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Pericyte Electrical Signalling and Brain Haemodynamics

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ABSTRACT

Dynamic control of membrane potential lies at the nexus of a wide spectrum of biological processes, ranging from the control of individual cell secretions to the orchestration of complex thought and behaviour. Electrical signals in all vascular cell types (smooth muscle cells, endothelial cells and pericytes) contribute to the control of haemodynamics and energy delivery across spatiotemporal scales and throughout all tissues. Here, our goal is to review and synthesize key studies of electrical signalling within the brain vasculature and integrate these with recent data illustrating an important electrical signalling role for pericytes, in doing so attempting to work towards a holistic description of blood flow control in the brain by vascular electrical signalling. We use this as a framework for generating further questions that we believe are important to pursue. Drawing parallels with electrical signal integration in the nervous system may facilitate deeper insights into how signalling is organized within the vasculature and how it controls blood flow at the network level.

1 | Introduction

Neurons and glia that are located far from arterioles and the proximal capillary bed are presented with the problem of communicating the need for local blood flow alterations over long distances. Occlusion of a single cortical penetrating arteriole (PA) in the cortex causes neuronal death over a radius of ~200–300 μm from the occluded vessel [1, 2], suggesting that the supply territory of a single PA is about 400–600 μm in diameter. Pure diffusion of signalling molecules from neurons and glia to PAs is unlikely to explain neurovascular coupling (NVC) over these distances, as neuronal activity would need to elicit the release of mediators that are (a) potent enough to affect the PA supplying their nearby capillaries; (b) able to readily navigate the extreme tortuosity of the brain parenchyma and (c) able to overcome their exponential decay in concentration over distance and any local consumption/degradation processes occurring as they spread from their point source of generation. On the other hand, a sensor and transmitter embedded in the vasculature capable of reporting neural activity could relay signals back to the arteriole from the capillaries through the vessels themselves, requiring neurons and glia instead to signal only short distances to nearby capillaries to initiate an augmentation of blood flow.

Evidence for cells of the vasculature performing both local sensing and long-distance transmission roles in the brain is accumulating, and we review here our understanding of the current mechanisms enabling both of these aspects of blood flow control. Indeed, the collective works of many groups has led to remarkable advances in our understanding of how vascular cells interact with their local environment and with one another to continuously regulate blood flow using electrical

Summary

Brain blood vessels are responsible for the delivery of nutrients to populations of active brain cells. Pericytes, which reside upon capillaries (the smallest blood vessels in the brain), are emerging as key players in this delivery system. Pericytes initiate electrical signals that travel along the capillaries to indicate the need for energy to upstream muscularized arterioles, causing them to dilate to increase blood flow and deliver more nutrients. Together, pericytes, capillaries and their upstream arterioles form a functional unit that tracks the activity of local neurons, sends electrical signals, and delivers more blood very precisely to working regions.

signals. We aim to illustrate the core principles that have emerged from this body of work and highlight some of the exciting questions that remain to be addressed to further advance our understanding in this area. We focus in particular on the emerging role of thin-strand pericyte electrical signalling from the deep capillary bed and we explore the concept of pericytes as integrators of the activity and metabolic state of the local tissue within the territories they occupy. By encoding this information as electrical signals, thin-strand pericytes can stream these data over long distances via the underlying capillaries to be factored into computations being continually performed and read out by the upstream blood flow control apparatus.

1.1 | The Vascular System Is a Cooperative of Cells Engaged in Constant Communication

The vasculature, comprised primarily of smooth muscle cells (SMCs), endothelial cells (ECs), and pericytes, forms a body-wide functional syncytium that delivers blood to and from all cells. In humans this network is composed of thousands of miles of arteries, capillaries and veins—numbering hundreds of billions of cells [3]—and these are required to dynamically regulate energy substrate delivery, waste clearance, blood pressure and temperature on a moment-to-moment basis in response to a huge array of input stimuli (e.g., chemical mediators, shear forces, changes in temperature and deviations in intravascular pressure). These diverse input modalities are distilled into changes in cellular membrane potential, which spread as messages encoding information on local blood flow needs at their point of origin. These signals are continually integrated and read out by the contractile cells on arteries and proximal regions of the capillary bed (i.e., SMCs and ensheathing pericytes, respectively) and converted into deviations in lumen diameter which adjust local blood flow on a moment-to-moment basis.

In the brain, pial arteries coursing over the cortical surface give rise to orthogonal PAs that dive into the tissue. From here, these branch again to give rise to capillaries that ramify further before joining with draining penetrating venules [4–6]. A widely accepted labelling system [7–11] that enables ready orientation in this tortuous network takes the PA as the 0-order vessel, and an initial capillary branching from this is referred to as a '1st order capillary', which typically bifurcates giving rise to two daughter branches labelled '2nd order capillaries'. With each consecutive branching, the ordering number of the daughter segments increases by one. The capillaries ramify extensively to form a vast and interconnected plexus through which blood must be efficiently directed to nourish the local neural tissue, and these are then drained by numerous penetrating venules (Figure 1).

Arteries and arterioles consist of an inner lining of ECs that directly contact the blood, and these are encircled by SMCs that regulate vessel diameter and therefore blood flow. The majority (~70%) of PAs have at least one specialized pre-capillary sphincter [12–14], which are poised to tightly control blood entry into the capillaries and are predicted to shape the pressure gradient from PA to the capillary bed [13]. The capillaries are composed of a molecularly-distinct set of ECs that organize into a continuum with varying gene expression along the arterio-venous axis [15–17], and their abluminal surface is decorated by the cell bodies and processes of pericytes, which may be divided into the major categories of ensheathing (in the initial ~3–4 capillary branch orders) and thin-strand (~5th order and above) subtypes [7, 8, 18]. Draining venules and veins consist of ECs and surrounding ramified, non-contractile venous SMCs. This syncytium of vascular cells is embedded within the computing brain parenchyma consisting of networked neurons alongside a range of glial cell types. Multiple mechanisms have been proposed through which increased neuronal energy demand is communicated to the vasculature to control blood flow [19–22], including the generation of diffusible mediators [10, 23–32], direct synapsing of axons onto SMCs [33] and metabolic sensing mechanisms [34–36] (n.b. [37, 38]). It is thought that these mechanisms are converted to electrical signalling throughout the vasculature which ultimately relaxes smooth muscle and ensheathing pericytes to cause vasodilation [31, 35, 39–42]. Below, we focus our attention on this latter aspect of brain blood flow control and aim to integrate established findings with emerging data to provide a current understanding of how electrical signalling throughout the cerebrovascular network controls blood distribution.

1.2 | Electrical Connectivity Between Cells of the Cerebrovascular Network

Ultrastructural data, dye transfer studies, functional analyses and molecular experiments together support the conclusion that each of the vascular cell types described above are connected to one another via gap junctions (GJs). In arteries and arterioles, ECs and SMCs are separated from one another by an internal elastic lamina. This sheet of connective tissue is fenestrated, and the resulting gaps allow ECs and SMCs to make direct synapse-like connections, known as myo-endothelial projections (MEPs; Figure 2), at which their membranes come into contact and GJs are formed [43, 46–48]. This allows for the rapid exchange of signalling molecules and electrical charge between these two cell types. Similarly, pericytes and ECs are connected via peg-socket junctions (PSJs) [45, 49, 50]—sites of direct contact between these two cell types which allow for the rapid interchange of information. PSJs have been documented at the level of thin-strand pericytes [45], found in the deeper reaches of the capillary

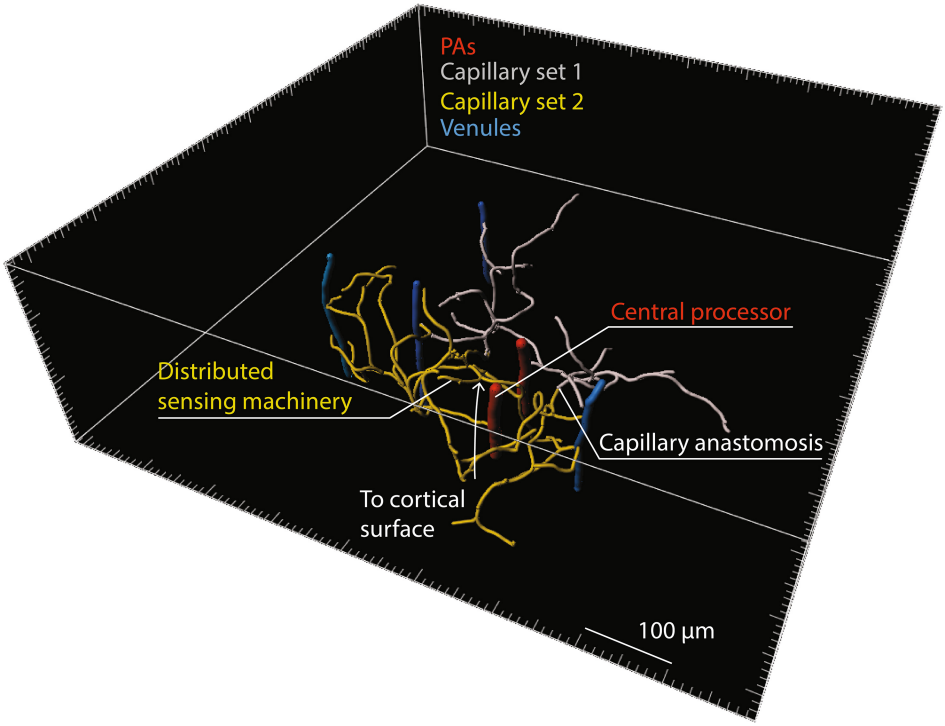

FIGURE 1 | Conceptualization of capillaries as a distributed sensor array for penetrating arterioles. Two PAs (red) are shown diving into the cortex. From these extend a wide net of branching and anastomosing capillaries (grey/yellow) terminating at a number of draining venules (blue). This arrangement effectively increases the sensing range and input to the contractile SMCs that control PA diameter and ensheathing pericytes that control proximal capillary diameter. Accordingly, the deep capillaries can be viewed as distributed sensing machinery that continually streams signals to the PA and proximal capillaries where these signals are processed to output a blood flow response.

network, and are presumed to occur at the level of ensheathing pericytes in the proximal capillary bed also, although extensive study of these is currently lacking (Figure 2). Notably, GJs have been observed in human cerebral capillaries at pericyte-EC PSJs [51], suggesting that this anatomy is conserved between research animals and humans. Longitudinal connectivity is also provided via GJs between adjacent ECs [52–55], which is supported by functional evidence [56–58] and dye-transfer studies [59, 60]. MEPs have also been observed in saphenous veins [46] and, by extension, likely also occur in the cerebral venular system, but this possibility has yet to be directly addressed to our knowledge. Together, these structures convert the vasculature into a continuum allowing for the spread of electrical signals originating in response to an increase in network activity or metabolic need at a particular locus. Ultimately, electrical signals are transmitted to, and interpreted by, the SMCs and ensheathing pericytes to control blood flow into the capillary bed.

1.3 | SMC and Ensheathing Pericyte Membrane Potential Dictates Blood Flow

It is emerging that SMCs and ensheathing pericytes express similar molecular machinery [8, 16–18, 61], which enables rapid modulation of the diameter of the underlying vessel and thereby tight control of blood flow. In SMCs, work over the past several decades has delineated the molecular controllers of contractile state in detail. At the heart of this is the contractile apparatus, consisting of α-smooth muscle actin and heavy and light myosin chains which interact through crossbridge cycling to control cell shape. This process is dependent on phosphorylation of the myosin headgroup by myosin light chain kinase which in turn is activated by calmodulin, the key sensor of intracellular calcium (Ca^{2+}) levels. (Note also that phosphorylation state can be controlled through Ca^{2+}-independent mechanisms, recently reviewed by Touyz et al. [62]). Modulation of intracellular Ca^{2+} is primarily the result of the opening and closing of the large population of L-type voltage-dependent Ca^{2+} channels (VDCCs) in the membrane, the activity of which is dictated by membrane voltage (Vm) [63]. Accordingly, the combined activity of the rich repertoire of ion channels in the membrane of an individual SMC and the electrically connected cells nearby is continually read out by the VDCCs to tune SMC Ca^{2+} levels and contractile state [64, 65].

There are a broad range of stimuli that influence channel activity and SMC Vm, including local agonists acting on membrane proteins (e.g., G-protein coupled receptor (GPCR) activity leading to channel phosphorylation to modulate open probability), and physical forces such as intravascular pressure sensed by SMCs or shear stress on ECs. These inputs are continually integrated by the cell membrane and, while extremely important, they form the backdrop for the present discussion. Our focus is on another important source of modulatory voltage input for SMCs in the form of electrical signals transmitted through the vasculature from the capillary bed [31, 35, 40, 56, 66–69]. Indeed, beyond their well appreciated roles in nourishing and cleaning the local milieu around the neurons that they supply,

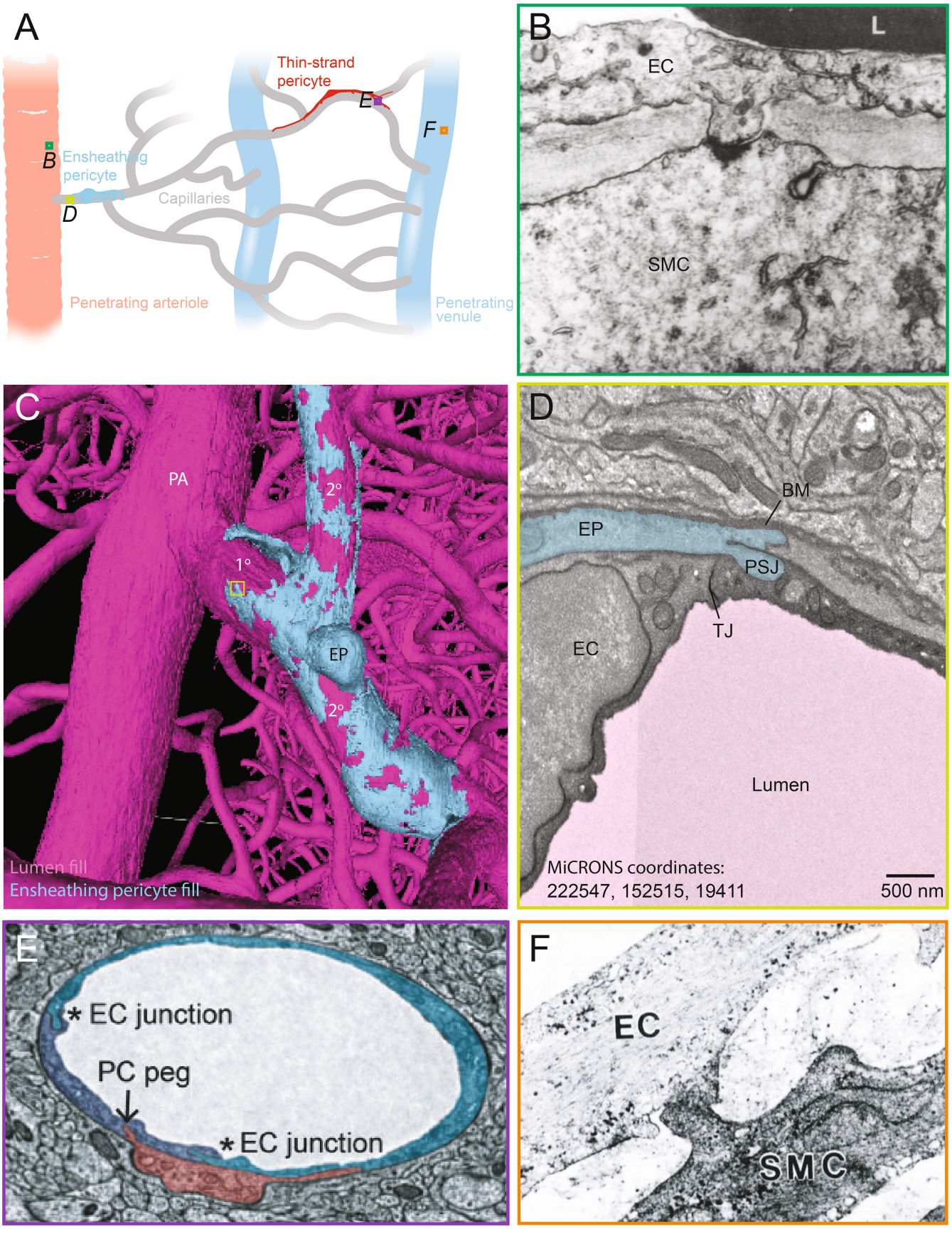

FIGURE 2 | Legend on next page.

FIGURE 2 | Connectivity along the arterio-capillary-venous continuum. (A) Cartoon depicting the representative locations of the projections identified in ultrastructural data in (B–F) throughout penetrating arterioles, capillaries and venules. **B.** Myoendothelial projection between a human penetrating arteriole endothelial cell (EC) and an adjacent smooth muscle cell (SMC). Reproduced with permission [43]. (C) 3-D render extracted from the MiCRONS [44] dataset of a mouse penetrating arteriole (PA) and its branching downstream capillaries with an ensheathing pericyte (EP) highlighted. (D) Scanning electron micrograph from the MiCRONS dataset at the location indicated by the yellow box in *C*, showing a likely peg-socket junction (PSJ) between an ensheathing pericyte (EP) and underlying capillary endothelial cell (EC). The capillary basement membrane (BM) in which the pericyte is embedded is also visible, as is a tight junction (TJ) which forms the blood brain barrier at EC borders. (E) Peg-socket junctions are also found between thin-strand pericytes (red) and ECs (blue). Reproduced with permission [45]. (F) A myoendothelial projection between a venous SMC and EC in a human saphenous vein. Reproduced with permission [46].

the capillaries can be viewed as a distributed sensor array (Figure 1) which continually streams information to the upstream arteriole on the activity state of neural networks within their flow domain. Given access to this information, the PA can then optimize blood delivery to its various downstream capillary offshoots by summating the incoming voltage inputs and altering the contractile state of cells feeding those branches to match demand.

The positioning of ensheathing pericytes just downstream of the PA on the initial few branches of the capillaries [8] allows them to fill a separate blood flow control niche [11]. By operating in a similar manner to SMCs and integrating signals from the downstream capillaries, the ensheathing pericytes are positioned to provide fine-grained control of blood flow into their downstream capillary network [18]. This conceptualization relies on differences in the magnitude of signals being generated in the deeper reaches of the capillary bed which then spread upstream to these sites. Strong signals will not only pass via PSJs from ECs into electrically coupled ensheathing pericytes (Figure 2) and cause them to dilate, but will also reach beyond these to the PA to cause SMC relaxation and an increase in arteriole diameter. The result will be a large increase in blood flow through the dilated vessels to the capillary bed. On the other hand, weaker signals that reach the ensheathing pericytes but do not extend as far as the PA would be expected to cause dilation of the underlying capillary alone. This scenario will create a path of least resistance for flowing blood which is diverted towards the active network. By extension, the weaker the signal—and therefore, the fewer ensheathing pericyte-covered capillaries it affects—the subtler the blood flow diversion to the region in need of hyperemia [36].

Discernment of the contractile machinery and membrane protein complement of ensheathing pericytes is an ongoing effort in the field. Evidence to date indicates that the molecular toolkit of these cells is similar to SMCs [16, 17], although there are key differences emerging which have implications for the control of blood flow. In keeping with SMCs, ensheathing pericytes express the same actin and myosin components that enable rapid modulation of cell shape and therefore lumen diameter [8, 18, 61]. However, the levels of actin and myosin, quantified from immunohistochemical staining, appears to decrease progressively with increasing distance into the transitional zone of the capillary bed [18]. Similar experiments reveal a decrease in phalloidin staining of filamentous actin in ensheathing pericytes compared to SMCs, a lack of the actin-binding protein calponin and an absence of staining for polymerized microtubules despite expression of tubulin genes [16–18]. Other studies have noted shared expression of desmin by SMCs and ensheathing pericytes, with desmin noted to be more abundant in ensheathing pericytes [61], and possibly shared expression of caldesmon [70] and vimentin [71]. The expression of platelet-derived growth factor receptor β (PDGFRβ) and the cell surface proteoglycan NG2 are also shared in both SMCs and pericytes, again with higher levels in the latter [61], and these have been used to drive mural cell-specific expression of tools. Both cell types produce and secrete the basement membrane protein collagen IV [61, 72] but a further distinction between ensheathing pericytes and SMCs is expression of the aminopeptidase CD13, found at high levels in the former but not the latter [16, 17, 61]. It is noteworthy that not all findings in this area are universal and, while there is general agreement between independent observers, the choice of fixatives, markers and various other aspects of preparations may influence experimental outcomes (see, e.g., [73]). Further studies utilizing orthogonal approaches are expected to continue to refine our knowledge of the similarities and differences between these cells.

Electrophysiological and imaging investigations are adding an important functional layer to our understanding in this area. Indeed, several Ca^{2+} imaging studies have yielded further insights that differentiate ensheathing pericytes from SMCs. An interesting distinction in this regard is the predominance of inositol-1,4,5-triphosphate receptor (IP_3R)-dependent Ca^{2+} signals in ensheathing pericytes [18], and the lack of a prominent role for ryanodine receptors (RyRs) here, which in contrast are an important player in SMC Ca^{2+} handling [74]. Both SMCs and ensheathing pericytes possess functional L-type voltage-dependent Ca^{2+} channels (VDCCs), and both respond to the thromboxane analog U46619 with robust elevations of Ca^{2+} signalling [18, 75, 76]. Ensheathing pericytes also possess functional Ca^{2+}-activated Cl^- channels (CaCCs) [77], in similarity with cerebral SMCs [78, 79]. Interestingly, CaCC current density appears to be higher in ensheathing pericytes compared to SMCs [77–79], which could suggest distinctions in the contribution of these channels to control of Vm in either cell type.

Our understanding of the ion channels functionally expressed by ensheathing pericytes certainly lags our more extensive understanding of SMC ion channels at present, and thus, further work is needed to continue to develop our understanding in the former. In SMCs, there are several important classes of K^+ channel which make varied contributions to Vm control. Large-conductance Ca^{2+}-activated K^+ (BK) channels are found in PA SMCs [80] and play an important role in SMC physiology through

their responses to both Ca^{2+} and membrane voltage. These channels are organized into microdomains with local RyRs on the ER where RyR-mediated Ca^{2+} sparks drive BK channel activity to provide feedback control of Vm, and their activity can also be modulated by a range of other signalling events and modifications [81]. Voltage-gated K^+ (Kv) channels are another important source of feedback control in PA SMCs [82], which become more active at increasingly depolarized potentials to resist contraction. Kv1.2 and Kv1.5 are thought to be the predominant SMC Kv isoforms that constitute these channels in vascular SMCs, with Kv2 and Kv7 also contributing to Vm control [83]. Also notable is the contribution of strong inward-rectifier ($K_{IR}2$) channels to the control of PA SMC Vm [84], which can amplify arriving hyperpolarizing signals through their relief of voltage-dependent block of the channel by positively-charged species such as polyamines [85]. Whether these K^+ channels are functional in ensheathing pericytes specifically remains to be addressed, to our knowledge, although a recent study provided evidence for these conductances in isolated brain pericytes generally without making subtype distinctions [86]. Further work is thus needed to determine the function and roles of these channels in different pericyte subtypes along the arterio-venous continuum.

The field is also beginning to explore transient receptor potential (TRP) channels in ensheathing pericytes, and a recent study indicated that Ca^{2+} events in these cells in the retina are sensitive to the non-selective TRPC channel-antagonist SKF-96365 [76]. This creates a path for further studies using precision approaches to delineate the exact channel isoforms that are functionally present in these cells, along with exploration of the presence of other potential TRP family members [87]. Interestingly, TRPC channels (in particular isoforms 3 [88] and 6 [89]) play an important role in cerebral SMC function, and other functionally important members of this family for SMCs include TRPM4 [89, 90] and TRPV4 [91]. Thus, it is important to determine whether these same isoforms are similarly expressed in the neighbouring ensheathing pericytes and whether they perform the same functions.

Accordingly, a picture is emerging of ensheathing pericytes as cells that operate in a similar manner to SMCs, but the emerging subtle differences between these cell types could lead to distinct dynamics of contraction and relaxation to influence their contribution to blood flow control (Figure 3). Indeed, one careful study documented distinct response properties of units of the vasculature defined primarily by the type of mural cell they possess (i.e., SMCs, ensheathing pericytes, or thin-strand pericytes) [11]—the emerging differences in protein and gene expression between these different mural cells may provide a molecular basis for these observations.

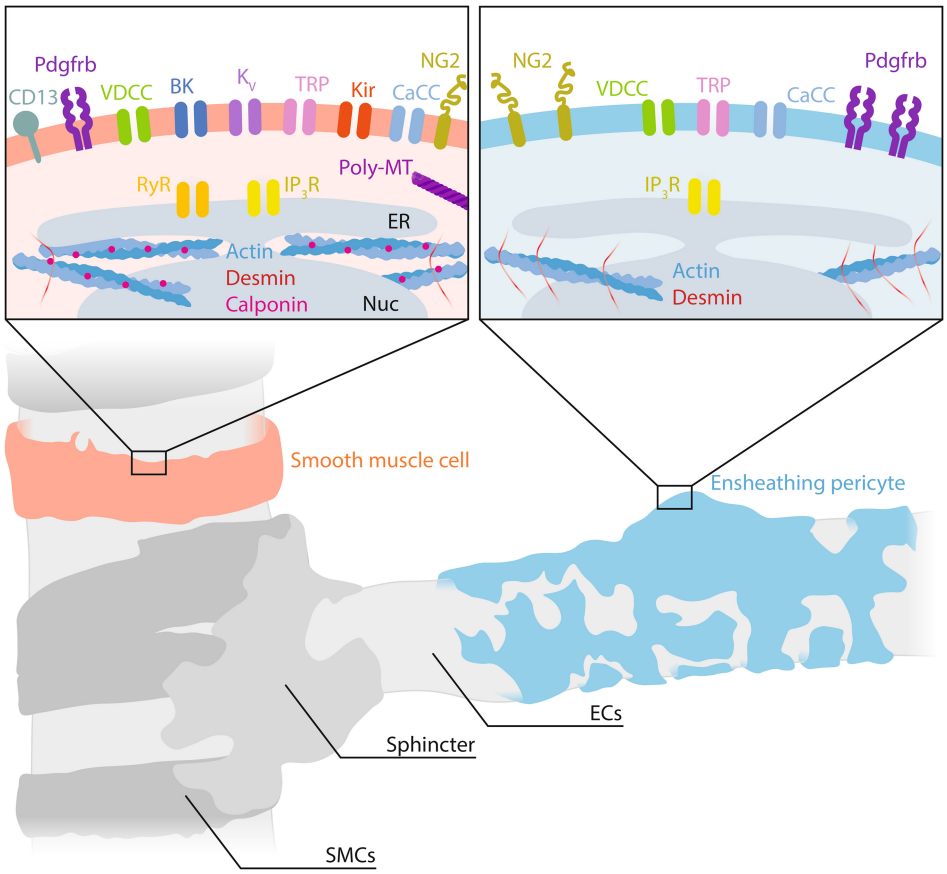

FIGURE 3 | Current understanding of similarities and differences in the contractile machinery, Ca^{2+} signalling apparatus and membrane ion channel complement of SMCs and ensheathing pericytes, see the main text for details. Further work is needed to advance our understanding of the differences between these cells which will illuminate shared and individual roles in blood flow control.

1.4 | ECs Exert Short- and Long-Range Control Over SMC Membrane Potential

Work over several decades has established that ECs are key conduits for electrical transmission of signals throughout the vasculature. In arteriolar ECs, electrical signals spread bidirectionally and over long distances with minimal degradation [92–94]. Here, the K_{IR} channel plays a major role in that it acts as a signal booster and aids in regeneration and propagation of the signal [95]. At the level of capillaries, early studies determined that these cells respond to hyperpolarizing and depolarizing stimuli with membrane potential changes [96, 97] and blood flow changes [68, 69, 98, 99], providing a foundation for potential capillary communication with upstream arterioles as a mechanism to control blood flow. More recent studies show that focal damage to capillaries leads to long lasting constrictions of remote upstream PAs and ensheathing pericytes [100], supporting the idea that events occurring in this interconnected network are transmitted over long distances. Our recent work examined this concept in detail in the capillary bed of the brain [31, 40, 66, 67, 85] and established that these vessels transmit electrical signals from the computing brain parenchyma to the sparse upstream arterioles. Here, inward-rectifier K^+ (K_{IR}) channels [85, 101] operate as neuronal activity sensors (detecting K^+ elevations) and transduce this into membrane hyperpolarizations that can then be passed to adjacent capillary ECs, presumably via EC-EC gap junctions [31]. Upon arriving in the next cell, hyperpolarization can activate K_{IR} channels to regenerate the signal by relieving voltage-dependent block of the K_{IR} channel pore [85]. In this way, the signal can be transmitted over long distances to the upstream arteriole, and there it can be passed into smooth muscle to modulate tone as described above.

Additional hyperpolarizing electrical input can be provided by other K^+ channels, such as small- (SK) and intermediate-conductance Ca^{2+} activated K^+ (IK) channels, which respond to Ca^{2+} elevations in arteriolar ECs mediated primarily by IP_3Rs and TRPV4 channels [102–104] and can also be engaged in capillaries by TRPA1-mediated signalling [42]. An interesting emerging concept is the possibility of hyperpolarization of the EC membrane causing an increase in driving force for Ca^{2+} entry, which at the arteriolar level may activate SK and IK channels to further amplify electrical signals. Indeed, recent evidence from the mesenteric arteries of the peripheral vasculature shows that gain of function mutations in resident K_{ATP} channels amplifies Ca^{2+} signalling [105]. Evidence for this idea has also begun to emerge from recent work in capillaries showing Ca^{2+} signalling in capillary ECs is partially dependent on electrical signalling via K^+ channels [106, 107].

It will be informative to determine the degree of signal degradation as a function of distance in capillaries and to assess whether this is influenced by architectural features such as the presence of branches, which could act as sinks. This would be best addressed with optical voltage-sensor tools (see last section) which could help to further inform models for how endothelial electrical signalling over large territories is coordinated. Together, ECs in both capillaries and arterioles are thought to play a key role in transmitting signals and ensuring that they reach SMCs where they can invoke changes in tone and subsequently drive changes in vessel diameter and blood flow.

1.5 | Thin-Strand Pericytes Communicate Electrically With the Capillary Endothelium

Our recent work has aimed to help address the long-standing question of whether pericytes control blood flow by specifically assessing whether thin-strand pericytes of the deep capillary bed are electrically coupled to the capillary network and capable of modulating ongoing electrical signalling. Pericytes were first observed over 150 years ago, and their morphology and positioning immediately evoked the question of whether they control blood flow. Until recently, a lack of tools and techniques to address their functions stymied progress on this issue. However, previous evidence from elegant studies of the retinal vasculature has supported this concept, where electrical signals originating in pericytes can be transmitted to adjacent and upstream cells [108]. In the cortex, we observed that hyperpolarizing signals generated selectively in thin-strand pericytes using an optogenetic approach can be transmitted over long distances to upstream arterioles, where they promote vasorelaxation and dilations [109].

Thin-strand pericyte–generated electrical signals are likely passed into capillary ECs via gap junctions at PSJs [45, 49, 50]. Indeed, as noted above, ultrastructural data suggest that gap junctions form at these locations [50] (Figure 2) and this is supported by molecular data which indicate the expression of several connexin genes by thin-strand pericytes [16, 17, 110]. PSJs are analogous to the myo-endothelial projections that form between arteriolar ECs and SMCs and prior findings indicate that myo-endothelial projections are hubs at which proteins are selectively organized to optimize communication between cells. For example, endothelial SK and IK channels cluster in these structures and generate hyperpolarizations in response to local Ca^{2+} events that can then be immediately passed to the overlying smooth muscle with minimal signal loss [102, 104, 111]. Are thin-strand pericyte PSJs similarly organized? It would be intriguing to determine whether proteins that participate communication between cell types (e.g., K_{ATP} channels [35, 112]) are preferentially positioned at PSJs. In-depth exploration of this possibility could be made with a reliable marker of PSJs, such as staining for a protein that preferentially localizes to these sites.

Notably, pericytes maintain distinct territories from one another (see next section) and are not thought to form direct membrane contacts with one another except in the relatively rare cases of interpericyte tunnelling nanotubes [113, 114]. The latter are extremely thin connections which reach through the parenchyma and contact pericytes on adjacent capillaries and thus may link up pericytes that are distant from one another in terms of the linear vascular length separating them. Imaging studies suggest that gap junctions form at these points of contact [114] and nanotube structures have been observed in 28% of pericytes of the retina [114]. Accordingly, nanotubes may add a layer of complexity to capillary electrical signalling to enable modes of communication that would otherwise not be possible. Two potentially interesting scenarios that emerge from this organization, among many, are the possibility that nanotubes allow for electrical signals to skip tortuous bends of the vasculature and take a more direct path to the upstream arteriole, thus speeding transmission and limiting leak, and for passage of electrical signals between discrete areas that are not otherwise connected to

allow sharing of information on electrical state between distant regions of the capillary network.

Our recent data demonstrated that hyperpolarizing signals generated by an exogenous opsin in thin-strand pericytes can be transmitted to upstream arterioles [109], which naturally evokes the question of how pericytes might generate these signals in the physiological context. Work by a number of groups is now determining the landscape of ion channels that thin-strand pericytes are equipped with. A growing body of work supports the functional expression of K_{ATP} channels, VDCCs, IP_3Rs, RyRs and orai channels in thin-strand pericytes [35, 76, 109, 112, 115]. RNA-seq data indicate the expression of genes encoding a wide range of further K^+, Ca^{2+} and Cl^- channel genes which likely expands the functional repertoire of channels in the thin-strand pericyte plasmalemma [110], though many of these await experimental confirmation. These channels in turn are known to be controlled by an array of signalling pathways, implying a rich palette of mechanisms by which pericyte membrane potential may be modulated to influence the electrical state of the capillary network.

We have explored one such mechanism in depth and have discovered that K_{ATP} channels in thin-strand pericytes are activated by depletion of local glucose [35] and in response to local neuronal activation [109]. This led us to propose an electro-metabolic signalling [36] role for these cells in the control of brain blood flow. Our data support the concept that pericytes operate as metabolic sentinels monitoring local energy substrate availability. In this case, when local glucose drops below a key threshold of ~1 mM, this switches on K_{ATP} channels to hyperpolarize the pericyte membrane [35]. This in turn can be passed into the capillary endothelium to engage K_{IR} channels to aid in transmitting the signal over long distances to drive upstream dilations to increase local blood flow, replenishing local substrate levels and clearing waste products to homeostatically reset local tissue metabolic state [35]. Strikingly, we have also found that thin-strand pericyte K_{ATP} channels participate in the rapid neurovascular coupling response to network activity [109], which exists in part to satisfy the enhanced metabolic demands of increased neuronal computation. Whether or not this is due to a mechanism for rapid translation of dips in external glucose to K_{ATP} activation remains to be determined, but excellent recent work from Santana and colleagues [116], indicating that ATP fluctuates rapidly during cardiac activity, opens the door for similar possibilities in other cell types—potentially providing a mechanistic means to couple changes in external substrate availability to rapid metabolic changes that then engage K_{ATP} channels. Further work addressing these possibilities is needed.

Changes in glucose represent just one of many inputs that pericytes likely respond to with Vm changes that can then be passed into the underlying ECs to modulate capillary electrical signalling [36]. Elucidation of these various inputs is an important endeavour that will allow us to gain deeper insights into the mechanisms of pericyte electrical control of blood flow. This work could ultimately yield pericyte-focused therapeutic targets for novel clinical approaches that aim to maintain or restore blood flow in pathological contexts.

1.6 | Pericyte Territories

Recent imaging studies have revealed that thin-strand pericytes establish and negotiate territorial domains through the coverage of their processes and have shown that the processes of neighbouring cells do not impinge on one-another's domains [117]. When considered from the perspective of information processing, this evokes the possibility that a given pericyte integrates information about the local activity state of the parenchymal cell bodies and processes within its domain and then produces a smoothed voltage output that reflects the needs of the local tissue at any one time. This voltage can then be passed into the capillary endothelium to be added to the stream of information travelling to the arteriolar SMCs (Figure 4). Given that a pericyte territory likely covers thousands of local neuronal and astrocytic elements, proximity of these structures to the vasculature would allow for greater influence on vascular signalling. As the information processed by the pericyte is then transmitted upstream to the arteriole, it will be folded into other signals from other pericyte territories (along with signals from independent processing occurring in the endothelium) that add and subtract from it, such that the ultimate signal from any one pericyte will contribute only fractionally to the behaviour of upstream SMCs (Figure 4). Consistent with this idea, data from our recent studies [109] have suggested that signals originating in pericytes that are a greater distance from the upstream arteriole have a weaker effect on the contractile state of SMCs compared with pericytes that are closer. This decrement in 'influence' over PA vasomotion with capillary path distance is likely due to other conductance's within the network capable of innately reestablishing resting SMC membrane potential which may be difficult to outcompete for a single distal pericyte. Accordingly, the contribution of any one pericyte to blood flow control will be defined by a complex mix of its physical location, the nature of local processes signalling to it, and the competing influence of other cells contributing to the electrical state of the vascular network at any given moment.

1.7 | Computation in Arterioles: Integration of Incoming Electrical Signals From Capillaries

Compared to the extensive coverage of the capillary bed, arterioles are relatively sparse and are outnumbered by penetrating venules ~3 to 1 [4]. The capillaries can thus be conceptualized as a distributed sensor network that greatly extends the sensing range of each arteriole, allowing it to gather information from a wider area that can then be processed in the smooth muscle to modulate contractile state, and thus blood flow (Figure 4). SMCs receive this input through the underlying arteriolar endothelium which is connected electrically via gap junctions at myoendothelial projections. As noted above, the arteriolar endothelium is optimized to enable the spread of charge over long distances, as demonstrated by classic experiments indicating that arteriolar ECs are well coupled and readily pass charge laterally along the vessel lumen, whereas the smooth muscle layer is more poorly coupled and thus does not pass charge as readily along the long axis of the vessel [93, 118]. This means that signals are primarily transmitted into the SMCs 'vertically' from the endothelium in

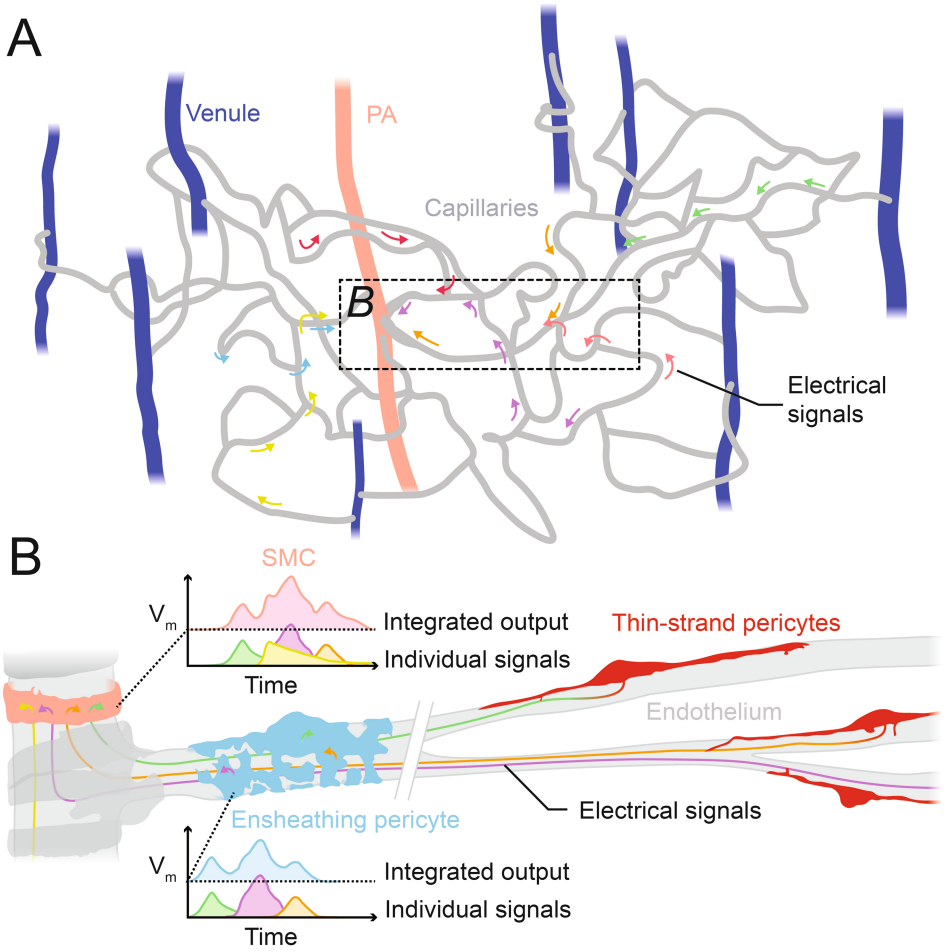

FIGURE 4 | Integration of electrical signals by mural cells to control blood flow. (A) Illustration of a capillary network emanating from a cortical PA and its draining venules. Coloured arrows represent the paths of electrical signals arising over time throughout different parts of the capillary network. (B) Detailed view corresponding roughly to the outlined box in *A*, showing arriving electrical signals from the capillaries being integrated over time by an ensheathing pericyte and a SMC, producing slightly differing voltage outputs in either cell which will dictate differences in contractile state, vessel diameter, and ultimately blood flow.

a roughly simultaneous fashion, which can then modulate the contractile state of a many adjacent cells simultaneously to produce uniform changes in lumen diameter. This is important to ensure that flow changes smoothly and is not adversely affected by uneven diameter changes, which could introduce turbulence. Accordingly, the cobblestone sheet of networked endothelial cells that line the arteriole represents a form of central processing unit which integrates the many inputs from the capillary offshoots of a given arteriole and then converts this into output signals that control SMC tone. Such a computational role for the arteriolar endothelium in cell–cell signalling has been explored for a range of signalling molecules in an elegant series of papers by McCarron and co-workers [119–122].

The SMCs of any tissue exist in a complex environment with many potential sources of input. Thus, in addition to the electrical control from the endothelium described here, it is important to note that these signals could also be overwritten by others stemming from the actions of exogenous molecules and other inputs. For example, the release of nitric oxide by local neurons [24, 123] could diffuse to SMCs and directly activate guanylate cyclase to increase the open probability of various K^+ channels in the membrane and promote dilations that are independent of the electrical state of the underlying endothelium.

1.8 | Parallels With Neuronal Electrical Signalling

Dendriform motifs are repeated throughout nature at many scales. In the brain, the arborescent pattern of the vasculature is reflective of the branching of dendrites on neurons, which invites potentially instructive comparisons for understanding information processing in the vasculature.

Dendrites are long and highly branching cables which transmit signals originating in synapses along their length to inform computation occurring at the level of the cell body. At the synapse, the actions of neurotransmitters generate postsynaptic currents that are passed into the dendrite. The number of synaptic inputs per dendrite can be vast, numbering tens of thousands in total per cell [124], and any one signal originating at a synapse adds to or subtracts from the voltage information being passed to the cell body. Synapses relative to one another can contribute to action potential generation based on local properties such as

membrane and/or axial electrical resistance, ion channel composition and physical distance from the soma. The cell body is the site of central integration which continuously combines information from all its upstream dendrites in real time. This controls the membrane potential and influences whether a spike is generated as a result.

Similarities can be drawn with vascular structures. Here, the cable-like capillaries could be analogous to dendrites, with the pericytes acting as the synapse—a point for current injection into the underlying wire. Thus, the voltage activity of any one electrically-coupled pericyte could be passed into the underlying capillaries and added to the stream of electrical information being transmitted to the arteriole. Like the neuronal cell body, the arteriolar ECs sum the voltage input from the range of downstream capillaries and this information is then passed to the SMCs where it informs Vm to influence whether the vessel changes diameter. We hypothesize further that pericytes, like synapses, have a large variance in their ability to transmit these hyperpolarizing signals to the ensheathing pericytes and arteriolar SMCs (dictated by their expression of the relevant sensing and electrical signal-generating machinery). Individual high-order capillary branches could also present a high-resistance path for current flow, providing some electrical isolation to perform spatially localized computation, like high resistance dendrites which allow for computations to occur somewhat independently of other dendrites, with the resultant output then spreading to the soma.

1.9 | Key Outstanding Questions

Our understanding of the bioelectric processes controlling vascular tone and cerebral blood flow has expanded greatly in recent decades. Understanding how these processes contribute to the mechanisms of brain blood flow control is critically important, as deficits in blood flow are associated with ischemic stroke, cognitive decline and dementia, cerebral small vessel disease and diabetes, among others [125–129]. Thus, a deeper understanding of the mechanisms underlying blood flow control could help to focus therapeutic development, create standardized tests, and ultimately inform clinical decision making. Developing this complete picture of brain blood flow control requires an understanding of cell-type specific contributions to the overall response, and there exist many pressing questions to further advance our knowledge. Below we outline three of these which we believe will lead to compelling advances, and outline the tools needed to rigorously address these questions.

1. How do pericyte-derived electrical signals contribute to blood flow control?

We have just begun to scratch the surface of how pericytes generate and transmit electrical signals to control blood flow and many avenues for further exploration remain open. Indeed, defining the range of stimuli (and resultant mechanisms) that capillaries respond to with electrical signals will greatly extend our understanding of how electrical signalling is organized in the vasculature. Furthermore, determining the reset and opposing mechanisms for the hyperpolarizing electrical signalling described here is important for understanding how signalling is balanced, and how the electrical state of the network as a whole is prevented from locking itself at hyperpolarized potentials. The recent discovery of depolarizing conductances through TRPV4 [130] and Piezo1 [131, 132] in capillary ECs could hold keys to understanding this aspect of electrical control, and extending our understanding other depolarizing conductances in ECs and pericytes will enrich understanding. Another intriguing aspect of pericyte morphology and signalling are the interpericyte tunnelling nanotubes that were first described in the retina [114] and are also found in heart [39]. From the perspective of electrical signalling these connections could allow for direct and rapid interactions between pericytes that are close in physical space but otherwise distant from one another in terms of the length of vasculature between them.

2. Is there specialized organization of signalling proteins in pericytes at PSJs?

The organization of proteins at myo-endothelial projections has been explored in detail, and studies have revealed preferential signalling at these structures which reflects their important role in EC-SMC communication. Similar explorations of signalling events and protein organization at PSJs will help to illuminate pericyte mechanisms for control of electrical signalling and blood flow. For example, determining whether Ca^{2+} events and other signals occur preferentially in these regions may point to mechanisms for cell–cell communication, and exploration of whether ion channels and GPCRs localize to these regions could provide further insights into how signalling is organized and optimized. A key breakthrough needed to enable this analysis is a reference protein or structure that is positioned exclusively or preferentially in PSJs. In ECs, gaps in the internal elastic lamina (which is intrinsically auto-fluorescent) are readily visible in isolated vessel preparations [102, 104] and indicate the locations of MEPs. Developing similar approaches to visualize gaps in the capillary basement membrane that have been documented in ultrastructural studies [49] may enable inroads into these questions.

3. How is electrical signalling from the capillary bed to arterioles coordinated in space and time?

Asking how pericyte electrical signalling contributes to blood flow control naturally leads to broader questions of how electrical signalling is organized throughout the capillary bed. The capillaries form a vast and anastomosing plexus of interconnected vessels and as such have the task of integrating many parallel—complementary and opposing—signals at any given time (Figure 4). Our model is similar to a previously proposed model of capillary-dependent neurovascular coupling [133]. In their ‘proximal integration model’, Itoh and Suzuki argue haemodynamic signals generated by neural activity in the periphery could be detected by capillaries which then signal upstream through unknown mechanisms. These signals can then be integrated at the root arteriole and 1st order capillary segments, providing a way to relate the neural activity along a particular arteriole-capillary bed with blood delivery. This general framework is well supported by the increasing evidence for capillary-to-arteriole electrical signalling in the brain described here. A key test to implicate retrograde signals controlling energy delivery through cerebrovascular networks

is whether haemodynamics at the level of the arteriole are better correlated to distal neural activity from neurons supplied by the arteriole's capillary bed than neural activity in neurons supplied by a different capillary bed at a similar Euclidean distance, which would align with data from an elegant recent multimodal imaging study [134]. A further key test that would implicate pericyte-generated signals and their ultimate integration at the arteriole would be to recruit many pericytes simultaneously in different capillaries served by the same parent PA. Presently most approaches do not allow for pericytes to be genetically targeted in isolation, though a recent study has identified an ATP binding cassette highly specific to pericytes that may be useful in enabling such experiments [135].

This gives rise to many further interesting scenarios and questions:how are two signals arriving at the same time at a parent branch from two separate daughter branches integrated? How are two signals travelling in opposite directions handled? Is there preferential conduction or directionality to signalling through the capillary bed? How do signals change (through decay, amplification, etc.) as they traverse the capillary network? Do capillaries communicate electrically with veins, and to what end? Is it possible for electrical signals to be generated in veins and communicated to capillaries and PAs? Direct answers to these questions will rely on the development of increasingly sensitive voltage sensor tools [136] and their selective expression in ECs, pericytes, and SMCs together and separately. Combining these tools with technologies for rapid three-dimensional imaging [137] will allow for studies characterizing the electrical signalling in native vascular networks in unprecedented detail, and this will doubtless yield breakthrough insights.

Addressing the above questions will both broaden and deepen our knowledge substantially and may lay the groundwork for the development of therapeutics that are able to protect or rescue electrical communication in the vasculature. Such advances hold the promise of expanding the health span of many tissues and may aid in the prevention or treatment of diseases with a vascular component.

Author Contributions

T.A.L. and D.I. wrote the manuscript. D.I. performed image analysis. Both authors reviewed the manuscript and approved its submission.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated during the current study.

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