Chapter 12

Experience Mediated Development of the Visual Cortex Vascularization

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http://dx.doi.org/10.5772/48749

1. Introduction

The vascular system of every organ is composed of an afferent arterial system that ensures metabolic support, and an efferent venous drainage system that evacuates the substances produced by the organ as well as the catabolites that are generated. Both systems communicate via a terminal network in which the arterial capillaries anastomose with the venous ones. Vascular organisation depends on the structure and function of each organ, thus there is not a general vascular system, but an organ-specific one. The large blood vessels supplying the brain are the carotid and vertebral arteries, which then branch to form the network of pial arteries covering the surface of the brain. In the cerebral cortex, the pial vessels branch into smaller arteries, which enter the brain tissue itself and are called the penetrating arterioles. These arterioles branch into secondary and tertiary arterioles, until they reach the smallest vessel supplying the brain tissue, the capillary, which is only wide enough for one red blood cell to pass through it at a time. The capillaries then feed into the venules and veins, which carry the blood away [1].

Brain vascularisation is especially important due to brain metabolic peculiarities. Although the brain represents only 2% of the body weight and vascularisation is only 1% of brain size, it receives 20% of the cardiac output, 20% of total body oxygen consumption, and 25% of total body glucose utilization [2, 3]. As the brain lacks a glucose storage system, most of it has to be supported by a constant blood supply. Within the brain vascularisation, the vascularisation of the cerebral cortex has differential features compared not only to other body regions, but also to other brain areas. The two main differential features are the Blood Brain Barrier function and the dense capillary network.

2. Blood brain barrier

The endothelium of the CNS vessels is a structure specialised in the maintenance of the homeostasis of the internal environment of the brain parenchyma. This function is crucial in the CNS, as its function requires a strict regulation of the neuronal environment, which is extremely sensitive to ionic and/or metabolic changes. In fact, most of the substances freely available in blood from daily food intake that are constantly metabolised and excreted, are potentially neurotoxic [4]. This function is known as the Blood Brain Barrier, and was first described in 1885 by Paul Ehrlich, who found that soluble dyes injected into the system stained all organs but the brain and the spinal cord [5].

The Blood Brain Barrier is the set of mechanisms (physical and metabolic) that regulate the passage of compounds from blood to brain, allowing the regulation of the internal environment of the CNS with independence of fluctuations in blood composition. These mechanisms include, among others, the enzymatic systems that transform some compounds during their passage through the endothelium, as well as the specific transporters of some substances, such as glucose or aminoacids. Bigger molecules, such as insulin, use exocytosis systems mediated by receptors. Some of these enzymatic mechanisms have been used to quantify vascular distribution, e.g., butyryl cholinesterase histochemistry [6], alkaline phosphatase histochemistry [7], LEA lectin histochemistry [8-10] and immunohistochemistry against antigens such as glucose transporter-1 (GluT-1) [11], PECAM [12], RECA-1 [13] and the endothelial barrier antigen (EBA) [11, 14-18].

Among the cellular components of the BBB, we could mention the following:

a. Endothelial cells. The cortical endothelium is formed by a layer of endothelial cells with a higher mitochondrial component, almost complete absence of pinocytic activity, absence of fenestrations and presence of interendothelial junctions [19, 20].
b. Pericytes. Joined to the abluminal membrane of the endothelium, pericytes are included in the same basal membrane as the endothelium. The pericyte is a cell of heterogeneous origin that is related to macrophages, muscle cells, etc. Despite the fact that they have been largely neglected, recent studies show that they play a relevant role in angiogenesis and in the BBB function, by, among other mechanisms, inhibiting apoptosis of endothelial cells. Apart from their vascular role, recent studies have demonstrated a crucial role of pericytes in the formation of the glial scars produced after brain injuries, thus linking the maintenance of the Blood Brain Barrier with scarring and tissue repair, a role that has long been attributed to astrocytes [21].
c. Astrocytes. These play a relevant role in the dual nature of the BBB, physical and metabolic. On the one hand, their prolongations ensheathing the endothelial wall (astrocytic endfeet) are closely related to the basal membrane. Astrocytes induce several metabolic BBB properties, such as the enzymatic activity of the capillary wall, the uptake of glucose and the establishment of tight junctions. In general, astrocytes play a key role in the induction, expression and maintenance of the BBB. Although astrocytes are implicated in flow regulation and microvascular permeability by elevating calcium

levels in endothelial intervention, they are less involved than expected in the structural properties of the BBB [4].

d. Extracellular matrix. Apart from the afore-mentioned cells and neurons, that also regulate blood flow to cope with energy requirements and that even regulate vascular permeability, the extracellular matrix plays a key role in the interaction with vascular permeability, even regulating the expression of proteins that constitute the TJ of the BBB. On the other hand, the ECM has to be digested in order to allow angiogenesis, thus liberating non-soluble VEGF. This function is performed by the matrix metalloproteases [22].

Due to the cellular heterogeneity that constitutes the BBB, all the elements can be described as a neurogliovascular unit, where all elements are interrelated, as can be seen in pathological processes such as Alzheimer's, Parkinson's or stroke, where all elements are implicated [4, 23].

Among the structural elements of the BBB, tight junctions play a crucial role in the control of the paracellular diffusion of blood compounds to brain parenchyma. Tight Junctions coexist with other junction structures, such as belt desmosomes and gap junctions; nevertheless, tight junctions are still the main ones [23].

The proteins that constitute the tight junctions share a common cytoplasmatic location, and are linked to the actin cytoskeleton (ZO-1 and 2, cingulin, AF-6 and 7H6). The transmembrane proteins are JAM-1, occludin and claudin [24]. Actin plays an even greater role than TJ proteins in the maintenance of BBB integrity [22].

Although the development of cortical vascularisation is closely related to the development of the cortical function, in previous work we have demonstrated that despite the effects of sensorial deprivation on the development of the vascularisation of the visual cortex, the maturation of the BBB is not related to the functional maturation of the cortex, as neither visual deprivation nor environmental enrichment induced changes in the maturation of early and late markers of barrier maturation [14]. As most of the barrier structural and functional markers are fully developed prior to the beginning of the critical period, experience-mediated modifications do not appear to influence barrier maturation.

3. Capillary network

On the other hand, 90% of the cortical vascularisation is constituted by a fine capillary network that spreads all over the cortex and whose density is related to local neuronal activity. This network was described by Galen, who called it *rete mirabile* [25, 26]. Estimations suggest that the human brain contains up to 100 billion vessels, suggesting a ratio of one vessel per neuron [3]. In prior work, we have demonstrated that this relationship is maintained in the visual cortex despite deprivation of visual inputs. Indeed, both the number of vessels per neuron and the vascular surface per neuron maintain similar values when comparing normal and visually deprived rats [27]. In contrast, visual environmental enrichment does increase the ratio of vessels per neuron in response to the increased demand [28, 29].

The vascular system of the rat cerebral cortex is organised from the penetrating vessels perpendicular to the surface, emanating from the leptomeningeal vascular system. These vascular trunks branch, forming the capillary network that is the essential nutritional sector of the cortex, although metabolic exchange also takes place in the microcirculation sectors preceding the capillary network in small calibre arterioles [6].

When determining the three types of intracerebral vessels, although the vessel size is not a firm criterion for differentiating between capillary venules and arterioles, in general it can be established that the arterioles are vessels of 10 to 100 microns gauge. Arterioles with a caliber between 50 and 100 microns are called large arterioles, and arterioles smaller than 50 microns are called terminals. The capillaries are vessels under 10 microns, while venules are vessels of about 30 microns in diameter.

The main differences between these two types of vessel are in the structure of the wall:

- Arterioles lack a complete internal elastic membrane despite having a middle layer consisting of three layers of smooth muscle. A distinctive feature of the arteriolar wall structure is the adventitia, much thinner in cerebral arterioles than in the arterioles of the rest of the body, becoming discontinuous in some points, thus allowing the exchange of nutrients.
- Venules lack a distinct muscular middle layer. In its place is a layer of periendotelial cells. Periendotelial cells are a cell type that does not correspond clearly to smooth muscle cells or to pericytes, and that play a phagocytic function. The endothelium is structurally similar to the capillary structure.
- The capillary wall thickness is 4 to 10 times lower than the arteriolar wall thickness. The capillary wall has no muscle layer and is in close relationship with glia, physically structuring the blood brain barrier.

The study of the vascular system of the cerebral cortex requires the establishment of the topographical relationships between neurons, and blood vessels must provide a sufficient supply. Vascular density is closely related to local metabolic activity and oxygen consumption of different cortical regions. In areas with increased activity, characterised by an increase in mitochondrial volume density and increased local consumption of glucose, there was an increase in capillary density [2].

The differences in capillary density between different areas are mainly due to neuronal density and activity, and more specifically due to synaptic density. Being structurally similar, the differences will be based solely on the degree of activity. Furthermore, the development of the vascular bed is the result and runs parallel to the development of the cortex. Thus, in animal species in which the cerebral cortex is not fully mature at birth, there is little cortical vascularisation, and as the cortex matures, it develops its vascular architecture. This does not happen in lower species that are born relatively mature in which we see a vascular network similar to the adult animal brain. Similarly, phylogenetically older regions such as the entorhinal cortex have a more primitive vascular structure [30].

4. Development of the vascularisation of the visual cortex

The development of new blood vessels can occur via two mechanisms: vasculogenesis and angiogenesis. Vasculogenesis is the development of new vessels from differentiated endothelial cells in situ. Angiogenesis is the development of capillaries from preexisting vessels and is the way that cerebral vessels develop. Angiogenesis is a process that coordinates the precise timing and location of all the cells belonging to the NeuroGlioVascular unit to form a hierarchical vascular network with CNS specifications, including BBB function, reciprocal interactions between neurons, glia and pericytes and a vascular niche for neural stem cells [31]. The angiogenic process starts when one endothelial cell, in response to the VEGF secreted following local hypoxia, differentiates into a tip cell that advances according to the VEGF signal. The adjacent endothelial cells are destined to become stalk cells that follow the tip cell, providing a lumenized endothelial cell chain. The signal to inhibit tip cell differentiation and to become stalk cell is mediated by the Notch pathway [32, 33]. This process is functionally similar to axon growth cones [34, 35].

The development of intracortical vascularisation starts at 12 days post coitum with radially penetrating stem vessels following the pattern of neural tube growth, and is completed when these vessels form new cortical branches terminating in different cellular layers with 'en bouquet' [36] terminations. This is the vascular pattern of the mature neocortex.

The first draft of brain vessels is formed starting from where the aortic arches approach the ventral neural tube to form a primary vascular plexus. Vessels from the primary plexus reach the vesicles developing in the telencephalic basolateral surface, and vascular buds penetrate perpendicularly to the walls of the hemisphere [3, 37]. The development of the cerebral vasculature is divided into two stages, one extracerebral and the other intracerebral.

a. Extracerebral vascularisation (leptomeningeal)

The arterial and venous vessels that cover the entire cerebral cortex are formed from undifferentiated capillary plexus. This perineural vascular system ends when the development of intracerebral vascularisation finishes. At first, the density of leptomeningeal arteries decreases, and then the veins, which indicates that the venous system reaches its final pattern after the arterial [36].

b. Intracerebral vascularisation

The vascularisation of the cerebral cortex begins with the development of vascular trunks penetrating radially, perpendicular to the cortical surface. The development of these early intracerebral vessels begins in the early days of embryonic development and is completed during postnatal life [38].

The first vascular sprouts penetrate the cortex before the development of the cortical plate, so that the first vessels reach the ventricular zone perpendicularly opposite the leptomeningeal plexus. The earliest vessels that arise during development penetrate the cortical plate throughout its depth. This is reflected in the vascular structure of the adult cortex, where we can distinguish the radial trunks originated at first and going through all

cortical layers without collateral branches. During development of the cortex, the vascular leptomeningeal system issues new branches that penetrate the cortical plate, covering a territory that corresponds to their age so that the first vessels supply the deeper layers and the later ones supply the outermost layers. In the rat, from the second week of postnatal life there is no new perpendicular growth that enters the cortex from the leptomeningeal vascular system. The volume and surface area of the cortex continue to increase, so the vessel density decreases in proportion, penetrating perpendicularly to the cortical surface.

The cerebral cortex, in addition to thickening, undergoes a three-dimensional growth process accompanied by vascular arborization involving penetrating vessels. As was the case with the increase in thickness, the most ancient vessels provide greater horizontal branches due to the fact that older vessels occupy a larger area.

After maturation of the capillary network there is no budding of new vascular branches, indicating that the process of budding of new vascular branches ends when the vascular wall has fully developed. During the first phase of vascular development, a basic pattern is provided to suit the needs of each cortical area, showing specific regional differences in relation to the further development of different areas. During the second phase of vascularisation, a large increase in the vascular bed density occurs which corresponds to a large increase of the capillary surface closely associated with increased metabolic demands of the tissue. Between 8 and 20 days of postnatal life the microvascular endothelial cells proliferate rapidly. It is during this period that virtually all vascular branches form. The mitotic activity of endothelial cells drops sharply during the third week of postnatal life, as can be verified by tritiated thymidine incorporation into endothelial cells [36].

The third phase of vascular development is the elongation of existing branches. Originally, successive vascular branches are established, and then the vessels grow in length to cover the whole territory they serve. This elongation stage of pre-existing capillaries extends from day 20 until adulthood. This is the physiological pattern of development. In animals kept under normal conditions the definitive pattern of vascularisation culminates in the third week of postnatal life. Subsequent changes in transient local metabolic demands are supplied by local flow changes, but there are special circumstances that occur in which definitive increases in metabolic demand are offset by extending or adapting the normal pattern of development, as happens in adaptation to altitude hypoxia [39].

The development of the vascularisation of the visual cortex has one main specificity in rats, as the first phases of cortical development occur prior to eye opening, and thus are not experience-mediated. So, the experience-mediated vascular development happens during the so-called critical period. Sensory modifications during the early critical period result in substantial plasticity and are a crucial factor in establishing the mature circuitry [hooks]. This time window of postnatal life is specific for each brain area, and is experience-mediated. The critical period in the rat visual system is located between the 3rd and 5th postnatal weeks with a peak at the 4th [40]. Previously, we have shown that vascular density is closely related to neuronal activity, and by increasing and decreasing visual experience, we have found that the peak of VEGF expression is also at the 4th postnatal week [41], corresponding to the activity peak.

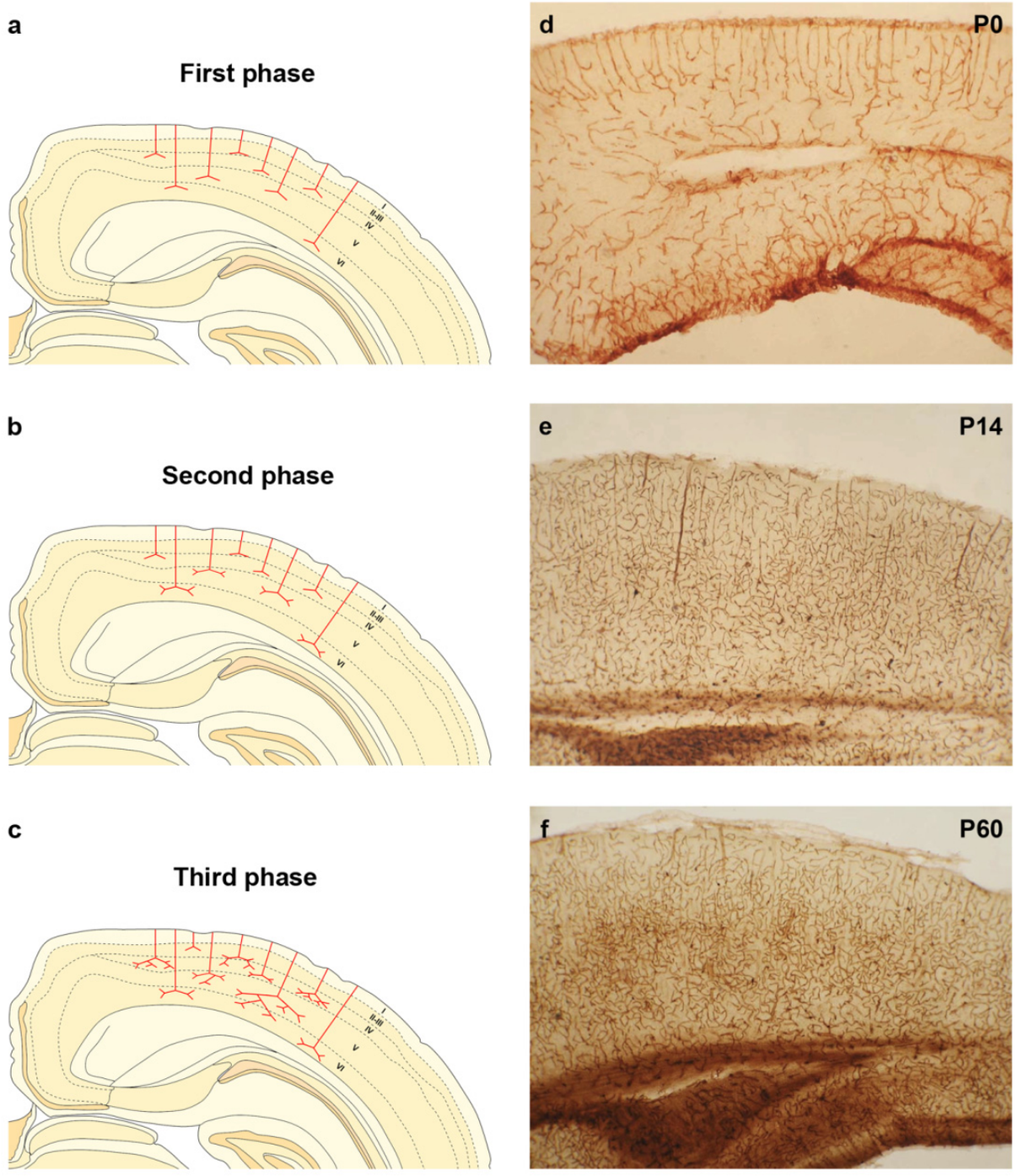

Figure 1. Schematic representation and histochemical sections of the development of the vascularisation of the visual cortex: a) first phase where vascular sprouts penetrate the cortex; b) during the second phase an increase in the vascular bed occurs; c) in the third phase the vessels grow in length and cover the whole territory they serve; d,e,f) show visual cortex vascularisation by butyrylcholinesterase histochemistry during these phases at P0 (first phase), P14 (second phase) and P60 (third phase), when the rats reach adulthood.

Thus, during the first two weeks of postnatal development, most of the vessels are the perpendicularly penetrating vessels, and this terminates at the end of the second postnatal week, just at the opening of the eyes. At this point, the adult pattern starts, characterised by a dense capillary network that is thicker in the most active areas, such as layer IV [6, 30]. The influence of experience can be demonstrated in visually-deprived dark-reared rats, that have an immature vascular pattern characterised by a higher prevalence of perpendicularly penetrant trunks and a much sparser vascular network. In contrast, environmental enrichment induces just the opposite, a faster development of the mature pattern and a higher vascular density [14]. These changes occur in parallel to the development of the rest of the elements of the neurogliovascular unit, and we have demonstrated that the development of the astroglial population in layer IV of the visual cortex mirrors the vascular behaviour [42, 43]. In a similar way, vascular density is also higher in layer IV of the auditory cortex and lower in layer I [30]. On the other hand, we have recently described upregulated neuropeptides in the visual system, and as some of them have also strong angiogenic properties, and as neuropeptides are among the main regulators of the critical period in the visual cortex along with angioglioneurins, this finding is also consistent with the common patterns for neural and vascular development [44].

There is a direct relationship between vascular density and metabolic activity. This is evidenced by the correlation between vascular density and mitochondria. Local capillary density, local utilization of glucose and local cerebral blood flow [45, 46] have also been found to be correlated. The relationship between the increase in neural activity and the increase in perfusion to supply this requirement is the so-called neurovascular coupling, that is the basis of modern neuroimaging techniques and that has been recently described as neurogenic instead of metabolic [47].

5. Angioglioneurins

Neovascularisation is mediated by a variety of cytokines, including Vascular Endothelial Growth Factor (VEGF). VEGF is a hypoxia-inducible secreted homodimeric glycoprotein of 45,000 daltons that plays a major role in developmental [48-51] and pathological angiogenesis [52-57]. Five major isoforms of human VEGF exist, of which VEGF165 is the predominant one in most mammals. However, VEGF164 is shorter by one amino acid in rodents. VEGF isoforms are differentially expressed in disease, suggesting differences between pathological entities in the mechanisms of VEGF up-regulation as well as in their employment of distinct isoforms for neovascularisation [58]. The main receptors for the Vascular Endothelial Growth Factor (VEGF) family are the feline sarcoma virus-like tyrosine kinase receptor (Flt-1 or VEGFR-1) and the fetal liver kinase receptor (Flk-1) or VEGFR-2, also known as KDR [49, 51, 59, 60].

VEGFR-2 plays a critical role in some permeability-enhancing effects of VEGF [54]. In pathological conditions, VEGFR-2 mediates an antiapoptotic effect via Phosphoinositide 3-kinase (PI3K)-dependent signalling pathways which promote the survival of endothelial

cells induced by VEGF [61-63] and is related to the blood-brain barrier (BBB) opening in brain injury [54, 64, 65]. A neuroprotective role for VEGF via VEGFR-2 has also been described [59, 66-68], which occurs via the PI3k/Akt and the mitogen-activated protein kinase/ERK kinase/extracellular signal-regulated protein kinase (MEK/ERK) pathways [69, 70].

But despite VEGF being the main angiogenic molecule, there are others that also play a multicellular role. Molecules that affect both neural and vascular cell processes have recently been termed angioneurins [71]. Angioneurins include molecules first described as vascular growth factors, such as Vascular Endothelial Growth Factor (VEGF), molecules first described as neurotrophins such as Brain-Derived Neurotrophic Factor (BDNF), and other factors such as Insulin-Like Growth Factor-1 (IGF-I) or Erythropoietin (EPO). Independently of their origin, all angioneurins share a common action on vascular and neuronal function. As most of these molecules also have effects on the third component of the neurogliovascular unit, the glia, we propose the term angioglioneurins to describe them [39, 72].

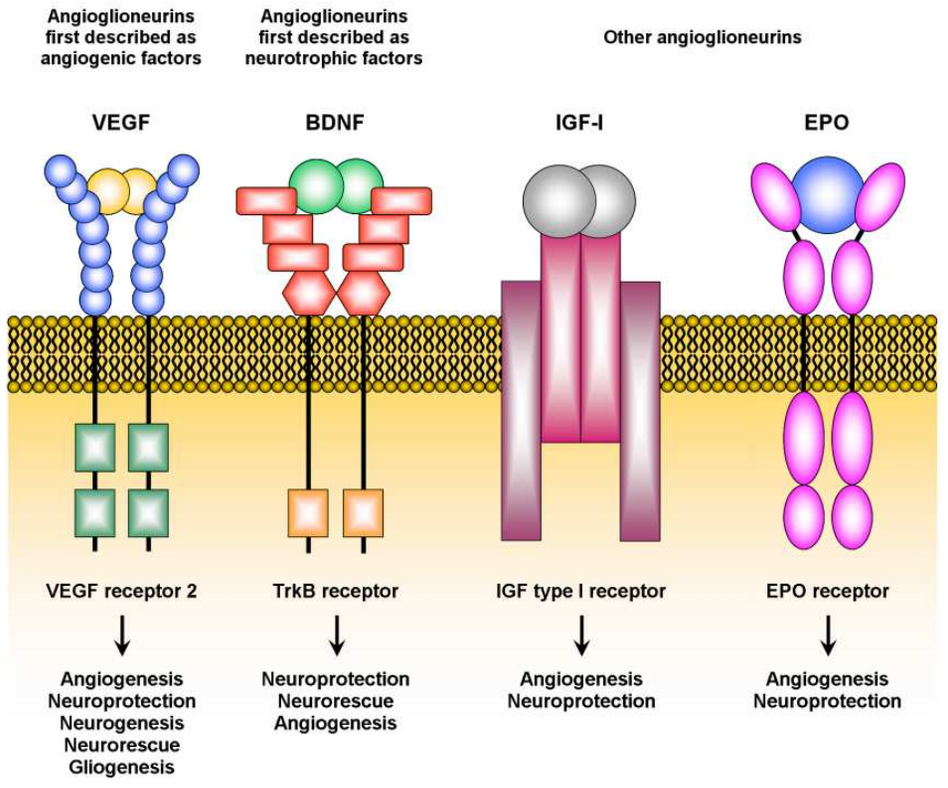

Figure 2. Schematic representation of referred angioglioneurins and their receptors. Some angioglioneurins were originally discovered through their angiogenic effects and then later found to also have neuroprotective activity (for example, vascular endothelial growth factor (VEGF)); some neurotrophic factors were originally discovered through their neuronal effects and then later found to also have angiogenic activity (for example, brain derived neurotrophic factor (BDNF)); others are more pleiotropic but still have relevant neurovascular activities, being involved in both angiogenesis and neuroprotection (for example, insulin-like growth factor 1 (IGF1) and erythropoietin (EPO)) (modified from Zacchigna et al., Nat Rev Neurosci; 9(3):169-81. Review).

6. Conclusions

The brain is highly vascularised, containing a very intricate network of capillaries (nearly every brain cell is located within 20 μm of a capillary). The endothelial cells that form the brain capillaries are sealed together by tight junctions, and have no fenestrations and very low pinocytosis. This combination of features creates the BBB, which is both a physical and enzymatic barrier.

Angiogenesis is one of the main adaptive mechanisms of brain microcirculation to changing needs of the CNS, as happens in the development of the visual cortex under the influence of visual activity, mainly during the critical period.

The nervous system needs a stable internal environment, which is created by the Blood Brain Barrier (BBB). The BBB function is related to structural and functional features of the vascular endothelium. Both the development of angioarchitecture and the functional maturation of the BBB occur postnatally and are regulated by tissue microenvironment and external environment. The development of the vascular tree, the acquisition of functional competence by the BBB and the induction and modulation of neoangiogenesis are closely dependent on the changes in metabolic demand induced by functional modifications (increases or decreases of stimuli).

The vascular network plays a crucial role in development and function of the CNS. It adapts to specific changes of metabolic demand and local flow modifications. However, if these changes become permanent, the supply is ensured by neoangiogenesis. Angiogenic, neurotrophic and neuroprotective factors participate in the development and maintenance of vascular, astroglial and neuronal structures, suggesting that the neurogliovascular unit preserves brain integrity. The improvement of the neurogliovascular unit by mechanisms such as the direct administration or the indirect stimulation of secretion of angioglioneurins could be an efficient strategy in brain diseases.

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