HHS Public Access

Author manuscript

Curr Opin Immunol. Author manuscript; available in PMC 2021 February 10.

Published in final edited form as:

Curr Opin Immunol. 2020 February ; 62: 123–130. doi:10.1016/j.coi.2020.01.003.

Innate sensing of mechanical properties of brain tissue by microglia

Pinar Ayata[1], **Anne Schaefer**[1,*]

[1]Nash Family Department of Neuroscience, Center for Glial Biology, Ronald M. Loeb Center for Alzheimer's Disease, Precision Immunology Institute, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY

Abstract

External organic or inorganic objects (foreign bodies) that are inadvertently or purposefully placed in the human or animal tissues can trigger local tissue responses that aim at the elimination and/or segregation of foreign bodies from the tissue. The foreign body response (FBR) may have major implications for neurodegeneration associated with the formation of aberrant protein-based aggregates or plaques. The distinct physical features of the plaques, including high rigidity and varying surface properties, may trigger microglial mechanosensing of the plaque as a foreign body. The microglial FBR may have a dual function by promoting and/or suppressing the plaque driven neurodegeneration. Microglial contact with the plaque may trigger inflammatory activation of microglia and support microglia-driven neuronal damage. Conversely, persistent microglial activation may trigger the formation of a microglia-supported cell barrier that segregates and compacts the plaques thus preventing further plaque-induced damage to healthy neurons.

Keywords

Microglia; Alzheimer's disease; neurodegeneration; mechanosensing; foreign body response; amyloid plaque; plaque-associated microglia; barrier microglia; microglia region-specific heterogeneity

Adaptive nature of tissue-specific macrophage diversity

The macrophage owes its name to their initial discovery by Eli Metchnikoff, who observed the engulfment of a splinter—artificially placed into the star fish tentacle—by large cells that congregated around the foreign object [1]. During the following century, our knowledge of the macrophages' oeuvre of functions expanded significantly and well beyond their role as "phagocytes" [2]. The myeloid lineage is represented by circulating monocytes and tissue-

*Corresponding author.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

resident macrophages that fight infectious agents both by using potent innate response mechanisms and by facilitating adaptive immune responses via antigen presentation and cytokine production that recruit other immune cells [2]. Tissue-resident macrophages can also operate as accessory cells supporting highly specialized parenchymal cells, such as hepatocytes, cardiomyocytes, and neurons [2]. This supportive function relies on the ability of macrophages to sense chemically distinct cues derived from neighboring cells and to respond to them in a variety of ways that can range from cell killing to the production of ligands supporting cell survival and specialized functions. Macrophages display a tissue-specific heterogeneity that is exemplified by their polymorphic morphology, and their distinct patterns of cell surface receptor proteins and gene expression [2]. This tissue specific heterogeneity implies either a developmentally predetermined ability of macrophages to operate within a specific tissue setting and/or a remarkably stable macrophage adaptation to a particular environment. The latter mechanism is supported by findings that show the phenotypic plasticity of macrophages when transplanted into a “new” tissue environment [3-5].

The mechanism of macrophage diversity is particularly puzzling in the brain, where resident macrophages—termed microglia by Rio del Hortega [6]—may adjust not only to specific cell types, i.e. neurons or oligodendrocytes, but also to functionally distinct neurons in different brain areas (Fig 1). The progenitor cells of microglia are produced in the yolk sac and arrive in the brain alongside the developing blood vessels during early embryonic development [7-9]. The microglia population subsequently expands during brain development and maintains itself as a self-renewing population throughout life [10,11]. As the brain develops and many of the newly generated neurons that fail to establish connection to other neurons die, microglia scavenge dying cells *en mass* [12]. Microglia further contribute to the robustness of neuronal networks by pruning weak/afunctional synapses [13-15]. These clearing functions of microglia are thought to be essential for brain development as ablation of microglia leads to impaired brain formation and premature death [16-18].

Microglia clearing activities generally subside in the adult brain, except in the cerebellum, where neurons die continuously—albeit at a low rate—throughout life (e.g. ~4% of Purkinje cells are lost per month in mice between 4-12 months [19]) (Fig 1). The physiological attrition of the cerebellar neurons is associated with increased phagocytic activity of microglia that is reflected by increased lysosomal load [20], directed motility [21], and the expression of genes encoding proteins associated with lysosomal/clearance functions [20,22,23] (Fig 1). Similar to the cerebellum, microglia in the subventricular zone, hippocampus, and the olfactory bulb—sites of *de novo* neurogenesis and associated neuron death—are maintained in a state of heightened clearance activity [12,24]. Collectively, these observations suggest that clearance phenotype of microglia occurs in response to dying neurons. Indeed, independent studies showed that the phagocytic phenotype of microglia can be induced by mere exposure to apoptotic neurons *in* and *ex vivo* [20,25].

The clearance potential of microglia becomes particularly apparent during ageing and neurodegenerative diseases. The analyses of gene expression changes in isolated microglia and brain tissue of mice and humans affected by Alzheimer’s disease (AD), Parkinson’s

disease, Huntington's disease, amyotrophic lateral sclerosis, frontotemporal dementia or Pick's disease, show up-regulation of genes that are linked to microglia clearance activity and inflammation [25-31].

Cellular and extracellular triggers of microglial activation during neurodegeneration.

Dying neurons, however, are not likely to be the only signal that triggers microglia activation during neurodegeneration. Many neurodegenerative diseases are associated with the emergence of extracellular protein aggregates that share common features with foreign bodies [32]. Indeed, the aberrant protein/lipid-rich plaques in neurodegenerative conditions are densely surrounded by microglia (Fig 2), evoking Metchnikoff's description of macrophages as a scavenger of foreign bodies [1]. Metchnikoff's original experiments as well as numerous follow-up studies [33] show that the encounter of a macrophage with a foreign object will first lead to the migration and polarization of the macrophage toward the object followed by its engulfment [34]. A failed attempt to phagocytose (and/or an object size > 10 μm in diameter) will trigger macrophage fusion, inflammatory activation, and the secretion of bioactive molecules [34-36] to mitigate the extracellular breakdown the object. In cases when the foreign substance cannot be eradicated, the persistent inflammatory activation of macrophages will lead to the recruitment of other immune and stromal cells [36] and the subsequent isolation and neutralization of the foreign material. Microglia responses to plaques are highly reminiscent of the described foreign body response (FBR) (Fig 2) and involve microglial migration and cell polarization toward the plaques [37,38], microglial adhesion to the plaque surface [39], inflammatory activation [40], and the physical insulation and potential compaction of the plaques (Fig. 2).

There are many components of the plaque that may trigger microglia recruitment and activation (Fig 3), similar to those elicited by foreign bodies. The FBR is triggered by recognition of molecular signals, such as pathogen-associated patterns (PAMPs) and damage-associated patterns (DAMPs) [41-45], as well as mechanical cues [46] (Fig. 3). In addition to localized tissue damage caused by plaque aggregation, amyloid plaques contain proteins, carbohydrates, nucleic acids, lipids, and metal ions, many of which have opsonizing and clearance- or inflammation-inducing activities on macrophages [47,48]. These molecules together with a soft lipid-rich halo plaster the rigid core of the plaques, which is formed by densely folded amyloid β (Aβ) sheets [49]. Physical measurements reveal that the level of stiffness of the plaque's core is similar to the stiffness of the bone or extracellular collagen fibers [49-51], which is more than 10^6-fold above the stiffness of the brain, one of the softest tissues in the entire body [52]. Amyloid plaques as well as other pathogenic protein aggregates further possess the capacity to produce different aggregation forms with variable surface structures and roughnesses [53]. Based on these characteristics of the plaques, it is plausible that microglia deem the plaques as *bona fide* foreign objects, not overly dissimilar from the splinter inserted into the tissue of a starfish [1] or any other foreign object placed in the human tissue including the brain [54]. The clustering of microglia around the plaques was originally thought to reflect the process of microglia-mediated phagocytosis of the plaques. However, multiple recent studies assessing microglia-

mediated plaque clearance suggest that microglia, while engulfing oligomeric Aβ [55-57], are rather inefficient/unable to remove fibrillar Aβ deposits [58,59] and conversely, may even contribute to dense-core plaque formation [60-62]. Reminiscent of FBR in other well described scenarios [34-36], the inability of microglia to clear the plaques may trigger the formation of a microglia-supported barrier around the plaques [63,64] (Fig. 2). Microglia barrier formation can lead to the physical isolation/segregation of the plaques and by preventing plaque growth and facilitating its compaction may reduce plaque-induced toxicity to healthy neurons [63-65].

Mechanosensing by microglia

The stark disparity between the physical features of the plaque and the brain tissue suggests that mechanosensing could be a potential integral part of plaque-driven microglia activation. Microglia, similar to other cells, have the ability to sense changes in mechanical forces and can adjust their morphology according to the stiffness of their environment [66]. Microglial mechanosensing may allow microglia to discriminate between cellular densities and the associated tissue stiffness [67] and may contribute to microglia region-specific morphological and functional specification in the different brain regions [68,69] in the healthy and diseased brain (Fig. 1). If given a choice, microglia *in vitro* and *in vivo* display a preference toward stiff materials [66]. This preference, which is called durotaxis, could explain the recruitment of microglial to the amyloid plaques. Moreover, the degree of environmental stiffness is directly correlated with the inflammatory activity of macrophages [70], microglia and astrocytes [71].

While the underlying molecular mechanism for mechanically induced macrophage/microglia activation remains to be determined, various studies have implicated molecules regulating adhesion signaling, cytoskeleton signaling [72], and podosomes [73]. Primary mediators of cellular mechanosensing, however, are mechanically-gated channels that are located in lipid bilayers of cell membranes and that can undergo conformational changes upon shifts in the direction of the mechanical forces applied on the bilayer [46]. Even though the presence of mechanically-activated sensors were first described in 1979 in vertebrate hair cells [74], their identity was not known until the discovery of the Piezo channels in 2010 [75]. Today, multiple families of mechanically-gated ion channels are identified, including two-pore-domain potassium channels TREKs and TRAAK [76], transient receptor potential (TRP) channels [76], and degenerin/epithelial sodium channels [76], in addition to Piezo channels [75]. One of the mechanically-gated channels that has been shown to directly regulate innate immune functions is TRPV4, which can tune inflammatory response of macrophages to the stiffness of their microenvironment [77]. Moreover, two recent studies implicated mechanical sensing through PIEZO1 in both peripheral innate immune functions and oligodendrocyte progenitor cell (OPC) differentiation [78,79]. In one study, the authors use an *in vitro* model to mimic the pressure changes in the lung and show that mechanical forces acting on monocytes drive a calcium influx via PIEZO1 that leads to an HIF1α-mediated inflammatory response [79]. The activation of PIEZO1 dictates the level of systemic immune response both in the context of pathogens and autoinflammation [79]. The second study shows that aging leads to the

stiffening of the OPC microenvironment, which impairs the proliferation and differentiation rates of OPCs through mechanosensing by PIEZO1 [78].

Based on their distinct physical properties, it is plausible that amyloid plaques present a mechanical stimulus to microglia that is sensed through mechanically-gated ion channels or yet to be identified mechanosensing properties of other immunoreceptors [80]. Among the well-studied mechanically-gated ion channels, PIEZO1 in the brain is highest expressed in endothelial cells and microglia [81] and displays increased expression in astrocytes around the plaques in a rat model of AD [82]. Even though the function of PIEZO1 in plaque-associated microglia remains unknown, it is an exciting candidate for microglial mechanosensing of dense-core plaques. Notably, AD patients display a significant dysregulation of the unsaturated fatty acid metabolism [83], which may have a direct impact on lipid membrane composition and fluidity, and subsequently the sensitivity of mechanically-gated channels. Recent data show that lateral membrane tension activates PIEZO1 [84]. These findings suggests that the composition of lipid bilayers—which directly affects membrane fluidity [85]—may play a role in regulating PIEZO1-mediated mechanosensation. Accordingly, a recent study showed that saturated lipids, which decrease membrane fluidity, lead to inhibition of PIEZO1 function, which can be rescued by an increase in unsaturated fatty acids [85]. Therefore, one of the direct effects of lipid imbalance during AD could be alterations in microglial mechanosensing.

The mechanosensing by macrophages involves the production of the peptide ligand EDN1 that mediates the PIEZO1-driven impact on HIF1α stability and supports inflammatory gene expression [79]. Provided that similar mechanisms can operate in the brain, the pharmacological manipulation of the ligand/receptor pair induced by mechanical stress may prevent microglial inflammatory responses and attenuate neuronal degeneration. The next exciting steps will be to investigate the specific extrinsic signals, receptors, and downstream signaling pathways that govern microglial mechanosensing. Elucidating the regulatory mechanisms underlying microglial mechanosensing and its contribution to microglia activation states will expand our understanding of how microglia contribute to neurodegeneration and has the potential to reveal novel therapeutic avenues in the treatment of neurodegenerative diseases.

Acknowledgements:

We would like to thank A. Tarakhovsky for inspiration and discussions and A. Tarakhovsky and A.T. Chan for comments on the manuscript. We apologize to authors whose works we could not cite due to space limitations.

This work was supported by the National Institutes of Health (NIH) R01MH118329 (A.S.), R21MH115353 (A.S.), RF1AG054011 (A.S.), U01AG058635 (A.S.), DA047233 (A.S.), and NARSAD Young Investigator Award #25065 (P.A.).

References:

1. Metchnikoff E: Sur la lutte des cellules de l'organisme contre l'invasion des microbes. Annales de L'Institut Pasteur 1887, 1:321–336.
2. Murray PJ, Wynn TA: Protective and pathogenic functions of macrophage subsets. Nature Reviews Immunology 2011, 11:723.

3. Gosselin D, Link VM, Romanoski Casey E, Fonseca Gregory J, Eichenfield Dawn Z, Spann Nathanael J, Stender Joshua D, Chun Hyun B, Garner H, Geissmann F, et al.: Environment Drives Selection and Function of Enhancers Controlling Tissue-Specific Macrophage Identities. Cell 159:1327–1340.

4. 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. [PubMed: 25480296]

5. Hasselmann J, Coburn MA, England W, Figueroa Velez DX, Kiani Shabestari S, Tu CH, McQuade A, Kolahdouzan M, Echeverria K, Claes C, et al.: Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. Neuron 2019.

6. P RH: Estudios sobre la neuroglia. La microglía y su transformación en células en bastoncito y cuerpos gránulo•adiposos. Trab Lab Invest Biol Univ Madrid 1920, XVIII:37–82.

7. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, et al.: Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. Science 2010, 330:841–845. [PubMed: 20966214]

8. Schulz C, Perdiguero EG, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SEW, Pollard JW, et al.: A Lineage of Myeloid Cells Independent of Myb and Hematopoietic Stem Cells. Science 2012, 336:86–90. [PubMed: 22442384]

9. Tay TL, Savage JC, Hui CW, Bisht K, Tremblay M-È: Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. The Journal of Physiology 2017, 595:1929–1945. [PubMed: 27104646]

10. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FMV: Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 2007, 10:1538–1543. [PubMed: 18026097]

11. Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch U-K, Mack M, Heikenwalder M, Bruck W, Priller J, Prinz M: Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. Nat Neurosci 2007, 10:1544–1553. [PubMed: 18026096]

12. Sato K: Effects of Microglia on Neurogenesis. Glia 2015, 63:1394–1405. [PubMed: 26010551]

13. 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.

14. Hong S, Dissing-Olesen L, Stevens B: New insights on the role of microglia in synaptic pruning in health and disease. Current Opinion in Neurobiology 2016, 36:128–134. [PubMed: 26745839]

15. Gunner G, Cheadle L, Johnson KM, Ayata P, Badimon A, Mondo E, Nagy A, Liu L, Bemiller SM, Kim K-w, et al.: Sensory lesioning induces microglial synapse elimination via ADAM10 and fractalkine signaling. 2019:551697.

16. Erblich B, Zhu L, Etgen AM, Dobrenis K, Pollard JW: Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. PloS one 2011, 6:e26317–e26317. [PubMed: 22046273]

17. Guo L, Bertola DR, Takanohashi A, Saito A, Segawa Y, Yokota T, Ishibashi S, Nishida Y, Yamamoto GL, Franco JFdS, et al.: Bi-allelic CSF1R Mutations Cause Skeletal Dysplasia of Dysosteosclerosis-Pyle Disease Spectrum and Degenerative Encephalopathy with Brain Malformation. The American Journal of Human Genetics 2019, 104:925–935. [PubMed: 30982609]

18. Oosterhof N, Chang IJ, Karimiani EG, Kuil LE, Jensen DM, Daza R, Young E, Astle L, van der Linde HC, Shivaram GM, et al.: Homozygous Mutations in CSF1R Cause a Pediatric-Onset Leukoencephalopathy and Can Result in Congenital Absence of Microglia. The American Journal of Human Genetics 2019, 104:936–947. [PubMed: 30982608]

19. Woodruff-Pak DS, Foy MR, Akopian GG, Lee KH, Zach J, Nguyen KPT, Comalli DM, Kennard JA, Agelan A, Thompson RF: Differential effects and rates of normal aging in cerebellum and hippocampus. Proceedings of the National Academy of Sciences 2010, 107:1624–1629.

20. 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. Nature Neuroscience 2018, 21:1049–1060. [PubMed: 30038282]

21. Stowell RD, Wong EL, Batchelor HN, Mendes MS, Lamantia CE, Whitelaw BS, Majewska AK: Cerebellar microglia are dynamically unique and survey Purkinje neurons in vivo. Developmental Neurobiology 2017:n/a–n/a.

22. Grabert K, Michoel T, Karavolos MH, Clohisey S, Baillie JK, Stevens MP, Freeman TC, Summers KM, McColl BW: Microglial brain region-dependent diversity and selective regional sensitivities to aging. Nat Neurosci 2016, 19:504–516. [PubMed: 26780511]

23 **. Masuda T, Sankowski R, Staszewski O, Böttcher C, Amann L, Sagar, Scheiwe C, Nessler S, Kunz P, van Loo G, et al.: Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 2019, 566:388–392. [PubMed: 30760929] Using single-cell analysis, this study revealed specific time- and brain region-specific subtypes of microglia in the healthy, demyelinating, and degenerating adult mouse brain. It also found corresponding microglia clusters in healthy individuals and those with multiple sclerosis.

24. Fourgeaud L, Través PG, Tufail Y, Leal-Bailey H, Lew ED, Burrola PG, Callaway P, Zagórska A, Rothlin CV, Nimmeijahn A, et al.: TAM receptors regulate multiple features of microglial physiology. Nature 2016, 532:240–244. [PubMed: 27049947]

25 *. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, Beckers L, O'Loughlin E, Xu Y, Fanek Z, et al.: The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. Immunity 2017, 47:566–581.e569. [PubMed: 28930663] Using compliant substrate cultures, this study found that microglia adapt their morphology to the stiffness of the environment. Microglia preferentially migrate towards stiffer substrates, a process called durotaxis, which becomes further amplified upon microglia activation.

26. Mathys H, Adaikkan C, Gao F, Young JZ, Manet E, Hemberg M, De Jager PL, Ransohoff RM, Regev A, Tsai L-H: Temporal Tracking of Microglia Activation in Neurodegeneration at Single-Cell Resolution. Cell Reports 21:366–380. [PubMed: 29020624]

27. Holtman IR, Raj DD, Miller JA, Schaafsma W, Yin Z, Brouwer N, Wes PD, Möller T, Orre M, Kamphuis W, et al.: Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis. Acta Neuropathologica Communications 2015, 3:31. [PubMed: 26001565]

28. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, et al.: A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell 2017, 169:1276–1290.e1217. [PubMed: 28602351]

29. Yang H-M, Yang S, Huang S-S, Tang B-S, Guo J-F: Microglial Activation in the Pathogenesis of Huntington's Disease. Frontiers in aging neuroscience 2017, 9:193–193. [PubMed: 28674491]

30. Joe E-H, Choi D-J, An J, Eun J-H, Jou I, Park S: Astrocytes, Microglia, and Parkinson's Disease. Experimental neurobiology 2018, 27:77–87. [PubMed: 29731673]

31. Haukedal H, Freude K: Implications of Microglia in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. Journal of Molecular Biology 2019, 431:1818–1829. [PubMed: 30763568]

32. Aguzzi A, O'Connor T: Protein aggregation diseases: pathogenicity and therapeutic perspectives. Nature Reviews Drug Discovery 2010, 9:237–248. [PubMed: 20190788]

33. Gordon S: The macrophage: Past, present and future. European Journal of Immunology 2007, 37:S9–S17. [PubMed: 17972350]

34. Sheikh Z, Brooks PJ, Barzilay O, Fine N, Glogauer M: Macrophages, Foreign Body Giant Cells and Their Response to Implantable Biomaterials. Materials (Basel, Switzerland) 2015, 8:5671–5701.

35. Sridharan R, Cameron AR, Kelly DJ, Kearney CJ, O'Brien FJ: Biomaterial based modulation of macrophage polarization: a review and suggested design principles. Materials Today 2015, 18:313–325.

36. Ode Boni BO, Lamboni L, Souho T, Gauthier M, Yang G: Immunomodulation and cellular response to biomaterials: the overriding role of neutrophils in healing. Materials Horizons 2019, 6:1122–1137.

37. Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, Klunk WE, Kohsaka S, Jucker M, Calhoun ME: Dynamics of the Microglial/Amyloid Interaction Indicate a Role in Plaque Maintenance. The Journal of Neuroscience 2008, 28:4283–4292. [PubMed: 18417708]

38. Hefendehl JK, Wegenast-Braun BM, Liebig C, Eicke D, Milford D, Calhoun ME, Kohsaka S, Eichner M, Jucker M: Long-Term *In Vivo* Imaging of β-Amyloid Plaque Appearance and Growth in a Mouse Model of Cerebral β-Amyloidosis. The Journal of Neuroscience 2011, 31:624–629. [PubMed: 21228171]

39. Navarro V, Sanchez-Mejias E, Jimenez S, Muñoz-Castro C, Sanchez-Varo R, Davila JC, Vizuete M, Gutierrez A, Vitorica J: Microglia in Alzheimer's Disease: Activated, Dysfunctional or Degenerative. Frontiers in aging neuroscience 2018, 10:140–140. [PubMed: 29867449]

40. Cuello AC: Early and Late CNS Inflammation in Alzheimer's Disease: Two Extremes of a Continuum? Trends in Pharmacological Sciences 2017, 38:956–966. [PubMed: 28867259]

41. Colonna M: TREMs in the immune system and beyond. Nature Reviews Immunology 2003, 3:445–453.

42. Elward K, Gasque P: "Eat me" and "don't eat me" signals govern the innate immune response and tissue repair in the CNS: emphasis on the critical role of the complement system. Molecular Immunology 2003, 40:85–94. [PubMed: 12914815]

43. Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD: The role of complement in biomaterial-induced inflammation. Molecular Immunology 2007, 44:82–94. [PubMed: 16905192]

44. Li W: Eat-me signals: keys to molecular phagocyte biology and "appetite" control. Journal of cellular physiology 2012, 227:1291–1297. [PubMed: 21520079]

45. Christo SN, Diener KR, Bachhuka A, Vasilev K, Hayball JD: Innate Immunity and Biomaterials at the Nexus: Friends or Foes. BioMed Research International 2015, 2015:23.

46. Lim C-G, Jang J, Kim C: Cellular machinery for sensing mechanical force. BMB reports 2018, 51:623–629. [PubMed: 30293551]

47. Drummond E, Nayak S, Faustin A, Pires G, Hickman RA, Askenazi M, Cohen M, Haldiman T, Kim C, Han X, et al.: Proteomic differences in amyloid plaques in rapidly progressive and sporadic Alzheimer's disease. Acta neuropathologica 2017, 133:933–954. [PubMed: 28258398]

48. Stewart KL, Radford SE: Amyloid plaques beyond Aβ: a survey of the diverse modulators of amyloid aggregation. Biophysical reviews 2017, 9:405–419. [PubMed: 28631243]

49. Mattana S, Caponi S, Tamagnini F, Fioretto D, Palombo F: Viscoelasticity of amyloid plaques in transgenic mouse brain studied by Brillouin microspectroscopy and correlative Raman analysis. Journal of innovative optical health sciences 2017, 10:1742001. [PubMed: 29151920]

50. Tjhia CK, Stover SM, Rao DS, Odvina CV, Fyhrie DP: Relating micromechanical properties and mineral densities in severely suppressed bone turnover patients, osteoporotic patients, and normal subjects. Bone 2012, 51:114–122. [PubMed: 22561877]

51 *. Fitzpatrick AWP, Park ST, Zewail AH: Exceptional rigidity and biomechanics of amyloid revealed by 4D electron microscopy. Proceedings of the National Academy of Sciences of the United States of America 2013, 110:10976–10981. [PubMed: 23784773] Using compliant substrate cultures, this study found that microglia adapt their morphology to the stiffness of the environment. Microglia preferentially migrate towards stiffer substrates, a process called durotaxis, which becomes further amplified upon microglia activation.

52. Budday S, Nay R, de Rooij R, Steinmann P, Wyrobek T, Ovaert TC, Kuhl E: Mechanical properties of gray and white matter brain tissue by indentation. Journal of the mechanical behavior of biomedical materials 2015, 46:318–330. [PubMed: 25819199]

53. Di Carlo M: Beta amyloid peptide: from different aggregation forms to the activation of different biochemical pathways. European Biophysics Journal 2010, 39:877–888. [PubMed: 19305989]

54. Lotti F, Ranieri F, Vadalà G, Zollo L, Di Pino G: Invasive Intraneural Interfaces: Foreign Body Reaction Issues. Frontiers in Neuroscience 2017, 11.

55. Chung H, Brazil MI, Soe TT, Maxfield FR: Uptake, Degradation, and Release of Fibrillar and Soluble Forms of Alzheimer's Amyloid β-Peptide by Microglial Cells. Journal of Biological Chemistry 1999, 274:32301–32308. [PubMed: 10542270]

56. Mandrekar S, Jiang Q, Lee CYD, Koenigsknecht-Talboo J, Holtzman DM, Landreth GE: Microglia mediate the clearance of soluble Abeta through fluid phase macropinocytosis. The Journal of

neuroscience : the official journal of the Society for Neuroscience 2009, 29:4252–4262. [PubMed: 19339619]

57. Lee CYD, Landreth GE: The role of microglia in amyloid clearance from the AD brain. Journal of neural transmission (Vienna, Austria : 1996) 2010, 117:949–960.

58. Wang Y, Cella M, Mallinson K, Ulrich Jason D, Young Katherine L, Robinette Michelle L, Gilfillan S, Krishnan Gokul M, Sudhakar S, Zinselmeyer Bernd H, et al.: TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer's Disease Model. Cell 2015, 160:1061–1071. [PubMed: 25728668]

59 *. Zhao R, Hu W, Tsai J, Li W, Gan W-B: Microglia limit the expansion of β-amyloid plaques in a mouse model of Alzheimer's disease. Molecular Neurodegeneration 2017, 12:47. [PubMed: 28606182] This study provides functional evidence that the neuroprotective barrier function of microglia is dependent on TREM2 signaling. Loss of TREM2 function results in reduced plaque compaction and increased neuritic damage.

60 **. Baik SH, Kang S, Son SM, Mook-Jung I: Microglia contributes to plaque growth by cell death due to uptake of amyloid β in the brain of Alzheimer's disease mouse model. Glia 2016, 64:2274–2290. [PubMed: 27658617] Using longitudinal two-photon imaging, this study reveals the existence of microglia that uptake Aβ and subsequently die, forming the seed for bona fide plaques. NO HIGHLIGHT- remove

61 **. Sosna J, Philipp S, Albay R 3rd, Reyes-Ruiz JM, Baglietto-Vargas D, LaFerla FM, Glabe CG: Early long-term administration of the CSF1R inhibitor PLX3397 ablates microglia and reduces accumulation of intraneuronal amyloid, neuritic plaque deposition and pre-fibrillar oligomers in 5XFAD mouse model of Alzheimer's disease. Molecular neurodegeneration 2018, 13:11–11. [PubMed: 29490706] This is the first study to show that pharmacological ablation of microglia during early diseases stages in the 5XFAD model of AD results in decreased plaque formation.

62 **. Spangenberg E, Severson PL, Hohsfield LA, Crapser J, Zhang J, Burton EA, Zhang Y, Spevak W, Lin J, Phan NY, et al.: Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. Nature Communications 2019, 10:3758.This study shows that microglia are necessary for the seeding of dense-core amyloid plaques in the brain. The pharmacological ablation of microglia in the 5XFAD model leads to a failure in plaque formation and subsequent Aβ deposition in blood vessel. The repopulation of the brain with microglia is associated with the appearance of the plaques.

63 **. Condello C, Yuan P, Schain A, Grutzendler J: Microglia constitute a barrier that prevents neurotoxic protofibrillar Aβ42 hotspots around plaques. Nature communications 2015, 6:6176–6176.This study provides strong evidence for the neuroprotective barrier function of microglia. The encapsulation of plaques by microglia renders them inert, such that they are resistant to plaque expansion and are associated with reduced dystrophyc neurites.

64 **. Yuan P, Condello C, Keene CD, Wang Y, Bird TD, Paul SM, Luo W, Colonna M, Baddeley D, Grutzendler J: TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. Neuron 2016, 90:724–739. [PubMed: 27196974] This study provides functional evidence that the neuroprotective barrier function of microglia is dependent on TREM2 signaling. Loss of TREM2 function results in reduced plaque compaction and increased neuritic damage.

65. Ulland TK, Song WM, Huang SC-C, Ulrich JD, Sergushichev A, Beatty WL, Loboda AA, Zhou Y, Cairns NJ, Kambal A, et al.: TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. Cell 2017, 170:649–663.e613. [PubMed: 28802038]

66 **. Bollmann L, Koser DE, Shahapure R, Gautier HOB, Holzapfel GA, Scarcelli G, Gather MC, Ulbricht E, Franze K: Microglia mechanics: immune activation alters traction forces and durotaxis. Frontiers in cellular neuroscience 2015, 9:363–363. [PubMed: 26441534] Using compliant substrate cultures, this study found that microglia adapt their morphology to the stiffness of the environment. Microglia preferentially migrate towards stiffer substrates, a process called durotaxis, which becomes further amplified upon microglia activation.

67. Shawky JH, Balakrishnan UL, Stuckenholz C, Davidson LA: Multiscale analysis of architecture, cell size and the cell cortex reveals cortical F-actin density and composition are major contributors to mechanical properties during convergent extension. Development 2018, 145:dev161281. [PubMed: 30190279]

68. von Bartheld CS, Bahney J, Herculano-Houzel S: The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. The Journal of comparative neurology 2016, 524:3865–3895. [PubMed: 27187682]

69 *. Keller D, Erö C, Markram H: Cell Densities in the Mouse Brain: A Systematic Review. Frontiers in Neuroanatomy 2018, 12.This study shows that TRPV4 sensing of environmental stiffness in the range seen in inflamed lung synergizes with chemical sensing of inflammatory stimuli and promotes secretion of anti-inflammatory/pro-resolution cytokines and phagocytosis.

70. Patel NR, Bole M, Chen C, Hardin CC, Kho AT, Mih J, Deng L, Butler J, Tschumperlin D, Fredberg JJ, et al.: Cell elasticity determines macrophage function. PloS one 2012, 7:e41024–e41024. [PubMed: 23028423]

71 **. Moshayedi P, Ng G, Kwok JCF, Yeo GSH, Bryant CE, Fawcett JW, Franze K, Guck J: The relationship between glial cell mechanosensitivity and foreign body reactions in the central nervous system. Biomaterials 2014, 35:3919–3925. [PubMed: 24529901] This study elegantly showed that material stiffness influences microglia and astrocyte morphology and inflammatory gene expression. Increasing material stiffness enhances the tone of foreign body response in the brain involving both microglia and astrocytes.

72. Ohashi K, Fujiwara S, Mizuno K: Roles of the cytoskeleton, cell adhesion and rho signalling in mechanosensing and mechanotransduction. The Journal of Biochemistry 2017, 161:245–254. [PubMed: 28082721]

73. Jain N, Moeller J, Vogel V: Mechanobiology of Macrophages: How Physical Factors Coregulate Macrophage Plasticity and Phagocytosis. Annual Review of Biomedical Engineering 2019, 21:267–297.

74. Corey DP, Hudspeth AJ: Response latency of vertebrate hair cells. Biophysical Journal 1979, 26:499–506. [PubMed: 318064]

75. Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A: Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels. Science 2010, 330:55–60. [PubMed: 20813920]

76. Ranade SS, Syeda R, Patapoutian A: Mechanically Activated Ion Channels. Neuron 2015, 87:1162–1179. [PubMed: 26402601]

77 **. Scheraga RG, Abraham S, Niese KA, Southern BD, Grove LM, Hite RD, McDonald C, Hamilton TA, Olman MA: TRPV4 Mechanosensitive Ion Channel Regulates Lipopolysaccharide-Stimulated Macrophage Phagocytosis. Journal of immunology (Baltimore, Md. : 1950) 2016, 196:428–436.This study shows that TRPV4 sensing of environmental stiffness in the range seen in inflamed lung synergizes with chemical sensing of inflammatory stimuli and promotes secretion of anti-inflammatory/pro-resolution cytokines and phagocytosis.

78 * . Segel M, Neumann B, Hill MFE, Weber IP, Viscomi C, Zhao C, Young A, Agley CC, Thompson AJ, Gonzalez GA, et al.: Niche stiffness underlies the ageing of central nervous system progenitor cells. Nature 2019, 573:130–134. [PubMed: 31413369] This study shows that the microenvironment stiffening of oligodendrocyte progenitor cells (OPCs) impairs the function of OPCs, a phenomenon associated with aging.

79 *. Solis AG, Bielecki P, Steach HR, Sharma L, Harman CCD, Yun S, de Zoete MR, Warnock JN, To SDF, York AG, et al.: Mechanosensation of cyclical force by PIEZO1 is essential for innate immunity. Nature 2019, 573:69–74. [PubMed: 31435009] This study showed that mechanosensing by myeloid cells in the lung occurs via the ion channel PIEZO1 and dictates the level of systemic immune response in the context of pathogens or auto-inflammation.

80. Zhu C, Chen W, Lou J, Rittase W, Li K: Mechanosensing through immunoreceptors. Nature Immunology 2019, 20:1269–1278. [PubMed: 31534240]

81. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, et al.: An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex. JNeurosci 2014, 34.

82 **. Velasco-Estevez M, Mampay M, Boutin H, Chaney A, Warn P, Sharp A, Burgess E, Moeendarbary E, Dev KK, Sheridan GK: Infection Augments Expression of Mechanosensing Piezo1 Channels in Amyloid Plaque-Reactive Astrocytes. Frontiers in aging neuroscience 2018, 10:332–332. [PubMed: 30405400] This study found that PIEZO1 expression is induced in the astrocytes near the amyloid plaques upon aging and inflammation.

83 * . Snowden SG, Ebshiana AA, Hye A, An Y, Pletnikova O, O'Brien R, Troncoso J, Legido-Quigley C, Thambisetty M: Association between fatty acid metabolism in the brain and Alzheimer disease neuropathology and cognitive performance: A nontargeted metabolomic study. PLoS medicine 2017, 14:e1002266–e1002266. [PubMed: 28323825] This study shows that lipid composition influences membrane fluidity. The increase of saturated fatty acids leads to membrane stiffening and PIEZO1 de-sensitization, which is rescued by the increase of unsaturated fatty acids that increase membrane fluidity.

84. Lin Y-C, Guo YR, Miyagi A, Levring J, MacKinnon R, Scheuring S: Force-induced conformational changes in PIEZO1. Nature 2019, 573:230–234. [PubMed: 31435018]

85 **. Romero LO, Massey AE, Mata-Daboin AD, Sierra-Valdez FJ, Chauhan SC, Cordero-Morales JF, Vásquez V: Dietary fatty acids fine-tune Piezo1 mechanical response. Nature communications 2019, 10:1200–1200.This study shows that lipid composition influences membrane fluidity. The increase of saturated fatty acids leads to membrane stiffening and PIEZO1 de-sensitization, which is rescued by the increase of unsaturated fatty acids that increase membrane fluidity.

86. Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, et al.: Intraneuronal β-Amyloid Aggregates, Neurodegeneration, and Neuron Loss in Transgenic Mice with Five Familial Alzheimer's Disease Mutations: Potential Factors in Amyloid Plaque Formation. The Journal of Neuroscience 2006, 26:10129–10140. [PubMed: 17021169]

Highlights:

- Microglia display diverse and brain region-specific phenotypes.
- Microglial response to protein aggregates/plaques during Alzheimer disease resembles macrophage response to foreign bodies.
- Microglial mechanosensing of the physical properties of a plaque as a potential driver of microglial activation.

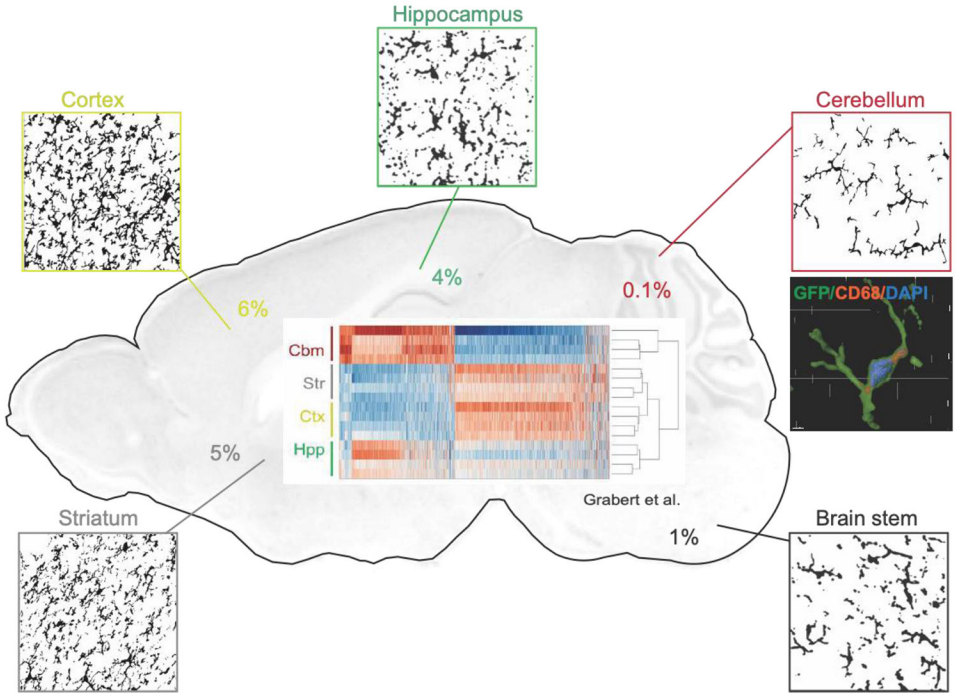

Figure 1: Brain region specific microglia specification.

Microglia display region specific differences in morphology, density, and gene expression patterns. Schematic shows the mouse brain with digitized immunofluorescence images of IBA1+ microglia from the cortex (yellow), striatum (gray), hippocampus (green), cerebellum (red), and brain stem (black), percentages indicate the number of microglia as compared to total cell number per corresponding brain region [68,69]. A representative image of an individual cerebellar IBA1+ microglia (green) containing two large lysosomal structures (CD68: red) is shown (Imaris, DAPI: blue) [20]. Heat map shows the differential gene expression pattern of microglia by brain regions [22].

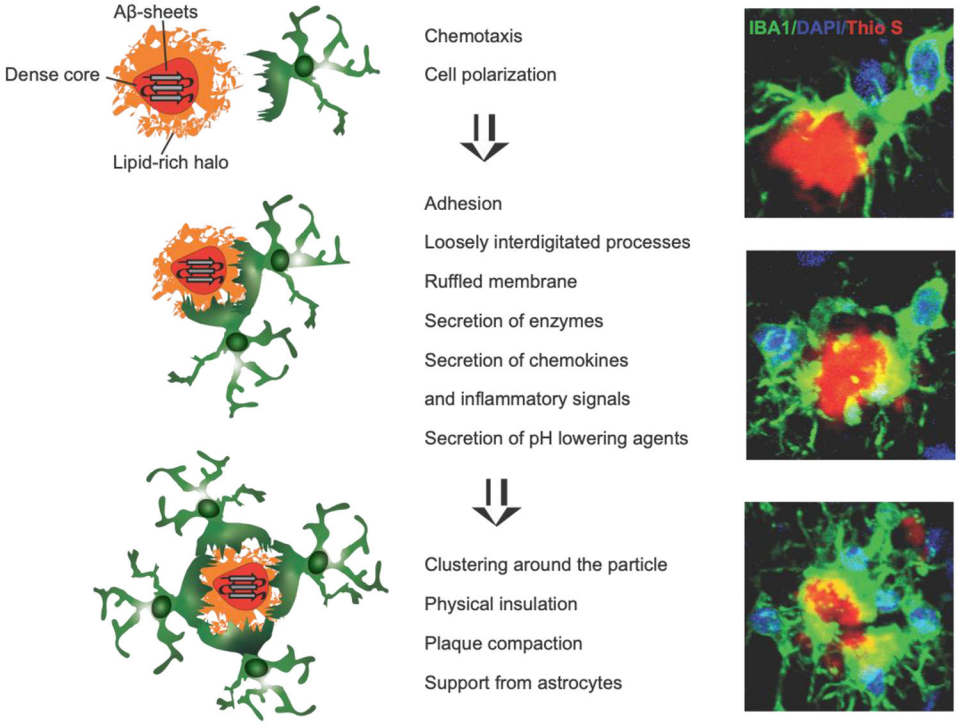

Figure 2: Microglial response to amyloid plaques resembles macrophage response to foreign bodies.

(left) Schematic shows microglia (green) responses to amyloid plaques (orange/red), amyloid plaques are characterized by a soft lipid-rich halo (orange) surrounding the rigid core of the plaques (red), which is formed by densely folded amyloid β (Aβ) sheets [49]. (right) Representative images of IBA1+ microglia (IBA1: green) interactions with amyloid plaques (Thioflavin S (Thio S): red) in the *5xfAD* mouse model [86] of Alzheimer disease are shown (DAPI+ nuclei: blue). Similar to macrophage FBR, microglia polarize and migrate toward the plaque [37], display ruffling of cell membranes and adhesion to the plaque surface, release enzymes for extracellular degradation [57], produce an inflammatory response [57], and have their processes loosely interdigitated [34-36]. The inability of microglia to clear the plaque may trigger the formation of a microglia-supported barrier leading to the physical isolation/segregation of the plaques [63,64]. Microglia barrier formation has been suggested to prevent plaque growth, facilitate its compaction, and reduce plaque-induced toxicity to healthy neurons [63-65].

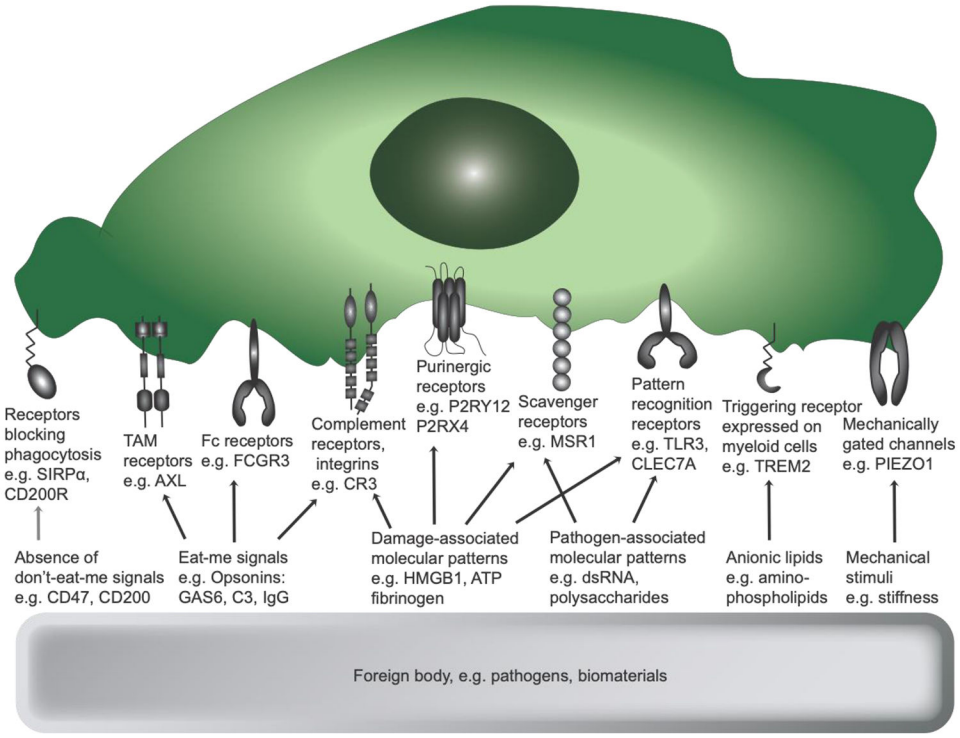

Figure 3: Cellular and extracellular triggers of microglial activation during neurodegeneration.

Schematic displays the variety of molecular signals that can be involved in mediating FBR including activation of pathogen-associated pattern recognition receptors, such as toll-like receptors and scavenger receptors, to initiate inflammatory or phagocytic response [44,45]. The same receptors can also recognize damage-associated molecular patterns, that are released or exposed upon host danger, such as HMGB1 (released upon necrotic cell death) and fibrinogen (exposed upon disruption of extracellular matrix [44,45]. Another potent signal representing cellular damage is ATP, which is recognized by purinergic receptors expressed on microglia [45]. Identification of foreign particles can be mediated by the presence of "eat-me" or the lack of "don't-eat-me signals". These are pairs or macrophage-expressed receptors that bind their cognate ligands [42]. "Don't-eat-me" signals can be mediated e.g. by the expression of CD47 on functional neurons and synapses, which by binding to SIRPα on myeloid cells triggers inhibition of (unwanted) phagocytosis. Conversely, opsonization of pathogen and foreign bodies by the complement system [43] can mediate strong "eat-me" signals via the activation of Fc receptors, such as FcγR3. A more recently characterized family of receptors are called triggering receptor expressed on myeloid cells (TREMs) which can recognize anionic lipids and ApoE found on plaques [41]. Given the chemically complex composition of amyloid plaques that can contain a variety of lipids, nucleic acids, carbohydrates, and misfolded proteins [47,48], it is likely that many of these receptors are simultaneously activated to drive a microglia response. In addition to chemical cues, mechanical cues, such as stiffness and surface roughness, are also potent triggers of FBR [46] and can directly affect the level of inflammatory response elicited [36]. Mechanical cues can be detected among others by specific mechanosensors expressed on microglia [46,76].