Neurovascular crosstalk coordinates the central nervous system development
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Abstract
Purpose of the review: The synchronic development of vascular and nervous systems is orchestrated by common molecules that regulate the communication between both systems. The identification of these common guiding cues and the developmental processes regulated by neurovascular communication are slowly emerging. In this review, we describe the molecules modulating the neurovascular development and their impact in processes such as angiogenesis, neurogenesis, neuronal migration, and brain homeostasis.

Recent findings: Blood vessels not only are involved in nutrient and oxygen supply of the central nervous system (CNS) but also exert instrumental functions controlling developmental neurogenesis, CNS cytoarchitecture, and neuronal plasticity. Conversely, neurons modulate CNS vascularization and brain endothelial properties such as blood–brain barrier and vascular hyperemia. Summary: The integration of the active role of endothelial cells in the development and maintenance of neuronal function is important to obtain a more holistic view of the CNS complexity and also to understand how the vasculature is involved in neuropathological conditions.

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Introduction: Neurovascular development of the central nervous system
The central nervous system (CNS) originates from the neural plate, a specialized region in the dorsal ectoderm. During early embryogenesis, the neural plate folds to form the neural tube in a process called neurulation that culminates with the closure of the neural tube between embryonic day 8.5 (E8.5) and 10 (E10) in mouse (around 28 days after conception in humans) (reviewed in the studies by Blom et al. [1 and Nikolopoulou et al 2]). Shortly after the closure of the neural tube, the primary brain vesicles (forebrain, midbrain, and hindbrain) and spinal cord are differentiated along the antero—posterior axis of the neural tube. This incipient neural tube is avascular; however, concomitant to the closure of the neural tube, endothelial cell precursors (angioblasts) from the adjacent presomitic mesoderm are recruited by the neural tube and form a primitive vascular network around the neural tissue named perinatal vascular plexus (PNVP) (reviewed in [3]) (Figure 1). Pioneering work identified vascular endothelial growth factor A (VEGF-A) as the proangiogenic signal produced by the neural tube which triggers the vasculogenesis of the neural tube [4,5]. This initial neurovascular communication event will be followed by a plethora of cellular and molecular interactions coordinating the development and homeostasis of the CNS.

At around E10.5, one day after the formation of PNVP, angiogenic sprouts from PNVP ingress radially from the pial (basal) surface of the neural tube toward its luminal (apical) part in a stereotypical manner forming the intraneural vascular plexus (INVP) (Figure 1). Concurrent to the initiation of neural tube vascularization, neuroepithelial cells transform into radial glia cells (RGCs), neural progenitors that will give rise to the neurons and glial cells of the CNS (reviewed in [6]). RGCs have a bipolar morphology and differentiate into several neural cell lineages, which ultimately migrate to their final destination from the germinal zone, following a complex multistep regionalization process along the anteroposterior and dorsoventral axes. In general, the vascular patterning of
the INVP is driven by multiple angiogenic sprouts that follow the RGC fibers and laterally branch to anastomoses in the ventricular zone, forming the periventricular vascular plexus (PVP) [7]. As a particularity, in the forebrain, the PVP derives from the vessels in the basal telencephalic floor that progress tangentially from the ventral to the dorsal telencephalon [8]. Interestingly, RGC fibers attach to the CNS vasculature in the PVP and in the pial surface, and disruption of such anchorage leads to defects in neural precursor mitogenesis and neuronal migration [9,10].

As soon as vessels ingress into the neural tube, pericytes (perivascular cells) are recruited by endothelial cells (ECs) and the vasculature acquires blood–brain barrier (BBB) properties to assure a highly selective molecular transport between the blood and the brain parenchyma [11]. During the first postnatal week [11], astrocytic processes start ensheathing the vasculature and contribute to the maintenance of the BBB. The cellular entity formed by ECs, perivascular cells, and astrocytes, which also connects to neurons, is named the neurovascular unit (NVU). The crosstalk of NVU cellular components is essential to maintain CNS homeostasis in health and disease [12].

**Neuronal guided CNS angiogenesis**

Since the discovery of neural derived VEGF-A as the initial driving force for neural tube vascularization [4,5], numerous studies have elucidated its role in CNS angiogenic processes. Of particular interest is its crucial role in the specification and guidance of the endothelial tip cell, a specialized cell at the leading edge of growing blood vessels [13]. In addition, VEGF-A controls the proliferation of cells following the tip cell in a growing sprout, the stalk cells [13]. This dual function leading vessel sprouts to avascular areas is mediated by the vascular endothelial growth factor receptor 2 (VEGFR2) which also cooperates in some instances with the noncatalytic coreceptor Neuropilin-1 (Nrp1) expressed in ECs (reviewed in [14]).

CNS-resident neural progenitor cells are the initial main source of VEGF-A for the neural tube vascularization [4,15]. Concurrently, soluble VEGF-A decoy receptor fms-related tyrosine kinase 1 (sFLT1) is also expressed during spinal cord development and regulates the VEGF-A bioavailability. In zebrafish, this attenuation mechanism of VEGF-A signaling is not cell autonomously regulated by RGCs [16] and has been shown to be a key modulator of radial vessel ingestion and

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**Neural tube vascularization:** (a) Graphic representation of the developing perineural vascular plexus (PNVP, red) around the neural tube. Simultaneously to the closure of neural tube, starting around embryonic day (E) 8.5 in mouse, VEGF-A (light orange gradient) produced by this structure recruits angioblasts (red cells) from the adjacent presomitic mesoderm. This first neurovascular communication event constitutes the initiating proangiogenic signal triggering neural tube vasculogenesis. The ectoderm (blue) and notochord (pink) are located dorsal and ventral, respectively, to the neural tube. (b) Closer view of the neural tube during intraneural vascular plexus (INVP, red) formation. Approximately one day after the establishment of the PNVP, growing vessels stereotypically ingress into the neural tube from the pial (basal) toward the ventricular (apical) surface. A, anterior; D, dorsal; P, posterior; V, ventral; VEGF-A, vascular endothelial growth factor A.
sprouting in the spinal cord parenchyma [16,17]. An analogous system has been identified in the vascularization of the mammalian spinal cord [18], where postmitotic motor neurons express high VEGF-A but have a local delayed vascularization due to concomitant sFLT1 expression in a Nrp1-dependent manner. In addition, tissue hypoxia and the hypoxia-inducible factor (HIF) are positive modulators of VEGF-A signaling in the CNS and direct blood vessel formation toward areas with low oxygenation [19]. In the spinal cord, HIF contributes to balance signals conducting vessel ingression into motor neuron columns [18]. Moreover, hypoxia and HIF signaling in oligodendrocyte precursor cells (OPCs), CNS myelinating cells, were also reported to couple postnatal white matter angiogenesis in the mammalian forebrain [20]. HIF-mediated VEGF-A upregulation in oligodendroglia has later been proposed to promote postnatal CNS angiogenesis [21].

VEGF-A pathway is also important for postnatal hippocampal development. Hippocampal pyramidal neurons express VEGFR2 which is necessary for the development of dendritic arbors, maturation of dendritic spines, and axonal branching [22,23].

Wnt ligands expressed in CNS-resident progenitor cells also stimulate the ingrowth of vessels from the PNVP into the neural tube and successive vessel maturation [24]. The expression of the two main CNS vascular Wnt ligands, Wnt7a and Wnt7b, correlates with enriched downstream molecules of Wnt signaling, such as β-catenin, exclusively in CNS ECs [24]. Canonical Wnt signaling plays an important role in initiating CNS angiogenesis, and it has also been linked to VEGF-A/VEGFR2 signaling in ECs [25]. Moreover, recent reports have suggested the involvement of noncanonical Wnt signaling in vessel sprouting, remodeling [26], and, ultimately, regulating coupling between EC adherens junctions and actin cytoskeleton [27]. The G-protein–coupled receptor 124 (Gpr124) and the reversion-inducing cysteine-rich protein with Kazal motifs (Reck) have also been identified as receptor coactivators required for canonical Wnt signaling in ECs [28]. Novel studies have further characterized how Gpr124 and Reck interact with canonical Wnt receptors (Frizzled and Lrp5/6) in higher-order complexes [29,30], leading to distinct functions during CNS angiogenesis and BBB development and maintenance [31,32]. Besides the role of Reck in ECs, a non-cell-autonomous function of Reck expressed in neural progenitor cells has also been proposed to be essential for forebrain vascular development by enhancing endothelial Wnt [33]. In addition, other critical molecular players were found to fine-tune Wnt singling-derived vascular functions. This is the case of the Wnt/β-catenin negative regulator Apcdd1, which has been shown to coordinate vessel pruning and barrier maturation [34], or the sphingosine-1-phosphate receptor (S1pr) signaling, counteracted by Wnt to coordinate brain angiogenesis and BBB formation [35]. Remarkably, S1pr is also involved in a brain region-specific mechanism of neurovascular communication. S1pr is expressed in the RGCs from the germinal matrix, primordium of the striatum, and locally modulates blood vessel development by regulating integrin-β8 RGC expression and, subsequently, transforming growth factor β (TGF-β) signaling in blood vessels [36].

Upstream of both VEGF-A and Wnt endothelial signaling, retinoic acid (RA) is necessary to ensure proper vascular development, with proangiogenic and antiangiogenic roles described up to now (reviewed in [37]). Cerebral meninges surrounding the brain are a major source of RA in the CNS. Interestingly, the PNVP develops within the pia mater, the innermost layer of the meninges, rich in fibroblasts. Rhdi10 and Foxc1 mutant mice have a reduced activity of the first enzyme required for RA synthesis (retinol dehydrogenases) and show defects in cerebral meninges, respectively. The two mutants result in decreased RA levels, leading to a hyperplastic PNVP in correlation with decreased canonical Wnt signaling in this vascular structure [38,39]. Furthermore, RA modulates cerebrovascular development by suppressing the expression of Wnt inhibitors and stimulating VEGF-A expression in neocortical progenitors [38,39]. A cell-autonomous function of RA signaling in ECs has also been reported, limiting Wnt signaling by promoting β-catenin degradation [38,40] and influencing pericyte recruitment and vessel stability [40].

The four classical axonal guidance ligands (semaphorins, ephrins, netrins, and slits), with their respective receptors, have been found to exert regulatory functions in the vascular system (reviewed in the studies by Segarra et al [12] and Paredes et al. [41]). Owing to their dual function in both systems, these guidance cues are named angioneurins. Recent studies have unveiled new angioneurins, such as fibronectin leucine-rich transmembrane proteins (FLRTs) [42], Nogo-A [43], and Reelin [9]. FLRTs mediate analogous adhesion and repulsion mechanisms in neurons and ECs [42]. Particularly, FLRT3 expressed in the neuroretina negatively influences postnatal retina vascularization through the binding to the uncoordinated-5 receptor B expressed in ECs, mediating repulsive responses in the developing blood vessels. The axonal growth inhibitor Nogo-A, expressed in the postnatal neuronal parenchyma, negatively regulates angiogenesis and vascular remodeling, as seen by the increased three-dimensional blood vessel volume exhibited in postnatal Nogo-A knockout mice [43]. Finally, the critical neuronal migration and brain layering modulator Reelin has emerged as a pivotal element synchronizing distinct neurovascular developmental processes. Reelin expression by Cajal–Retzius cells in the outermost layer of the cerebral cortex coincides with vessels sprouting from the...
meningeal PNVP. ApoER2/Dab1 (Disabled1) signaling initiated by Reelin in ECs converges with the VEGF/VEGFR2 pathway, which subsequently mediates cortical and retina proangiogenic responses and supports BBB development [9].

The processes described in this section are summarized in Table 1.

**Developmental regulation of neurogenesis by the vascular niche**

During early cerebral cortex development, RGCs, neural stem cells (NSCs) located in the ventricular zone, switch from the cell expansion fate to the differentiation program to generate the neurogenic progenitors that ultimately will give rise to neurons and glial cells [44] (Figure 2). Interestingly, the onset of angiogenesis in the neurogenic niche correlates in a spatiotemporal manner with RGC differentiation and generation of the neurogenic progenitors [45]. This correlation is driven by a change in tissue oxygenation regulating RGC fate as shown in the Gpr124 CNS-specific vascular mouse mutants. Gpr124-null embryos fail to form the PVP and this favors the expansion of NSCs at expenses of a decreased level of neurogenesis. Exposure to high oxygen levels rescues NSC differentiation in Gpr124 mutants indicating that hypoxia determines the RGC expansion switch [45]. In agreement to that, HIF-1α loss-of-function and gain-of-function experiments demonstrated that relief of hypoxia by CNS vascularization regulates RGC differentiation [45]. In the cerebellum, which develops mostly postnatally, perinatal low oxygenation levels trigger HIF-1α activation which in turn restrains the differentiation of granule cell progenitors and their exit of the germinal zone until vascularization progresses [46]. However, not all neurogenic niches are regulated by hypoxia as in the developing hindbrain the vasculature of the germinal zone regulates neurogenesis independently of the oxygenation levels [47]. In this case, Nrp1-ko embryos failed to properly form the PVP resulting in defects in neural progenitor mitosis, but such deficiency was not
| Neural source | Neural signal | CNS region (organism) | Vascular function | Model | Reference |
|---------------|---------------|-----------------------|-------------------|-------|-----------|
| Vasculogenesis and angiogenesis | Neural progenitors | VEGF-A | Neural tube (mouse/quail) | Neural tube vascularization | Avian and murine neural tube explants | Vegfa<sup>120/120</sup><br>Gfap:Pgfa<sup>a</sup> | [4]<br>[5] |
| | Astrocytes | VEGF-A | Retina (mouse) | Guidance of developmental angiogenesis | Vegfa<sup>120/120</sup><br>aA-Crystallin:Vegf120<sup>120</sup><br>aA-Crystallin:Vegf164<sup>α</sup>| [13] |
| | RGCs | VEGF | Spinal cord (zebrafish) | Radial glia controlled PNVP formation | TgBAC(gfap:gal4ff) | [15] |
| | RGCs | VEGF/sFlt1 | Spinal cord (zebrafish) | Vessel ingression in the spinal cord parenchyma | Tg(gfap:NTR) Tg(elavl3:NTR) Tg(hsp70:slt1) Tg(hsp70:slt4) ifB mutants | vegfa<sup>Δ</sup>1 mutants<br>vegfat<sup>Δ</sup> mutants<br>vegfc<sup>Δ</sup> mutants<br>flt1<sup>Δn29</sup> mutants | [16] |
| | Neurons | VEGF/sFlt1 | Spinal cord (zebrafish) | Vessel ingression in the spinal cord parenchyma | Tg(gfap:NTR) Tg(elavl3:NTR) ift<sup>Δn29</sup> mutants<br>ift1h<sup>Δn90</sup> mutants<br>ift1h<sup>Δk05</sup> mutants<br>ift1h<sup>Δn14</sup> mutants<br>vegf<sup>Δ</sup> mutants<br>sFlt1 loss of function and Vegfa gain of function | [17] |
| | Neurons | VEGF/sFlt1 | Spinal cord (mouse/chick) | Vessels patterning around motor neuron columns | Nse:Vegfa<sup>Δ</sup> Slt1and Nrp1 loss-of-function | [18] |
| | OPCs | HIF-z | Corpus callosum (mouse) | White matter angiogenesis | Pip-cre:Vhl<br>Sox10-cre:Vhl<br>Olig1-cre:Hif1α<br>Olig1-cre:Hif2α | [20] |
| | OPCs | HIF-z/VEGF-A | CNS (mouse) | White matter angiogenesis | Cnp-cre:Hif1α<br>Cnp-cre:Vhl<br>Pdgfra-cre:Vhl<br>Pip-cre:Vhl | [21] |
| | NPCs | Wnt7a/b | Forebrain and spinal cord (mouse) | Guidance of developmental CNS angiogenesis | Tek-cre:Ctnnb1<br>Wnt7a-KO<br>Wnt7b-KO | [24] |
| | Neural cells | Wnt/Norrin | Brain and retina (mouse) | Crosstalk between the Wnt/β-catenin and the Notch and VEGF-A signaling in CNS angiogenesis | Cdh5-cre:Ctnnb1<br>Cdh5-cre:Ctnnb1<sup>△143</sup><br>Cdh5-cre:R26-Δxin1-IRES2-LacZ<br>B6.Cg(Tg(ROSA)<sup>666</sup>2Gtros(ES7G5))/J | [25] |
| | NPCs | Wnt7 | | | (kdr:EGFP)<sup>Δ</sup>842<sup>Δ</sup> | [29] |
| Tissue/Region | Gene(s) | Function/Effect |
|--------------|---------|-----------------|
| Midbrain and hindbrain (zebrafish) | ECs selective recognition of Wnt7 ligands through the assembly of Reck/Gpr124/Frizzled/Lrp5/6 complexes | (kdrl:HRAS-mCherry)s896 gpr124s896 |
| NPCs | Wnt7a/b | Brain and spinal cord (mouse) | Wnt7a/b-Reck-Gpr124 in mammalian CNS angiogenesis and BBB formation |
| NPCs | Reck/Wnt7a/b | Forebrain (mouse) | Noncell-autonomous function of neuronal Reck in angiogenesis |
| RGCs | S1pr/Integrin-b8 | Germinal matrix (mouse) | Region-specific modulation of blood vessel development |
| RGCs | S1pr/Integrin-b8 | Germinal matrix (mouse) | Region-specific modulation of blood vessel development |
| Meninges | RA/WNT/VEGF-A | Brain (mouse) | Role of meninges in brain vascular development |
| Neurons | FLRT3 | Retina (mouse) | Guidance of developmental angiogenesis |
| Neurons | Nogo-A | Brain (mouse) | Guidance of developmental angiogenesis |
| Neurons | Reelin | Forebrain and retina (mouse) | Guidance of developmental angiogenesis and BBB development |

CNS, central nervous system; ECs, endothelial cells; RGCs, radial glia cell; VEGF-A, vascular endothelial growth factor A; GABA, gamma-aminobutyric acid; NMDAR, N-methyl-D-aspartate receptor; OPCs, oligodendrocyte precursor cells; BBB, blood–brain barrier; NPCs, neural progenitor cells.
| Vascular source | Vascular signal | CNS region (organism) | Neuronal function | Model | Reference |
|-----------------|-----------------|-----------------------|-------------------|-------|-----------|
| **Developmental neurogenesis** | | | | | |
| ECs | Dab1/laminin-\(\alpha\)4 | Forebrain (mouse) | RGC attachment to the pial surface | Cdh5-cre:Dab1 | [9] |
| Blood vessels | Laminin | Ventral telencephalon (mouse) | RGC division and interneuron production | Tek-cre:A14tdTomato | [10] |
| Blood vessels | CNS defective vascularization/hypoxia changes during vascularization | Forebrain (mouse) | Switch from RGC expansion to neurogenesis | Gpr124-KO | [44] |
| Blood vessels | Hypoxia | Cerebellum (mouse) | Cerebellar granule cell differentiation and exit from germinal zone | Atoh1-cre:Hif1a | [46] |
| ECs | NRP1/defective subventricular vascular plexus | Hindbrain (mouse) | Neural progenitor cell cycle | Nrp1-KO; Tek-cre:Nrp1; Nes-cre:Nrp1 | [47] |
| ECs | Vascular filopodia | Ventral telencephalon (mouse) | Neural progenitor cell cycle and differentiation | Cdh5-cre:S1p1 | [48] |
| ECs | Dab1/laminin-\(\alpha\)4 | Forebrain (mouse) | RGC attachment and neuronal migration | Cdh5-cre:Dab1 | [9] |
| ECs | VEGF-A | Forebrain (mouse) | Cortical cytoarchitecture; interneuron migration | Tie2-cre:Vegf; Vegfa\(^{120120}\) | [52] |
| ECs | GABA | Forebrain (mouse) | Interneuron migration and establishment of cortical circuits | Tie2-cre:Gabrb3 Tie2-cre:Gvat | [54] |
| ECs | NMDAR/MMP-9 | Forebrain (mouse) | Glutamate controlled interneuron migration through a vascular mediated mechanism | t-PA-KO | [56] |
| Blood vessels | Aberrant vascularization | Forebrain (mouse) | Modulation of RMS postnatal neurogenesis | Angiogenesis inhibition | [57] |
| Blood vessels | – | Forebrain (human) | Postnatal interneuron migration to the frontal lobe | Postmortem infant human brain | [58] |
| Blood vessels | Aberrant vascularization | Optic tract (mouse) | Axonal organization | Bm3b-cre:Nrp1 Tek-cre:Nrp1 Vegfa\(^{120120}\) Vegfa\(^{88188}\) | [59] |
| Blood vessels | – | – | Axonal projection and guidance | In vitro microvessels and rat spinal cord injury systems | [60] |
| Blood vessels | CXCL12 | Brain and spinal cord (mouse) | OPC migration and differentiation | Gpr124-KO Pdgfrb-KO Cxcr4-KO Cdh5-cre:Gpr124 Olig2-cre:Apc | [61] |
| **Homeostasis** | ECs | Barrel cortex (mouse) | Functional hyperemia | Tek-cre:Grin1 Camkii-cre:Kdr | [66] [67] |
compensated by exposing Nrp1-ko mice to hyperoxia, suggesting that other vascular-derived factors must regulate neurogenesis [47]. Remarkably, a recent study has fine-tuned the role of ECs in embryonic neurogenesis [48]. Blood vessels from the PVP of the ventral telencephalon project EC tip cells toward apical progenitors, and this interaction regulates the cell division of the NSC. Synergistically, mitotic apical RGCs produce VEGF-A which modulates the formation of vascular filopodial extensions [48].

At the anatomical level, the vasculature in the neurogenic niche also physically interacts with the NSCs. In the hindbrain, the PVP extends along the germinal zone and RGCs attach their processes to vessels from the pial surface and also from the PVP [47]. In the dorsal telencephalon, the basal processes of RGCs attach to the pial vessels. This adhesion is regulated by Reelin signaling via the endothelial secretion of laminin-α4 triggering the activation of integrin-β1 in the glial cells [9]. In the ventral telencephalon, where RGC progenitors produce neocortical interneurons, the radial glia fibers of RGC progenitors attach to the periventricular vasculature. Interestingly, this interaction is also mediated by integrin-β1, and its disruption interferes with RGC progenitor cell division [10]. Recently, radial glia (RG)-like cells residing in the meninges have been identified. This neurogenic population migrates from the meninges to the neocortex where it differentiates into cortical neurons [49]. It will be very interesting to investigate whether the blood or the lymphatic meningeal vasculature also participates in the regulation of such novel neurogenic processes.

NSCs persist in certain areas of the adult brain and continue to generate glial cells and neurons which maintain also a crosstalk with the vasculature. The vascular role on adult neurogenesis has been discussed in recent reviews [50].

The neurogenic processes described in this section are summarized in Table 2.

**Role of CNS vasculature in neuronal migration and axon pathfinding**

During embryonic cerebral cortex development, newborn projection neurons migrate radially along RGC fibers from the ventricular zone toward the pia surface (Figure 2). As a result of successive migration waves, six cortical layers are formed in an inside-out manner (reviewed in [51]). Remarkably, endothelial dependent Dab1 signaling is not only important for vascular development but also a central hub for neuro—glia—vessel communication and, ultimately, supports proper cortical neuronal migration [9]. In particular, Dab1-regulated deposition of laminin-α4 in the vascular basal lamina and the consequent activation of integrin-
β1 in RGCs have been suggested to be essential for neuronal migration during cortical layering.

Simultaneously to excitatory neuronal migration, gamma-aminobutyric acid (GABA)ergic inhibitory interneurons originate and migrate from the ventral telencephalon ganglionic eminences during cortical development (Fig. 2). Specified interneurons migrate first tangentially along defined streams into the cortex, and they later radially spread within the cortical layers (reviewed in [51]). Despite neural cells being the major source of VEGF-A during CNS formation, ECs also express VEGF-A and its tissue specific deletion from the vessels severely affected cerebral cortex cytoarchitecture [52]. Thus, EC-secreted VEGF promotes correct early interneuron migration and positioning, together with interneuron spatial association to blood vessels during this process [53]. GABA can also be secreted by the vasculature and influences cortical interneuron migration and distribution during embryogenesis [54]. Importantly, the disruption of GABA signaling in neurons and ECs upon loss of vascular GABA secretion has been shown to lead to persistent neuronal and behavioral changes [54,55]. Furthermore, the communication between GABAergic interneurons and projection neurons is essential to ensure proper inhibitory interneuron migration. Recent work has implicated glutamate release by excitatory cells as a modulator of interneuron migration in adjacency to blood vessels. Particularly, this neurovascular communication involves the endothelial expression of N-methyl-D-aspartate receptor (NMDAR) along with the modulation of vascular proteases [56].

Interestingly, postnatal vascular rearrangements also play a role in the tangential migration of young inhibitory interneurons traveling from the subventricular zone to the olfactory bulb through the rostral migratory stream [57]. This finding supports the idea of blood vessels modulating the addition of newborn neurons into specific cortical circuits after birth, mediation that has also been suggested in the infant human brain [58].

Notably, axonal pathfinding is associated with CNS vascular development, as observed in Nrp1 and VEGF-A mutant mice with blood vessel aberrations linked to disrupted axonal tracts in the optic chiasm and the spinal cord [18,59]. The aberrant vascularization displayed by these mouse models was suggested to act as a physical obstacle for growing axonal tracts rather than exerting a direct axonal guidance function. Nonetheless, the ability of microvessels promoting aligned neural progenitor cell axonal projection and guidance has been proposed using a tridimensional in vitro system and in a rat spinal cord injury model [60]. Moreover, during developmental CNS axonal myelination, OPCs originated in the subventricular zone also require extensive migration to reach their final positions. A physical interaction with the nearby blood vessels modulated by Wnt—chemokine receptor 4 signaling has been proposed as a regulator of OPC migration and oligodendrocyte differentiation [61].

The neuronal processes described in this section are summarized in Table 2.

**Neurovascular crosstalk regulates CNS homeostasis**

The communication between neurons and ECs is important not only for CNS development but also for the maintenance of its homeostasis. Neuronal activity regulates postnatal angiogenesis, BBB integrity, and vascular hyperemia, that is, repetitive auditory stimulation causes the reduction of the capillary vascular network in the primary auditory cortex, and conversely, hypoxia produced by deficient vessel density triggers the loss of dendritic spines in the areas distant from the vessels [53]. In line with this, modulation of neuronal activity induced by whisker stimulation or plucking influences the vascularization of the barrel cortex [63]. It has been also shown that, using a combination of chemogenetic tools, behavior paradigms, and transcriptomics in ECs, neuronal activity regulates the expression of circadian clock genes in the vasculature which in turn modulate the expression of BBB efflux transporters [54]. Moreover, synaptic activity triggered by odor stimulation induces a synchonic drop in calcium signaling in mural cells and subsequent vasodilation along the vasculature upstream of the activated synapses all the way to the pial vessels [65].

Neurons and ECs share a signaling vocabulary important for the coupled communication at the neurovascular interface, that is, ECs express NMDAR which are involved in the regulation of local functional hyperemia in response to neuronal stimulation of the barrel cortex [66]. In addition, VEGF-A induces a crosstalk between VEGFR2 and NMDAR in hippocampal neurons which regulates hippocampal plasticity in the Schaffer collaterals, and it is involved in emotional memory [67]. In line with an active neurovascular communication, coculture of brain ECs and neurons indicates that soluble factors released by ECs influence in the maturation and synapse formation of neurons, process being mediated by VEGF/VEGFR2 pathway [68].

ECs also regulate neuronal plasticity highlighting the relevance of blood vessels for higher-order brain function. ECs secrete Semaphorin3G (Sema3G), which regulates the synaptic strength in the CA1-CA3 hippocampal circuit and the dendritic spine density in the CA1 neurons [69]. These effects are mediated by the Sema3G receptors Nrp2/PlexinA4 in the postsynaptic compartment of excitatory neurons. Loss of endothelial
Sema3G results in memory impairment, but these cognitive defects can be compensated by Sema3G overexpression.

The neuronal processes described in this section are summarized in Table 2.

Conclusions
Neuronal and vascular signals converge in the development of the CNS and synergistically coordinate the complex morphological and functional arrangements of this organ. Recent discoveries have shed light on the molecular mechanisms that orchestrate the CNS development and highlighted the instructional role of the endothelium in this process. The study of the bidirectional neurovascular communication is essential to fully understand how the brain is constructed and to translate this knowledge to discern the pathomechanisms of neuronal dysfunctions.

Conflict of interest statement
Nothing declared.

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Key points
- Emerging new neuronal signals regulate the vascularization of the central nervous system (CNS).
- The vascular niche has an instructional role in neural progenitors’ fate during CNS development.
- Blood vessels contribute actively in the migration of different populations of neurons and glial cells.
- The bidirectional neurovascular communication is also relevant in the CNS homeostasis in functions such as vascular hyperemia, blood–brain barrier, and neuronal plasticity.

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