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► **To cite this version:**

Laetitia Travier, Roshani Singh, Daniel Sáenz Fernández, Aleksandra Deczkowska. Microbial and immune factors regulate brain maintenance and aging. *Current Opinion in Neurobiology*, Elsevier, 2022, 76, pp.102607. 10.1016/j.conb.2022.102607 . pasteur-03746573

HAL Id: pasteur-03746573

<https://hal-pasteur.archives-ouvertes.fr/pasteur-03746573>

Submitted on 5 Aug 2022

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Microbial and immune factors regulate brain maintenance and aging

Laetitia Travier¹, Roshani Singh¹,
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Abstract

Tissue aging can be viewed as a loss of normal maintenance; in advanced age, the mechanisms which keep the tissue healthy on daily bases fail to manage the accumulating “wear and tear”, leading to gradual loss of function. In the brain, maintenance is provided primarily by three components: the blood-brain barrier, which allows the influx of certain molecules into the brain while excluding others, the circulation of the cerebrospinal fluid, and the phagocytic function of microglia. Indeed, failure of these systems is associated with cognitive loss and other hallmarks of brain aging. Interestingly, all three mechanisms are regulated not only by internal conditions within the aging brain, but remain highly sensitive to the peripheral signals, such as cytokines or microbiome-derived molecules, present in the systemic circulation. In this article, we discuss the contribution of such peripheral factors to brain maintenance and its loss in aging.

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Current Opinion in Neurobiology 2022, 76:102607

This review comes from a themed issue on **Crosstalk between neural and immune systems**

Edited by **Kelly Jordan-Sciutto** and **Anthony Filiano**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online xxx

<https://doi.org/10.1016/j.conb.2022.102607>

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Introduction

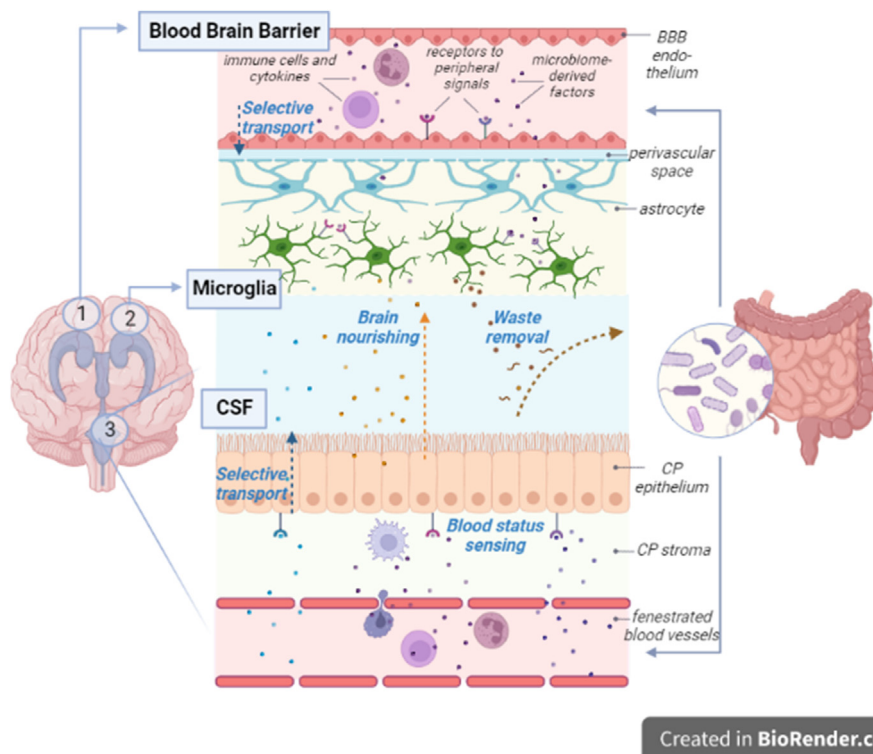
As in other tissues, aging of the brain can be defined as a dysfunction of tissue maintenance systems, physiological mechanisms keeping the tissue nourished and clean from any toxic material, cellular debris or waste. In the brain these functions are fulfilled by three main

systems: the blood-brain barrier (BBB), the phagocytic activity of the microglia and the flow of cerebrospinal, interstitial and perivascular fluids (which we will call altogether cerebrospinal fluid; CSF) throughout and around the brain. Inefficiency of these mechanisms in aging is linked to the accumulation of apoptotic and myelin debris which normally should be swiftly removed, presence of toxic protein aggregates, such as amyloid beta (A β), excessive accumulation of extracellular matrix, neuroinflammation and excessive synaptic clearance. These conditions create an ideal environment for the neurodegenerative diseases to arise; indeed, aging is the top risk factor of Alzheimers disease (AD), the most prevalent form of dementia, which is becoming extremely common in our aging society, but remains incurable. Therefore, the development of interventions that are able to counteract brain aging is urgently needed.

Interestingly, various systemic interventions, such as dietary change, exercise regime, heterochronic parabiosis or injections of plasma of young animals to old ones were able to restore brain function of aging animals in experimental settings [1], suggesting that the brain can not only be revitalized, but that its aging can be in fact induced by aging-related changes in the systemic environment. Indeed, aging-related changes in other systems which can shape brain activity, such as the immune system and the microbiome, have been recorded. For example, aging is associated with dysbiosis, characterized by reduced microbiome diversity, which correlates with the overall increased host frailty and reduced cognitive ability in the elderly [2–5]. Simultaneously, normal activity of the peripheral immune cells and pathways plays an important role in regulating the brain, therefore aging of the immune system may also contribute to a decline in brain function in old age and promote neurodegenerative disease [6–9].

In this Opinion article, we discuss the existing and potential mechanistic links between the dysfunction of the brain maintenance mechanisms in aging and the aging-associated changing conditions in the periphery (Figure 1). Development of next-generation therapeutic strategies aiming to preserve brain health and delay aging-related neurodegeneration may emerge from

Figure 1

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Peripheral immune cells and microbiome-derived molecules shape brain maintenance systems. The cross-talks between peripheral signals and the three main systems involved in maintenance of the brain homeostasis: (1) the blood-brain barrier (BBB) composed by a layer of endothelial cells constituting a barrier towards blood circulation, covered by a layer of astrocytic endfeet, (2) the microglia populating the brain parenchyma and (3) the circulation of the cerebrospinal fluid (CSF) produced at the choroid plexus (CP), which are composed of a layer of epithelial cells creating a barrier between the CSF-filled ventricles and the peripheral blood circulation irrigating the CP stroma through fenestrated blood vessels.

answering questions such as whether aging-related systemic changes in the composition and activity of the immune cells and microbiome promote dysregulation of brain maintenance mechanisms in aging and can we restore the function of these brain maintenance pathways by peripheral modulation?

BBB regulation and function in health and aging

BBB, the vascular interface of the central nervous system (CNS), is constructed of a layer of endothelial cells interconnected by specialized tight junctions, covered by a basal membrane and a layer of astrocytic endfeet [10]. The BBB is vital for brain maintenance by providing an extremely selective pathway of exchange between the blood and the brain. The plasma proteins that cross the BBB, and may have key regulatory roles in brain homeostasis, pass it via specialized, ligand-specific, receptor-mediated clathrin-dependent transcytosis [11]. In aging, transcytosis becomes largely replaced by unregulated caveolar transport. This shift is promoted by structural changes in the BBB; loss of pericytes and

thickening and altered composition of the basal membrane; phenomena described both in mouse and human aging [11]. Consequently, factors normally crossing the BBB are lost and others leak into the brain territory in an uncontrolled fashion, altogether dysregulating this pathway of peripheral-brain communication. In addition, aging in humans is associated with cerebral microbleeds and increased risk of hemorrhagic stroke; cases of complete breach of the normal separation between the brain and the periphery [12]. It is still largely unknown which regulatory factors pass through the BBB to the brain under physiological conditions and what their roles in CNS' health are, but aging is associated with increased BBB permeability for albumin, fibrinogen, antibodies and other proteins, which promote neuroinflammation and accelerate aging-associated brain function decline [13–16]. For example, the fibrinogen interaction with microglia via CD11b specifically promotes their phagocytic activity towards synaptic spines; a genetic perturbation of fibrinogen-CD11b interaction protected from cognitive decline in a model of aging-associated AD [14]. Simultaneously, the BBB's ability to remove waste, such

as A β via the Low-density lipoprotein Receptor-related Protein 1, declines in aging [17].

The aging-associated dysfunction of the brain vasculature is in large part induced by systemic factors; plasma exchange experiments between young and old mice showed that aging peripheral factors promote inflammation-related activation of the cerebral endothelium, but young plasma can partially reverse this effect in old mice [18]. Heterochronic parabiosis and treatment with a “rejuvenating” Growth Differentiation Factor 11, in addition to boosting neurogenesis in old mice, lead to vascular remodeling and increased cerebral blood flow, a parameter that declines in aging [19]. Conversely, plasma of aged mice induced endothelial expression of Vascular Cell Adhesion Molecule 1 (VCAM1), a factor which promotes interaction of the vessels with immune cells, and accelerated brain aging in otherwise young animals, whereas VCAM1 blockade in old mice reversed age-related impairments and improved hippocampal-dependent learning and memory [20]. VCAM1 expression may be induced by aging-associated infiltration of CD8⁺ T cells, the presence of which, in the CSF and brain parenchyma, was associated with pathological aging in mice and humans [21,22].

Microbial factors can also shape BBB properties; in embryos and adult mice, germ-free status is linked to a decreased expression of tight junction proteins at the BBB and increased permeability to dyes, which partially normalizes upon dietary supplementation with short-chain fatty acids (SCFA), one of the bacterial products [23]. Interestingly, Parkinson’s disease was associated with changes in microbiome composition characterized by decreased SCFA production [24,25], and SCFA treatment ameliorated brain pathology in Parkinson’s disease animal models [25–28] and in models of stroke [29,30]. Various other microbiome and diet-derived factors were shown to modulate BBB integrity [reviewed in [31]], and further understanding of their specific effects in aging will illuminate new intervention strategies to promote brain health into old age. The remaining key questions in the field are— what are the peripheral factors which cross the BBB in physiology, what is their role in brain maintenance and how aging changes the peripheral-brain communication via this pathway.

CSF flow from choroid plexuses to meninges – regulation and function in brain maintenance and aging

At all times the brain and spinal cord are bathed in specialized fluids; the interstitial fluid which drains into perivascular spaces, and the CSF in the brain ventricles, connected and constantly exchanging via the glymphatic pathway [32–37], collectively referred to here as CSF.

The CSF is produced largely by the choroid plexus (CP), a tissue forming a border in each of the brain’s ventricles [38,39]. Structurally, each CP is composed of a single layer of epithelial cells, interconnected by tight and adherens junctions, which create a barrier between the CSF-filled ventricles and the peripheral blood circulation irrigating the CP through fenestrated blood vessels [40]. The space between the epithelial and endothelial layers, the CP stroma, is populated by mesenchymal cells, rare neuronal cells and a diverse population of immune cells [40–43]. The CP controls the CSF properties by managing the blood-to-CSF passage of water, ions, nutrients and regulatory molecules, such as leptin, folate and copper [44–47]. In addition, the CP epithelium produces various CSF-enriching factors, such as insulin, which is crucial for glucose metabolism, Platelet-derived growth factors, key regulators of brain stem cells, and Transforming Growth Factor β , which displays various neuroprotective and immune-regulatory functions [48–51].

The apical side of the CP epithelium is ciliated, and the cilia as well as the cardiorespiratory pulsation of brain’s vasculature maintain the fluid in constant motion, which allows distribution of the CSF through the brain, but also clearance of the waste metabolites and toxins, which the brain produces, such as A β and excess neurotransmitters [52–55]. Flushed from the brain parenchyma such factors later leave the brain territory together with the CSF via arachnoid villi, along spinal and cranial nerves, and via the meningeal lymphatics (reviewed in: [56]). Altogether, the CSF composition and flow, largely dependent on the CP activity, is key to brain maintenance.

In aging, these various CP mechanisms which keep the brain healthy in young individuals, underperform. This effect can be, at least in part, promoted by peripheral immune and microbial factors. In elderly patients, slower CSF flow was associated with cognitive deficits [57]. Imaging of the dye drainage from the CSF to the deep cervical lymph nodes showed slower CSF flow in naturally aging mice and models of AD, which was at least in part caused by a dysfunction of the meningeal lymphatic; restoration of the dural lymphatic structure using VEGFC treatment improved the CSF flow and decreased A β accumulation and neuroinflammation in mouse AD models [58,59].

Changes in diet and loss of healthy microbiota diversity may alter the CSF flow and composition. In models of germ-free and antibiotic-treated mice, the absence of microbiota increased the gut-extrinsic sympathetic activity [60], which may favor stimulation of the superior cervical ganglia and the sympathetic nerves innervating CP in the vicinity of its epithelium and lead to the

reduction of CSF production [61,62]. Dysbiosis has been linked to reduced cardiovascular health, with lower vascular barrier function, remodeling, contractility and blood flow [63–65], which may alter the CSF movement as well [55]. Furthermore, studies aiming to explain the link between dysbiosis and mood disorders, showed that microbiota-mediated intestinal inflammation may favor episodes of “closure” of the CP vasculature, thereby leading to chronic brain deprivation from essential nutrients filtered from the blood to the CSF at this site [66]. Further research will reveal whether similar mechanisms contribute to the CP dysfunction in aging.

Studies in mice and humans showed that in aging, the composition of the CSF is also altered, in part due to changes in the CP secretory activity. Secretion of neurotrophic factors from the CP decreases, such as Fibroblast Growth Factor 17 promoting oligodendrogenesis and memory [40,67–69], but high expression of brain aging-linked factors, such as β 2-microglobulin, eotaxin (CCL11) or Interferon (IFN) β is observed [8,68,70–72]. In part these changes are dependent on peripheral signals; for example, expression of CCL11 in the CP epithelium in aging is induced by locally increased levels of cytokine interleukin 4 (IL-4), derived from Th2 CD4⁺ T cells, and lower levels of counteracting cytokine IFN- γ , linked to Th1 CD4⁺ T cell activity [8]. CCL11 is elevated in the CSF of older individuals and CCL11-treated animals showed reduced neurogenesis and accelerated cognitive loss [8,72]. In parallel, innate lymphoid cells 2 (ILC2) accumulate at the aging CP in both mice and humans [73]. In young and old mice these cells are dormant but could be activated to produce IL-5 and IL-13 upon IL-33 stimulation. Systemic IL-33 treatment and injection of ILC2s to the brain ventricles improved learning and memory in old mice in an IL-5-dependent manner, but it is unclear whether ILC2 cells cross the CP and enter the brain territory to confer this protective effect under conditions of normal aging as well [73].

Immune cells residing at the meninges were also implicated in directly regulating behavior by stimulating cytokine sensing neurons, e.g. meningeal-derived IL-4, IL-17 and IFN- γ were shown to shape murine learning and memory, anxiety and social behavior [74–76]. IL-17 production was lower in meningeal $\gamma\delta$ T cells in germ-free and broad-spectrum antibiotic-treated animals, which show, in general, decreased anxiety phenotype [75]. Analysis of meningeal T cells in old mice revealed that the frequency of T regulatory cells increases with age, while effector cells, which produce such neuron-stimulating cytokines, are relatively less abundant. Blockade of T regulatory cells with anti-CD25 antibody lead to improved cognition in old mice, while loss of CCR7, a chemokine receptor key for T cell function, which is naturally lower in aging, promoted AD pathology in a mouse model [7]. Interestingly, CCR7-

deficient mice also showed meningeal lymphatic dysfunction, revealing an unexpected link between immune cell-expressed receptor and CSF flow regulation [7].

Microglia – peripheral regulation and local function in brain maintenance and aging

Microglia are specialized CNS macrophages and professional phagocytes. Microglial phagocytosis is key mechanism for the removal of myelin debris [77], soluble forms of A β by engulfment and packing it into less toxic plaques [78], as well as excess extracellular matrix which allows for remodeling of the synapses [79]. The A β removal activity is key for brain fitness and impairment of microglial machinery involved in A β sensing for phagocytosis leads to spontaneous occurrence of AD in mice [80], a species which otherwise never develops this disease [81]. Further, microglia remove dead cells and excessive synaptic elements via sensing of the phosphatidylserine and complement proteins which mark such inactive domains for elimination [82–85]. Microglial pruning of excessive synapses is a key step in shaping of the brain architecture during development [86].

In aging, arising accumulation of waste material and loosening of the brain barriers leads to uncontrolled leakage and increased local production of complement proteins, among others, which now decorate otherwise functional synapses marking them for microglia-mediated clearance; mice deficient of complement cascade keep young-like number of synapses and maintain cognitive ability in old age [87,88]. Simultaneously, microglia lose their phagocytic ability towards extracellular waste [89–91], leading to the accumulation of A β , myelin and other cellular debris and abundant extracellular matrix, which may interfere with tissue function [77,92]. Importantly, under physiological conditions, microglia are extremely long-lived and are only replenished locally, without input from the bone-marrow-derived monocytes [93–95]. Therefore, in aging, when their activity is altered, microglia cannot be easily replaced by new, better-performing phagocytes.

This shift in microglial function can be partially induced by cellular aging [96], but microglial phenotype, as is the case of all macrophages, is critically dependent on signals they sense from their environment [97–99]. For example, microglia in different brain regions display slightly different transcriptomic signatures and the differences are further emphasized in aging [100]. Can peripheral factors contribute to this diversity as well? Studies in germ-free and antibiotic-treated mice showed that microbiome colonization promotes microglial maturation [101–103]. Interestingly, microglia of mice deficient in Free Fatty Acid Receptor 2 (FFAR2), a SCFA receptor also showed immature phenotype, similar to antibiotic-treated or germ-free mice, yet

expression of FFAR2 on microglia was not detected, suggesting an indirect impact of the microbiome metabolic product on microglial phenotype [101]. Studies in an Experimental Autoimmune Encephalomyelitis, a model of multiple sclerosis, showed that tryptophan metabolites directly modulate microglial Aryl Hydrocarbon Receptor to promote production of Transforming Growth Factor α , a cytokine having an anti-inflammatory effect on astrocytes [104]. Both models describe microbial contribution to the regulation of microglial activity under conditions of compromised brain barrier integrity [23,105], but similar impairment in barrier function is present in aging [11,16]. In addition, the aging-associated production of the IFN-I at the CP further promotes microglial immune activation [70], especially in the brain regions adjacent to the ventricles [100].

Further, physiological microglia maturation during development is also promoted by brain-resident CD4⁺ T cells; in CD4⁺ T cell-depleted mice, microglia are not able to prune synapses [106]. In aging, another population of T cells was shown to infiltrate the brain – the CD8⁺ T cells, usually associated with the killing of virus-infected and tumor cells and promoting cellular defense systems [21]. These cells can further redirect microglial activity towards immune activation and away from normal physiological function. Therefore, both aging-associated changes in the local environment in the brain, and systemic changes, which may “leak” to the brain through compromised barriers, can contribute to impairment of microglial ability to maintain brain function in aging.

Concluding remarks

Tissues operate in two states: either promoting homeostatic performance under normal conditions or amplifying inflammation, in the case of a detected danger signal, which is a mechanism aiming to eliminate the stressor [107]. Aging is a slow progression from physiology towards chronic inflammatory state, both induced by accumulating “wear and tear”, and leaving the maintenance mechanisms unable to preserve their normal activity, leading to further buildup of damage and waste material. The inflammation-promoting signal in brain aging comes, at least in part, from the aging environment beyond the brain territory, such as microbiome and the immune cells and signals. Future research is needed to identify precise anatomical and molecular pathways mediating the effects of the peripheral factors on the brain. In the future, precise manipulation of such pathways from the periphery to revive the brain maintenance systems could be applied as a brain rejuvenation strategy.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank members of the Deczkowska lab for fruitful discussions and Kyle Everton Lamber for editing the manuscript. The figures were prepared with BioRender.com. Research in Deczkowska lab is supported by core G5 funding from Institut Pasteur, Ville de Paris EMERGENCE(S) grant and Alzheimer’s Association Research Grant (AARG-22-917964). RS is a student from the AIRE master program funded by the Bettencourt Schueller foundation and the EURIP graduate program (ANR-17-EURE-0012). DSF is supported by the cooperative Erasmus⁺ Program developed by Universitat de Barcelona and Paris-Sorbonne Université.

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