVN2) unique paths connecting DA and AV, for 2-in-2-out, we have 297 (MVN1) and 115 (MVN2) unique paths. Generally, the described trends are consistent for MVN1 and MVN2. However, it is noteworthy that the overall average flow rate is larger in MVN2 and the number of paths per vessel is larger in MVN1. The latter is likely caused by a higher density of penetrating vessels in MVN2, which reduces the number of highly interconnected flow paths through the capillary bed.

Subsequently, we asked whether the frequency of MSC-types varied over cortical depth (Supplementary File 1b) or along the pathway between DA and AV (Figure 5c, Figure 5 - figure
supplement 1 d-k). The latter investigation was performed by analyzing the frequency of occurrence of the four MSC-types for different AV-factors (Figure 5c). With respect to cortical depth, the frequency of the different MSC-types showed the same characteristics. For the distribution of the MSC-types along the pathway between DA and AV we observed that 1-in-2-out capillaries are more frequent on the arterial side of the capillary bed, while 2-in-1-out are more common towards the AVs. This is plausible because at the arterial end blood is distributed to the capillary bed, while it is re-collected close to the AVs. No significant differences could be observed for the distribution of 1-in-1-out and 2-in-2-out capillaries along the capillary path. Notably, 93% (MVN1) and 64% (MVN2) of all paths between DA and AV contain each MSC-type at least once. MSC-type 2-in-2-out is most frequently missing along a path between DA and AV.

As previously mentioned, the median flow rate decreases significantly over cortical depth (-66% and -80% for MVN1 and MVN2, respectively). This is consistent for all MSC-types (Figure 5 - figure supplement 2 d-f). No consistent trend as to how the supplied tissue volume changes over depth could be identified (Figure 5 - figure supplement 2 g-i). Important to note, is that the largest supplied tissue volume is found for 1-in-1-out capillaries in all ALs and the relative supplied tissue volume (Figure 5 - figure supplement 2 j-l) does not vary significantly with depth. Consequently, our conclusion holds that 1-in-1-out capillaries might be key capillaries for nutrient and oxygen discharge.

[PLACEHOLDER FIGURE 5]

Figure 5 Characteristics of the four topological configurations at a microstroke capillary (MSC) for both microvascular networks (MVNs). a) Schematic of the four topological configurations at a MSC. The MSC is color coded in accordance with subfigures b)-k). b) Grid representation of the tissue in which realistic MVN1 is embedded. The tissue points are color-coded based on the closest MSC-type. c) Relative frequency of occurrence of the different MSC-types along the capillary path (AV-factor, Methods). The number of occurrence per MSC-type is normalized by the total number of capillaries per AV-factor bin. Upper row: MVN1 (n = 2968). Lower row: MVN2 (n = 6571). d)-e) Frequency of occurrence of the four MSC-types (d: MVN1, e: MVN2). f)-g) Median supplied tissue volume for the four MSC-types (Methods, f: MVN1, g: MVN2). h)-i) Median flow rate for the four MSC-types (h: MVN1, i: MVN2). j)-k) Median number of paths leading through a MSC from the descending arteriole (DA) to the ascending venule (AV). l) Median flow rate decrease (Methods). The Kruskal-Wallis test confirms that the differences between the MSC-types are significant for the supplied tissue volume, the flow rate and the number of paths (p<0.001 for all quantities in each MVN). P-values for pairwise comparison with the Mann-Whitney U Test are listed in Supplementary File 1e.

3.7 A microstroke locally reduces the number of available flow paths

To further investigate the redistribution of flow during a microstroke we analyzed the number of flow paths leading from DA to AV. To this end, we followed the flow downstream from the main branch of a DA until it reaches an AV main branch (Methods). Importantly, due to the finite size of the MVN, various flow paths do not start at a DA or do not end at an AV. These flow paths are not considered (Methods). In a first step we computed the total number of unique flow paths between DA and AV main branch (Figure 6b and e). The total number of flow path during baseline in MVN1 is 139,399 and does not change significantly for single or multi-capillary occlusion. This large number highlights the interconnectivity of the capillary bed. Nonetheless, it is important to keep in mind that some flow paths only differ by one or a few vessel segments.

Subsequently, we investigated if single capillary occlusion reduces the number of unique DA-AV-endpoint-pairs, i.e. the total number of fluid dynamically connected pairs between DA and AV endpoints. Our results show that in response to a microstrokes new DA-AV-endpoint-pairs have been connected and existing DA-AV-endpoint-pairs have been lost (Figure 6 - figure supplement 1b). However, with respect to the total number of DA-AV-endpoint-pairs these changes are small (~0.2%). As such, our results suggest that single capillary occlusion and the occlusion of up to
nine proximal capillaries, does not reduce the overall number of flow paths and the number of unique **DA-AV-endpoint-pairs** in MVN1.

To investigate changes in flow paths in the vicinity of the MSC, we count the number of paths going through a predefined capillary (Figure 6 - figure supplement 1c) and analyzed the change in the number of unique flow paths going through: 1) capillaries upstream and downstream of the MSC (up to generation 3), 2) parallel to the MSC and 3) distant to the MSC (Figure 2f, Methods). Based on the results presented in Figure 2g-i, where we detected an increased flow in the parallel vessels, we expected to see an increase in the number of paths going through the parallel vessels. However, no consistent trend could be observed for the three different vessel categories (Figure 6 - figure supplement 1d). This suggests that an increase in flow does not necessarily cause an increase in the number of flow paths through the respective capillary.

In our last analysis we examined the number of possible flow paths between given **DA-AV-endpoint-pairs**. Therefore the **DA-AV-endpoint-pairs** are assigned to two categories (Figure 6c, Methods): 1) before stroke there is at least one path that leads from the DA- to the AV-endpoint through the MSC and 2) none of the paths between the given **DA-AV-endpoint-pair** go through the MSC. For **DA-AV-endpoint-pairs** of category 1 we note a decrease in the number of available flow paths between the respective **DA-AV-endpoint-pairs** for all MSC-types (Figure 6d). The mean ratio of unique flow paths before and after microstroke at the respective **DA-AV-endpoint-pair** is between 0.67 and 0.75. If more than five capillaries are occluded in the vicinity of the MSC, the relative frequency of **DA-AV-endpoint-pairs** with a decrease in available flow paths rises (Figure 6f). Interestingly, the ratio of unique flow paths before and after microstroke is not affected by the number of occluded capillaries and remains at ~0.7. For the majority of **DA-AV-endpoint-pairs** of category 2, we do not observe a change in the number of unique flow path in response to single or multi-capillary occlusion. Nonetheless, ~10% of **DA-AV-endpoint-pairs** of category 2 experience an in- or decrease in the number of connecting pathways, which underlines that the impact of capillary occlusion goes beyond directly connected vessels.

Taken together, the decrease in the number of pathways between **DA-AV-endpoint-pairs** of category 1 shows that a microstroke locally reduces the number of available paths. Based on the results shown in the preceding sections (Figure 2h), we suggest that the average flow rate likely increases along some of the remaining paths.

[PLACEHOLDER FIGURE 6]

**Figure 6** Changes in flow paths in response to single and multiple microstrokes in MVN1. a) Schematic of the four topological configurations at the microstroke capillary (MSC). The MSC is color coded in accordance with subfigures b) and d). b) Total number of flow paths connecting a descending arteriole (DA) to an ascending venule (AV) in MVN1. The bar plot depicts the results for the baseline simulation [base] and the median for the each microstroke case. The spheres show the total number of flow paths for each microstroke simulations (p=0.4, Kruskal-Wallis test). c) Schematic to introduce the two categories of **DA-AV-endpoint-pairs** (Methods). Each subplot shows all flow paths between one **DA-AV-endpoint-pair**. Flow paths that do not go through the MSC are labeled by the dotted line. d) Relative frequency of **DA-AV-endpoint-pairs** with an increase, a decrease or no change in the number of unique flow paths for the four MSC-types. The microstroke simulations per MSC-type are combined before the relative frequency is computed. Upper plot: **DA-AV-endpoint-pairs** belonging to category 1. Lower plot: **DA-AV-endpoint-pairs** belonging to category 2 (see c). e) Total number of flow paths connecting DA and an AV in MVN1 for an increasing number of occluded capillaries. The bar plot depicts the median of six simulations per number of occluded capillaries (No. occluded caps). The spheres show the total number of flow paths for each simulation (p=0.6, Kruskal-Wallis test). f) Relative frequency of **DA-AV-endpoint-pairs** with an increase, a decrease or no change in the number of unique flow paths for different numbers of occluded capillaries. The microstroke simulations per number of occluded capillaries are combined before the relative frequency is computed. Upper plot: **DA-AV-endpoint-pairs** belonging to category 1. Lower plot: **DA-AV-endpoint-pairs** belonging to category 2. Abbreviations of the four MSC-types: 2-2: 2-in-2-out, 2-1: 2-in-1-out, 1-2: 1-in-2-out, 1-1: 1-in-1-out.

4 Discussion

By performing blood flow simulations in realistic MVNs for 167 of single capillary occlusions we show that the severity of a microstroke depends on the local vascular topology and on the
Nonetheless, saturation in proximal capillaries is to causes hypoxic conditions i and the redistribution of flow to neighboring vessels, single capillary deficits in neuronal activity a impede susceptibility for occlusion has line with the occlusion of single capillaries at a MSC with a divergent bifurcation upstream and a convergent bifurcation. As the volume of the affected area increases and the local drop in perfusion is more severe, no significant reduction in flow rate is visible until the 10\textsuperscript{th} downstream vessel and where the occluded capillary volume is as large as 220 nl [1, 2, 25]. For scenarios in which multiple proximal capillaries are occluded the volume of the affected area increases and the local drop in perfusion is more severe.

Our observation that the severity of a microstroke is affected by the baseline flow rate of the occluded vessels is in agreement with previous \textit{in vivo} and \textit{in silico} observations for the occlusion of penetrating vessels [2, 4, 13, 25]. Additionally, Nishimura et al. [13] report an RBC velocity reduction in response to single capillary occlusion of 93\% and 55\% in downstream vessels of generation 1-2 and 3-4, respectively. Although the \textit{in vivo} velocity reductions are slightly higher, they generally compare well with our results for the occlusion of a 2-in-2-out and a 1-in-2-out MSC. However, Nishimura et al. [13] do not observe flow reversals and velocity reductions in upstream and parallel vessels. These differences are likely due to the fact that many of the occluded vessels in Nishimura et al. [13] are direct offshoots of the DA main branch. Due to significantly larger flow velocities in the DA main branch, velocity reductions and reversals upstream of the site of occlusion are not to be expected.

To be highlighted is that for all MSC-types the effects of single capillary occlusions are spatially constrained. To be more precise, no significant reduction in flow rate is visible 5 generations away from the MSC and in a tissue volume of 0.3 nl around the MSC the perfusion drops maximally by 10\% for all MSC-types. This is in contrast to the occlusion of DAs where the flow rate does not fully recover until the 10\textsuperscript{th} downstream vessel and where the infarct volume is as large as 220 nl [1, 2, 25]. For scenarios in which multiple proximal capillaries are occluded the volume of the affected area increases and the local drop in perfusion is more severe.

As the supplied tissue volume of a 1-in-1-out MSC is 0.055 nl, which is approximately 15 times smaller than the infarct volume observed for the occlusion of a DA offshoot [2], we postulate that the occlusion of single capillaries does not directly cause tissue damage. This hypothesis is in line with the results of Shih et al. [2]. Nonetheless, our results show that single capillary occlusion has a strong impact on the local flow field. As such, it seems plausible that the altered flow field is a possible mechanism by which to affect Aβ deposition [14] or solute clearance via the perivascular space in general [51]. The local disturbances in the flow field might increase the susceptibility for vessel ruptures [6] or additional occlusions, which subsequently might further impede clearance [52, 53] as well as oxygen and nutrient supply in an increasing area around the MSC.

For the occlusion of larger caliber vessels it has been shown that proximal microinfarcts are likely to coalesce [2] and that BBB leakage and intravascular platelet aggregation [4], as well as deficits in neuronal activity and functional vasodynamics [5] are also observed beyond the microinfarct border. However, whether comparable effects can be triggered by the occlusion of a single capillary remains unknown. Likewise, we don’t know whether single capillary occlusion induces local tissue hypoxia or if proximal vessels and increased gradients in tissue pO2 compensate for the lack of perfusion in the occluded capillary. Due to the dense capillary bed and the redistribution of flow to neighboring vessels, a single capillary occlusion likely only causes hypoxic conditions if tissue pO2 is already low during baseline and if the oxygen saturation in proximal capillaries is too low to compensate for the drop in perfusion.

Nonetheless, because of the significant impact on the flow field, single capillary occlusions might lead to a local drop in tissue oxygenation and oxygen saturation within RBCs, which might provoke a cascade of consecutive responses in the affected tissue around the MSC or downstream of the occluded area.
Furthermore, the impact of a microstroke on tissue oxygenation can be affected by the level of oxygen within the occluded capillary and by the local arrangement of arteriole- and venule-side capillaries with respect to each other. We hypothesize that arteriole-sided capillaries with a high oxygen content might be distributed in a convenient fashion throughout the vasculature to enhance the robustness of oxygen delivery throughout the tissue. Indeed, Nishimura et al. [15] provided supporting evidence for this hypothesis by showing that capillaries with varying topological distances to the DA can be in spatial proximity. However, further studies resolving oxygen partial pressure within capillaries in microvascular networks are necessary to confirm this hypothesis.

The remaining unknowns clearly underline the need for an in-depth in vivo quantification of the impact of single capillary occlusion. Based on our results we suggest that the focus of future in vivo microstroke studies should be on tissue oxygenation, Aβ deposition and long-term changes in the vicinity of the MSC. In these investigations it is important to keep in mind that the severity of the microstroke is affected by the local vascular topology and the baseline perfusion of the MSC. Consequently, care should be taken that effects are analyzed in an MSC-type specific manner. In addition to in vivo approaches, in silico studies resolving oxygen discharge from individual RBCs [54] are a convenient tool to improve our understanding of the impact of single capillary occlusions on tissue oxygenation.

As previously stated the severity of a microstroke depends on the local vascular topology. Worthy of note, we observe significant differences in the frequency and the characteristics of the local vascular topologies (MSC-types). 1-in-1-out is by far the most frequent MSC-type and supplies the largest tissue volume. At the same time it is characterized by having the smallest average flow rate and by containing the smallest number of unique paths connecting DAs and AVs. In contrast 2-in-2-out is the rarest MSC-type and contains the largest number of flow paths connecting DAs and AVs. We postulate that MSC-type 2-in-2-out is responsible for distributing blood within the capillary bed and that MSC-type 1-in-1-out is designed to enable efficient oxygen and nutrient discharge to the tissue. In vivo evidence supporting this hypothesis is not yet available. Here, the first step would be to confirm the characteristics of the different MSC-types in vivo and to subsequently study the role of these differences on oxygen and nutrient supply.

The frequency of 2-in-2-out MSCs is low and in a volume of 0.2 nl around the MSC, 27% of vessels show no flow decrease after a microstroke. These two features suggest that the capillary bed offers an inherent level of robustness towards single capillary occlusion and they agree well with the reported highly interconnected nature of the capillary bed that allows efficient rerouting of blood flow [23, 25, 33-35, 55, 56]. Nonetheless, the significant differences between the characteristics of the MSC-types raise further questions regarding the origin and the severity of microstrokes.

First of all: Due to the larger supplied tissue volume, might the occlusion of a 1-in-1-out MSC be more severe for oxygen and nutrient supply, while the occlusion of a 2-in-2-out MSC has a larger impact on the flow field? Here, in vivo studies monitoring tissue oxygenation in response to capillary occlusion or combined blood flow and oxygen transport simulations could provide insights into the most critical MSC-type for oxygen and nutrient supply. Secondly: Would a microstroke be more probable in a 1-in-1-out MSC? This idea is based on the lower average flow rate in 1-in-1-out MSCs, which implies that the vessel might be blocked more easily [44, 45]. However, to answer this question we need to improve our understanding of the mechanisms that cause capillary occlusions. In healthy mice stalls occur with a frequency < 1% [28, 29, 44, 57]. This number increases to 1.8% in AD [28] and to 30% in the core of the stroke [29]. For both pathological conditions the majority of stalls are caused by neutrophils adhering to the vessel wall and occurred across all capillary diameters (~4-10µm) [28, 29]. Besides this aspect, no capillary phenotype could be identified in which stalls are more prominent [44, 57]. Next to...
the phenotype of individual capillaries, the type of bifurcation might be an important factor for the likelihood of occlusion. For example, convergent bifurcations are likely more susceptible to blood particles getting stuck, which might cause an occlusion in capillaries up- and downstream of the bifurcation. However, if the origin of occlusions does not come from blocked particles but from plaque deposits or mural cell activity, then the situation is less clear and all MSC-types are likely affected to similar extent.

Studying the effect of single capillary occlusions in an isolated manner in our in silico model is advantageous on the one hand, but limited on the other hand. For example, our simulation model does not account for dynamic responses of the vasculature. It has been shown that single DA occlusion induces a heterogeneous response in the capillary bed comprising capillary dilations and constrictions [4, 15]. Nonetheless, the in silico approach enables us to perform an in-depth study of fluid dynamical changes in response to single capillary occlusion detached from external and internal influences. Moreover, our observations can be conveniently linked to the surrounding vascular topology. These insights can subsequently be used to distinguish changes observed in in vivo studies.

Taken together, we show that for 57% of all capillaries an occlusion significantly reduces the flow rate in the directly neighboring capillaries. Consequently, we conjecture that a single capillary occlusion can be the starting point of a cascade of small consecutive disturbances, which might be relevant for the development of larger microinfarcts and for the progression of pathologies. In addition, we reveal novel features of the capillary bed, which are relevant for the robustness of perfusion and for advancing our understanding of topological characteristics of this highly interconnected network. Importantly, resolving the smallest scale of disturbance is not only essential to improve our understanding of microinfarct development, but might eventually offer novel possibilities for therapeutic treatment and prevention.

5 Methods

The presented results are based on a computational model to simulate blood flow in realistic MVNs. The model has been published previously [31] and is briefly revised here. We start by giving a summary of the numerical framework to simulate blood flow in realistic MVNs with tracking of discrete RBCs [31, 40]. Subsequently, we provide more details on the analyses used in the current study.

5.1 Blood flow modeling with discrete RBC tracking

The microvascular network is represented as a graph structure, i.e. it consists of a set of nodes $n_i$ connected by a set of edges $e_{ij}$. The subscript $ij$ indicates that edge $e_{ij}$ is connecting node $n_i$ and $n_j$. Anatomically accurate microvascular networks (MVN) have been acquired by Blinder et al. [25] from the mouse somatosensory cortex by two-photon laser scanning microscopy. They are embedded in a tissue volume of ~1.6 mm$^3$ (MVN1) and ~2.2 mm$^3$ (MVN2) and contain ~12,100 and ~19,300 vessels, respectively.

The vessels are labeled as pial arteries (PAs), descending arterioles (DAs), capillaries (Cs), ascending venules (AVs) and pial veins (PVs). For the penetrating vessels, i.e. DAs and AVs, we additionally differ between the main branch and the offshoot vessels. The vessel type is assigned by following the vessels from the cortical surface and by applying a diameter criterion which requests that two subsequent vessels have a diameter smaller than 6 µm in order to change the vessel type from DA to C [31]. The equivalent criterion is applied on the venule side. To differentiate between the main branch of the penetrating vessels and the offshoots we use a criterion that is based on the branching angle and the length of the resulting main branches. This approach ensures that short offshoots are not labeled as main branch.

To compute the pressure field and the blood flow rates in the realistic MVN we employ the continuity equation at every node and Poiseuille’s law along the vessels. This approach is valid...
due to the small Reynolds numbers in the cortical microvasculature (Re < 1.0 for all vessels). To account for the presence of RBCs the vessel resistance is multiplied by the relative effective viscosity $\mu_{rel}$. Taken together, Poiseuille’s law reads

$$q_{ij} = \frac{p_i - p_j}{R_{ij}^2} = \frac{\pi D_{ij}^4}{128 L_{ij} \mu \mu_{rel}} (p_i - p_j)$$

where $D_{ij}$ and $L_{ij}$ are the vessel diameter and the length and $p_i$ and $p_j$ are the pressure at node $i$ and $j$, respectively. $\mu$ is the dynamic plasma viscosity and $\mu_{rel}$ the relative effective viscosity, which is computed as a function of the hematocrit and the vessel diameter as described in Pries et al. (in vitro formulation) [58].

The hematocrit of individual vessels is computed from the discretely tracked RBCs. In order to correctly model the motion of RBCs we account for the Fahraeus effect [58, 59] and the phase-separation. The phase-separation in vessels with a diameter larger than 10 µm is described based on the empirical relation by Pries et al. [58]. In vessels with a diameter < 10 µm, single file flow can be assumed and consequently, we postulate that the RBC follows the path of the largest pressure force [31, 40, 60, 61]. The unequal partitioning of RBCs at divergent bifurcations and their impact on the vessel resistance cause a fluctuating flow and pressure field. In the current study we focus on the analysis of the time-averaged flow field of the statistical steady state. Our average is computed over ten turnover times (15.4 s), where one turnover time is defined as the time until 85% of all vessels have been completely perfused at least once.

The pressure boundary conditions are assigned as described in Schmid et al. [31]. In brief, at the pial vessels we make use of existing experimental data and assign a diameter-dependent pressure value. The pressure values at capillary in- and outlets are set based on the simulation results of the hierarchical boundary conditions approach. Here, the realistic MVN is implanted into a large artificial MVN. Subsequently the flow and pressure field for a constant hematocrit is computed and the resulting pressure values are assigned as boundary conditions. The pressure boundary conditions are kept constant for each microstroke scenario.

### 5.2 Microstroke simulations

The microstroke simulations are performed in MVN1. To mimic a microstroke the diameter of a single capillary is set to 0.01 µm. For all investigated scenarios the resulting flow rate in the microstroke capillary (MSC) is $< 10^{-10} \mu m^3 ms^{-1}$. The average flow rate in a capillary in MVN1 is 4.2 $\mu m^3 ms^{-1}$. This proves that the flow rate in the MSC approaches 0 $\mu m^3 ms^{-1}$ and therewith confirms the validity of our microstroke model.

In total there are 11,386 capillaries in realistic MVN1. To ensure that we choose representative MSCs the following selection criteria have to be fulfilled:

1. The MSC should be located in a cylinder with a radius of 444 µm around the x-y-center of the MVN (number of possible MSCs: 8,718).
2. The average flow rate in the MSC has to be $> 0.16 \mu m^3 ms^{-1}$ (95% of all capillaries, number of possible MSCs: 8,237). In MVN2 this corresponds to 98% of all capillaries.
3. The average hematocrit needs to be $> 0.02$ (95% of all capillaries, number of possible MSCs: 7,824).
4. The flow rate in the MSC and its upstream and downstream neighbors should be stable, i.e. frequent flow direction changes should not occur. To be more precise, we allow 5%, 10% and 30% flow direction changes in the MSC, in the first upstream and downstream vessels and in the second and third upstream and downstream vessels, respectively (number of possible MSCs: 6,307). The relative number of flow direction changes is computed by dividing the number of time steps with a flow direction change by the total number of simulated time steps.
5. The MSC is located approximately in the center of the capillary bed, i.e. it is at least three segments apart from the main branch of the DA and AV (number of possible MSCs: 3,543).
6. The MSC has to fit into a bounding box with a volume of 0.2 nl (number of possible MSC: 3,440).

It should be noted that the "number of possible MSCs" is computed by subsequently considering an additional selection criterion.

One of our goals is to comment on factors influencing the severity of a microstroke. To analyze the impact of different factors, e.g. the baseline flow rate in the MSC, additional selection criteria may be prescribed. These criteria are defined in more detail in the related results sections.

Supplementary File 1a provides an overview of all selection criteria. In total we analyzed twelve different cases. For each case eight microstroke simulations have been performed.

5.3 Thresholded relative change

A major challenge in comparing blood flow simulations in realistic MVNs is the large variety in flow rates, which ranges from 0.09 $\mu m^3 ms^{-1}$ to values as large as 26.76 $\mu m^3 ms^{-1}$ in the capillary bed (minimum and maximum of 95% of all flow rates in the capillary bed, median: 1.99 $\mu m^3 ms^{-1}$). In such a flow field a large relative change in a vessel with a small baseline flow rate might be negligible, while a small relative change in a vessel with a large baseline flow rate might be significant. To improve the comparability of simulation results, we introduce an absolute threshold, $t h^{abs}$. If the absolute change is smaller than $t h^{abs}$ the relative change is set to 0%.

To choose an appropriate threshold value we compare the average flow rate at two points in time for three different averaging intervals (ten, five and three turnover times). The absolute flow change between the two time points is characteristic for the fluctuations of the baseline flow field. Thus, it can be used as a reference of how large the absolute flow change needs to be such that it is likely to be caused by the microstroke and not by baseline fluctuations. The difference between the two time points is 20 s.

It becomes apparent that for each of the three averaging intervals > 87% of the capillaries change their flow rate by less than 0.1 $\mu m^3 ms^{-1}$ (Supplementary File 1f). Consequently, in the microstroke simulations a flow rate change > 0.1 $\mu m^3 ms^{-1}$ is likely caused by the impact of the microstroke and not by baseline fluctuations. Accordingly, we set the absolute threshold $t h^{abs}$ to 0.1 $\mu m^3 ms^{-1}$.

The relative change in flow rate can either be computed directly from the flow rate in the vessel

$$\Delta^{dir} q_{ij} = \frac{q_{ij}^{stroke} - q_{ij}^{base}}{q_{ij}^{base}}$$

or from the absolute flow rates in the vessel

$$\Delta q_{ij} = \frac{|q_{ij}^{stroke} - |q_{ij}^{base}|}{|q_{ij}^{base}|},$$

where $q_{ij}^{base}$ and $q_{ij}^{stroke}$ are the flow rates in vessel $ij$ for the baseline and the simulation with microstroke, respectively. Even though the second formulation neglects changes in flow direction, it is more suitable for comparing the total perfusion of individual capillaries. As the total perfusion is more relevant for oxygen and nutrient supply, we employ the second expression and analyze flow direction changes separately. Taken together the thresholded relative change is computed as

$$\Delta^{dir} q_{ij} = \begin{cases} \frac{|q_{ij}^{stroke} - |q_{ij}^{base}|}{|q_{ij}^{base}|} & \text{for } |q_{ij}^{stroke} - |q_{ij}^{base}| \geq t h^{abs} \\ 0.0 & \text{for } |q_{ij}^{stroke} - |q_{ij}^{base}| < t h^{abs} \end{cases}.$$

The same approach is used to compute the relative change in RBC flux. Here, the threshold is set to 2 RBCs/s.
5.4 Investigating differences over cortical depth

To analyze differences over cortical depth the realistic MVN is divided into five analysis layers (ALs) each 200 µm thick (Figure 2 - figure supplement 4). This analysis approach was first introduced by Schmid et al. [31]. To assign a vessel to an AL, either the source or the target vertex of the vessel has to be within the upper and lower bound of the AL (Supplementary File 1b). The second end point of the vessel is required to be within ±50 µm of the bounds of the AL.

5.5 Analysis of total inflow and total flow in an analysis box around MSC

To comment on the blood supply of a tissue volume around the MSC we compute the total inflow into an analysis box around the MSC. The volume of the smallest analysis box is chosen such that each MSC fits into the smallest analysis box (Figure 2a). This results in an initial box volume of 0.2 nl (200,000 µm³), which would be equivalent to a cube with a side length of 58.48 µm. Moreover, for each MSC we have at least 6 capillaries in the initial analysis box and at least 5 capillaries crossing the border of the analysis box. The chosen initial box volume is a compromise between having the smallest possible analysis box around the MSC and ensuring at the same time that sufficient capillaries are within the analysis box to perform a quantitative investigation. The side lengths of the analysis box vary for the different MSC capillaries. To increase the box volume, the side lengths of the smallest analysis box are increased by the same distance in all three directions until we reach the desired box volume.

To compute the relative inflow change in response to a microstroke we add up all inflows during baseline and during stroke and calculate the relative difference between the total inflow during baseline and during stroke. It is important to note that due to flow reversals in response to a microstroke the number of inflow vessels can change for the baseline and the microstroke case. The equivalent analysis is repeated for increasing box volumes. The relative inflow change per analysis box is depicted in Figure 2b-e, Supplementary Figure 2 - figure supplement 2 c-d, Figure 2 - figure supplement 3 c-d and Figure 2 - figure supplement 5 c-d.

The change in total flow rate per analysis box is computed comparably to the inflow change in an analysis box. Here, instead of computing the total inflow during baseline and during stroke we add up the length weighted total flow rates in the analysis box for baseline and during stroke by summing up the flow rate of all vessels in the analysis box. We consider the vessel tortuosity to compute the vessel length within the analysis box. The total flow rate change per analysis box is used in Figure 2g-i.

5.6 Definition of vessels parallel and distant to the MSC

To study the redistribution of flow in an analysis box we introduce three vessel categories: 1) Vessels upstream and downstream of the MSC. 2) Vessels that branch off/into an upstream/downstream vessel of generation 1 or 2 of the MSC (parallel vessels). Here, we follow each parallel vessel of generation 1 three segments downstream/upstream to create the entire set of parallel vessels. 3) All other vessels in the analysis box (i.e. neither upstream, downstream nor parallel vessels) are called distant vessels. A schematic drawing of these vessel categories is provided in Figure 2f and Figure 2 - figure supplement 1e.

As the whole MVN is connected, distant vessels are also connected to the MSC. However, for this vessel category the point of connection is relatively far upstream or downstream. This approach allows us to study changes in response to a microstroke with respect to the topological distance from the MSC. Note that the concept of parallel vessels has also been used in Nishimura et al. [13]. However, their definition of parallel vessels is different from that used in our analysis. Nishimura et al. [13] consider parallel vessels to be only those vessels that have the same source vertex as the occluded vessel.
5.7 Multi-capillary occlusion scenarios

As we hypothesize that the occlusion of a single capillary might trigger an accumulation of additional microstrokes around the initial MSC, we designed a simulation approach where we sequentially occluded more capillaries around the MSC. From the 27 2-in-2-out simulations eight MSCs have more than twelve capillaries in the analysis box around the MSC with a volume of 0.3 µl (volume factor = 1.5). From this subset we randomly picked six MSC capillaries to study the effect of multi-capillary occlusions. Based on the time-averaged blood flow rates for the simulation with N occlusions, we chose the two capillaries with the lowest blood flow rate in the analysis box around the MSC. These two capillaries will subsequently be occluded and the time-averaged flow field will be re-computed for the new setup with N+2 occlusions. This sequential approach is repeated until we reach nine occlusions in the analysis box of 0.3 µl around the MSC (Figure 3a).

5.8 Computation of AV-factor and related investigations

The AV-factor is computed to distinguish between capillaries that are close to DAs (arteriole-sided capillaries) and those that are close to AVs (venule-sided capillaries). For each capillary $ij$ we computed all paths to all DA endpoints and all paths to all AV endpoints (Figure 4a). This results in a set of path lengths on the arteriole side of the capillary ($dist_{ij}^{DA}$) and a set of path lengths on the venule side ($dist_{ij}^{AV}$). To compute the AV-factor for capillary $ij$ we use the median of each set of path lengths, i.e.

$$AV-factor_{ij} = \frac{\text{median}(dist_{ij}^{DA})}{\text{median}(dist_{ij}^{DA}) + \text{median}(dist_{ij}^{AV})}.$$  

The resulting AV-factor lies between 0 and 1 and approaches 0 on the arterial side and 1 on the venule side of the capillary path.

To investigate the distribution of arteriole- and venule-sided capillaries within the vascular network the following investigations have been performed. First for each venule-sided capillary we computed the shortest distance to any vessel (Figure 4b) and to the closest arteriole-sided capillary (Figure 4c). For this analysis each vessel is split into multiple discretization points, which are on average 1.3 µm apart and which are used to compute the shortest distance. Note that the AV-factor can only be assigned to capillaries along a flow path from DA to AV. Due to the finite size of our simulation domain the AV-factor is only assigned to 60% and 63% of all capillaries in MVN1 and MVN2, respectively. In consequence, the calculated shortest distance to an arteriole-sided capillary might be slightly overestimated.

In the second investigation, an analysis sphere with a radius of 50 µm is moved along the discretization points of every venule-sided capillary (Figure 4d). All capillary points within these analysis spheres are identified and used to calculate the average AV-factor within the analysis spheres of the venule-sided capillary. To guarantee a representative analysis only venule-sided capillaries are considered if at least 50% of all capillary points within the analysis sphere have been assigned an AV-factor.

For the third study on the distribution of AV-factors within the vascular network we employ analysis cubes of varying size (side length 30 – 120 µm). For each cube size the vascular network is discretized by the analysis cubes and the average AV-factor per analysis cube is computed (Figure 4 - figure supplement 1). Here, an overlap of half the cube side length is used between neighboring analysis cubes. The analysis cube is only considered if it contains at least four capillaries with AV-factor and if at least 50% of the capillaries within the analysis cube could be assigned an AV-factor. The analysis cube with a side length of 30 µm contains on average $4.6 \pm 0.9$ (MVN1) and $4.7 \pm 1.0$ (MVN2) capillaries with AV-factor. For the analysis cube with a side length of 120 µm we find $35.7 \pm 8.9$ (MVN1) and $51.0 \pm 11.6$ (MVN2) capillaries with AV-factor within the cube. This results in $7,336$ (MVN1) and $17,915$ (MVN2) analysis cubes with a side length of 30 µm and in $1,316$ (MVN1) and $1,700$ (MVN2) analysis cubes for a side length of 120 µm.
5.9 Computation of the topological supplied tissue volume

To comment on the infarct volume of a microstroke and for further topological studies we compute the supplied tissue volume for each vessel. To do this, the tissue is discretized on a Cartesian grid, in which the realistic MVNs are embedded. One grid cell spans 4 x 4 x 4 µm³, which results in ~11.6 million grid cells for MVN1 and ~15.3 million grid cells for MVN2. Each cell center is assigned to the closest vessel. By summing over all cells assigned to one vessel we obtain the topological supplied tissue volume per vessel. It is important to note that the topological and the effective supplied tissue volume can differ significantly [46, 47, 54, 62]. This is because of different oxygen levels along the capillary path. Consequently, for vessels with high oxygen levels the effective supplied tissue volume is likely larger than the topological supplied tissue volume and vice versa. Nonetheless, we believe that the topological supplied tissue volume is a representative characteristic for the study of topology and perfusion related aspects of the cortical microvasculature. Please note that for simplicity the topological supplied tissue volume is called supplied tissue volume throughout this manuscript.

5.10 Flow paths between descending arterioles and ascending venules

Flow paths between the penetrating vessels are computed by following the flow from the DA to the AV. For this investigation a DA endpoint is defined as the first branch point after the main branch of the DA arteriole. The equivalent definition is used for AVs, i.e. the endpoint of an AV is the point proximal to the capillary bed and the start point of the AV is the root of the penetrating tree at the cortical surface.

To compute all paths between DA and AV we first identify all DA- and AV-endpoints. Subsequently, for each DA-AV-endpoint-pair we compute all unique connecting flow paths. Note that some DA- and AV-endpoints are not fluid dynamically connected. Additionally, multiple paths enter/leave the MVN across its boundaries. As these paths do not connect a DA with an AV they are not considered any further for this analysis.

The resulting flow path data allows for various investigations:

1. The computation of the total number of flow paths in the MVN (Figure 6b and e).
2. The computation of the number of flow paths per capillary and how this number changes during a microstroke (Figure 6 - figure supplement 1d). We focus on the relative change in the number of paths during baseline and during stroke. This is calculated as 100% · \( \frac{n_{\text{stroke}} - n_{\text{baseline}}}{n_{\text{baseline}}} \), where \( n_{\text{baseline}} \) and \( n_{\text{stroke}} \) are the number of flow paths through an individual capillary during baseline and during stroke, respectively.
3. Instead of looking directly at the flow paths, we can also analyze the number of unique DA-AV-endpoint-pairs. Here, we are interested in total number of unique DA-AV-endpoint-pairs. As before, this quantity can be compared between the baseline and the stroke simulation. In Figure 6 - figure supplement 1b we compare the difference of the total number of DA-AV-endpoint-pairs before and after stroke.
4. Lastly, we count the number of unique flow paths between a given DA-AV-endpoint-pair (Figure 6c). To comment on the redistribution of flow with respect to the MSC we introduce two categories to classify DA-AV-endpoint-pairs (Figure 6c): Category 1) before stroke there is at least one path that leads from the DA- to the AV-endpoint through the MSC and Category 2) none of the paths between the given DA-AV-endpoint-pair go through the MSC. Each DA-AV-endpoint-pair is assigned to the according category and the ratio of the number of unique flow paths is computed \( \frac{n_{\text{stroke}}}{n_{\text{baseline}}} \), where \( n_{\text{DA-AV-paths}}^{\text{baseline}} \) and \( n_{\text{DA-AV-paths}}^{\text{stroke}} \) are the number of unique flow paths between a given DA-AV-endpoint-pair during baseline and during stroke. In Figure 6d and f we show the relative frequency of DA-AV-endpoint-pairs with an increase in the number of unique flow paths (i.e. ratio > 1), a decrease in the number of unique flow paths (i.e. ratio < 1) or no change in the number of unique flow paths (i.e. ratio=1) in response to a single...
5.11 Statistics

Depending on the underlying data different statistical tests have been employed. The statistical tests have been performed in R or with the Python Library Stats. The statistical output is summarized in the Figure legends and in Supplementary File 1c-e. For the relative changes presented in Figure 1-3 and Figure 2 - figure supplement 3 and Figure 2 - figure supplement 4 we use a two-way mixed ANOVA with Bonferroni correction (Supplementary File 1c-d). As the data in Figure 2 - figure supplement 2 is paired we compare the grouped data with the paired Wilcoxon test (grouping based on generation and volume factor, respectively). For differences in the characteristics of the four MSC-types we use the Kruskal-Wallis and the Mann-Whitney U test (Figure 5 and Supplementary File 1e). To test for a statistical significant difference in the total number of unique flow paths (Figure 6b and e) we employ the Kruskal-Wallis test.

6 Acknowledgements

We thank David Kleinfeld, Philbert Tsai and Pablo Blinder for sharing the realistic microvascular networks with us. Moreover, we are grateful for the fruitful discussions of our results with Eva Erlebach, Robert Epp and Jacqueline Condrau. Additionally, we thank Eva Erlebach for her feedback on our manuscript. We thank Karen Everett for editorial help with the manuscript.

7 Competing interests

The authors declare that no competing interests exist.

8 References


1188 58. Pries AR, Secomb TW. Microvascular blood viscosity in vivo and the endothelial surface
1189 layer. American Journal of Physiology-Heart and Circulatory Physiology. 2005;289(6):H2657-
1190 H64. doi: 10.1152/ajpheart.00297.2005.
1192 60. Fung Y-C. Stochastic flow in capillary blood vessels. Microvascular research.
1194 61. Yen R, Fung Y. Effect of velocity of distribution on red cell distribution in capillary blood
1199 PMCPMC5932636.

9 Supplementary figure and supplementary file legends

Figure 1 - figure supplement 1 Absolute flow changes in response to occlusions of different MSC types and
frequency of flow decreases. a) d) Average relative absolute change in flow rate ∆qij for capillaries up- and
downstream of the MSC. For each topological configuration the flow field for >=20 microstrokes has been computed
(n: number of microstroke simulations). The average relative change per generation for each simulation is depicted by
the color- and marker-coded symbols. Note that the number of up- and downstream vessels per generation varies
between MSCs. The boxplots are based on the data for each generation. The red line indicates the median relative
change if only capillaries with flow decrease are considered (equivalent to Figure 1). e) f) Frequency of vessels with a
flow decrease for the different MSC-types and different generations. The relative frequency is computed by dividing
the number of capillaries with a flow decrease by total number of capillaries per generation. The bars and error bars
depict mean ± std for >= 20 microstroke simulations.

Figure 1 - figure supplement 2 Flow direction changes for the four MSC types. a) Percentage of flow direction
changes for the four possible topological configurations at a MSC (Figure 1a-d). Only percentage values >2% are
annotated. For each topological configuration >=20 microstroke simulations have been performed. The percentage is
computed by identifying all vessels with a flow direction change for each generation across microstroke simulations
and setting it in relation to the total number of vessel per generation. Abbreviations of the four MSC-types: 2-2: 2-in-
2-out, 2-1: 2-in-1-out, 1-2: 1-in-2-out, 1-1: 1-in-1-out. b) Illustration of the specific vascular configuration for the 2-in-1-
out MSC that leads to a cessation of flow in the generation -1 vessels. A schematic (upper left) and a realistic example
(lower right) are provided. The MSC (dark) and its adjacent vessels (grey, generation -1 and 1) are depicted. The
schematic also shows the joint inflow at generation -2.

Figure 2 - figure supplement 1 Occurrences of different vessels categories within the analysis box around the MSC.

a)-d) Percentage of vessel within the analysis box which are positioned differently with respect to the 2-in-2-out MSC.
2-2 a) Percentage of vessels upstream and downstream of the MSC for an increasing analysis box volume. b) Percentage of
parallel vessels (Methods). c) Percentage of vessels directly connected to the MSC, i.e. upstream, downstream and
distant vessels. d) Percentage of distant vessels, i.e. vessels that are neither upstream, downstream nor parallel. e)
Schematic to illustrate the concept of vessels upstream, downstream, parallel and distant to the MSC (Methods). f)-h)
Percentage of upstream and downstream vessels of the MSC for an increasing analysis box volume for different cases.
f) 2-in-1-out, g) 2-in-2-out close to a descending arteriole (DA) and h) 2-in-2-out far away from a DA. Further details on
the selection criteria are provided in Supplementary File 1a. The initial box volume, i.e. volume factor = 1, is 0.2 nl The
>=20 microstrokes per case are depicted by the colour- and marker-coded symbols. The boxplots are based on the
available data for each volume factor.

Figure 2 - figure supplement 2 Differences between the relative change in flow rate and in red blood cell (RBC) flux
at a 2-in-2-out MSC. a)-b) Average relative decrease in flow rate ∆qij (a) and in RBC flux ∆qij\text{RBC} (b) for capillaries up-
and downstream of the MSC (only capillaries with a flow decrease are displayed). Only at generation -1 there is a
significant differences between the relative change in flow and RBC flux (p=0.018, paired Wilcoxon test). c)-d)
Relative inflow difference for an increasing box volume around the MSC for the flow rate (c) and the RBC flux (d). The
initial box volume, i.e. volume factor = 1, is 0.2 nl. For all volume factors there is significant difference between the
relative inflow rate change and the relative RBC flux change (p < 0.0138 for all volume factors, paired Wilcoxon test).
a) and c) are also depicted in Figure 1a and 2b and are only provided to facilitate comparison between the changes
observed for the flow rate and the RBC flux. The >=20 microstrokes per case are depicted by the colour- and marker-
coded symbols. The boxplots are based on the available data for each generation/volume factor.
Figure 2 - figure supplement 3 Impact of the baseline flow rate on the severity of a microstroke in a 2-in-2-out microstroke capillary (MSC). a)-b) Average relative change in flow rate $\Delta q_y$ for capillaries up- and downstream of the MSC. The red line indicates the median relative change if only capillaries with flow decrease are considered. The two-way interaction between case and generation is not significant (Upstream: $F(1.37,61.84)=0.93$, p=0.37, Downstream: $F(1.37,61.75)=3.2$, p=0.065) and there is no main effect of case (Upstream: $F(1.45)=1.58$, p=0.22, Downstream: $F(1.45)=0.25$, p=0.62, two-way mixed ANOVA). c)-d) Relative inflow difference for an increasing box volume around the MSC. The initial box volume, i.e. volume factor = 1, is 0.2 nl. The relative inflow difference is computed by summing up the inflows across the borders of the box for the baseline and the stroke simulation (Methods). While the two-way interaction between case and volume factor is not significant (F(1.59,71.45)=0.582, p=0.52), there is a statistical significant main effect of case and volume factor on the relative change (Case: $F(1.45)=13.97$, p < 0.001, Volume factor: $F(1.59,71.45)=28.4$, p=0.011, two-way mixed ANOVA). a) and c) show the results for a high baseline flow rate (6.6-25.0 $\mu$m$^3$s$^{-1}$) and b) and d) for a lower baseline flow rate (0.1-4.0 $\mu$m$^3$s$^{-1}$). Further details on the selection criteria are provided in Supplementary File 1a. The >=15 microstrokes per case are depicted by the colour- and marker-coded symbols. The boxplots are based on the available data for each generation/volume factor.

Figure 2 - figure supplement 4 Impact of the cortical depth on the severity of a microstroke in a 2-in-2-out microstroke capillary (MSC). a)-d) Upper panel: Schematic of a 2-in-2-out and the realistic microvascular network, which has been divided into 5 analysis layers (AL) each 200 $\mu$m thick. The arrow indicates the AL for which the results are depicted below. Lower panel: Average relative change in flow rate $\Delta q_y$ for capillaries up- and downstream of the MSC. For each cortical depth the flow field for twelve MSCs has been computed. The average relative change per generation for each of the twelve simulations is depicted by the colour- and marker coded symbols. The boxplots are based on all available data for each generation. The red line indicates the median relative change if only capillaries with flow decrease are considered. Further details on the selection criteria are provided in Supplementary File 1a. The two-way interaction between cortical depth and generation is not significant (Upstream: $F(5.37,81.95)=0.288$, p=0.87, Downstream: $F(6.27,95.68)=0.54$, p=0.79) and there is no main effect of cortical depth (Upstream: $F(4,61)=0.47$, p=0.76, Downstream: $F(4,61)=0.77$, p=0.55, two-way mixed ANOVA).

Figure 2 - figure supplement 5 Impact of the distance of the microstroke capillary (MSC) to the penetrating vessels on the severity of a microstroke in a 2-in-2-out a)-b) Average relative change in flow rate $\Delta q_y$ for capillaries up- and downstream of the MSC. The red line indicates the median relative change if only capillaries with flow decrease are considered. The two-way interaction between distance to descending arteriole (DA) and generation is not significant (Upstream: $F(2.17,69.47)=0.3$, p=0.83, Downstream: $F(2.76,86.34)=0.38$, p=0.7) and there is no main effect of distance to DA (Upstream: $F(1.32)=0.25$, p=0.62, Downstream: $F(1.32)=0.25$, p=0.62, two-way mixed ANOVA). c)-d) Relative inflow difference for an increasing box volume around the MSC. While the two-way interaction between distance to DA and volume factor is not significant (F(1.55,49.6)=1.22, p=0.295), there is a statistical significant main effect of distance to DA on the relative inflow change (Case: $F(1.32)=8.27$, p = 0.007chr, two-way mixed ANOVA). The upper panel shows a schematic of the location of the MSC capillary along an exemplary capillary path between descending DA and ascending venule (AV). The initial box volume, i.e. volume factor = 1, is 0.2 nl. The relative inflow difference is computed by summing up the inflows across the borders of the analysis box for the baseline and the stroke simulation (Methods). Further details on the selection criteria are provided in Supplementary File 1a. The >=15 microstrokes per case are depicted by the colour- and marker coded symbols. The boxplots are based on the available data for each generation/volume factor. a) and c) show the results for MSC close to the DA and b) and d) for MSCs distant to the DAs.

Figure 4 - figure supplement 1 Average AV-factor in analysis cubes of varying size for microvascular network 1 (MVN1, a) and MVN2 (b). To compute the average AV-factor the MVNs are discretized by analysis cube of varying size (Methods). Neighbouring analysis cubes overlap by half their side length. This results in 7,336 (MVN1) and 17,915 (MVN2) analysis cubes with a side length of 30 $\mu$m and 1,316 (MVN1) and 1,700 (MVN2) analysis cubes for a side length of 120 $\mu$m.

Figure 5 - figure supplement 1 Length and distance to penetrating vessels of microstroke capillaries (MSC) of different types. a) Schematic of the four topological configurations at the MSC. The MSC is colour coded in accordance with subfigures b)-k). b)-c) Median vessel length of the four MSC types (b: MVN1, c: MVN2). d)-g) Median length of the minimum distance of all paths leading from a MSC to descending arteriole (DA, d: MVN1, e: MVN2) and ascending venule (AV, f: MVN1, g: MVN2) main branches. h)-k) Median length of the median distance of all paths leading from a MSC to DA (h: MVN1, i: MVN2) and AV (j: MVN1, k: MVN2) main branches. The error bars show the 95%-confidence interval. Abbreviations of the four MSC-types: 2-2: 2-in-2-out, 2-1: 2-in-1-out, 1-2: 1-in-2-out, 1-1: 1-in-1-out. The statistics are based on all capillaries that fulfill the general selection criteria described in the Methods. The fifth selection criterion is less strict for the current analysis, i.e. the capillary only has to be two segments apart from the DA/AV, and the sixth criterion is not applied. This results in 4,794 and 8,517 capillaries for analysis for MVN1 and
MVN2, respectively. The analysis on the path lengths is based on 2968 and 6571 capillaries for MVN1 and MVN2, respectively.

Figure 5 - figure supplement 2 Median flow rate, median and total relative supplied tissue volume for the four microstroke capillary (MSC) types over cortical depth. a) Schematic of a realistic microvascular network, which has been divided into 5 analysis layers (AL) each 200 µm thick. The arrow indicates for which AL the results are depicted in the subplots below. d) Median flow rate for the different MSC-types over cortical depth. g) Median supplied tissue volume for the different MSC-types over cortical depth. The relative supplied tissue volume is calculated by summing up the supplied tissue volume for each MSC-type and dividing it by the total tissue volume per AL. The results for AL 1, 3 and 5 are displayed. The results are shown separately for microvascular network 1 (MVN1) and MVN2. Abbreviations of the four MSC-types: 2: 2-in-2-out, 2: 2-in-1-out, 1: 1-in-2-out, 1: 1-in-1-out. The statistics are based on all capillaries that fulfill the general selection criteria described in the Methods. The fifth selection criterion is less strict for the current analysis, i.e. the capillary only has to be two segments apart from the DA/AV, and the sixth criterion is not applied.

Figure 6 - figure supplement 1 Changes in the number of flow paths and the number of DA-AV-endpoint-pairs in response to a microstroke. a) Schematic of the four topological configurations at the microstroke capillary (MSC). The MSC is colour coded in accordance with subfigures b)-d). b) Difference (Diff.) in the total number of unique DA-AV-endpoint pairs (Methods, Diff. < 0: Decrease in the number of unique DA-AV-endpoint pairs with respect to baseline). The data of all microstroke simulations per MSC-type is shown in the scatter points and summarized in the boxplot right of it. Six data points are not displayed because the absolute difference is >60. c) Schematic to introduce DA-AV-endpoint-pairs and the concept of flow paths through a MSC. The illustrated example has 15 flow paths going through the MSC and 6 DA-AV-endpoint-pairs. Only paths through the MSC are depicted. d) Relative change in the number of flow paths through upstream and downstream, parallel and distant of capillaries (Methods). The relative change is computed from the total number of paths during baseline and during stroke (Methods). Only capillaries that are at least along one flow path between DA and AV during baseline are considered. The data of all microstroke simulations per MSC-type is shown in the scatter points and summarized in the boxplot right of it. Triangles: Capillaries upstream and downstream of the MSC (3 generations). Squares: Capillaries parallel to the MSC. Circles: Capillaries distant to the MSC. 0.1% of the data is not displayed (change > 800%). Abbreviations of the four MSC-types: 2: 2-in-2-out, 2: 2-in-1-out, 1: 1-in-2-out, 1: 1-in-1-out.

Supplementary File 1a Overview of the eight selection criteria used to analyse the impact of structural and functional characteristics on the severity of a microstroke. The different microstroke capillary (MSC) types are depicted in Figure 1a-d. For cases 1-7 the cortical depth selection criterion requires that only the source of the MSC be within the given range. For cases 8-12 at least one of the vertices should be within the given range, while the second one may be ±50 µm outside the given range. The mean and standard deviation (std) are calculated from the results of the baseline simulation for the eight chosen MSC per case. For the mean and std of the cortical depth the values of the source and the target vertex are both considered. The definition of the main branch is provided in the methods. DA: descending arteriole. AV: ascending venule. n: simulated number of MSCs per case.


Supplementary File 1c Statistical results for the effect of the MSC-type on the changes observed at different generations (Figure 1). The effect of the MSC-type has been analysed separately for the generations up- (to -5) and downstream (1 to 5) of the MSC. The statistical test has been performed in R with the function anova.test() as a two-way mixed ANOVA with Bonferroni correction. Upper table: There is a significant simple main effect of the factor MSC-type at all generations except generation ±5. Lower table: Pairwise t-test to determine for which MSC-types there is a significant difference in the changes observed per generation. Only pairs with a significant difference are listed. Case 1: 2-in-2-out, Case 2: 2-in-1-out, Case 3: 1-in-2-out, Case 4: 1-in-1-out. p-adj.: adjusted p-value, sign: significance.
Supplementary File 1d Statistical results for the effect of the MSC-type on the changes in inflow rate for analysis boxes of different volumes (Figure 2b-e). The statistical test has been performed in R with the function anova_test() as a two-way mixed ANOVA with Bonferroni correction. Upper table: There is a significant simple main effect of the factor MSC-type for all volume factors <2.75. Lower table: Pairwise t-test to determine for which MSC-types there is a significant difference in the changes observed per volume factor. Only pairs with a significant difference are listed. Case 1: 2-in-2-out, Case 2: 2-in-1-out, Case 3: 1-in-2-out, Case4: 1-in-1-out. p-adj.: adjusted p-value, sign: significance.

Supplementary File 1e Statistical results for the characteristics of different MSC-types (Figure 5f-k). The statistical test has been performed in with the Python library scipy.stats. The Kruskal-Wallis test showed a significant difference between supplied tissue volume, flow rate and number of paths in both microvascular networks (MVNs, all p-values <0.001). Below the p-values of the pairwise comparison with the Mann-Whitney U test are listed. Upper table: p-values for MVN1. Lower table: p-values for MVN2. Abbreviations for the MSC-types: 2-2: 2-in-2-out, 2-1: 2-in-1-out, 1-2: 1-in-2-out, 1-1: 1-in-1-out. ns: not significant.

Supplementary File 1f Absolute differences between averaged flow rates in all capillaries at two time points t1 and t2. The time difference between the two time points is 20s. In the left panel the absolute differences for an averaging interval of 10 turnover times (ToT) are displayed. In the middle and the left panel the differences for averaging intervals of 5 ToTs and 3 ToTs are shown. The absolute differences between the averaged results increase for smaller averaging intervals. For an averaging interval of 10 ToT for 94% of all vessels the absolute difference is smaller than 0.1 μm²ms⁻¹. This value decreases to 91% and 87% for an averaging interval of 5 ToT and 3 ToT, respectively.
a) 2-in-2-out
b) 2-in-1-out
c) 1-in-2-out
d) 1-in-1-out

Relative flow change [%] vs. Generation

- a) n= 27
- b) n= 20
- c) n= 22
- d) n= 20
a) Frequency of direction changes

<table>
<thead>
<tr>
<th>Generation</th>
<th>Downstream MSP</th>
<th>Upstream MSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-2</td>
<td>5%</td>
<td>46%</td>
</tr>
<tr>
<td>2-1</td>
<td>16%</td>
<td>42%</td>
</tr>
<tr>
<td>1-2</td>
<td>6%</td>
<td>19%</td>
</tr>
<tr>
<td>1-1</td>
<td>6%</td>
<td>8%</td>
</tr>
</tbody>
</table>

b) Flow direction in MSC

Joint inflow at generation -2
Volume factor [-]

Relative flow difference [%]

Volume factor = 1
V = \(V_{\text{init}}\) = 0.2 nl

Volume factor = 1.25
V = 1.25*\(V_{\text{init}}\) = 0.25 nl

1-in-1-out

Upstream and downstream vessels
Parallel vessels
Distant vessels

MSC
US & DS

\(\text{relative inflow difference [%]}\)

2-in-2-out

2-in-1-out

1-in-2-out

2-in-2-out
Analysis layers

2-in-2-out

upstream MSC downstream

2-in-2-out

upstream MSC downstream

2-in-2-out

upstream MSC downstream

2-in-2-out

upstream MSC downstream

Relative flow change [%]

n= 12

-5 -4 -3 -2 -1 1 2 3 4 5

Generation

Relative flow change [%]

n= 12

-5 -4 -3 -2 -1 1 2 3 4 5

Generation

Relative flow change [%]

n= 12

-5 -4 -3 -2 -1 1 2 3 4 5

Generation

Relative flow change [%]

n= 12

-5 -4 -3 -2 -1 1 2 3 4 5

Generation
Occluded capillaries

- 126 µm
- 92 µm
- 138 µm
- 133 µm
- 114 µm
- 107 µm

Relative flow difference [%]

Volume factor [-]

Vessels with flow decrease [-]

Occluded capillaries

1 3 5 7 9
a) Set of paths to penetrating vessels for capillary \( ij \)
\[ \text{dist}_{ij}^{\text{DA}} = \{p_1, p_2, p_3\} \]
\[ \text{dist}_{ij}^{\text{AV}} = \{p_1, p_2, p_3, p_4, p_5\} \]

AV-factor = \[ \frac{\text{median} (\text{dist}_{ij}^{\text{DA}})}{\text{median} (\text{dist}_{ij}^{\text{DA}}) + \text{median} (\text{dist}_{ij}^{\text{AV}})} \]

b) Relative frequency of shortest distance [\( \mu \text{m} \)]

| 20 | 0.10 |
| 40 | 0.10 |
| 60 | 0.10 |
| 80 | 0.10 |
| 100| 0.10 |

MVN1 | MVN2 | AV-factor > 0.5
---------------------
68.08% | 31.92% |

c) Relative frequency of distance factor [\( \mu \text{m} \)]

| 5  | 0.30 |
| 10 | 0.30 |
| 15 | 0.30 |
| 20 | 0.30 |
| 25 | 0.30 |

MVN1 | MVN2
-----|-----
2.73  | 2.78

e) MVN1

| -0.5 | Delta AV-factor |
| 0.00 | 0.00 |
| 0.50 | 0.50 |

Caps with AV-factor decrease: 68.08%
Caps with AV-factor increase: 31.92%

MVN2

| -0.5 | Delta AV-factor |
| 0.00 | 0.00 |
| 0.50 | 0.50 |

Caps with AV-factor decrease: 60.39%
Caps with AV-factor increase: 38.70%
Relative frequency [-]

2-in-2-out
2-in-1-out
1-in-2-out
1-in-1-out

a) base

b) Number of paths from DA-AV [-]

c) Category 1 - Baseline

MSC

Category 1 - After microstroke

Category 2 - Baseline

and after microstroke

Number of paths from DA-AV [-] × 10^4

No occluded caps [-]

f) Decrease
Increase
no change

Category 1

Category 2

Decrease
Increase
no change

Number of paths from DA-AV [-] × 10^4

No. occluded caps [-]