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The neurovascular unit in healthy and injured spinal cord

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Abstract

The neurovascular unit (NVU) reflects the close temporal and spatial link between neurons and blood vessels. However, the understanding of the NVU in the spinal cord is far from clear and largely based on generalized knowledge obtained from the brain. Herein, we review the present knowledge of the NVU and highlight candidate approaches to investigate the NVU, particularly focusing on the spinal cord. Several unique features maintain the highly regulated microenvironment in the NVU. Autoregulation and neurovascular coupling ensure regional blood flow meets the metabolic demand according to the blood supply or local neural activation. The blood–central nervous system barrier partitions the circulating blood from neural parenchyma and facilitates the selective exchange of substances. Furthermore, we discuss spinal cord injury (SCI) as a common injury from the perspective of NVU dysfunction. Hopefully, this review will help expand the understanding of the NVU in the spinal cord and inspire new insights into SCI.

Keywords

Blood–spinal cord barrier, neurovascular coupling, neurovascular unit, spinal cord, spinal cord injury

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Introduction

Proper structural and functional homeostasis of the central nervous system (CNS) requires precise regulation of blood perfusion, oxygen delivery, and energy supply.[1,2] The CNS has two unique vascular features to maintain homeostasis: the neurovascular unit (NVU) ensures adequate blood supply according to neural activation and metabolic waste removal, and the blood–central nervous system barrier (BCNSB) partitions the circulating blood from the parenchyma to permit the selective exchange of substances.[3] The blood–brain barrier (BBB) and blood–spinal cord barrier (BSCB) have similar barrier abilities and are sometimes referred to as BCNSB.[4] These key features in the NVU are maintained by the highly coordinated activity of multiple cell types.[5–7] The NVU has attracted enthusiastic interest in the scientific community for its status in brain function. However, the spinal cord, another essential organ in the CNS, has received less attention. The current understanding of the NVU in the spinal cord typically has to follow the paradigm obtained from the brain.

It has been widely accepted that BCNSB disruption contributes to neurological deficits.[2,8,9] Extreme alterations of the NVU are thought to result in tissue ischemia and energy supply deficits, further promoting neurological disorders.[10] Diverse immune cells are strategically distributed within the NVU and associated with neuroinflammatory response in diseases.[11] Thus, studies of the BCNSB and NVU can help in understanding the mechanisms of neurological disorders and may provide novel treatments for these diseases, such as ischemic stroke, traumatic brain injury, and vascular dementia.[12–14] However, to date, interpretation of the mechanisms of the NVU and BSCB are insufficient in injured spinal cord. Spinal cord injury (SCI) is a devastating injury that causes long-term disability.

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The primary insult initiates a sustained impairment cascade called secondary injury, which continues to exacerbate the degree of SCI.[15] Insults to the spinal cord can disrupt the functional or structural integrity of the NVU and BSCB, thus playing a vital role in secondary injury.[16,17] Therefore, we will discuss the status of the research on the NVU and BSCB dysfunction in SCI.

The neurovascular unit

It was observed that the blood supply to the CNS was not exclusively controlled by systemic circulation; the brain could regulate its blood supply and metabolic needs in active brain regions by neural activity. The concept of the NVU was proposed to study the pathological mechanisms of stroke by the National Institute of Neurological Disorders and Stroke Progress Review Group in 2001, emphasizing the unique relationship between brain cells and cerebral vasculature.[10] This concept emerged as a new paradigm for investigating physiology and pathology in the CNS.

Today's view of the NVU has been broadly amplified. BCNSB is currently preferred as the main functional configuration of the NVU.[1] In addition to its barrier function which preserves the normal function of the CNS, the BCNSB also actively serves as a communication interface for the NVU between the periphery and the CNS. In contrast, interactions in the NVU are critical for the formation and maintenance of the BCNSB.[5,10] The role of the NVU in regulating cerebral blood flow has been broadly categorized as (1) autoregulation, the response of the cerebral vasculature to changes in perfusion pressure;[18,19] (2) neurovascular coupling, the local blood flow response to changes in neural activity;[3,20] (3) vascular reactivity to metabolic stimuli, such as pH or CO_2 content;[21] and (4) endothelium-dependent responses, such as hemodynamic stimuli (e.g., shear stress), neurotransmitters, and pharmacological agents.[22] Nevertheless, some functions of the NVU are difficult to distinguish clearly in experiments and therefore are often misidentified in studies.

At different levels of the vascular network, diverse types of cells and associated structures play diverse roles in cerebrovascular function and homeostasis synergistically throughout the CNS.[23] Collectively, the BCNSB comprises endothelial cells (ECs), basement membrane (BM), pericytes, smooth muscle cells (SMCs), and glia limitans. Interactions among these different vascular cells with neurons and astrocytes refine the functions of the BCNSB and act as a whole NVU (Figure 1).[24] In the following section, we discuss the common features of cells in the NVU, supplemented by available studies from the perspective of the brain.

Neurons

Neurons are protected by the BCNSB to ensure normal function.[2] Neurons can regulate nearby blood vessels by generating signals directly or via interposed cells based on their oxygen or metabolic demands. Neuron terminals can trigger calcium signaling in astrocyte processes via several mechanisms, thus preceding the onset of vascular responses.[25] Classically, neurons release glutamate to activate the ionotropic and metabotropic glutamate receptors on astrocytes, evoking Ca^{2+} release from internal stores and increasing the intracellular Ca^{2+}, leading to activation of downstream Ca^{2+}-dependent enzymes and generation of vasodilatory substances.[26] Thus, astrocytes take over to adjust the blood supply by modulating local vascular cells.

Moreover, studies have shown that neurons release various neurotransmitters directly or indirectly via astrocytes and pericytes to mediate vascular changes in the brain.[27] Diverse signaling cascades may involve this process at the capillary or arteriole levels.[26] Neuronal activity-induced capillary dilation classically relies on glutamate receptor (mainly metabotropic) activated Ca^{2+}-dependent signaling in astrocytes. In contrast, neurovascular coupling-evoked arteriole dilation in the brain depends on NMDA receptor activation and Ca^{2+}-dependent NO generation by interneurons.[26] Different types of interneurons have been suggested to control local blood flow responses differently.[27] More information is needed to identify the exact function of these neurotransmitter signals in the NVU at the level of the spinal cord.

Astrocytes

Small arteries penetrating the neural parenchyma are completely enveloped by astrocytic endfeet, which occupy a larger surface area of the microvasculature than the neural processes.[28] Astrocytes also ensheath capillaries to constitute the most abluminal layer in the NVU.[29] In the human cortex, a single astrocyte might sense the activity of more than one million neuronal synapses in its domain by the fine endfeet.[30] Moreover, each astrocyte has at least one process with its endfeet surrounding the cerebral blood vessel.[31] Characterized by highly ramified processes contacting the neural processes and microvessels, astrocytes are well positioned to link neural activity to microvascular function.[32] Astrocytes are expected to modulate local perfusion by neuron-to-astrocyte signaling.[33] In vivo imaging has verified a frequency-related correlation between neuronal activity, Ca^{2+} transients in astrocytes, and vasomotility.[34] Astrocytic Ca^{2+} transients are less synchronized with low-frequency neuronal Ca^{2+} transients and do not seem to contribute to low-frequency

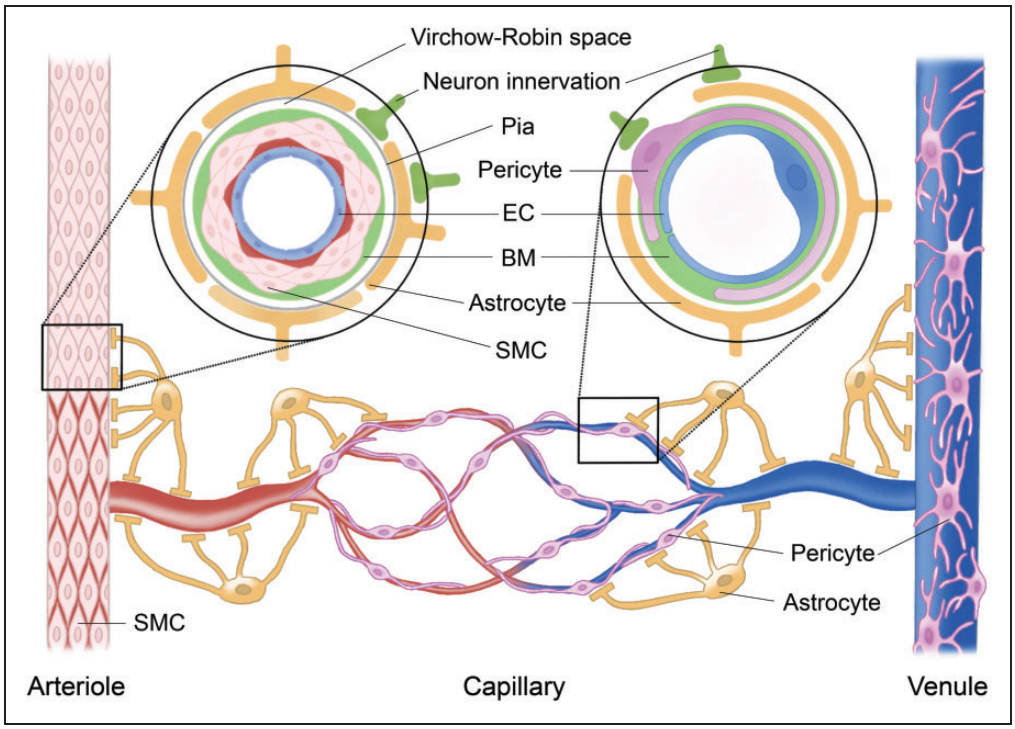

Figure 1. The typical components of the neurovascular unit (NVU) in the central nervous system (CNS). The NVU comprises (1) vascular cells, including endothelial cells (ECs) and mural cells, such as pericytes on capillaries and venules, or smooth muscle cells (SMCs) on arterioles and small arteries; (2) neuroglial cells, such as astrocytes; and (3) neurons. ECs form the luminal layer of the vessel wall. The space between adjacent ECs is held by paracellular junctions. The expression of some junctional proteins (including claudin-11, ZO-1, occludin, catenin, and VE-cadherin) is lower in the BSCB than in the BBB. At the arteriolar level (left inset), the basement membrane (BM) and SMC envelope endothelium. Astrocytic endfeet insert into the BM to regulate SMCs through the pia and the glia limitans. The capillary and venule (right inset) lack SMCs, where pericytes embed in the BM and wrap around the ECs on the abluminal side. The astrocytic endfeet attach to the pericytes at both venules and capillaries. Pericyte coverage is less on the BSCB than on the BBB. SMCs, pericytes, and astrocytes all receive neuronal innervation to control their function. In addition, these conformations of cells are also the major cellular structure of the blood–central nervous system barrier.

hemodynamic changes. However, under high-frequency-stimulated neuronal Ca^{2+} responses, astrocytic Ca^{2+} transients are cumulative and temporarily correlated with vasoconstriction.[35] In the CNS, astrocytes can detect changes in both blood and neurons through several neurotransmitters, mainly glutamate and γ-aminobutyric acid, and evoke downstream calcium signaling.[36] These calcium signals can activate smooth muscle cells via potassium signaling and the Na^{+}-K^{+} ATPase,[37] causing local vasodilation and then propagating away to induce vasomotion in adjacent arterioles.[32,38] Moreover, astrocytes also produce and release various molecular mediators, such as prostaglandins, nitric oxide (NO), and arachidonic acid, to regulate local blood flow in a coordinated manner.[39,40]

Astrocytes alone are not to determine barrier properties because astrocytes appear postnatally in the brain, well after the BBB has already been sealed.[41] Nevertheless, astrocytes still contribute to BBB properties by releasing molecules to promote BBB repair, restrict peripheral immune cells, and assist in the resolution of inflammation.[42] Astrocyte-derived factor induces endothelial polarization and produces glycocalyx on the CNS endothelium.[29] Astrocyte-derived Sonic hedgehog signaling stimulates the expression of tight junction (TJ) proteins such as claudin-5 and occludin in the CNS endothelium.[43] In addition, astrocytes can also generate and release vascular endothelial growth factor, which downregulates claudin-5 and occludin through activation of endothelial nitric oxide synthase (NOS) and increases BBB permeability.[44]

Endothelial cells and smooth muscle cells

The ECs lining the lumen of vessels are the core anatomical unit of the barrier directly facing the blood flow, regulating transportation and communication between the CNS and blood circulation. Many features of ECs contribute to the barrier property. The paracellular junctions, mainly including tight junctions (TJs) and adherens junctions, hold the space between adjacent ECs and limit the paracellular flux of solutes.[45]

These paracellular junctions primarily confer low paracellular permeability and high electrical resistance to the barrier in the CNS.[6,46] The majority of the regulated transportation occurs through the paracellular route.[47] In addition, the ECs in BCNSB offer low-rate vesicle-mediated transportation across the endothelium, called transcytosis.[45,48] This form of transportation is designed to transport macromolecules mediated by specialized structures, including clathrin, caveolae, and vesiculo-vacuolar-organelles.[49] Vesicles are likely to be a highly efficient and economical way of exchanging information across the BBB.[50] However, transcytosis is understudied in the spinal cord.

ECs and SMCs are major vasomotion effectors in the NVU that regulate blood flow in response to signals from neurons and astrocytes. ECs are equipped with a repertoire of voltage-gated ion channels, ligand-gated ion channels, and G-protein-coupled receptors, which provide the ability to respond to multiple physiological inputs in the CNS.[3,51] Diverse bioactive molecules can elicit endothelial functions to regulate hemodynamics in the brain, and many of them are autocrine, such as adrenomedullin, angiotensin, NO, prostanoids, and several others.[52] ECs also propagate vasomotor responses to regulate blood flow in the CNS in response to both chemical and mechanical signals from the luminal side.[53] Shear stress evoked by flowing blood and impinging on the endothelial surface can induce the release of dilatory NO.[53] Therefore, there is likely a positive feedback mechanism in which dilatation increases the blood flow at arterioles; thus, increased shear stress and release of NO can cause further vascular dilatation downstream.[54] The mechanosensory effect of shear force is probably mediated by the endothelial glycocalyx with changing oncotic pressure gradients at the endothelium surface,[55] transmitted by the pathway platelet endothelial cell adhesion molecule-1, vascular endothelial cell cadherin, and PI3 kinase.[56] Alternatively, the mechanosensory effect may be directly transduced by mechanosensitive ion channels, such as inwardly rectifying potassium channels (KIRs) and transient receptor potential cation channels V4 and Piezo-1.[57]

The primary function of SMCs is to maintain the vascular tone or narrow vessel diameter to increase intravascular pressure,[58] by receiving signals from ECs, tissue metabolism, humoral stimuli, and neural stimuli.[59] ECs can directly affect SMCs via several mechanisms, including the release of signaling molecules such as NO, or electrical signals via myoendothelial gap junctions between ECs and SMCs.[60] In addition to directly responding to neurotransmitters released by neurons, the SMC also responds to the modulated signaling within astrocytes in the CNS. Neuronal activity can promote a calcium signal in astrocytic endfeet decoded by Ca^{2+}-sensitive K^+ channels, which locally release K^+ into the perivascular space to activate SMC inward rectifier K^+ channels and cause vasodilation.[37] ATP from both neurons and astrocytes can activate purinergic receptors on SMCs. The activation of purinergic receptors P2X and P2Y in SMCs has been shown to cause an increase in Ca^{2+}, thus causing SMC contraction.[61] The influx of Ca^{2+} across the plasmalemma or the release of Ca^{2+} stores from the sarcoplasmic reticulum in SMCs could activate contractile proteins and narrow the vascular diameter.[58] In capillaries and postcapillary venules that lack SMCs, pericytes are thought to be the major regulators that replace SMCs.[62] However, vessel dilation in capillaries occurs seconds prior to arterioles mediated by neurovascular coupling in the cerebral cortex.[34] Whether there is a functional relationship between SMCs and pericytes remains unclear. Furthermore, the contraction and relaxation of SMCs are simultaneously governed by multiple mechanisms from different cells in the NVU, which appears redundant and inconvenient, yet the physiological significance of this type of overlapping control on SMCs is not well understood.

Pericytes

Pericytes are embedded in the basement membrane at the capillary abluminal side.[29] Several neurovascular functions necessary for CNS homeostasis are regulated by pericytes, including BCNSB maintenance, vascular angioarchitecture, vesicle trafficking, and neurovascular coupling.[63,64] Recruitment of pericytes to the capillary wall is a critical marker for maturity of the BCNSB.[5] The pericyte synthesizes many elements of the basement membrane, such as proteoglycans and laminal proteins, which are thought to be critical in the differentiation of BCNSB.[65] Unlike astrocyte endfeet, pericyte remodeling is likely nonexistent in the brain.[66] This property may be essential in restoring capillary flow in injury or aging but has not been verified in the spinal cord. Pericytes regulate the function of BCNSB in at least two ways: regulating BCNSB-specific gene expression in ECs and inducing polarization of astrocyte endfeet surrounding blood vessels.[67] Pericytes probably control the expression of endothelial paracellular junctions, the alignment of TJs, and bulk flow transcytosis of vesicles across the BCNSB.[68] Coculture of pericytes with ECs has shown enhanced barrier function with increased expression of TJs and decreased permeability under healthy and hypoxic conditions.[69] Pericyte-deficient mouse models have increased permeability of the BBB,[64,67] and reduced TJ proteins expression.[70]

Pericytes are major regulators that adjust blood flow in the capillaries and postcapillary venules,[62] by vasomotion according to neuronal activity changes in the CNS.[34] Pericytes possess contractile proteins, including the mesenchymal intermediate filament proteins vimentin and microfilaments that are assembled to actin and myosin, and to the contraction-related proteins tropomyosin and desmin.[71] Pericytes have a Ca^{2+}-dependent contraction in response to neurotransmitters, neuronal activity, or electrical stimulation in neurovascular coupling.[27,72] Neurotransmitters such as norepinephrine cause pericyte contraction, while gamma-aminobutyric acid, adenosine, glutamate, and dopamine cause pericyte relaxation.[68] These signals activate purinergic receptors or voltage-gated Ca^{2+} channels expressed on pericytes to increase intracellular Ca^{2+} and cause pericyte contraction.[68,73] Activating K^+ channels decreases Ca^{2+} influx and causes pericyte relaxation in response to high lactate and carbon dioxide levels.[27,40,74] Moreover, capillary dilation is reduced by blocking NOS, suggesting a role for NO in pericyte-induced capillary dilation and contraction.[62] Such signals in neurovascular coupling for contraction can propagate between pericytes in the brain, probably transmitting through gap junctions between pericytes or ECs.[40] Hence, it has been theorized that the vascular responses may be initiated by pericytes in capillaries and then propagated to upstream arterioles, because pericytes are closer to the active neurons than arterioles in the brain, and the neuronal activity activates an outward membrane current in pericytes that dilate capillaries before the arterioles.[40,62]

In pericyte-deficient mice with decreased pericyte coverage, cerebral microcirculation perfusion, oxygenation levels, and neurovascular coupling are diminished,[72] and the BBB is dysfunctional resulting in an accumulation of neurotoxic substances in the brain.[70] Physiologically, the number of pericytes and their capillary coverage in the spinal cord are lower than those in the brain,[75] probably indicating that the regulating ability of capillaries is weaker in the spinal cord. In chronic SCI, the paradoxical excess activity of monoamine receptors on pericytes causes local capillaries to constrict abnormally and reduce blood flow.[76]

Basement membrane

The BM is an essential accessory structure to seal the BCNSB, provide structural support and allow intercellular signaling communication in the NVU.[29] The space of discontinuous pericyte coverage and the space between astrocytes and endothelium are padded by BM. The BM is an extracellular matrix that in itself does not prevent small molecules from entering the CNS. Instead, it probably serves to restrict the passage of immune cells and provides a specific perivascular microenvironment for substance exchange and signaling communication.[77] Disruption of the BM can lead to alterations in the cytoskeleton of the endothelium, in turn affecting TJ proteins and barrier integrity.[65]

The BM in the CNS is mainly comprised of type IV collagen, fibronectin, laminins, and other glycoproteins. Collagen IV and fibronectin are secreted by multiple cells in the NVU, while deletion of either one is embryonic lethal.[78] Laminin contributes to BCNSB regulation and pericyte differentiation,[29] in addition to repairing BBB after ischemic brain injury.[79] Conditional deficiency of laminin leads to compromised BBB integrity with decreased pericyte coverage, diminished aquaporin-4 and TJs expression, and enhanced transcytosis.[80] Two main families of ECM receptors predominate in the BM, including dystroglycans and integrins. Laminin α2–dystroglycan receptor interactions regulate the maturation and function of the BBB.[81] Integrin β1 is indispensable for proper VE-cadherin signaling and paracellular junction organization in the BBB.[82] The degradation of the BM opens a conduit that enhances the migration of inflammatory cells into the parenchyma to aggravate the secondary injury.[83]

Multiple proteases regulate the extracellular matrix (ECM) in the BM, such as matrix metalloproteinases (MMPs).[84] Currently, twenty-four human MMP homologs have been categorized into six subfamilies: collagenases, gelatinases, stromelysins, matrilysins, membrane-type metalloproteinases, and others.[85] MMPs are typically maintained at low expression levels in the adult CNS, which is necessary for maintaining normal functions of the ECM and is modified in pathological conditions.[85,86] Some MMPs, such as MMP-1, -2, -3, -7, and -9, have been found to upregulate and break the BM in CNS injury.[87] These MMPs cleave the ECM, diminish signaling in the ECM, and degrade the paracellular junction proteins, thus disrupting BCNSB.[88] MMP-2 and MMP-9 can cleave dystroglycan to break the anchors of astrocyte endfeet.[89] MMP-8 and MMP-13 are also reported to be upregulated during neuroinflammation and are associated with BBB damage.[90] In addition, increased MMP-12 regulates ECM remodeling by inactivating some proteases, which is correlated with the development and myelination of the CNS.[84] The upregulation of MMPs has been widely reported to deteriorate the BSCB in SCI,[9,16] mainly including MMP-2, -3, and -9.[91] MMP-9 is the main contributor to disrupting the BSCB after SCI because MMP-9 inhibition or deficiency can reduce barrier disruption.[92] However, MMP-2 and MMP-9 likely support tissue repair by ECM remodeling to facilitate angiogenesis and glial scar formation in chronic SCI.[93] MMP-2 deficiency leads to vascular instability, a decline in vascularity, and prolonged MMP-9 upregulation in SCI.[94]

Moreover, MMP-12 knockout mice showed reduced permeability of the BSCB and improved functional recovery after SCI.[92]

Other cells

Oligodendrocytes and microglia constantly reside around the NVU but are not traditionally considered part of it. The role of oligodendrocytes or oligodendrocyte precursor cells in the NVU is partially conclusive. Oligodendrocytes ensheath axons and are adjacent to astrocytes, pericytes, and endothelial cells in cerebral white matter.[95] The possible role of oligodendrocytes in the NVU may be to engage in cerebral vascular remodeling or regeneration through Wnt or Nogo signaling and release matrix metalloproteinase.[96] Perivascular microglia are immune cells in the CNS that inhabit the NVU and respond to changes in the NVU in disease. These microglia probably have dual-directional effects on the NVU, protecting NVU integrity in acute injury but engulfing astrocytic end-feet and increasing barrier permeability in chronic injury.[97] In addition, microglia probably have an unrecognized role in modulating blood flow and neurovascular coupling in the brain through P2RY12 signaling to interact with other cellular components in the NVU.[98,99]

Autoregulation

Autoregulation is a self-adjusted mechanism involving constriction or dilation of cerebral resistance vessels to maintain constant tissue perfusion within a range of systemic mean arterial blood pressure (MAP). The vessels in the CNS constrict autonomically to impede blood flow when MAP increases, or dilate to facilitate blood flow when MAP decreases or the partial pressure of CO_2 increases.[23] This buffering system in the CNS is designed to stabilize blood perfusion to match metabolic need and maintain neural function during systemic blood pressure fluctuations. Lassen et al. first proposed this concept and described a plateau region wherein cerebral blood flow remains relatively stable across a range of MAP (60–150 mmHg).[100] Interestingly, Birch et al. identified that the relative capacity to buffer changes in cerebral blood flow is strongly dependent on the speed of changes in MAP. The slower the change in MAP, the smaller the impact on cerebral blood flow, to a point where cerebral blood flow becomes almost unaffected.[101] Autoregulation ability of the spinal cord mirrors that of the brain, providing a similar mechanism for maintaining balance for spinal cord blood flow (SCBF).[102]

However, the mechanism of autoregulation in the spinal cord seems more complicated. The autoregulation is still preserved after operationally sacrificing the segmental arteries of the spinal cord.[103] Autoregulation remains intact in the thoracic spinal cord after the high cervical spinal cord is completely severed.[104] SCBF and local field potentials are not significantly affected and remain coupled in the preserved spinal cord segments, indicating that spinal cord autoregulation allows a potent adaptation when significant fluctuation in MAP occurs or supraspinal control is lost.[105]

Autoregulation in the CNS is now divided into two categories: "static" and "dynamic" autoregulation.[18,21] The former refers to blood perfusion or blood flow in the CNS maintaining a relatively steady state when MAP varies under physiological conditions.[106] Dynamic autoregulation refers to the ability of autoregulation to respond to rapid changes in blood pressure from seconds to minutes. The effectiveness of static autoregulation can be even more prominent with lower frequency changes in MAP. The progressively slower changes in MAP result in more minor changes in cerebral blood flow.[18] Therefore, the MAP and cerebral blood perfusion are usually measured and averaged over longer time intervals (10 min or more) in experiments to make the resulting values adequately represent static autoregulation.[18] In contrast, dynamic autoregulation requires high temporal resolution techniques to measure the fast changes in blood flow.[107] During more rapid changes in MAP, there is much greater variability in cerebral blood flow than the assumption based on Lassen's concept of static autoregulation.[18]

The relationship between static and dynamic autoregulation has not been clearly identified. Tiecks et al. reported a robust linear relationship between static and dynamic autoregulation in the brain, probably denoting that static autoregulation is additionally maintained based on the constant and rapid adjustment of dynamic autoregulation.[108] Otherwise, they are opposite types of adaptive responses. As de Jong et al. suggested, there is a weak relationship between static and dynamic autoregulation in the brain, which has significant variability between individuals and sex.[109] In addition, after high-level SCI, static autoregulation is well maintained, while dynamic cerebral autoregulation is markedly altered, supporting the view that static and dynamic autoregulation are separated.[110]

Anatomical basis of spinal cord autoregulation

In humans, the parenchyma of the spinal cord is supplied by branches of several major arteries, including the vertebral and posterior-cerebellar arteries. The anterior radicular artery enters the spinal cord

bilaterally, with each anterior root joining the anterior spinal artery, which descends on the ventral surface of the spinal cord. Small branches of the vertebral or posterior-inferior cerebellar arteries continue caudally over the dorsal spinal cord, usually forming two small trunks known as the posterior spinal arteries (Figure 2). While the venous drainage of the spinal cord may be variable, its anatomic pattern often runs parallel to the paired artery.

Cardiac pulsatile flow can cause fluctuating hemodynamic changes in the arterial vessels of the spinal cord. Moreover, the vessels entering the spinal cord lack the siphon structure seen in the brain (such as the internal carotid artery siphon) to attenuate pulsatile variation of blood velocity and pressure.[111] How autoregulation of the spinal cord adjusts to these pulsatile hemodynamic changes is unclear. Theoretically, the pulsatile pressure load from blood flow in the spinal cord is supposed to be alleviated by large elastic arteries, as the arteries distend when the blood pressure rises during systole and recoil when the blood pressure falls during diastole, called Windkessel effect.[112] There is also a novel hypothesis that an extensive array of highly innervated arteriovenous glomeruli found in the spinal cord might be involved in the autoregulation of SCBF, because these arteriovenous anastomoses are similar in the pads of mammalian extremities or penis to bypass the capillaries,[113] but more data are needed to prove this hypothesis.

Signal transmission in autoregulation

Autoregulation in the CNS is considered to operate through multiple mechanisms including myogenic, metabolic, neurotransmitter-mediated, neurogenic, and systemic control (Figure 3).[20,51] The myogenic mechanism encompasses the direct reflex response of vessels to variations in mechanical force caused by blood flow, such as perfusion pressure, shear force, and stretch force, which dictate the responses of ECs and SMCs.[51] The metabolic mechanism of blood flow control in the CNS involves metabolic byproducts, such as lactate or CO_2, to regulate blood perfusion.[51] Accumulating extracellular lactate can induce vessel relaxation which adjusts to the metabolic needs of neurons.[114] Gap junction-coupled astrocytes can remove lactate from the extracellular fluid and discharge it through their endfeet into the perivascular fluid.[115] The released lactate then causes vasodilation by some different mechanisms, either by releasing endothelial vasoactive substances such as NO and hindering the transport of extracellular prostaglandin,[27,40] or by interfering with the metabolism of surrounding cells via the lactate/pyruvate ratio to release vasoactive substances.[74,115]

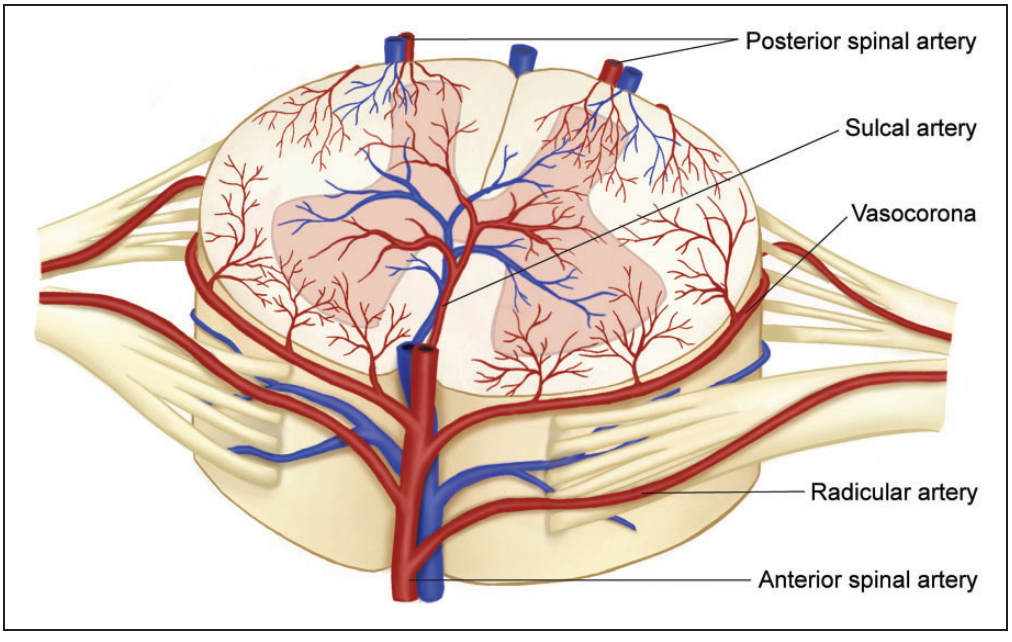

Figure 2. A dorsal view of the vascular anatomy of the spinal cord. The spinal cord is supplied by a net-like anastomosing vascular system from the branches of longitudinal anastomotic trunks: one anterior spinal artery and two posterolateral spinal arteries. The pattern of venous return is often parallel to the anatomic arteries. The spinal arteries are supplied by the aorta and lack the siphon structure as in the brain to attenuate cardiac pulsatile blood flow variation. The blood flow direction can change in the median spinal vessels and vasocoronas due to multiple traffic branches. Theoretically, there is a dead point where the opposing pressures stop the blood flow. The location of the dead point is constantly changing due to physical activity or blood pressure fluctuation, indicating that autoregulation in the spinal cord is more sophisticated than in the brain.

The vasculature in the CNS is known to be innervated by (1) sympathetic nerves, (2) parasympathetic nerves, and (3) sensory nerves.[116] Sympathetic control plays a vital role in the neurogenic autoregulation of the spinal cord. Autoregulation can be eliminated in animals receiving paravertebral sympathectomies, causing the SCBF to vary in a linear relationship with the changes in MAP.[117] The neurotransmitter theory of autoregulation is very complicated. Neurons can release multiple neurotransmitters from nerve endings via the nervi-vasorum,[51] or hierarchically through astrocytes and pericytes,[32,118] to adjust vascular reactivity to control vascular reactivity.[119] Several independent studies have reported that many neurotransmitters are involved in autoregulation (Figure 3). Some neurotransmitters are directly vasoactive, such as adenosine, acetylcholine, catecholamines, and neuropeptides;[37] other neurotransmitters are not vasoactive but stimulate the production of vasodilators, including NO, metabolites of cyclooxygenase-2 (COX-2) and P450 epoxygenases, through downstream enzymatic activation in astrocytes and vascular cells.[33,119]

However, it is difficult to discern the independent autoregulation mechanism in the CNS through inhibition or activation in experimental investigations because overlapping effects in the NVU provide redundant or synergistic effects in autoregulation.[20] Moreover, despite their sound theoretical background,

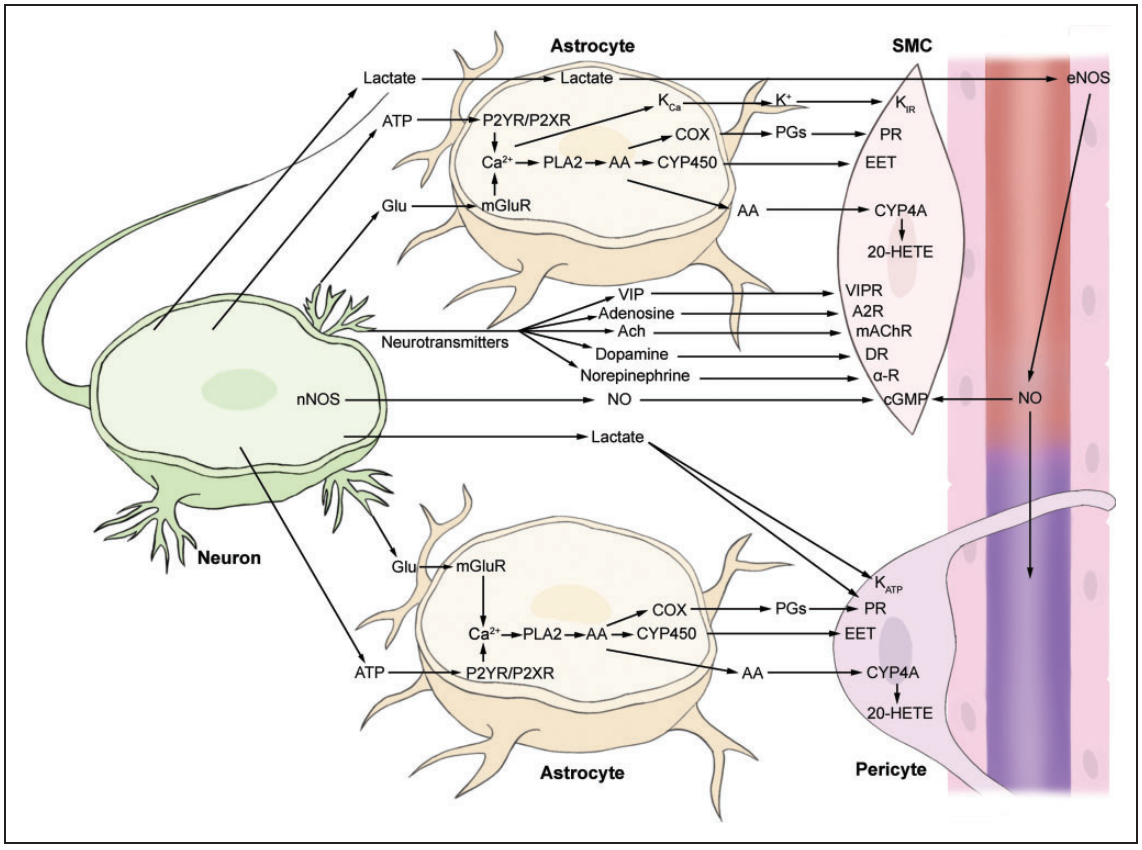

Figure 3. Simplified schematic diagram of the major pathways that regulate blood flow in the cerebral NVU. Neuronal presynaptic release of glutamate (Glu) is taken up by astrocytic processes and acts on metabotropic glutamate receptors (mGluR) to raise [Ca^{2+}], inducing the generation of arachidonic acid (AA) from phospholipase A2 (PLA2). The ionotropic glutamate receptors seem less important in controlling blood flow in the spinal cord, and this signaling is not shown in the figure. AA can be converted to multiple prostaglandins (PGs) (by cyclooxygenase, COX), epoxyeicosatrienoic acid (EET) (by cytochrome P450 epoxygenase, CYP450) to dilate vessels, or 20-hydroxyeicosatetraenoic acid (20-HETE) (by ω-hydroxylase, CYP4A) to constrict vessels. Raised [Ca^{2+}] in astrocyte endfeet may activate Ca^{2+}-gated K^+ channels (K_{Ca}) to release K^+ and activate inward rectifier K^+ channels (KIRs) on SMCs. The metabolic process of neurons releases adenosine triphosphate (ATP) acting on metabotropic purinergic receptors (P2XR or P2YR) on astrocytes to increase intracellular Ca^{2+}. The metabolic byproducts adenosine (to adenosine 2 receptor, A2R) and lactate activate ATP-sensitive potassium (K_{ATP}) channels, causing vessel relaxation; lactate inhibits PG transporters to cause PG accumulation or activate endothelial nitric oxide synthase (NOS) to release nitric oxide (NO), subsequently causing vasodilation. Neurons release diverse vasoactive neurotransmitters to control the NVU directly, such as vasoactive intestinal peptide (VIP) (to VIP receptor, VIPR), acetylcholine (ACh) (to muscarinic acetylcholine receptor, mAChR), dopamine (to dopamine receptor, DR), norepinephrine (to α-adrenoceptor, α-R), and NO (to cyclic guanosine monophosphate, cGMP).

these signaling molecules have broad communication and sometimes are able to activate each other. Thus, no single method has been universally accepted as a gold standard.[19] In addition, side effects may interfere with results in different pharmacological experiments,[21] easily disrupting the precise balance and confusing the understanding of this autocontrol mechanism in the NVU.

Neurovascular coupling

One vital role in the prototypical functions of the NVU is neurovascular coupling, previously called functional hyperemia.[10] neurovascular coupling refers to the link between neural activity and blood flow, resulting in a rapid dilatation of blood vessels and blood flow acceleration near the activated neurons with high spatial and temporal correspondence.[3,20,23] In animal models, focal spinal cord perfusion was observed to increase markedly during peripheral nerve stimulation.[120] These results suggest that neurovascular coupling in response to increased metabolic demands of neurons also exists in the spinal cord.

Neurovascular coupling is mainly regulated by neuron-astrocytic interactions in the NVU. Neurons release glutamate to activate metabotropic glutamate receptors on astrocytes and release Ca^{2+}, leading to the activation of downstream Ca^{2+}-dependent enzymes and the generation of vasodilatory substances.[26] In addition, ionotropic NMDA glutamatergic receptors activate NOS and release NO to regulate blood flow.[23] However, pharmacologically inhibiting NMDA receptors does not significantly affect blood flow in the spinal cord, indicating that these signals are not predominant in the spinal cord.[121] Moreover, neurovascular coupling probably induces a remote response transmitted upstream where multiple cells release mediators to engage signaling pathways and activate effectors across the NVU network in a highly orchestrated manner.[10,23] These signaling pathways in neurovascular coupling probably involve a feed-forward mechanism, resulting in the release of vasoactive substances, such as extracellular K^+, NO, and prostanoids in the brain,[34,40] but there is still not enough data to support its existence.[10,20] There could also be a relationship between neurovascular coupling and dynamic autoregulation.[122] The activity-induced retrograde propagation of vasodilation may also involve the dynamic autoregulation of the intravascular pressure changes induced by downstream vasodilatation in the brain,[23] but more data are needed to support this possibility.

The data regarding neurovascular coupling in the spinal cord are insufficient. Some reports have shown that task-related or peripheral nerve electrical stimulation can induce a noticeable increase in SCBF.[123] Interestingly, SCBF monitored by fMRI still remains coupled with local field potentials after spinal transection to abolish the changes in MAP.[105] Some BOLD-fMRI reports have detected neuronal activity-related blood flow changes in the spinal cord using averaged values per unit time or the flow-rephasing compensation method to eliminate the influence of pulsatile variation in blood flow.[124] However, these methods may not be suitable for detecting rapid blood flow changes, such as those in functional neurovascular coupling, because these averaged values may reflect the changes caused by dynamic autoregulation.

In addition, various methods quantifying the efficacy of NVU mechanisms accompany their respective limitations, contributing to results that sometimes appear conflicting.[18] New imaging methods have recently provided in vivo evidence that neurovascular coupling exists in the spinal cord. Functional ultrasound imaging detects hemodynamic changes responding to electrical epidural stimulation that reflect neurovascular coupling in the spinal cord.[120] Intrinsic optical signals and light reflectance spectroscopy reveal that the light scattering of vessels and hemoglobin oxygen saturation in the lumbar spinal cord change simultaneously with peripheral electrical stimulation.[125] These results confirm that neurovascular coupling exists in the spinal cord.

Blood–central nervous system barrier

The modern concept of BCNSB was described by Davson et al. as limiting the entrance of plasma components, red blood cells, leukocytes, and foreign pathogens into the neural parenchyma in healthy conditions.[126] Moreover, the CNS parenchyma is thought to lack a potent innate immune response related to this barrier function, termed "immune privilege". Pathogens introduced directly into the CNS parenchyma evade systemic immunological recognition.[127] Local cell death in the CNS parenchyma does not elicit a stereotypic response of myelomonocytic cells.[128] Despite some controversy regarding whether CNS parenchyma lacks humoral immunity surveillance,[129] the immune privilege or immunologically unique property of the CNS is primarily due to the BCNSB.[128]

More functions have been subsequently found in the BCNSB. The BCNSB responds to stimuli that arise within both the CNS parenchyma and blood compartments.[130] In addition, the BCNSB serves as a key homeostatic site for the NVU to regulate nervous tissue perfusion,[2,3,5] and a relay station for immune signaling between the blood and CNS via cytokines.[24] Furthermore, the BCNSB acts as both a secretory and endocrine target tissue with endocrine-like properties to enable substance exchange control

and communication.[131] The BCNSB releases hormones and neuropeptides into the blood or the interstitial fluid,[132] and is also a target for some hormones, such as leptin and insulin, to regulate glucose metabolism.[133]

The endothelium in the CNS lacks membrane fenestrations and pinocytic vesicles (transcellular pathway) when compared to peripheral organs and has extensive paracellular junctions to limit the flux between ECs (paracellular pathway).[65] The paracellular pathway is occupied by paracellular junctions, including TJs, to regulate barrier permeability and provide mechanical stability.[2,6,45] The majority of trans-endothelium transport occurs through the paracellular route.[47] TJs are comprised of the claudin family, zonula occludens (ZO) family, TJ-associated MARVEL domain-containing proteins (TAMPs), and junctional adhesion molecules (JAMs).[6,46] Claudins comprise TJ strands and play pivotal roles in regulating paracellular permeability.[134] Claudins interact with ZO proteins, which are scaffolding proteins with a cytoplasmic region that anchor to the cytomembrane and are essential for TJ assembly.[6] In addition to TAMPs (including occludin and tricellulin), other members of the immunoglobulin superfamily, such as JAMs, endothelial cell-selective adhesion molecule (ESAM), and nectins, are also associated with TJs.[130] Adherens junctions involve the cadherin family and platelet endothelial cell adhesion molecules.[8] These endothelial junctions impose a high trans-endothelial resistance of 1500–6000 Ω/cm^2 in cerebral capillaries and 20–50 Ω/cm^2 in the placental barrier.[135] In addition, the barrier has a negative surface charge to repulse anionically charged compounds,[65] which mainly come from the glycocalyx, a negatively charged dense layer of carbohydrates on the luminal side, acting as the first line of barrier and preventing immune cell entry.[29,136] Some early studies utilized the loss of anionic charge to reflect BSCB disruption in SCI.[137] However, the disruption of the BCNSB still lacks a direct detection method.[138] In many studies, the common method used to evaluate BCNSB function is to measure the entry of serum proteins or intravenously injected tracers into the CNS.[130]

The BCNSB contains multiple substrate-specific transport systems that control the transport of nutrients, energy metabolites, and other essential molecules transported between the interstitial fluid in the CNS and the blood,[5] through passive diffusion, facilitated diffusion, and active transport.[24] The forms of exchanges interact broadly with hemodynamic changes and NVU functions.[47] The intravascular pressure gradient between arterioles and venules is the primary regulator of luminal flow.[135] Dilation of upstream resistance arterioles increases the pressure gradient, thus increasing blood flow in the capillary, which is critical for regulating transportation across the barrier.[135] The physiological shear force corresponding to the increased blood flow in capillaries maintains the barrier phenotype through regulation of junctional protein and substance transportation.[135] BCNSB dysfunction-related blood flow reductions can further contribute to neurological deficits.[2]

Comparison of the BSCB and BBB

Traditionally, the characteristics of the BSCB are believed to be similar to the BBB,[4,139] despite several minor structural and functional differences.[17] The number of pericytes covering the BSCB is less than that covering the BBB.[75] The large superficial vessels in the spinal cord contain more glycogen deposits, which are not generally seen in the brain.[140] Compared to the BBB, the permeability of the BSCB is considered to be higher in the physiological state because the spinal cord generally takes up more tracers than the brain.[141] In multiple sclerosis or experimental allergic encephalomyelitis affecting both the brain and spinal cord, more blood-derived interferons and tumor necrosis factors reach the spinal cord.[139] Compared to conditioned brain injury, the duration of the BSCB remains highly permeable after conditioned SCI, and the area of BSCB leakage is 2–3 times greater than that of BBB leakage.[139] These differences between the BBB and BSCB may be due to lower expression of TJ proteins (claudin-11, ZO-1, and occludin), adherens junction-associated proteins (beta-catenin and VE-cadherin), or efflux transporters (such as P-glycoprotein) in the spinal cord vasculature compared to the brain.[142,143]

Advanced approaches to study the NVU in the spinal cord

The first quantitative study to calculate blood perfusion in the spinal cord with an autoradiographic technique by computing the intracardiac injected microspheres lodging in the spinal cord in proportion to the cardiac output.[144] Similar measurements using fluorescent or radioactive microspheres have reported the autoregulation ability in the spinal cord.[145] Later, various methods were developed to measure the SCBF, such as the hydrogen electrode technique, radioactivity or fluorometric angiography technique, Doppler flowmetry, and magnetic resonance imaging (MRI). However, these methods have a common drawback that is inappropriate for measuring NVU elements at high resolution. Some technological advances with high spatial and temporal resolution have recently been applied to measure neuronal and vascular dynamics in the brain,[146] but they have scarcely been used for investigating the spinal cord. Next, we review the methods that can be applied to monitoring NVU changes:

(1) Intrinsic optical signal (IOS) imaging. The first intraoperative IOS imaging of the human brain was reported by Haglund's group to monitor stimulation-evoked epileptiform discharges and cognitively evoked functional activity in the cerebral cortex.[147] IOS detects changes in light reflectance and the absorption rate of oxyhemoglobin and deoxyhemoglobin to provide information about SCBF, oxygenation, and metabolism. In particular, IOS is commonly used in vivo to provide hemodynamic information in response to simultaneous neuronal activity changes through neurovascular coupling. After electrically stimulating the peripheral nerves, changes in optical reflectance can be recorded simultaneously in the dorsal spinal cord.[148] The responses in the spinal cord increase gradually as the current intensity of the peripheral stimulus increases. Whereas the stimulus intensity has a maximal level, the relationship between stimulus and response is nonlinear out of this range.[149]

(2) BOLD-fMRI. The most common form of functional MRI relies on detecting changes in different magnetic properties of oxyhemoglobin and deoxyhemoglobin, called the blood oxygenation level-dependent (BOLD) signal. BOLD-fMRI provides indirect measurements of functional activation of neuronal networks through neurovascular coupling via direct measurement of transient changes in tissue perfusion, blood-volume changes, or oxygen concentration.[150] MRI is the most preferred noninvasive method applied clinically for the spinal cord, as gray and white matter and adjacent structures in the spinal cord can be visualized. Task-related or peripheral stimuli-related functional responses in the spinal cord can be observed under BOLD-fMRI in humans, which can be used to predict motor and sensory recovery after SCI.[123] However, spinal BOLD-fMRI has low spatial specificity because of physiological motion, such as breathing and heartbeat, and predominantly draining veins on the surface of the spinal cord.[151]

(3) Optical coherence tomography (OCT). An intrathecal OCT catheter can be used to observe the parenchyma, subarachnoid space, epidural vessels, dentate ligaments, and rootlets with an excellent minimally invasive visualization. Based on the interference pattern of low-coherence broadband light backscattered off the neural tissue, OCT can monitor the moving red blood cells as intrinsic contrast agents to directly measure SCBF. Furthermore, Doppler or speckle variance OCT imaging enables real-time visualization of SCBF in the posterior spinal venous vessels. OCT employing diffuse optical and correlation spectroscopies can also record the SCBF and oxygenation of the spinal cord in real time under various conditions.[152]

(4) Laser speckle contrast imaging (LSCI). LSCI detects the speckle contrast signals induced by the movement of red blood cells. This technique can monitor SCBF changes with an excellent spatial and temporal resolution by an ultrafast detector in a noncontact CCD camera. This wide-angle scanning technique can monitor spinal cord blood perfusion after SCI in animal models and patients in a full vision field intraoperatively.[153]

(5) Photoacoustic microscopy (PAM). PAM is a hybrid imaging technique based on the photoacoustic effect, combining the advantages of optical and ultrasound techniques and providing high ultrasonic spatial resolution.[146] A photoacoustic signal is generated after inducing pulsed laser energy, causing transient thermoelastic expansion of biological tissue that consequently emits high-frequency acoustic waves detectable by an ultrasound transducer. Imaging of blood optical absorption by PAM at multiple appropriately selected wavelengths can probe changes in hemoglobin concentration, blood volume, and hemoglobin oxygen saturation, along with functional hemodynamic responses to peripheral stimulation.[154] This method can also be utilized to assess tissue loss, guide microinjection, or trace exogenous contrast agents in the spinal cord.

(6) Functional ultrasound imaging (fUSI). fUSI is a recently developed Doppler ultrasound method using a single plane-wave emission as one focused beam to obtain centimeter-level images and coherently sum the set of images from tilted planar illuminations. This 'compound' ultrasonic image has better resolution and lower noise than other functional imaging. This device can image the microvascular topology and cerebral hemodynamics in vivo with 100 μm resolution, tens of frames per second, and a penetration depth of approximately 8–9 mm in the rodent brain.[155] The fUSI can record the hemodynamic response to neuronal activation induced by peripheral natural or electrical stimulations at a restricted area of approximately 1 mm in the contralateral dorsal horn of the spinal cord.[156]

(7) Two-photon fluorescent laser scanning microscopy (2PLSM). 2PLSM is based on the nonlinear excitation of a single fluorescent molecule by two nearly coincident photons, having a great advantage over single-photon fluorescence microscopy. Because of the longer wavelength and lower energy of exciting light, 2PLSM has less laser-induced damage to the tissue and less photobleaching of the imaged fluorophores. In addition, longwave

length and low-frequency light is less scattered in the tissue, which enables deeper tissue penetration. One major limitation of 2PLSM is that the observed object needs fluorescence labeling. By probes or transgenic labeling, the electrical activity of neurons can be monitored directly with voltage-sensitive indicators or indirectly with calcium-sensitive and neurotransmitter-sensitive indicators.[146] Methodologically, measurement of hemodynamic change can be classified into two main categories: changes in perfusion or oxygenation. 2PLSM can fulfill the measurement of blood flow velocity and vascular parameters by intravenously injected fluorescence dye (such as Q-dot or fluorescence-labeled dextran) or the measurement of oxygen saturation by a phosphorescent probe (such as PtP-C343).[157] 2PLSM can acquire information from fluorescently labeled elements of the NVU in the spinal cord with a very high spatial and temporal resolution, such as the velocity of a single red blood cell. A two-photon-excitable oxygen sensor probe, such as PtP-C343, can be injected intravenously and imaged under 2PLSM to enable quantitative measurement of oxygen metabolism changes in neurovascular coupling.[158]

All these methods have advantages and disadvantages and need to be chosen accordingly. Although having an unsurpassed spatial resolution, the 2PLSM can only image the dorsal spinal cord for limited penetration depth and relatively small imaging volume. PAM or fUSI provides deeper tissue penetration to reach the ventral side, so they are suitable for imaging cord-wide changes in NVU activity. MRI cannot provide sufficient spatial resolution to study individual vascular events. BOLD-fMRI is suitable for monitoring organic variation in blood perfusion but is limited by the long delays between sequential images.[157] 2PLSM can directly measure the changes in blood velocity or microvascular diameter and is therefore better for surveying neurovascular coupling. However, except for the LSCI and BOLD-fMRI, which give specific values that can be used to reference the changes in blood perfusion, the results detected by other methods require the assistance of formulas (Poiseuille's law) to estimate changes in blood perfusion.

NVU dysfunction in SCI

After SCI, the normal communication patterns within the NVU can be severely altered. The interruption of metabolism and angioarchitecture after injury can disturb the integrity of NVU function, leading to inappropriate blood perfusion changes[7] and BSCB disruption.[17] Amending NVU dysfunction after SCI, such as BSCB disruption and ischemia, is crucial in the secondary injury mechanism.[150]

Furthermore, assessment of small vessel disease in the spinal cord demonstrated a relationship between barrier impairment and lower tissue perfusion, suggesting that defects in functional elements in the NVU can affect each other.[159] Evidence shows that amyotrophic lateral sclerosis is a neurovascular disease, characterized by BSCB disruption, microhemorrhage, and microcirculation hypoperfusion,[160] similar to the secondary injury in SCI. However, NVU dysfunction in SCI may be more complicated, but related investigations are scarce.

Blood supply dysregulation in SCI

Disrupted autoregulation has been observed in early SCI reports.[161] However, whether this "autoregulation of blood perfusion" is an organic level autogenous perfusion control or managed by the NVU has not been confirmed. The systemic blood circulation deficiency after injury could overwhelm the autoregulation of the spinal cord, covering up the changes in this regulatory mechanism. Generally, despite traumatic blood loss, there is a transient state of spinal shock characterized by paralysis of the muscle pump and denervation of the cardiac pump to reduce effective blood circulation.[15] Severe systemic hypotension occurs with evident autonomic dysfunction and attenuated cardiopulmonary output, especially after cervical injuries, termed autonomic dysreflexia.[110] It is characterized by loss of supraspinal sympathetic control on the heart and decreased cardiac pump function. Systemic vasodilation secondary to loss of sympathetic tone further causes low effective circulating blood volume.[162] Profound hypotension leads to MAP failing to maintain autoregulation, continuously aggravating NVU dysfunction. SCI patients sometimes suffer cognitive impairments probably induced by NVU dysfunction caused by cerebral hypoperfusion, which could be reversed by increasing blood pressure.[163]

In addition to these systemic effects, the impaired autoregulatory capacity in spinal vasculature can further render the spinal cord vulnerable to systemic hypotension and contribute to ongoing ischemia.[15] Posttraumatic spinal cord ischemia worsens progressively after several hours and persists for weeks in SCI.[153] Hence, medical evidence-based guidelines suggest a level III recommendation to maintain the MAP between 85 and 90 mmHg for the first seven days following SCI.[164] Regardless of spontaneous neurologic recovery after spinal shock, management of MAP alone cannot provide a stable curative effect on the neurological prognosis of SCI patients,[165] making this issue more inexplicable.

Diverse mechanisms can affect blood flow in the spinal cord, including intraspinal cord pressure, which can even change the blood flow direction in venous vessels.[166] Given that the spinal cord is enveloped in the nonelastic dura and bony canal, it may obey the Monro-Kellie doctrine as in the brain.[167] Namely, the total volume of neural tissues, cerebrospinal fluid, and intraspinal blood are fixed. Increased intraspinal cord pressure after SCI caused by tissue swelling or bleeding may exhaust this compensatory ability. The higher the pathological intraspinal cord pressure is, the more deranged the autoregulation of the spinal cord. The intraspinal cord pressure increases significantly after SCI,[168] and the blood flow perfusion and oxygenation metabolism plummets after SCI and remains chronically diminished.[169]

Theoretically, when autoregulation is disturbed in SCI, the arterioles are maximally dilated due to local acidosis and hypoxia, and the perfusion of the spinal cord is directly proportional to the systemic blood pressure.[144] However, autoregulation in the injured spinal segment can be preserved for 1–2 hours after SCI.[113,161] During this period, a pathological hemodynamic change characterized as a significant blood flow acceleration in venous vessels occurs quickly after SCI and is maintained for approximately 1–2 hours. The pharmacologically elevating MAP only increases blood perfusion for one more hour before ischemia occurs, while for 4 hours, it is accompanied by cerebrospinal fluid drainage.[170] The time course of autoregulation dysfunction in SCI still needs to be elucidated.

Due to the distribution of radicular arteries, blood flow can ascend or descend within the spinal cord, creating numerous watershed areas of blood supply where opposing vascular currents meet. Watershed areas are more susceptible to ischemia.[166] Perfusion in the injured spinal cord is usually a patch-like pattern intersected by regions of low, intermediate, and high blood flow.[153] However, many reports find that hyperperfusion management by vasoconstriction agents after SCI has no benefit.[145] Some parts of the injury site were only perfused in the systolic period[153] and reversely exacerbated hemorrhage and tissue edema.[167] Despite the unfavorable systemic hypotension, vasodilatation agents can dilate the neighboring nondilated arterioles in the well-perfused regions and steal blood from the injured spinal cord (steal-phenomenon).[153] Interestingly, when a calcium channel blocker (nimodipine), which inhibits the contraction of vascular SMCs, is additionally administered for hyperperfusion management after SCI, local perfusion and electrophysiological output both improve.[171] These studies showed that autoregulation in SCI is far from understood, and the potential pharmacological treatment still needs further testing in SCI.

BSCB disruption in SCI

The BSCB disruption permits proinflammatory substances to enter the spinal parenchyma to amplify the pathophysiological cascades and magnify additional damage.[17] In the murine model, BSCB disruption in the acute SCI usually has biphasic peaks: immediate leakage at the epicenter and adjacent site within several hours and a delayed permeability increment with longer duration.[141] After mechanical impact, BSCB leakage generally starts in 5–15 min at the epicenter[17,47] and gradually appears in segments away from the epicenter, even along the whole spinal cord in some cases.[130] The murine models show that the segment located 1 cm away from the epicenter suffered BSCB leakage in 30 minutes and 2 cm away in approximately 3 hours.[172] The caudal segments seem more vulnerable to BSCB disruption than the rostral segments.[140] These phenomena suggest that the mechanism of BSCB disruption may be more complex in SCI. Moreover, the barrier disruption is difficult to quantitate except for measuring the leakage of hematogenous tracers where bleeding could also occur,[47] combining hemorrhage and BSCB disruption together to confuse matters. Some data indicate that BSCB disruption in the early period of SCI is a secondary change after mechanical damage. Pathological hemodynamic changes and leukocyte transmigration disrupt the BSCB after SCI and inhibiting these processes can also attenuate BSCB disruption. Much work is needed to clarify the mechanism of BSCB disruption in SCI according to the current view.

Some early studies suggested that BSCB leakage after SCI is caused by vesicular transport.[172] Electron microscopy showed that large numbers of vesicles appeared in the swollen ECs in capillaries and venules within 6 to 10 min after SCI, but without widening of the paracellular junctions.[173] Several forms of vesicle-based transcytosis exist and allow particular macromolecules to cross the endothelium, which can be classified into two categories: clathrin-mediated and caveolae-mediated.[47] The former is receptor-mediated to transfer insulin and transferrin; the latter is adsorptive transcytosis, where charged interactions between the molecule and plasma membrane facilitate its entry.[174] However, vesicular transport abnormalities seem not to be primarily responsible for BSCB disruption after SCI. Because transcytosis as an active transport process is low-rate, selective, and energy dependent,[48] it seems not to correlate with the extensive BSCB leakage of nonselective tracers of varied size in SCI.[17] Nevertheless, there are no further data to prove whether these vesicles are influx or efflux because vesicles are bidirectionally transported or whether these vesicles are caveolae,[175] which is closely related to neurovascular

coupling.[176] A recent report found many junctional discontinuities emerging on TJs 15 minutes after SCI, and reducing these junctional discontinuities can decrease BSCB leakage. Combined with the previous electron microscopy data demonstrating no widening of the paracellular junctions,[173] these results probably indicate that not all paracellular junctions are disintegrated in the BSCB leakage after SCI.

Many groups have suggested the degeneration of TJs as the reason for BSCB disruption in SCI, using the expression level of TJs as the indicator for BSCB function.[177] However, most results report that TJs are downregulated at approximately 12–24 h after SCI.[178] The expression of TJs remains unchanged in the early period of SCI when BSCB disruption occurs. Sometimes, a decrease in TJs expression may reflect that BSCB damage is more severe than paracellular leakage, such as whole endothelial cell loss.[130] Therefore, the related treatment to reverse the downregulation of TJs requires more caution when applied to clinical trials, especially in the acute period of SCI.

Conclusion and future directions

Today's view of the NVU has been broadly amplified. The BCNSB is constantly regarded as a functional configuration in the NVU. Despite the significant similarity of the NVU between the brain and spinal cord, investigation of the NVU in the spinal cord lags far behind that in the brain. Minor differences can be observed in the major elements of the NVU from the limited data available. The vascular anatomy of the spinal cord is markedly different from that of the brain, and the blood flow in the spinal cord is more variable and fluctuating, indicating a more sophisticated autoregulation mechanism in the spinal cord. NMDA receptor-mediated neurovascular coupling is less predominant in the spinal cord. Moreover, the permeability of the BSCB is physiologically higher than that of the BBB, probably because the coverage of pericytes and the expression of some paracellular junctional proteins are inherently lower than those of the BBB. More studies are needed to explore the spinal cord NVU.

Further, in SCI, impaired NVU function could be involved in secondary injury and exacerbate severity. Models of SCI should be weighed to explore the underlying mechanisms contributing to NVU dysfunction. The development of therapies to prevent and treat NVU dysfunction in SCI requires targeted exploration of the injury mechanisms of the NVU.

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