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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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Introduction

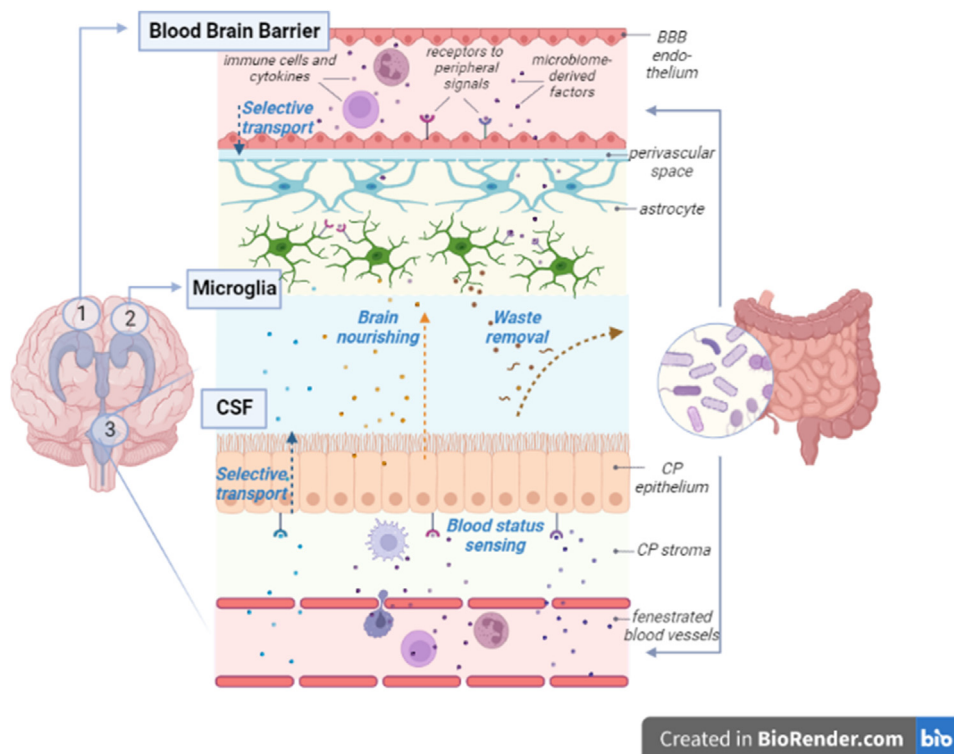
As in other tissues, aging of the brain can be defined as a dysfunction of tissue maintenance systems, physiological mechanisms keeping the tissue nourished and clean from any toxic material, cellular debris or waste. In the brain these functions are fulfilled by three main

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.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

- Horowitz AM, Villeda SA: **Therapeutic potential of systemic brain rejuvenation strategies for neurodegenerative disease.** *F1000Research* 2017, **6**:1291.
- Thevaranjan N, Puchta A, Schulz C, Naidoo A, Szamosi JC, Verschoor CP, Loukov D, Schenck LP, Jury J, Foley KP, *et al.*: **Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction.** *Cell Host Microbe* 2017, **21**: 455–466. e454.
- Claesson Marcus J, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, Marchesi Julian R, Falush D, Dinan T, Fitzgerald G, *et al.*: **Composition, variability, and temporal stability of the intestinal microbiota of the elderly.** *Proc Natl Acad Sci USA* 2011, **108**:4586–4591.
- Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HMB, Coakley M, Lakshminarayanan B, O'Sullivan O, *et al.*: **Gut microbiota composition correlates with diet and health in the elderly.** *Nature* 2012, **488**:178–184. This epidemiological study highlights correlations between the microbiome diversity and various measures of frailty in elderly individuals.
- Teng Y, Mu J, Xu F, Zhang X, Sriwastva MK, Liu QM, Li X, Lei C, Sundaram K, Hu X, *et al.*: **Gut bacterial isoamylamine promotes age-related cognitive dysfunction by promoting microglial cell death.** *Cell Host Microbe* 2022.
- Lima KAd, Rustenhoven J, Kipnis J: **Meningeal immunity and its function in maintenance of the central nervous system in health and disease.** *Annu Rev Immunol* 2020, **38**:597–620.
- Da Mesquita S, Herz J, Wall M, Dykstra T, de Lima Kalil A, Norris Geoffrey T, Dabhi N, Kennedy T, Baker W, Kipnis J: **Aging-associated deficit in CCR7 is linked to worsened glymphatic function, cognition, neuroinflammation, and β -amyloid pathology.** *Sci Adv* 2021, **7**, eabe4601.
- Baruch K, Ron-Harel N, Gal H, Deczkowska A, Shifrut E, Ndifon W, Miras-Neisberg N, Cardon M, Vaknin I, Cahalon L, *et al.*: **CNS-specific immunity at the choroid plexus shifts toward destructive Th2 inflammation in brain aging.** *Proc Natl Acad Sci U S A* 2013, **110**:2264–2269.
- Baruch K, Deczkowska A, Rosenzweig N, Tsitsou-Kampeli A, Sharif AM, Matcovitch-Natan O, Kertser A, David E, Amit I, Schwartz M: **PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease.** *Nat Med* 2016, **22**:135–137.
- Kadry H, Noorani B, Cucullo L: **A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity.** *Fluids Barriers CNS* 2020, **17**:69.
- Yang AC, Stevens MY, Chen MB, Lee DP, Stähli D, Gate D, Contrepois K, Chen W, Iram T, Zhang L, *et al.*: **Physiological blood–brain transport is impaired with age by a shift in transcytosis.** *Nature* 2020, **583**:425–430. Seminal paper identifying the molecular bases of transport across the BBB endothelium in physiology and aging.
- Wang HL, Zhang CL, Qiu YM, Chen AQ, Li YN, Hu B: **Dysfunction of the blood-brain barrier in cerebral microbleeds: from bedside to bench.** *Aging Dis* 2021, **12**: 1898–1919.

13. Parrado-Fernández C, Blennow K, Hansson M, Leoni V, Cedazo-Minguez A, Björkhem I: **Evidence for sex difference in the CSF/plasma albumin ratio in ~20 000 patients and 335 healthy volunteers.** *J Cell Mol Med* 2018, **22**:5151–5154.
14. Merlini M, Rafalski VA, Rios Coronado PE, Gill TM, Ellisman M, Muthukumar G, Subramanian KS, Ryu JK, Syme CA, Davalos D, *et al.*: **Fibrinogen induces microglia-mediated spine elimination and cognitive impairment in an Alzheimer's disease model.** *Neuron* 2019, **101**:1099–1108.e1096.
- A study identifying a mechanistic link between the loss of BBB integrity and progression of Alzheimer's Disease.
15. Shuken SR, Rutledge J, Iram T, Losada PM, Wilson EN, Andreasson KI, Leib RD, Wyss-Coray T: **Limited proteolysis—mass spectrometry reveals aging-associated changes in cerebrospinal fluid protein abundances and structures.** *Nature Aging* 2022.
16. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, *et al.*: **Blood-brain barrier breakdown in the aging human hippocampus.** *Neuron* 2015, **85**:296–302.
17. Storck SE, Meister S, Nahrath J, Meißner JN, Schubert N, Di Spiezo A, Baches S, Vandenbroucke RE, Bouter Y, Prikulis I, *et al.*: **Endothelial LRP1 transports amyloid- β (1-42) across the blood-brain barrier.** *J Clin Invest* 2016, **126**:123–136.
18. Chen MB, Yang AC, Yousef H, Lee D, Chen W, Schaum N, Lehallier B, Quake SR, Wyss-Coray T: **Brain endothelial cells are exquisite sensors of age-related circulatory cues.** *Cell Rep* 2020, **30**:4418–4432. e4414.
19. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL: **Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors.** *Science* 2014, **344**:630–634.
20. Yousef H, Czupalla CJ, Lee D, Chen MB, Burke AN, Zera KA, Zandstra J, Berber E, Lehallier B, Mathur V, *et al.*: **Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1.** *Nat Med* 2019, **25**:988–1000.
21. Gate D, Saligrama N, Leventhal O, Yang AC, Unger MS, Middeldorp J, Chen K, Lehallier B, Channappa D, De Los Santos MB, *et al.*: **Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease.** *Nature* 2020, **577**:399–404.
22. Unger MS, Li E, Scharnagl L, Poupardin R, Altendorfer B, Mrowetz H, Hutter-Paier B, Weiger TM, Heneka MT, Attems J, *et al.*: **CD8(+) T-cells infiltrate Alzheimer's disease brains and regulate neuronal- and synapse-related gene expression in APP-PS1 transgenic mice.** *Brain Behav Immun* 2020, **89**:67–86.
23. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, Korecka A, Bakocevic N, Ng LG, Kundu P, *et al.*: **The gut microbiota influences blood-brain barrier permeability in mice.** *Sci Transl Med* 2014, **6**:263ra158.
24. Unger MM, Spiegel J, Dillmann K-U, Grundmann D, Philippeit H, Bürmann J, Faßbender K, Schwiertz A, Schäfer K-H: **Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls.** *Park Relat Disord* 2016, **32**:66–72.
25. Aho VTE, Houser MC, Pereira PAB, Chang J, Rudi K, Paulin L, Hertzberg V, Auvinen P, Tansey MG, Scheperjans F: **Relationships of gut microbiota, short-chain fatty acids, inflammation, and the gut barrier in Parkinson's disease.** *Mol Neurodegener* 2021, **16**:6.
26. Laurent RS, O'Brien LM, Ahmad S: **Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease.** *Neuroscience* 2013, **246**:382–390.
27. Paiva I, Pinho R, Pavlou MA, Hennion M, Wales P, Schütz A-L, Rajput A, Szegő EM, Kerimoglu C, Gerhardt E: **Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage.** *Hum Mol Genet* 2017, **26**:2231–2246.
28. Liu J, Wang F, Liu S, Du J, Hu X, Xiong J, Fang R, Chen W, Sun J: **Sodium butyrate exerts protective effect against Parkinson's disease in mice via stimulation of glucagon like peptide-1.** *J Neurol Sci* 2017, **381**:176–181.
29. Lee J, d'Aigle J, Atadja L, Quaicoe V, Honarpisheh P, Ganesh BP, Hassan A, Graf J, Petrosino J, Putluri N, *et al.*: **Gut microbiota-derived short-chain fatty acids promote post-stroke recovery in aged mice.** *Circ Res* 2020, **127**:453–465.
30. Sadler R, Cramer JV, Heindl S, Kostidis S, Betz D, Zuurbier KR, Northoff BH, Heijink M, Goldberg MP, Plautz EJ, *et al.*: **Short-chain fatty acids improve poststroke recovery via immunological mechanisms.** *J Neurosci* 2020, **40**:1162–1173.
31. Knox EG, Aburto MR, Clarke G, Cryan JF, O'Driscoll CM: **The blood-brain barrier in aging and neurodegeneration.** *Mol Psychiatr* 2022.
32. Hauglund NL, Pavan C, Nedergaard M: **Cleaning the sleeping brain – the potential restorative function of the glymphatic system.** *Current Opinion in Physiology* 2020, **15**:1–6.
33. Abbott NJ, Pizzo ME, Preston JE, Janigro D, Thorne RG: **The role of brain barriers in fluid movement in the CNS: is there a 'glymphatic' system?** *Acta Neuropathol* 2018, **135**:387–407.
34. Jessen NA, Munk ASF, Lundgaard I, Nedergaard M: **The glymphatic system: a beginner's guide.** *Neurochem Res* 2015, **40**:2583–2599.
35. Rennels ML, Gregory TF, Blaumanis OR, Fujimoto K, Grady PA: **Evidence for a 'Paravascular' fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space.** *Brain Res* 1985, **326**:47–63.
36. Hladky SB, Barrand MA: **Fluid and ion transfer across the blood–brain and blood–cerebrospinal fluid barriers; a comparative account of mechanisms and roles.** *Fluids Barriers CNS* 2016, **13**:19.
37. Illiff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, *et al.*: **A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β .** *Sci Transl Med* 2012, **4**:147ra111–147ra111.
38. Fame RM, Lehtinen MK: **Emergence and developmental roles of the cerebrospinal fluid system.** *Dev Cell* 2020, **52**:261–275.
39. Saunders NR, Dziegielewska KM, Møllgård K, Habgood MD: **Physiology and molecular biology of barrier mechanisms in the fetal and neonatal brain.** *J Physiol* 2018, **596**:5723–5756.
40. Dani N, Herbst RH, McCabe C, Green GS, Kaiser K, Head JP, Cui J, Shipley FB, Jang A, Dionne D, *et al.*: **A cellular and spatial map of the choroid plexus across brain ventricles and ages.** *Cell* 2021, **184**:3056–3074. e3021.
41. Van Hove H, Martens L, Scheyltjens I, De Vlaminck K, Pombo Antunes AR, De Prijck S, Vandamme N, De Schepper S, Van Isterdael G, Scott CL, *et al.*: **A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment.** *Nat Neurosci* 2019, **22**:1021–1035.
42. Mrdjen D, Pavlovic A, Hartmann FJ, Schreiner B, Utz SG, Leung BP, Lelios I, Heppner FL, Kipnis J, Merkler D: **High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease.** *Immunity* 2018.
43. Korin B, Ben-Shaan TL, Schiller M, Dubovik T, Azulay-Debby H, Boshnak NT, Koren T, Rolls A: **High-dimensional, single-cell characterization of the brain's immune compartment.** *Nat Neurosci* 2017, **20**:1300–1309.
44. Di Spiezo A, Sandin ES, Dore R, Müller-Fielitz H, Storck SE, Bernau M, Mier W, Oster H, Jöhren O, Pietrzik CU, *et al.*: **The LepR-mediated leptin transport across brain barriers controls food reward.** *Mol Metabol* 2018, **8**:13–22.
45. Alam C, Aufreiter S, Georgiou Constantine J, Hoque Md T, Finnell Richard H, O'Connor Deborah L, Goldman ID, Bendayan R: **Upregulation of reduced folate carrier by vitamin D enhances**

- brain folate uptake in mice lacking folate receptor alpha. *Proc Natl Acad Sci USA* 2019, **116**:17531–17540.
46. Grapp M, Wrede A, Schweizer M, Hüwel S, Galla HJ, Snaidero N, Simons M, Bückers J, Low PS, Urlaub H, *et al.*: **Choroid plexus transcytosis and exosome shuttling deliver folate into brain parenchyma.** *Nat Commun* 2013, **4**:2123.
 47. Donsante A, Johnson P, Jansen LA, Kaler SG: **Somatic mosaicism in Menkes disease suggests choroid plexus-mediated copper transport to the developing brain.** *Am J Med Genet* 2010, **152a**:2529–2534.
 48. Delgado Ana C, Maldonado-Soto Angel R, Silva-Vargas V, Mizrak D, von Känel T, Tan Kelly R, Paul A, Madar A, Cuervo H, Kitajewski J, *et al.*: **Release of stem cells from quiescence reveals gliogenic domains in the adult mouse brain.** *Science* 2021, **372**:1205–1209.
 49. Silva-Vargas V, Doetsch F: **Exploring the source of human brain fluids.** *Science* 2020, **369**:143–144.
 50. Mazucanti CH, Liu QR, Lang D, Huang N, O'Connell JF, Camandola S, Egan JM: **Release of insulin produced by the choroid plexus is regulated by serotonergic signaling.** *JCI Insight* 2019, **4**.
 51. Shipley FB, Dani N, Xu H, Deister C, Cui J, Head JP, Sadegh C, Fame RM, Shannon ML, Flores VI, *et al.*: **Tracking calcium dynamics and immune surveillance at the choroid plexus blood-cerebrospinal fluid interface.** *Neuron* 2020, **108**:623–639. e610.
 52. Faubel R, Westendorf C, Bodenschatz E, Eichele G: **Cilia-based flow network in the brain ventricles.** *Science* 2016, **353**:176–178.
 53. Abdelhamed Z, Vuong SM, Hill L, Shula C, Timms A, Beier D, Campbell K, Mangano FT, Stottmann RW, Goto J: **A mutation in Ccdc39 causes neonatal hydrocephalus with abnormal motile cilia development in mice.** *Development* 2018:145.
 54. Dreha-Kulaczewski S, Konopka M, Joseph AA, Kollmeier J, Merboldt K-D, Ludwig H-C, Gärtner J, Frahm J: **Respiration and the watershed of spinal CSF flow in humans.** *Sci Rep* 2018, **8**:5594.
 55. Mestre H, Tithof J, Du T, Song W, Peng W, Sweeney AM, Olveda G, Thomas JH, Nedergaard M, Kelley DH: **Flow of cerebrospinal fluid is driven by arterial pulsations and is reduced in hypertension.** *Nat Commun* 2018, **9**:4878.
 56. Da Mesquita S, Fu Z, Kipnis J: **The meningeal lymphatic system: a new player in neurophysiology.** *Neuron* 2018, **100**:375–388.
 57. Attier-Zmudka J, Sérot J-M, Valluy J, Saffarini M, Macaret A-S, Diouf M, Dao S, Douadi Y, Malinowski KP, Balédent O: **Decreased cerebrospinal fluid flow is associated with cognitive deficit in elderly patients.** *Front Aging Neurosci* 2019, **11**.
 58. Da Mesquita S, Louveau A, Vaccari A, Smirnov I, Cornelison RC, Kingsmore KM, Contarino C, Onengut-Gumuscu S, Farber E, Raper D, *et al.*: **Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease.** *Nature* 2018, **560**:185–191.
 59. Da Mesquita S, Papadopoulos Z, Dykstra T, Brase L, Farias FG, Wall M, Jiang H, Kodira CD, de Lima KA, Herz J, *et al.*: **Meningeal lymphatics affect microglia responses and anti-A β immunotherapy.** *Nature* 2021, **593**:255–260.
 60. Muller PA, Schneeberger M, Matheis F, Wang P, Kerner Z, Ilanges A, Pellegrino K, del Marmol J, Castro TBR, Furuichi M, *et al.*: **Microbiota modulate sympathetic neurons via a gut–brain circuit.** *Nature* 2020, **583**:441–446.
 61. Lindvall M, Edvinsson L, Owman C: **Sympathetic nervous control of cerebrospinal fluid production from the choroid plexus.** *Science* 1978, **201**:176–178.
 62. Lindvall M, Owman C: **Autonomic nerves in the mammalian choroid plexus and their influence on the formation of cerebrospinal fluid.** *J Cerebr Blood Flow Metabol* 1981, **1**:245–266.
 63. Edwards JM, Roy S, Tomcho JC, Schreckenberger ZJ, Chakraborty S, Bearss NR, Saha P, McCarthy CG, Vijay-Kumar M, Joe B, *et al.*: **Microbiota are critical for vascular physiology: germ-free status weakens contractility and induces sex-specific vascular remodeling in mice.** *Vasc Pharmacol* 2020, **125–126**, 106633.
 64. Brown JM, Hazen SL: **Microbial modulation of cardiovascular disease.** *Nat Rev Microbiol* 2018, **16**:171–181.
 65. Travier L, Alonso M, Andronico A, Hafner L, Disson O, Lledo P-M, Cauchemez S, Lecuit M: **Neonatal susceptibility to meningitis results from the immaturity of epithelial barriers and gut microbiota.** *Cell Rep* 2021, **35**, 109319.
 66. Carloni S, Bertocchi A, Mancinelli S, Bellini M, Erreni M, Borreca A, Braga D, Giugliano S, Mozzarelli AM, Manganaro D, *et al.*: **Identification of a choroid plexus vascular barrier closing during intestinal inflammation.** *Science* 2021, **374**:439–448.
- An important study highlighting, for the first time, a dynamic response of the choroid plexus blood vessels to peripheral infection.
67. Iram T, Kern F, Kaur A, Myneni S, Morningstar AR, Shin H, Garcia MA, Yerra L, Palovics R, Yang AC, *et al.*: **Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17.** *Nature* 2022, **605**:509–515.
 68. Baruch K, Deczkowska A, David E, Castellano JM, Miller O, Kertser A, Berkutzki T, Barnett-Iltzhaki Z, Bezael D, Wyss-Coray T, *et al.*: **Ageing-induced type I interferon response at the choroid plexus negatively affects brain function.** *Science* 2014, **346**:89–93.
- This study highlights the changes the CP undergoes with aging and their (reversible) impact on the brain.
69. Silva-Vargas V, Maldonado-Soto AR, Mizrak D, Codega P, Doetsch F: **Age-dependent niche signals from the choroid plexus regulate adult neural stem cells.** *Cell Stem Cell* 2016, **19**:643–652.
- This work shows that choroid plexus-derived signals promote and regulate stem cell niche function in adult mice, yet this pathway is disrupted in aging.
70. Deczkowska A, Matcovitch-Natan O, Tsiou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, Singer O, David E, Winter DR, Smith LK, *et al.*: **Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN- λ dependent manner.** *Nat Commun* 2017, **8**:717.
 71. Smith LK, He Y, Park J-S, Bieri G, Snethlage CE, Lin K, Gontier G, Wabl R, Plambeck KE, Udeochu J, *et al.*: **β 2-microglobulin is a systemic pro-ageing factor that impairs cognitive function and neurogenesis.** *Nat Med* 2015, **21**:932–937.
 72. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, *et al.*: **The ageing systemic milieu negatively regulates neurogenesis and cognitive function.** *Nature* 2011, **477**:90.
- 71–72 are studies using heterochronic parabiosis and identifying CCL11 and B2M as peripheral pro-ageing factors.
73. Fung ITH, Sankar P, Zhang Y, Robison LS, Zhao X, D'Souza SS, Salinero AE, Wang Y, Qian J, Kuentzel ML, *et al.*: **Activation of group 2 innate lymphoid cells alleviates aging-associated cognitive decline.** *J Exp Med* 2020:217.
 74. Herz J, Fu Z, Kim K, Dykstra T, Wall M, Li H, Salvador AF, Zou B, Yan N, Blackburn SM, *et al.*: **GABAergic neuronal IL-4R mediates T cell effect on memory.** *Neuron* 2021, **109**:3609–3618. e3609.
 75. Alves de Lima K, Rustenhoven J, Da Mesquita S, Wall M, Salvador AF, Smirnov I, Martelossi Cebinelli G, Mamuladze T, Baker W, Papadopoulos Z, *et al.*: **Meningeal $\gamma\delta$ T cells regulate anxiety-like behavior via IL-17a signaling in neurons.** *Nat Immunol* 2020, **21**:1421–1429.
 76. Filiano AJ, Xu Y, Tustison NJ, Marsh RL, Baker W, Smirnov I, Overall CC, Gadani SP, Turner SD, Weng Z, *et al.*: **Unexpected role of interferon- γ in regulating neuronal connectivity and social behaviour.** *Nature* 2016, **535**:425–429.
- Studies 74–76 highlight the impact of T cell-derived cytokines on animal behavior and the mechanisms behind.
77. Safaiyan S, Kannaiyan N, Snaidero N, Brioschi S, Biber K, Yona S, Edinger AL, Jung S, Rossner MJ, Simons M: **Age-**

- related myelin degradation burdens the clearance function of microglia during aging.** *Nat Neurosci* 2016, **19**:995–998.
78. Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, Vieira-Saecker A, Schwartz S, Santarelli F, Kummer MP, *et al.*: **Microglia-derived ASC specks cross-seed amyloid- β in Alzheimer's disease.** *Nature* 2017, **552**:355–361.
79. Nguyen PT, Dorman LC, Pan S, Vainchtein ID, Han RT, Nakao-Inoue H, Taloma SE, Barron JJ, Molofsky AB, Kheirbek MA, *et al.*: **Microglial remodeling of the extracellular matrix promotes synapse plasticity.** *Cell* 2020, **182**:388–403. e315.
- Studies 77–79 highlight different aspects of microglia dysfunction in aging.
80. Heckmann Bradley E, Teubner Brett JW, Boada-Romero E, Tummers B, Guy C, Fitzgerald P, Mayer U, Carding S, Zakharenko Stanislav S, Wileman T, *et al.*: **Noncanonical function of an autophagy protein prevents spontaneous Alzheimer's disease.** *Sci Adv* 2020, **6**, eabb9036.
81. Geirsdottir L, David E, Keren-Shaul H, Weiner A, Bohlen SC, Neuber J, Balic A, Giladi A, Sheban F, Dutertre C-A, *et al.*: **Cross-species single-cell Analysis reveals divergence of the primate microglia program.** *Cell* 2019, **179**:1609–1622. e1616.
- By comparing transcriptomic and morphological features of microglia across species the authors highlight the relevance of specific animal models to human disease.
82. Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, Gulati G, Bennett ML, Sun LO, Clarke LE, *et al.*: **Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing.** *Neuron* 2019, **101**:207–223. e210.
83. Ayata P, Badimon A, Strasburger HJ, Duff MK, Montgomery SE, Loh Y-HE, Ebert A, Pimenova AA, Ramirez BR, Chan AT, *et al.*: **Epigenetic regulation of brain region-specific microglia clearance activity.** *Nat Neurosci* 2018, **21**:1049–1060.
84. Gunner G, Cheadle L, Johnson KM, Ayata P, Badimon A, Mondo E, Nagy MA, Liu L, Bemiller SM, Kim K-W, *et al.*: **Sensory lesioning induces microglial synapse elimination via ADAM10 and fractalkine signaling.** *Nat Neurosci* 2019, **22**:1075–1088.
85. Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B: **Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner.** *Neuron* 2012, **74**:691–705.
86. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L: **Synaptic pruning by microglia is necessary for normal brain development.** *Science* 2011, **333**:1456–1458.
87. Shi Q, Colodner KJ, Matousek SB, Merry K, Hong S, Kenison JE, Frost JL, Le KX, Li S, Dodart J-C, *et al.*: **Complement C3-deficient mice fail to display age-related hippocampal decline.** *J Neurosci: the official journal of the Society for Neuroscience* 2015, **35**:13029–13042.
88. Klaus C, Hansen JN, Ginolhac A, Gérard D, Gnanapragassam VS, Horstkorte R, Rossdam C, Buettner FFR, Sauter T, Sinkkonen L, *et al.*: **Reduced sialylation triggers homeostatic synapse and neuronal loss in middle-aged mice.** *Neurobiol Aging* 2020, **88**:91–107.
89. Pluvinage JV, Haney MS, Smith BAH, Sun J, Iram T, Bonanno L, Li L, Lee DP, Morgens DW, Yang AC, *et al.*: **CD22 blockade restores homeostatic microglial phagocytosis in ageing brains.** *Nature* 2019, **568**:187–192.
90. Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, Pluvinage JV, Mathur V, Hahn O, Morgens DW, *et al.*: **Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain.** *Nat Neurosci* 2020, **23**:194–208.
91. Daria A, Colombo A, Llovera G, Hampel H, Willem M, Liesz A, Haass C, Tahirovic S: **Young microglia restore amyloid plaque clearance of aged microglia.** *EMBO J* 2017, **36**:583–603.
92. Reed MJ, Damodarasamy M, Pathan JL, Erickson MA, Banks WA, Vernon RB: **The effects of normal aging on regional accumulation of hyaluronan and chondroitin sulfate proteoglycans in the mouse brain.** *J Histochem Cytochem: official journal of the Histochemistry Society* 2018, **66**:697–707.
93. Huang Y, Xu Z, Xiong S, Sun F, Qin G, Hu G, Wang J, Zhao L, Liang YX, Wu T, *et al.*: **Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion.** *Nat Neurosci* 2018, **21**:530–540.
94. Föger P, Hefendehl JK, Veeraraghavalu K, Wendeln AC, Schlosser C, Obermüller U, Wegenast-Braun BM, Neher JJ, Martus P, Kohsaka S, *et al.*: **Microglia turnover with aging and in an Alzheimer's model via long-term in vivo single-cell imaging.** *Nat Neurosci* 2017, **20**:1371–1376.
95. Réu P, Khosravi A, Bernard S, Mold JE, Salehpour M, Alkass K, Perl S, Tisdale J, Possnert G, Druid H, *et al.*: **The lifespan and turnover of microglia in the human brain.** *Cell Rep* 2017, **20**:779–784.
96. Hu Y, Fryatt GL, Ghorbani M, Obst J, Menassa DA, Martin-Estebane M, Muntslag TAO, Olmos-Alonso A, Guerrero-Carrasco M, Thomas D, *et al.*: **Replicative senescence dictates the emergence of disease-associated microglia and contributes to A β pathology.** *Cell Rep* 2021, **35**, 109228.
97. Deczkowska A, Amit I, Schwartz M: **Microglial immune checkpoint mechanisms.** *Nat Neurosci* 2018, **21**:779–786.
98. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S, Amit I: **Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment.** *Cell* 2014, **159**:1312–1326.
99. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, Stender JD, Chun HB, Garner H, Geissmann F: **Environment drives selection and function of enhancers controlling tissue-specific macrophage identities.** *Cell* 2014, **159**:1327–1340.
- 98-99 Seminal studies highlighting the critical role of local environment in establishment of macrophage identity across tissues.
100. Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang L-c, Means TK, El Khoury J: **The microglial sensome revealed by direct RNA sequencing.** *Nat Neurosci* 2013, **16**:1896–1905.
101. Emy D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, *et al.*: **Host microbiota constantly control maturation and function of microglia in the CNS.** *Nat Neurosci* 2015, **18**:965–977.
102. Thion MS, Low D, Silvin A, Chen J, Grisel P, Schulte-Schrepping J, Blecher R, Ulas T, Squarzone P, Hoeffel G: **Microbiome Influences prenatal and adult microglia in a sex-specific manner.** *Cell* 2017.
103. Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, Zelada Gonzalez F, Perrin P, *et al.*: **Microglia development follows a stepwise program to regulate brain homeostasis.** *Science* 2016, **353**:aad8670.
- 101-103 Seminal studies highlighting the contribution of microbiome to microglia maturation and identity.
104. Rothhammer V, Borucki DM, Tjon EC, Takenaka MC, Chao CC, Ardura-Fabregat A, de Lima KA, Gutiérrez-Vázquez C, Hewson P, Staszewski O, *et al.*: **Microglial control of astrocytes in response to microbial metabolites.** *Nature* 2018, **557**:724–728.
105. Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca MA: **Multiple sclerosis.** *Nat Rev Dis Prim* 2018, **4**:43.
106. Pasciuto E, Burton OT, Roca CP, Lagou V, Rajan WD, Theys T, Mancuso R, Tito RY, Kouser L, Callaerts-Vegh Z, *et al.*: **Microglia require CD4 T cells to complete the fetal-to-adult transition.** *Cell* 2020, **182**:625–640. e624.
107. Medzhitov R: **The spectrum of inflammatory responses.** *Science* 2021, **374**:1070–1075.