Made available by Hasselt University Library in https://documentserver.uhasselt.be

Microglia states and nomenclature: A field at its crossroads

Non Peer-reviewed author version

Paolicelli, Rosa C.; Sierra, Amanda; Stevens, Beth; Tremblay, Marie-Eve; Aguzzi, Adriano; Ajami, Bahareh; Amit, Ido; Audinat, Etienne; Bechmann, Ingo; Bennett, Mariko; Bennett, Frederick; Bessis, Alain; Biber, Knut; Bilbo, Staci; Blurton-Jones, Mathew; Boddeke, Erik; Brites, Dora; BRONE, Bert; Brown, Guy C.; Butovsky, Oleg; Carson, Monica J.; Castellano, Bernardo; Colonna, Marco; Cowley, Sally A.; Cunningham, Colm; Davalos, Dimitrios; De Jager, Philip L.; de Strooper, Bart; Denes, Adam; Eggen, Bart J. L.; Eyo, Ukpong; Galea, Elena; Garel, Sonia; Ginhoux, Florent; Glass, Christopher K.; Gokce, Ozgun; Gomez-Nicola, Diego; Gonzalez, Berta; Gordon, Siamon; Graeber, Manuel B.; Greenhalgh, Andrew D.; Gressens, Pierre; Greter, Melanie; Gutmann, David H.; Haass, Christian; Heneka, Michael T.; Heppner, Frank L.; Hong, Soyon; Hume, David A.; Jung, Steffen; Kettenmann, Helmut; Kipnis, Jonathan; Koyama, Ryuta; Lemke, Greg; Lynch, Marina; Majewska, Ania; Malcangio, Marzia; Malm, Tarja; Mancuso, Renzo; Masuda, Takahiro; Matteoli, Michela; McColl, Barry W.; Miron, Veronique E.; Molofsky, Anna Victoria; Monje, Michelle; Mracsko, Eva; Nadjar, Agnes; Neher, Jonas J.; Neniskyte, Urte; Neumann, Harald; Noda, Mami; Peng, Bo; Peri, Francesca; Perry, V. Hugh; Popovich, Phillip G.; Pridans, Clare; Priller, Josef; Prinz, Marco; Ragozzino, Davide; Ransohoff, Richard M.; Salter, Michael W.; Schaefer, Anne; Schafer, Dorothy P.; Schwartz, Michal; Simons, Mikael; Smith, Cody J.; Streit, Wolfgang J.; Tuan Leng Tay; Tsai, Li-Huei; Verkhratsky, Alexei; von Bernhardi, Rommy; Wake, Hiroaki; Wittamer, Valerie; Wolf, Susanne A.; Wu, Long-Jun & Wyss-Coray, Tony (2022) Microglia states and nomenclature: A field at its crossroads. In: NEURON, 110 (21) , p. 3458 -3483.

DOI: doi.org/10.1016/j.neuron.2022.10.020
Handle: http://hdl.handle.net/1942/39540

Microglia states and nomenclature: a field at its crossroads

Rosa C. Paolicelli[1,*], Amanda Sierra[2-4,*], Beth Stevens[5-7,*], Marie-Eve Tremblay[8-12,*], Adriano Aguzzi[13], Bahareh Ajami[14], Ido Amit[15], Etienne Audinat[16], Ingo Bechmann[17], Mariko Bennett[18], Frederick Bennett[19], Alain Bessis[20], Knut Biber[21], Staci Bilbo[22], Mathew Blurton-Jones[23], Erik Boddeke[24], Dora Brites[25], Bert Brône[26], Guy C. Brown[27], Oleg Butovsky[28], Monica J. Carson[29], Bernardo Castellano[30,31], Marco Colonna[32], Sally A. Cowley[33], Colm Cunningham[34,35], Dimitrios Davalos[36,37], Philip L. De Jager[38,39], Bart de Strooper[40,41], Adam Denes[42], Bart J.L. Eggen[43,44], Ukpong Eyo[45], Elena Galea[46,47], Sonia Garel[48,49], Florent Ginhoux[50], Christopher K. Glass[51], Ozgun Gokce[52], Diego Gomez-Nicola[53], Berta González[54], Siamon Gordon[55], Manuel B. Graeber[56], Andrew D. Greenhalgh[57], Pierre Gressens[58], Melanie Greter[59], David H. Gutmann[60], Christian Haass[61-63], Michael T. Heneka[64], Frank L. Heppner[65], Soyon Hong[66], David Hume[67], Steffen Jung[68], Helmut Kettenmann[69,70], Jonathan Kipnis[71], Ryuta Koyama[72], Greg Lemke[73], Marina Lynch[74], Ania Majewska[75], Marzia Malcangio[76], Tarja Malm[77], Renzo Mancuso[78,79], Takahiro Masuda[80], Michela Matteoli[81], Barry W. McColl[82], Veronique E. Miron[83,84], Anna Victoria Molofsky[85], Michelle Monje[6,86], Eva Mracsko[87], Agnes Nadjar[88,89], Jonas J. Neher[90,91], Urte Neniskyte[92,93], Harald Neumann[94], Mami Noda[95,96], Bo Peng[97], Francesca Peri[98], V. Hugh Perry[99,100], Phillip G. Popovich[101], Clare Pridans[102], Josef Priller[103-105], Marco Prinz[106-108], Davide Ragozzino[109,110], Richard M. Ransohoff[111], Michael W. Salter[112,113], Anne Schaefer[114,115], Dorothy P. Schafer[116], Michal Schwartz[117], Mikael Simons[118], Cody J. Smith[119], Wolfgang J. Streit[120], Tuan Leng Tay[121-125], Li-Huei Tsai[5,126,127], Alexei Verkhratsky[2,3,128], Rommy von Bernhardi[129], Hiroaki Wake[130], Valerie Wittamer[131,132], Susanne A. Wolf[133], Long-Jun Wu[134], Tony Wyss-Coray[86].

All authors are listed in alphabetical order

1. Department of Biomedical Sciences, Faculty of Biology and Medicine, University of Lausanne, Switzerland
2. Achucarro Basque Center for Neuroscience, Glial Cell Biology Lab, Leioa, Spain
3. Department of Neuroscience, University of the Basque Country EHU/UPV, Leioa, Spain
4. Ikerbasque Foundation, Bilbao, Spain
5. Broad Institute of MIT and Harvard, Cambridge, USA
6. Howard Hughes Medical Institute (HHMI), USA
7. Boston Children's Hospital, Boston, USA
8. Centre de recherche du CHU de Québec-Université Laval, Québec City, Canada
9. Department of Neurology and Neurosurgery, McGill University, Montréal, Canada
10. Division of Medical Sciences, University of Victoria, Victoria, Canada

11. Center for Advanced Materials and Related Technology (CAMTEC), University of Victoria, Victoria, Canada
12. Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada
13. Institute of Neuropathology, University of Zurich, Zurich, Switzerland
14. Department of Molecular Microbiology & Immunology, Department of Behavioral and Systems Neuroscience, Oregon Health & Science University School of Medicine, Portland, USA
15. Department of Systems Immunology, Weizmann Institute of Science, Rehovot, Israel
16. Institut de Génomique Fonctionnelle, Université de Montpellier, CNRS, INSERM, Montpellier, France
17. Institute of Anatomy, University of Leipzig, Leipzig, Germany
18. Children's Hospital of Philadelphia, Department of Psychiatry, Department of Pediatrics, Division of Child Neurology, Philadelphia, USA
19. Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, USA
20. École Normale Supérieure, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Paris Sciences et Lettres Research University, Paris, France
21. Neuroscience Discovery, AbbVie Deutschland GmbH, Ludwigshafen, Germany
22. Departments of Psychology & Neuroscience, Neurobiology, and Cell Biology, Duke University, Durham, USA
23. Center for the Neurobiology of Learning and Memory, UCI MIND, University of California, Irvine, USA
24. Department Biomedical Sciences of Cells & Systems, Section Molecular Neurobiology, University of Groningen, University Medical Center, Groningen, The Netherlands
25. Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal
26. BIOMED research institute, University of Hasselt, Hasselt, Belgium
27. Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom
28. Ann Romney Center for Neurologic Diseases, Dept Neurology, Brigham and Women´s Hospital, Harvard Medical School, Boston, USA
29. Center for Glial-Neuronal Interactions, Division of Biomedical Sciences, University of California Riverside School of Medicine, Riverside, USA
30. Unidad de Histología Medica, Depto. Biología Celular, Fisiología e Inmunología, Barcelona, Spain
31. Instituto de Neurociencias, Universidad Autónoma de Barcelona, Barcelona, Spain

32. Department of Pathology and Immunology, Washington University School of Medicine in St. Louis, St. Louis, USA
33. James and Lillian Martin Centre for Stem Cell Research, Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom
34. School of Biochemistry & Immunology, Trinity Biomedical Sciences Institute, Trinity College, Dublin, Republic of Ireland
35. Trinity College Institute of Neuroscience, Trinity College, Dublin, Republic of Ireland
36. Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, Cleveland, USA
37. Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, USA
38. Center for Translational & Computational Neuroimmunology, Department of Neurology, Columbia University Irving Medical Center, New York, USA
39. Taub Institute for Research on Alzheimer's disease and the Aging Brain, Columbia University Irving Medical Center, New York, USA
40. UK Dementia Research Institute at University College London, London, United Kingdom
41. Vlaams Instituut voor Biotechnologie at Katholieke Universiteit Leuven, Leuven, Belgium
42. "Momentum" Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary
43. Department of Biomedical Sciences of Cells & Systems, section Molecular Neurobiology, University of Groningen, Groningen, The Netherlands
44. University Medical Center Groningen, Groningen, The Netherlands
45. Department of Neuroscience, Center for Brain Immunology and Glia, University of Virginia School of Medicine, Charlottesville, USA
46. Institut de Neurociències and Departament de Bioquímica, Unitat de Bioquímica, Universitat Autònoma de Barcelona, Barcelona, Spain
47. ICREA, Barcelona, Spain
48. Institut de Biologie de l'ENS (IBENS), Département de biologie, École normale supérieure, CNRS, INSERM, Paris, France
49. College de France, Paris, France
50. Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore
51. University of California San Diego School of Medicine, La Jolla, USA
52. Institute for Stroke and Dementia Research, Ludwig Maximillian's University of Munich, Munich, Germany
53. School of Biological Sciences, University of Southampton, Southampton General Hospital, Southampton, United Kingdom

54. Unidad de Histología Medica, Depto. Biología Celular, Fisiología e Inmunología and Instituto de Neurociencias, Universidad Autónoma de Barcelona, Barcelona, Spain
55. Sir William Dunn School of Pathology, Oxford, United Kingdom
56. Ken Parker Brain Tumour Research Laboratories, Brain and Mind Centre, Faculty of Medicine and Health, The University of Sydney, Camperdown, Australia
57. Lydia Becker Institute of Immunology and Inflammation, Geoffrey Jefferson Brain Research Centre, Division of Infection, Immunity & Respiratory Medicine, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom
58. Université Paris Cité, Inserm, NeuroDiderot, F-75019 Paris, France
59. Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland
60. Department of Neurology, Washington University School of Medicine, St. Louis MO USA
61. Division of Metabolic Biochemistry, Faculty of Medicine, Biomedical Center (BMC), Ludwig-Maximilians-Universität Munchen, Munich, Germany
62. German Center for Neurodegenerative Diseases (DZNE), Munich, Germany
63. Munich Cluster for Systems Neurology (SyNergy); Munich, Germany
64. Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belvaux, Luxembourg
65. Department of Neuropathology, Charité - Universitätsmedizin Berlin, Berlin, Germany
66. UK Dementia Research Institute at University College London, London, United Kingdom
67. Mater Research Institute-University of Queensland, Brisbane, Australia
68. Department of Immunology and Regenerative Biology, Weizmann Institute of Science, Rehovot, Israel
69. Max-Delbrück Center for Molecular Medicine, Berlin, Germany
70. Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China
71. Center for Brain Immunology and Glia (BIG), Department of Pathology and Immunology, Washington University in St. Louis, St. Louis, MO, USA
72. Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan
73. MNL-L, The Salk Institute for Biological Studies, La Jolla, USA
74. Trinity College Institute of Neuroscience, Trinity College, Dublin, Republic of Ireland
75. Department of Neuroscience, University of Rochester, Rochester, New York, USA
76. Wolfson Centre for Age-Related Diseases, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom
77. University of Eastern Finland, Kuopio, Finland
78. Microglia and Inflammation in Neurological Disorders (MIND) Lab, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium

79. Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
80. Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, Japan
81. Humanitas University, Department of Biomedical Sciences, Milan, Italy
82. UK Dementia Research Institute, Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh BioQuarter, Edinburgh, United Kingdom
83. MRC Centre for Reproductive Health, The Queen's Medical Research Institute, Edinburgh BioQuarter, Edinburgh, United Kingdom
84. UK Dementia Research Institute at the University of Edinburgh, Edinburgh BioQuarter, Edinburgh, United Kingdom
85. University of California, San Francisco, USA
86. Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford University, Stanford, USA
87. Roche Innovation Center, Basel, Switzerland
88. Neurocentre Magendie, University of Bordeaux, Bordeaux, France
89. Institut Universitaire de France (IUF), France
90. German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
91. Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
92. VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center, Vilnius University, Vilnius, Lithuania
93. Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
94. Institute of Reconstructive Neurobiology, Medical Faculty and University Hospital of Bonn, University of Bonn, Bonn, Germany
95. Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan
96. Institute of Mitochondrial Biology and Medicine of Xi'an Jiaotong University School of Life Science and Technology, Xi'an, China
97. Department of Neurosurgery, Huashan Hospital, Institute for Translational Brain Research, State Key Laboratory of Medical Neurobiology, MOE Frontiers Center for Brain Science, Fudan University, Shanghai, China
98. Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland
99. UK Dementia Research Institute, University College London, London, United Kingdom
100. School of Biological Sciences, University of Southampton, Southampton, United Kingdom
101 Department of Neuroscience, College of Medicine, The Ohio State University, Columbus, USA

102. University of Edinburgh, Centre for Inflammation Research, Edinburgh, United Kingdom
103. Department of Psychiatry & Psychotherapy, School of Medicine, Technical University of Munich, Munich, Germany
104. Charité - Universitätsmedizin Berlin and DZNE, Berlin, Germany
105. University of Edinburgh and UK DRI, Edinburgh, United Kingdom
106. Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg, Germany
107. Center for Basics in NeuroModulation (NeuroModulBasics), Faculty of Medicine, University of Freiburg, Freiburg, Germany
108. Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Freiburg, Germany
109. Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy
110. Santa Lucia Foundation (IRCCS Fondazione Santa Lucia), Rome, Italy
111. Third Rock Ventures, Boston, USA
112. Hospital for Sick Children, Toronto, Canada
113. University of Toronto, Toronto, Canada
114. Nash Family Department of Neuroscience, Center for Glial Biology, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, USA
115. Max Planck Institute for Biology of Ageing, Koeln, Germany
116. Department of Neurobiology, Brudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, Worcester, USA
117. Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel
118. Institute of Neuronal Cell Biology, Technical University Munich, German Center for Neurodegenerative Diseases, Munich, Germany
119. Galvin Life Science Center, University of Notre Dame, Indianapolis, USA
120. Department of Neuroscience, University of Florida, Gainesville, USA
121. Faculty of Biology, University of Freiburg, Freiburg, Germany
122. BrainLinks-BrainTools Centre, University of Freiburg, Freiburg, Germany
123. Freiburg Institute of Advanced Studies, University of Freiburg, Freiburg, Germany
124. Department of Biology, Boston University, Boston, USA
125. Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, USA
126. Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA
127. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA

128. Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom
129. Faculty of Medicine and Science, Universidad San Sebastian, Santiago, Chile
130. Department of Anatomy and Molecular Cell Biology, Graduate School of Medicine, Nagoya University, Nagoya, Japan
131. Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles (ULB), Brussels, Belgium
132. ULB Institute of Neuroscience (UNI), Université Libre de Bruxelles (ULB), Brussels, Belgium
133. Charité Universitätsmedizin, Experimental Ophthalmology and Neuroimmunology, Berlin, Germany
134. Department of Neurology and Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

*Co-corresponding authors:
rosachiara.paolicelli@unil.ch (R.C.P.)
amanda.sierra@ehu.eus (A.S.)
beth.stevens@childrens.harvard.edu (B.S.)
evetremblay@uvic.ca (M.E.T.)

Abstract Word limit: 150

Microglial research has advanced considerably in recent decades yet has been constrained by a rolling series of dichotomies such as “resting *versus* activated” and “M1 *versus* M2”. This dualistic classification of good or bad microglia is inconsistent with the wide repertoire of microglial states and functions in development, plasticity, aging and diseases that were elucidated in recent years. New designations continuously arising in an attempt to describe the different microglial states, notably defined using transcriptomics and proteomics, may easily lead to a misleading, although unintentional, coupling of categories and functions. To address these issues, we assembled a group of multidisciplinary experts to discuss our current understanding of microglial states as a dynamic concept and the importance of addressing microglial function. Here, we provide a conceptual framework and recommendations on the use of microglial nomenclature for researchers, reviewers, and editors, which will serve as the foundations for a future white paper.

Abbreviations

AD – Alzheimer’s disease
ARM – activated response microglia
ATM – axon tract-associated microglia
BAM – border-associated macrophage
BBB – Blood-brain barrier
CAM – CNS-associated macrophages
CNS – central nervous system
CSF – cerebrospinal fluid
CSF1R – colony stimulating factor 1 receptor
DAM – disease-associated microglia
HAM – human AD microglia
iPSC – induced pluripotent stem cells
IRM – interferon-responsive microglia
ISF – interstitial fluid
LDAM – lipid-droplet-accumulating microglia in aging mice and humans
MGnD – microglial neurodegenerative phenotype
MIMS – microglia inflamed in multiple sclerosis
MS – multiple sclerosis
PAM – proliferative-region-associated microglia
ROS – reactive oxygen species
scRNASeq – single-cell RNA sequencing
WAM – white matter-associated microglia

Names, names, names

"If the names are unknown knowledge of the things also perishes."[1]
(Carolus Linnaeus)

And yet, we humans instinctively tend to name things and use that name to define their properties. Biologists are no exception: from the time of 18th century father of taxonomy Carolus Linnaeus, the main purpose of biology has been categorizing the natural world as a way of understanding it. Naming species and grouping them together into taxa served to define evolutionary relationships; even today taxonomy and phylogeny are closely interrelated. But we must never forget that nomenclatures and categories are artificial constructs and biology is seldom black and white, but rather an extended continuum of greys. While giving names is natural and useful, we need to be aware that categorization constrains our thinking by forcing us to fit our observations into established classes. As sociologists say, "categorization spawns expectations"[2]. This semantic issue has already been acknowledged by immunologists because, in fact, the given names have connotations that often imply a specific function[3]. In this paper, we extend similar initiatives on macrophages[4], dendritic cells[3], interneurons[5], and astrocytes[6] to discuss the widespread problems associated with categorization of microglia using outdated terms such as "resting *versus* activated" (**Box 1**) or "M1 *versus* M2" (**Box 2**).

Dichotomic, rigid categories convey a dualistic idea of good *versus* bad microglia and may actually impede scientific advancement. Widely used terms, such as "neuroinflammation" as a synonym of microglial reactivity (**Box 3**) and naming a panoply of presumed microglial populations and assumed functions arising from single-cell transcriptomics, are misleading and increasingly problematic, especially to those entering the field of glial biology and neuroimmunology. This nomenclature does not address the important question: what are the specific functions of microglia in the contexts of development, health, aging, and disease? It is now clear that microglia exist in diverse, dynamic, and multi-dimensional states depending on the context including local environment (**Figure 1**). We define dimensions as the key variables driving the phenotypic transformations of microglia. These variables are molecularly distinct signaling pathways regulated at multiple levels (e.g., transcriptional, epigenetic, translational, metabolic) that each give rise to distinct microglial functions or properties. In this manner, categorizing microglia based on a historical, one-dimensional nomenclature in the absence of functional data will constrain and stifle future progress and innovation.

To examine and address these issues, we assembled a team of international experts who have made major contributions to microglia research, inclusive of various groups, and

balancing gender, geographical distribution, and seniority. Authors from the fields of neuroscience, neurobiology, immunology, neuroimmunology, oncology, and neuropathology, both from academia and industry, discussed their perspectives on the current and future challenges in defining microglial states and nomenclature. A questionnaire (**Supplementary Data**) was created to collect all the authors' opinions on several nomenclature issues and the importance of directly addressing microglial function. The responses to the questionnaire, an online meeting held in June 2021 and an open session held at the EMBO meeting Microglia 2021 were used as a backbone to develop this paper.

Herein, we summarize our current knowledge about the identity of microglia and discuss best practices for how to define and study microglial state dynamics. We then outline "classical" microglial nomenclatures, highlighting some of the key discoveries that led to the above classifications and their limitations. We intentionally focus on citing studies related to the nomenclature, rather than providing a comprehensive review of the history of microglial research, as it has been done elsewhere[7,8]. We discuss the overall limitations and conclude with recommendations for the proper usage of microglial nomenclature as research evolves, provide a conceptual framework for discussing microglia, and offer perspectives on the future questions, gaps in knowledge, and challenges to tackle as a field.

Microglial identity: what we mean about when we talk about microglia

The origin and identity of microglia was for many years a matter of debate. In the dim and distant past, Ramón y Cajal´s disciple, Pío del Río-Hortega suggested that these cells were of mesodermal origin[9]. However, over time, an ectodermal origin was also proposed[10], sparking controversy until the 1980s. The mesodermal origin took solid hold later with the advance of technical approaches revealing more similarities than differences with the functions and features of macrophages. In 1999, microglia were reported to appear in the brain rudiment as early as embryonic day E8 in mice, and proposed to originate from yolk sac progenitors[11]. The recent combination of fate mapping studies and transplantation approaches this debate, revealing key aspects of microglial identity and plasticity. In mice, unlike other model organisms such as zebrafish[12,13], microglia are now considered to originate from a pool of macrophages produced during primitive hematopoiesis in the yolk sac, which start invading the neuroepithelium at E8.5[14-17]. In humans, microglial precursors invade the brain primordium around 4.5 to 5.5 gestational weeks[18].

One key signaling pathway critical for microglial development and maintenance is the CSF1R (colony stimulating factor receptor). Ligands of CSF1R that sustain this pathway include two cytokines with different origins and primary sequences, but similar tridimensional structures

and binding to CSF1R: IL-34 and CSF1[19]. IL34 is produced by neurons, while CSF1 is secreted primarily by oligodendrocytes and astrocytes. Accordingly, the two ligands have distinct and non-overlapping functions in the establishment and maintenance of microglia within the grey and white matter[20]. Microglia have the capacity for self-renewal in certain contexts, allowing them to repopulate the central nervous system (CNS) within one week of depletion, even when more than 99% of microglia are ablated with CSF1R antagonists[21,22] or diphtheria toxin[22]. This process, termed "microglial repopulation" or "microglial self-renewal"[23-25] is different from "microglia replacement" which, in contrast, occurs when endogenous microglia are replaced by exogenous cells that can include bone marrow-derived myeloid cells[26-29], peripheral blood cells[28,30], stem cell- or iPSC-derived peripheral blood cells[31], across various experimental or pathological conditions[31-33].

Our current definition is that mammalian microglia are yolk sac-derived, long-lived cells within the CNS parenchyma that persist into adulthood, and self-renew without any contribution from bone marrow-derived cells at steady-state.

The identification of microglia is currently based on the expression of specific genes highly enriched in microglia, which represent their transcriptional identity and are commonly employed as "microglial markers" (**Table 1. Microglial markers**). However, the expression of each marker alone is not sufficient to define microglial identity, as levels of expression may change depending on microglial adaptation to local signals. The present consensus is that mammalian microglia can be identified by the expression of transcription factors like Pu.1[16], cytoplasmic markers such as ionized calcium-binding adapter molecule 1 (IBA1), and surface markers including the purinergic receptor P2YR12, transmembrane protein 119 (TMEM119), and CSF1R[34]. Based on these markers, genetic tools (such as Cx3cr1^{CreERT2}, P2ry12^{CreERT2}, Tmem119^{CreERT2} and Hexb^{CreERT2} mouse lines) are available that allow for more specific manipulation or visualization of microglia, although they could also target other populations, including border-associated macrophages (BAMs), also named CNS-associated macrophages (CAMs) and other glial cells[35-40]. Most recently, a new binary transgenic model relying on co-expression of Sall1 and Cx3cr1 has been introduced that specifically targets microglia in a non-inducible way[41].

Nonetheless, many of these markers are downregulated in pathological states, and can be expressed by other brain macrophage populations such as BAMs residing in the perivascular space and leptomeninges[42,43], which also derive from the yolk sac[44]. In addition, caution must be exercised, because many classical microglial markers can also be expressed by cells originating from monocytes or iPSCs, and therefore their presence does not imply *bona fide*

microglia. These cells should be more accurately described as monocyte-derived microglia-like or iPSC-derived microglia-like cells (iMGL cells).

As resident macrophages of the brain parenchyma, microglia participate in many critical CNS functions ranging from glio-, vasculo- and neurogenesis to synaptic and myelination, through their process motility, release of soluble factors, and capacity for phagocytosis (**Figure 2**). These functions have been revealed using several constitutive and inducible knock-out models for microglial-specific genes[45] and by microglial-depletion paradigms in animal models[46], particularly rodents and zebrafish.

The key role of microglia in maintaining CNS health is also supported by the severe phenotype displayed by patients lacking microglia due to loss-of-function CSFR1 mutations. Heterozygous mutations, particularly in the kinase domain of *CSF1R* are associated with ALSP (adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, OMIM:221820) characterized by reduced microglial numbers and white matter atrophy that result in progressive cognitive and motor impairment, dementia, and early death[47]. Additionally, bi-allelic mutations are reported to cause complete absence of microglia with developmental brain malformation, hydrocephalus, bony lesions, and early death[48,49]. This phenotype, however, seems in apparent contradiction with the reported absence of gross neurological abnormalities at birth observed in mice with genomic deletion of FIRE, an intra-intronic super enhancer in the *Csfr1*gene enhancer region, whose brains lack microglia[50], though more nuanced analyses are needed. Nonetheless, FIRE mice have premature lethality and increased amyloid pathology as early as 5 months of age[51]. The source of discrepancy between the developmental impact of CSFR1 mutations in humans and mice is not yet fully understood. One possibility is that microglial developmental functions are partly redundant, modified by other environmental factors, or compensated in their absence by other cell types, such as astrocytes[52]. It will be important to determine how microglia communicate with other glial cells and immune cell populations to support CNS maturation and function in the future.

(Re)Defining microglial states: DAMs, HAMs, WAMs, and more

Core markers of cellular identity are useful to identify microglia, but are not necessarily informative about the functional “state” of microglia, which depends on the context (i.e., the physiological conditions in which microglia are found at any given CNS region and time). Microglia have a complex “sensome”[53], a series of surface receptors that allow them to detect changes in their environment. Microglial states are thus dynamic, and the outcome of the cell´s epigenome, transcriptome, proteome, and metabolome yields discrete morphological, ultrastructural and/or functional outputs (**Figure 3**). Microglia are anything but static, as they

are exceptionally responsive to alterations in their local environment. In the mature healthy CNS, the distribution of microglia is largely uniform and generally regular with little overlap between adjacent territories[54]. The cell bodies are largely sessile, but their processes are constantly moving and scanning the brain parenchyma[55,56]. Microglial functions adapt to their location and reciprocal interactions with nearby cells and structures. Their morphology, ultrastructure and molecular profile are similarly dynamic and plastic, resulting in many different cell states. As Conrad H. Waddington, founding father of systems biology, eloquently described: "*Cells are residents of a vast 'landscape' of possible states, over which they travel during development and in disease".*[57]

Single-cell technologies, multi-omics and integrative analyses of gene and protein expression have helped to not only locate cells on this landscape, but also provide new insight into the molecular mechanisms that shape the landscape and regulate specific cell states in a given context (e.g., development, adult, disease or injury model, etc.). Many diverse and context-dependent microglial states have been observed across species and models. Some examples of these states are the DAM (disease-associated microglia), originally associated with Alzheimer´s disease (AD) pathology models [58]; MGnD (microglial neurodegenerative phenotype) documented across several disease models[59]; ARM (activated response microglia) and IRM (interferon-responsive microglia) in an AD pathology mouse model[60]; HAM (human AD microglia)[61]; MIMS (microglia inflamed in multiple sclerosis (MS))[62]; and LDAM (lipid-droplet-accumulating microglia in aging mice and humans)[63], brain tumors (glioma-associated microglia, GAM)[64], amyotrophic lateral sclerosis (ALS)-associated signature[65] and Parkinson's disease (PD)-microglial signature[66]. In the developing and aging brain the WAM (white matter-associated microglia)[67]; ATM (axon tract-associated microglia)[68], and PAM (proliferative-region-associated microglia, related to phagocytosis of developing oligodendrocytes)[69], may share some features with the core DAM signature. In the developing human CNS, microglia also express some of the DAM/MGnD/ARM-like profiles[70].

While gene expression signatures indicate biological pathways, the functional implications of these states and relationship to one another remain unclear. In fact, the ever-growing list of branding clusters in single-cell RNA sequencing (scRNASeq) experiments and use of acronyms is not consistent across research groups and could hinder future advance of the field without validation and functional experiments to understand their meaning. Moreover, transcriptomic signatures depend on tissue dissection and gating strategies that can lead to isolation artifacts[71-74], which, when layered with the technical limitations of single-cell sequencing, can make it difficult to assign state identity across different studies. Another source of complexity comes from evident interspecies differences[75-77], which can further

hamper comparisons. Advances in computational tools and approaches, which enable the alignment and integration of single-cell datasets, can help solve some of these issues, providing a powerful way to determine microglial state similarities across contexts[78,79].

A practical limitation of solely defining functional states by their transcriptional signature is that mRNA expression may not directly predict protein levels[80]. Protein expression signatures obtained by methods, such as single-cell mass cytometry, have their own technical limitations[81] but may better represent true cell states[82,83]. Importantly, mRNA or protein expression alone do not necessarily predict microglial function, although they can be used to generate functional hypotheses that need to be experimentally tested. There are many methods that allow for the classification of microglia based on their constituent states, including gene expression, protein expression, post-translational modifications, mRNA profiling, morphology and ultrastructure. All these approaches can vary in coverage (e.g., expression of a single cell *versus* whole-transcriptome profiling), which has created overall confusion and mislabeling in the field. Presumably, each microglial state is associated with unique or specialized functions, although the unique roles of any observed state have so far remained elusive. Thus, it is critical that we begin to define microglial states taking into account their specific context within and between species, across sex, space and time (e.g., CNS region and biological age) as well as layers of complexity (e.g., epigenetic, transcriptional, translational, metabolic signatures), which ultimately determine together the cell's phenome (i.e., motility, morphology, ultrastructure) and function (**Figure 5**).

One major conceptual limitation of the various 'one-off' microglial acronyms (e.g., DAM, MGnD, etc.) is that they suggest stable states or phenotypes of microglia associated with a disease context, such as neurodegeneration. Intuitively, this classification system is similar to the concept of neuronal cell types, where neurons cluster into distinct subtypes based on their gene expression or neuroanatomy. However, contrary to microglia, neuronal groupings are considered fixed and terminally differentiated[5]. We do not know how temporally or spatially dynamic microglial states may be, as microglia are remarkably heterogeneous and plastic. Therefore, these cells are probably not permanently 'locked' into any single functional state. From the evidence available so far, microglial states appear dynamic and plastic, possibly transitory, and strongly dependent on the context[84]. New tools including imaging reporters for microglial states are needed to track transitions within individual cells over time and across the lifespan, following different challenges and perturbations, as well as in response to treatment.

Microglial heterogeneity: it all depends on the context

The term “homeostatic” is used to refer to microglia in physiological conditions but there are different interpretations of this nomenclature when describing microglia in health and disease. While homeostatic relates to the ‘physiological’ context assessed in space and time, it does not necessarily correspond to a unique molecular profile because, even without any perturbation, microglia display diverse morphological and functional states, depending on the signals from the CNS microenvironment. This continuous microglial sensing results in multiple transcriptional signatures from development to aging, depending on the specific local signals or challenges to the brain at each developmental stage[53]. A less responsive microglial state, which in other contexts would be considered more “homeostatic”, might be less effective at responding to damage or pathological cues in aging and disease contexts. For example, in aging and neurodegenerative disease, microglia may have reduced ability to rapidly respond to brain challenges (i.e., removing toxic amyloid, infected, damaged or degenerating neurons), leading to CNS dysfunction and disease progression. Microglia from adult TREM2 knockout mice have been described as ‘locked in a homeostatic state’ as they are less responsive to challenges (such as amyloid) and do not adopt a transcriptional DAM signature in disease contexts[85,86]. From this example, the term “homeostatic” is not informative if not well-defined and placed in the context of function.

Key modifying factors that lead to microglial heterogeneous states include age, sex, circadian time, local CNS signals and peripheral cues, such as the changes in the microbiota[87,88], or other systemic diseases (e.g., asthma)[89], in addition to the pathophysiological state of the CNS and overall organism (discussed in more depth in the next section). Age, indeed, has a key influence on the microglial homeostatic state, which goes through several distinct temporal stages (embryonic, perinatal, adult, and aging microglia), each notably characterized by an enrichment of defined regulatory factors and gene expression profiles[68,90]. After the initial establishment of microglial identity by a network of developmentally programmed and environment-dependent transcription factors[75,90], microglia become extremely heterogeneous in their transcriptome during early postnatal development, as determined by scRNASeq[68,69,91]. In contrast, microglia display a more limited transcriptomic heterogeneity in the adult CNS, where the different microglial scRNASeq clusters fall into a transcriptional continuum instead of representing distinct states[68,69,91]. Relatively small transcriptional differences may, however, lead to relevant functional differences, as exemplified by the functional variations between hippocampal and cerebellar microglia[92,93].

Sex differences due to sex chromosomes and/or gonadal hormones may also impact microglial states in different contexts. A growing body of evidence shows that male and female

microglia differ in their transcriptomic, proteomic, and morphological profiles, across brain colonization, maturation and function, in health and disease[88,94-96]. Of note, the microglial sex-specific transcriptomic signatures appear to be intrinsically determined, being maintained when microglia are transplanted into the brains of mice from the other sex[96]. Sexually differentiated roles of microglia could critically influence a variety of biological processes, in a time-dependent manner, and thus, emerge as key disease modifiers across various pathological conditions with sexual dimorphism in prevalence, manifestation, and response to treatment[97]. A well characterized example for sex-specific divergence is the purinergic receptor P2X4R, identified as the male-biased microglial mediator of chronic pain[98]. Sex differences in sexually dimorphic responses in physiology and pathology likely arise from a combination of Y chromosome-specific genes, sex hormones, neuronal circuit-related factors and epigenetic mechanisms[99].

Regardless of the reduced heterogeneity in the mature adult (compared to embryonic) CNS [7,68,90], microglia do differ among CNS areas in terms of their morphology and ultrastructure, transcriptional, proteomic, epigenetic profiles, and functional specialization, suggesting that microglial states are modulated by local cues[83,100,101]. However, local CNS signals are not sufficient to determine microglial identity because macrophages engrafted in the brain parenchyma can acquire a microglia-like morphology without reaching a transcriptomic signature identical to host microglia, even after prolonged CNS residence[26,102,103], supporting the idea that microglia are distinct from peripherally-derived macrophages, even when they colonize a similar niche. In addition, these findings suggest that once their identity is established, microglia assume different functional states in response to local CNS signals. Therefore, both the developmental genetic programs and CNS environment (nature and nurture) collaborate to dynamically determine microglial functional states.

Microglia not only respond to local cues within the brain, but they also receive continuous inputs from the periphery, including signals from the gastrointestinal tract[104]. In this context, the role of the host microbiota is gaining momentum in controlling microglial maturation and function in the CNS[88], with growing evidence that microbiota-derived short-chain fatty acids represent major mediators of the gut-brain axis[87,105]. Another example of cross-talk between microglia and the periphery is the so called “sickness behavior”, as a result of the central response to peripherally released cytokines produced by peripheral immune cells and tissue resident macrophages detecting specific pathogen-associated molecular patterns (PAMPs)[106]. This complex and coordinated response, in which the functional role of microglia remains poorly understood, gives rise to adaptive behavioral strategies, including lethargy.

Acute systemic inflammation, nevertheless, was extensively shown to impact on microglia[107,108] and induce a microglial state associated with robust IL-1β production[109].

The concept of the brain as an immune privileged organ has been challenged and definitely revisited in recent years. Indeed, peripherally produced cytokines and immune cells access the CNS and patrol the perivascular space in disease but also in health thus, playing important roles in coordinating central and peripheral immune responses[110]. It was also suggested that microglia require resident CD4+ T cells in the healthy developing brain for proper maturation and complete fetal-to-adult transition[111]. Microglia and T cell cross-talk was shown to help maintain homeostasis in the CNS, with dysfunctional regulation occurring in diseases, such as MS[112], ALS[113], AD[114], and encephalitis[115]. It will be important to continue investigating the influence of the peripheral immune system including B cells, NKs and other cells on microglial states and function in both health and disease.

Microglial states in the diseased CNS

Microglia are keen responders and critical players in numerous neurodevelopmental, neurological, and neurodegenerative conditions, as thoroughly reviewed elsewhere. Altered microglial states have been described in the diseased human brain and across various animal models of disease pathology based on morphology and gene expression signature. In addition, these states also differ depending on the timing (i.e., disease stage), genetic background, and local environment. Context-dependent signals vary dramatically during disease progression; they range from apoptotic cells, extracellular debris, toxic proteins (i.e., amyloid, α-synuclein), and signals resulting from blood-brain barrier disruption and altered function of neurons and other glial cells. Microglia respond to these challenges by changing their molecular profile, morphology and ultrastructure (**Box 3**), as well as motility and function.

The expression of core microglial markers is also altered over the course of disease, including downregulation of the “homeostatic” microglial signature. A prototypical example is P2RY12, one of the most widely used markers to discriminate microglia from other macrophages, with its reduced expression being one of the salient features of the microglial response to AD pathology and other disease conditions[116], as shown in several mouse models of disease (**Figure 4**). The apparent contradiction that core markers do not have a steady expression, as could perhaps be expected, is likely reflecting the functions those proteins have and how they change in the diseased brain. For instance, P2RY12 upregulation in epilepsy may relate to microglial sensing ATP and nucleotides released during seizures[117]. This seeming paradox strengthens the fact that determining microglial expression profile is far from attributing any

function to microglia, as it may only be suggestive of a potential functional identity, which – with unanimous consensus from all the authors– requires experimental validation using appropriate animal models and mutagenesis while using analyses that preserve the environmental influences shaping microglial function.

A microglial state that has received particular focus is the one denoted by the DAM signature, initially identified in a mouse model with mutations within five AD genes (5XFAD)[58] and later detected in other AD mouse models and samples from human AD (reviewed in [116]) and MS patients[62,118]. Single cell transcriptomic profiling of human microglial nuclei revealed a tau-associated microglia cluster that had not been identified in mice[119], reinforcing the idea that more human studies are needed. The shared DAM signature includes downregulation of CX3CR1 and P2RY12, and upregulation of APOE, AXL, SPP1, and TREM2[116], and it has been recently shown that it comprises two ontogenetically different cell lineages, both expressing TREM2: resident microglia and invading monocyte-derived cells (termed disease inflammatory macrophages, DIMs) that accumulate during aging[120]. Many questions remain open regarding the functional significance of the DAM signature.

Are DAM beneficial, detrimental or both? Several studies, in both mouse and human stem cell-differentiated microglia, demonstrated that the transition to a DAM state is dependent on TREM2[58,59,85,121]. How the TREM2 receptor drives the DAM transcriptional phenotype remains unclear, although the TREM2-ApoE signaling pathway is necessary for the switch from homeostatic to MGnD[59]. Many questions remain open on TREM2. For instance, is TREM2 a key sensor for amyloid-beta and other AD-related pathology or does its loss of function cause developmental defects in microglia that render them unable to change state? Is TREM2 controlling the microglial state by regulating their energetic and anabolic metabolism?[122,123] New bulk and single-cell epigenetic approaches[75,124-129] will help answer these questions and ultimately may provide a means to toggle microglial states at will, enabling the field to finally understand the function of distinct microglial states and their impact in different contexts.

Additionally, many genes of the DAM signature were identified across various contexts. For example, a common set of markers including (but not limited to) an upregulation of TREM2, APOE, CD11c, CLEC7A and LPL, and downregulation of TGFβ, CSF1R, P2RY12, and TMEM119 has been recently used to denote a microglial state that associates with myelinating areas in the developing brain, but also with aging and several models of degenerative diseases, such as AD, ALS[130], and MS[58,67,131]. These observations raise the question as to whether the DAM is a signature strictly associated with certain diseases, as the name implies, or perhaps represents a more universal core signature that appears in response to various

challenges and may differ between the young/developing *versus* aged/diseased CNS, and across distinct regions. Most likely, the same states that are beneficial in certain contexts may be detrimental in others, strictly depending on the complex interactions between microglia and their surrounding environment. One of the most relevant questions to be addressed is to which extent microglial states identified in the mouse brain are conserved and functionally relevant in the human brain.

Nomenclature troubles

Our current understanding of the plasticity of microglial states is at odds with the simplistic scenario established using outdated microglial nomenclature (resting *versus* activated and M1 *versus* M2, **Boxes 1 and 2**). Thus, a systematic, careful naming approach would greatly benefit microglial biology. As a first step to guide the field regarding the use of nomenclature, we generated a questionnaire (**Supplemental Data**) and collected the responses from the co-authors.

Surprisingly, there was more consensus than disagreement that the current nomenclature has severe limitations, and a more useful conceptual framework is needed to properly understand microglial states. There is also agreement that this framework is a first important step to guide the field and should be revisited every five to ten years by an international panel of experts as new discoveries are made. There is also a broad agreement that microglial responses should be framed in a multidimensional space, and should not be simplified as dichotomic good *versus* bad (**Figure 1**). Another point of strong agreement: abandon M1/M2 (and similar) nomenclature once and for all and generally avoid using the vague term 'neuroinflammation'. Most agree that inflammation is not always detrimental but, instead, represents an adaptive response to damage that can sometimes get out of control (**Box 4**). Quite importantly, a vast majority of authors support the use of "markers" (genes or proteins) to identify cell populations, but not as a readout of cell functions, which need to be addressed directly.

Nonetheless, there were a few points that are still under intense debate. The term "resting" microglia is strongly avoided by some authors, whereas others acknowledge that they still use it even with its limitations, for lack of a better term. "Homeostatic" has more acceptance, although it is recognized that it is based on a very particular gene signature not shared by microglia across all physiological contexts, such as embryonic and postnatal development, and that several homeostatic states likely exist. Thus, the term 'homeostatic' should always be accompanied by an accurate description of the context.

The opinion on use of the term “DAM”, on the other hand, is highly polarized. Many authors consider that a core set of transcripts in this signature is common to several pathological conditions and some physiological processes, including the development of white matter, whereas an equal number of authors state there is not enough evidence for “DAM” to be a universal signature of microglial response to damage. Finally, the extent to which microglia are unique or similar to other brain associated or tissue macrophages is evolving with new data and profiling methods: most agree that due to their lineage, microglia are to some extent similar to other macrophages but have unique functions resulting from their longer residence in the CNS environment.

Recommendations: DOs and DON’Ts

Based on the collective opinions from the authors, we provide a series of recommendations for researchers, reviewers, and editors. As the field has not yet reached a consensus on several nomenclature topics, including the appropriate use of descriptors for microglial states, it is premature to provide clearer recommendations. Nevertheless, we aim to raise awareness on these issues and stimulate the launch of further initiatives that will guide the field and allow to develop more specific guidelines.

Classic Nomenclature

- Consider microglia as highly dynamic and plastic cells that display multivariate morphological/ultrastructural, transcriptional, metabolic and functional states both in the healthy and pathological CNS.
- Describe microglia using as many as possible layers of complexity: ontogeny, morphology/ultrastructure, motility, -omics, and function, always placing them into a species and spatiotemporal context (**Figure 5**).
- Refer to microglia in basal conditions as “homeostatic”, instead of “resting” microglia, considering the limitations discussed above (i.e., that these terms refer to microglia under physiological conditions, not to the function of microglia). Use the term “surveillant/surveilling” to refer to microglia that are engaged in surveillance, but not as a synonym of microglia under normal physiological conditions.
- Refer to microglia in your experimental condition as “reactive to” or “responding to” while describing the particular signals they respond to (i.e., the context), instead of using the widely used broad term “activated”, as microglia are active in both health and disease.
- Disregard simplistic, dichotomic categorizations by providing the observed data and its context.

- Describe profiles of cytokine expression, considering that microglial complexity cannot be reduced to oversimplified and polarized “pro-inflammatory” *versus* “anti-inflammatory” categories. Similarly, do not use M1 *versus* M2 classification.
- When using the term “DAM”, do not use it as a universal term applicable to all diseases, models or challenges. The jury is still out to test whether its full or core signature is common to all or a subset of pathologies, particularly in the human brain.

Introducing New Terminology

- Until a consensus is reached about true subtype/s of microglia, with defined ontogeny, physical niches, functions, and transcriptional profiles (whether permanent or transient), use the term “state” rather than “subpopulation.
- Use combinations of gene or protein “markers” to identify putative supopulations but be aware that their expression is plastic and may change over time and under different experimental conditions. Use fate mapping approaches with lineage tracing to track individual microglial cells and assess possible intrinsic differences as well as changes in their state over time[84,132].
- In scRNASeq studies, describe the transcriptional signatures (sets or modules of expressed genes) that can be compared with other studies[116,133] To describe groups of transcriptionally similar cells in terms of signature, use the term “cluster”.
- Avoid the use of acronyms wherever possible, and only use these once multiple laboratories have defined a stable state with a clearly defined functional role.
- If new terminology needs to be introduced, follow FAIR principles: Findable, Accessible, Interoperable, and Reusable (https://neuronline.sfn.org/professional-development/data-sharing-principles-to-promote-open-science). An example of naming cell lines following these principles can be found here[134].

Microglial Markers and Function

- Use integrative methodological approaches that allow probing of microglia using different levels of analysis (**Figure 5**).
- Follow updated consensus guidelines when using methodologies such as scRNASeq[135], RTqPCR[136], or digital PCR[137].
- Do not use morphology or gene/protein expression as a substitute for directly assessing cell function. Morphology and expression can be used to generate hypotheses about function that need to be specifically tested.

Grammar Quandary:

- "Microglia" as a population is a plural noun in English but a singular noun in Latin-derived languages, which occasionally causes confusion. In English texts, microglial cells should always be referred to in the plural form unless referring to an individual cell. For example, "microglia are brain cells" but "this microglia is adjacent to a neuron".

Future questions and challenges

From words to action: A key challenge in the field is to match microglial morphological, ultrastructural, transcriptomic, proteomic, metabolomics and emerging lipidomic changes with functional responses (**Figure 3**). In the current single-cell era, an overwhelming wealth of data has been generated, profiling the expression of millions of microglia in different organisms, at different ages, across diverse brain regions. Yet, such 'omics' identities are not necessarily linked to functional states, and they often lack spatial resolution. Additionally, many widely used microglial markers are sensome genes, whose expression and activity at the microglial membrane may reflect functional adaptations to a changing environment, and are possibly more indicative of the microglial functional state than the transcription profile.

Transcriptional analysis will benefit from ribosome profiling by RiboSeq[138] and from gene-trap insertion profiling by TRAPSeq[139]. Proteomic approaches combined with *in situ* studies will provide better information in this respect, bridging the gap between expression and function. Further integration of complementary approaches, such as spatial transcriptomics, imaging mass cytometry, and correlative or conjugate electron microscopy in combination with other single-cell approaches, will provide a more comprehensive characterization of microglia. Ultimately, functional studies using specific pharmacological and transgenic approaches in animal models, as well as human-derived cells and organoids are indispensable to understand the multiple roles of microglia within specific spatiotemporal contexts of health and disease.

How are microglial states coordinated?

Even as we acquire more data about microglial states, there are still key questions remaining unanswered. To which extent are microglial states plastic and reversible? What is the relationship between microglial state and cellular function? These varied single-cell characterizations ultimately need to be linked to particular functions, to become relevant to development, health, and diseases. How do these states come about? How do signals from the CNS environment get integrated in microglia to produce specific states? New imaging tools and reporters that enable tracking and manipulation of specific microglial states are needed to address these questions.

How similar are peripherally-derived macrophages and microglia? A burning question that surely requires further investigation is related to the identity and function of microglia *versus* other brain macrophages. Although recent studies have provided evidence for an intrinsic unique core signature of microglia, their functional resemblances and differences remain undetermined. For instance, could engrafted parenchymal macrophages functionally replace the resident microglia, despite having a different molecular identity, and could they serve as therapeutic vectors?

The devil is in the details: Another major caveat is that microglia are incredibly reactive cells and evidence indicates that artifacts are often introduced during sample processing for a variety of methodologies, such as RNA profiling, immunohistochemistry, FACS, *in vivo* imaging, and so on. Hence, we may be missing or confounding important pieces of information because we unintentionally introduce changes in the parameters we are trying to measure. In addition, these artifacts are likely to generate variability across laboratories using different protocols. A future challenge is to increase reproducibility of data across laboratories, by coordinating a shared database of protocols and analysis pipelines curated using STAR methods guidelines. In addition, in the current single-cell multi-omics era, the challenges in big data analysis are exponentially growing[140]. Statistical methods (including multivariate statistics)[141] and artificial intelligence-based data mining approaches (such as machine learning)[142] will have to be introduced, to uniformly process and integrate large datasets, as well as extract the biological relevance of the findings.

Diversity as a source of richness: Many transcriptional states have been reported during embryonic development, aging, and disease. How many different microglial states can be identified? Within the homeostatic microglia, how many states exist? How do microglia navigate among their many states? Are they related through a transcriptional continuum, or perhaps as a hub-and-spoke set of states, as has been proposed for macrophages[4]? How dynamic are these states? And how spatially defined are they? Future research will need to address these important questions.

Male versus female microglia: Sex differences have been reported to affect the brain colonization, maturation, structure, transcriptomic, proteomic, and functional profiles of microglia, in a time-dependent manner. To what extent these differences may regulate the susceptibility to neurological diseases remains a fascinating question that urgently awaits answers. Investigating the molecular and cellular mechanisms underlying sex-mediated differences in microglial states would advance our understanding of microglial implication in

diseases with clear sex-related differences in their prevalence, symptoms, and progression, as well as response to treatments.

Relevance to humans: It will be imperative to study developmental and functional differences between human and animal model microglia. To date, most of the studies on microglia were conducted in mice and a direct comparison among brain regions is still missing. Whether microglial states identified in mice also exist in humans is still under debate. Translating and validating these findings across species is critical and will help prevent failure of clinical trials that stem from animal model limitations. In addition, most human microglial studies were performed in Caucasians and only recently data from other groups, such as African American individuals, are becoming available[143].

Towards a unified nomenclature: The conclusion of this paper is that the community has not yet reached an agreement on what defines microglial identity compared to other cell types; nor consensus on the number, dynamic nature, or definition of microglial states. The community advocates for creating harmonized, curated databases and guidelines for introducing novel terminology; to follow STAR methods; and share data as early as possible. Until such consensus is reached, the community urges all microglial studies to present data with all their layers of complexity and carefully define the context examined to offer clarity instead of confusion, thereby contributing to a more thorough understanding of the many facets of microglial biology. To establish new guidelines for microglial states and nomenclature we call for a community-based approach, whereby the issues and progress are discussed openly in workshops and meetings, with input from diverse researchers across fields and career stages. A useful model to look after are the 10 Human Leukocyte Differentiation Antigen workshops that have taken place since 1982, in charge of renaming CD (cluster of differentiation) antigens (https://www.sinobiological.com/research/cd-antigens/hlda1). We lastly advocate for the creation of an international panel/committee of experts in charge of overseeing the guidelines and establishing a specific roadmap to write a white paper in the nearest future.

We would like to conclude with the words of Río-Hortega, who sarcastically identified the problems of microglial nomenclature already 100 years ago: “If we were fond of introducing new nomenclature to describe microglia, as many modern histologists are, who think that enriching nomenclature resolves problems, we would find for microglia names that would indicate their origin, or morphology, or function, in addition to classify all the shapes that acquire when moving and evolving - resulting in the same absurdity that occurs in some branches of Histology and, particularly, Hematology.”[144]

Box 1. Resting *versus* activated microglia

The development of specific silver staining techniques in 1919 allowed Río-Hortega to clearly identify microglia and study their response to experimental manipulations[7,145]. Early on, Río-Hortega appreciated the striking morphological transformation of microglia following brain damage, but it was in the mid-1970s that the terms "resting" and "activated" microglia first appeared in the literature. These terms were used to morphologically describe cells with affinity for silver staining that were observed in physiological ("resting") *versus* pathological ("activated") conditions. This nomenclature consolidated in the 1980s and became widely used during the 1990s[146], in parallel with the development and use of histochemical and immunohistochemical techniques, such as lectin staining[147], detection of phosphatases and phosphorylases[148], and antibodies against the complement receptor CR3[7]. These techniques and nomenclature were pivotal in determining that "resting" microglia were unrelated to astrocytes, as some studies had wrongly concluded[149], and that "reactive" microglia shared many characteristics with the blood-borne monocytes[10].

As shown by a PubMed search with microglia in all fields, there were only few papers published on the topic before the 1990s, and then a steady increase until the beginning of our century, followed by an exponential growth[150]. There is a first inflexion point in 2005, with the seminal discovery using non-invasive two-photon *in vivo* imaging that microglia are extremely dynamic in the absence of pathological challenge, continuously surveying the parenchyma with their highly motile processes[55,56]. The development of non-invasive methods was necessary for our understanding of microglial roles in the healthy brain (reviewed in[151]). In 2005, microglial extreme dynamism in the intact brain was examined for the first time, through the skull of CX3CR1-GFP mice in which microglia are fluorescently labeled[55,56]. As a result, microglia are now considered to be the most dynamic cells of the healthy mature brain[151]. This seminal discovery prompted to rename quiescent or resting microglia as surveying[56,152] or surveillant (from the verb to survey)[153] microglia, and also led to propose the concept that microglia are never-resting[154]. Together, these and other *in vivo* two-photon imaging data put into serious doubt the concept of "activated" microglia, which suggests a unique form of response, as in fact microglia are always active, constantly responding (in different ways depending on the context) to the changes in their CNS environment, even under normal physiological conditions. Therefore, microglia do not switch from "resting" to "activated" in response to trauma, injury, infection, disease, and other challenges. Rather, microglia are continuously active and react to the stage of life, CNS region, species, sex, and context of health or disease by adopting different states and performing different functions. Thus, although still widely used, "resting" and "activated microglia" are labels that should be discontinued.

Box 2. M1 *versus* M2 microglia

Another terminology emerged in the early 2000s from immunologists classifying macrophages based on findings obtained using *in vitro* models: “M1”, the classical activation, considered pro-inflammatory and neurotoxic, as well as closely related to the concept of “activated” microglia, and “M2”, or alternative activation, considered anti-inflammatory and neuroprotective[155]. These responses were related to those of T helper lymphocytes (Th1 and Th2) based on their *in vitro* activation by specific immune stimuli that activated differential metabolic programs and changes in cytokine expression[156]. An associated term is “M0” microglia, which describes their state when cultured in the presence of TGFβ (transforming growth factor beta) and CSF-1 to mimic *in vivo* counterparts[157]. The terms became widely adopted in microglial research and the 2010s saw a boom of papers phenotyping macrophages and microglia into “M1” and “M2” based on the expression of markers related to these categories, used to indirectly assume a detrimental (“M1”) or beneficial (“M2”) microglial role[156]. In many cases, editors and reviewers have asked authors to comply with this nomenclature. However, it soon became evident that macrophage responses are more complex than simply “M1” and “M2”[158]. In the case of microglia, the advent of single cell technologies provided clear evidence that microglia in the living brain do not polarize to either of these categories, often co-expressing M1 and M2 markers[159], despite the continued use of M1 and M2 in the literature. We thus recommend to strictly avoid M1 and M2 labels and use more nuanced tools to investigate microglial function (reviewed in[160]).

Box 3. Microglial morphological responses across species

Microglial cells display a profusion of morphologies that have fascinated researchers since the early days of Río-Hortega. Many were tempted to equate morphology with function. Ramified microglia were traditionally associated with the “resting” state, although we now know that ramified microglia actively play many functions during normal physiological conditions. In contrast, “reactive” microglia (rounder cell body, generally with fewer and shorter processes) were called “activated” and equated with an inflammatory response. Only recently, however, a mechanistic link between microglial reduced branching and increased release of the inflammatory cytokine interleukin 1β was reported[161]. Activation of P2YR12 by tissue damage signals potentiates the tonically active potassium THIK-1 channel, expressed in microglia, leading both to decreased microglial ramifications and activation of the inflammasome machinery processing IL-1β precursors into their mature form[161]. Another morphology associated with functional changes is “ameboid” microglia, which were thought to be more “phagocytic”, but it is clear now that ramified microglia execute phagocytosis through their terminal or ‘en passant’ branches notably during adult neurogenesis[162,163], while in disease conditions such as epilepsy ameboid microglia can display reduced phagocytosis[164].

Therefore, morphological changes should not be interpreted in functional terms but, rather, taken as a suggestion prompting to investigate further the relationship between microglial structure and function. While the categorization described above is now outdated, the analysis of microglial morphology is considered valuable and still often used across animal model and human *post-mortem* brain studies.

Studies in *post-mortem* brain samples have revealed that human and mouse microglia can adopt similar morphologies. Using the now outdated terms “ramified”, “primed” (larger cell body, ramified processes), “reactive” (ameboid, few ramified processes), and “ameboid” (less than two unramified processes) microglia were described in middle-aged individuals[165]. In addition, “rod-shaped” microglia (elongated cell body, polarized processes) were found to become more abundant with aging[166]. Similarly, “dystrophic” microglia, presenting apparently fragmented (but still intact at the ultrastructural level) processes were reported in aging[167,168]. These different morphological types observed in humans were previously described in rodent models (reviewed in[169]). Nevertheless, a more sensitive quantitative microglial morphological assessment using a computational pipeline involving cluster analysis revealed differences between mouse and human, with distinct clusters found to be unique to each species[170]. Subsequently, a high-throughput comparative morphology analysis revealed a generally conserved evolutionary pattern, with some intriguing differences observed between the leech, zebrafish, axolotl, turtle, chicken, gecko, snake, bearded dragon, bat, boar, sheep, whale, hamster, rat, mouse, marmoset, macaque, and human, and across brain regions between mouse and human[76]. While detailed comparative ultrastructural analyses of microglia between species are currently lacking, the state of “dark microglia” (named based on their increased electron density giving these cells a dark appearance, compared to other microglial states) discovered in 2016, which is defined using electron microscopy by its markers of cellular stress in contexts of aging and disease, was found to be conserved across mouse, rat, and human[171,172]. New strategies are currently being developed to provide morphological data analyses based on automated pipeline, thus overcoming feature-selection-based biases[173]. Future studies will show how these varied morphologies correlate with transcriptional and proteomic profiles, and what they imply for the cell’s function. At the molecular level, recent single-cell transcriptome analyses also revealed that human microglia show multiple clusters that indicate a greater heterogeneity than in other mammalian species such as the mouse[76,91].

Box 4. Microglia and the term “neuroinflammation”

There is a long historical literature stating that inflammation is an important part of recovery from infection, injury, and disease, and it is the lack of resolution of this inflammatory response that is problematic in the context of CNS cell 'reactivity'. Therefore, when the term

"neuroinflammation" is encountered in the literature, the reader must be aware that it means different things depending on the context.

While the term "neuroinflammation" is widely used in the field as a synonym of microglial "activation"[174], its definition also varies dramatically among authors, according to our survey. Below are representative definitions which are currently used by the authors:

a. Neuroinflammation is inflammation of neural tissue particularly mediated by glial cells.
b. Neuroinflammation is strictly limited to conditions in which leukocytes enter CNS, e.g., in stroke and MS.
c. Neuroinflammation is a mixed cellular response to brain infection or damage involving innate and adaptive responses of resident brain cells and circulating immune cells.
d. The term neuroinflammation is too unclear and imprecise and should be avoided.

Considering that different definitions are used across authors, our main recommendation for the field is to liberate neuroinflammation from microglia and microglia from neuroinflammation, and to use both terms rigorously. The consensus among authors is four-fold. First, protection against tissue damage and extreme departures from homeostasis as well as repair (i.e., 'inflammation') encompasses, in the CNS, a highly complex set of local responses, and equally complex interactions with circulating immune cells or with immune cells residing in brain-blood and brain-cerebrospinal fluid interphases. In other words, 'neuroinflammation' is not a substitute for 'microglial reaction'. Second, there are numerous transcriptional states of microglia, astrocytes and oligodendrocytes. The functional outcomes of cells undergoing these transcriptional states remain incompletely understood. Furthermore, it is uncertain which transcriptional states are transient or represent durable cell fate choices. It is also unknown whether changes in states during diseases are 'inflammatory' or dedicated to maintaining microglial homeostatic functions. Taking these considerations together, one should exercise extreme caution in simplifying these phenomena as 'neuroinflammation', as at least some of these phenomena may represent alternative homeostatic or non-inflammatory reactive states. Third, it is not appropriate to imply that neuroinflammation is invariably deleterious. Rather, it should be recognized that each inflammatory response may exert adaptive or maladaptive effects, contingent on context. To be more specific, research is necessary to explore functions and distinct actions of cytokine-enriched microglia secretomes beyond binary characterizations such as 'pro-' and 'anti-inflammatory'. Fourth, with regards to nomenclature, we recommend the use of modest and precise terms to describe specific phenomena such as: microglial reaction; astrocytic reaction; molecules involved; loss of barrier function at the blood-brain barrier (BBB), etc. All in all, the main message we wish to convey is that

inflammation associated with the CNS follows unique rules that need to be fully discerned experimentally and not simply extrapolated from observations in non-nervous tissue.

TABLES AND FIGURE LEGENDS:

Figure 1. Microglial nomenclatures, past and future. Microglia have been traditionally framed into dichotomic categories but our current integration of epigenetic, transcriptomic, metabolomic and proteomic data favors a multidimensional integration of coexisting states.

Figure 2. Microglial core properties and functions: Phagocytosis, surveillance and capacity for releasing soluble factors (inner circle) are core properties through which microglia contribute to key biological functions (outer circle). Created with BioRender.com.

Figure 3. Microglial identity and states. The identity of microglia, compared to other CNS-associated macrophages in the perivascular space, choroid plexus and leptomeninges, is established early on from yolk sac-derived progenitors. Once they colonize the brain parenchyma and differentiate, they can adopt multiple states depending on the particular spatio-temporal context, as shown in more detail in **Figure 5**. Created with BioRender.com.

Figure 4. Microglial transcriptomic signatures. Recent scRNA-Seq studies have identified many microglial transcriptional signatures including but not limited to PAM and ATM in development; DAM, MgnD, ARM, MIMS in disease models of AD, MS, ALS and PD; and WAM, LDAM, HAM in aging, both in mice and human. The key upregulated (red) and downregulated (blue) genes in each signature are indicated. Created with BioRender.com.

Figure 5. Microglial states defined by their intrinsic and extrinsic determinants, spatiotemporal context, and layers of complexity. Microglial states depend on intrinsic determinants (such as species, ontogeny, sex, or genetic background) as well as the specific context they inhabit, including age, spatial location, and environmental factors (such as nutrition, microbiota, pathogens, drugs, etc.). All together, these factors impinge on microglia at multiple levels (i.e., epigenomic, transcriptomic, proteomic, metabolomics, ultrastructural and phenomic), which ultimately determine microglial functions. Created with BioRender.com

	Marker	Specificity	Labeled states	Staining patterns	Main applications	Ref.
Antibodies	F4/80 (EMR1)	Macrophages including microglia	Homeostatic conditions and disease-associated.	Does not provide a detailed cellular visualization, especially in homeostatic	Brightfield or fluorescence analysis of microglial density, distribution, and	[175-177]

			Expressed in rodents, but presence not yet confirmed in human.	conditions, due to its low basal expression. Its expression varies significantly between species and is low in human macrophages.	categorization into morphological states	
	CX3CR1	Macrophages including microglia	Homeostatic conditions and disease-associated, but downregulated by the DAMs, MGnD, dark microglia, and other pathological states.	CX3CR1-GFP reporter line generally used for visualization, with or without GFP immunostaining.	Brightfield or fluorescence analysis of microglial density, distribution, and categorization into morphological states.	58,59,178-180
	IBA1	Macrophages including microglia	Homeostatic conditions and disease-associated. Downregulated in some contexts (e.g., obesity and aging) and by some pathological states (e.g., DAM, dark microglia). Used to study microglia in early embryonic and	Provides exceptional visualization of microglial cell body and processes, including distal extremities. Diffuses throughout the cytoplasm. Staining can however be discontinuous in aging.	Brightfield or fluorescence analysis of microglial density, distribution, and morphology. Ultrastructural studies.	181,182 58,76,168,183-186

			postnatal development. Conserved across several species including human.			
	MerTK	Macrophages including microglia	Homeostatic conditions and disease-associated. Expressed in health and across various contexts of disease, notably in association with the phagocytosis of newborn neurons, amyloid, and myelin.	Partial visualization of microglial cell bodies and diffuse staining of their processes preventing a complete morphological visualization.	Brightfield or fluorescence analysis of microglial density, distribution. Morphological analysis or categorization into morphological states possible in combination with IBA1.	187-190
	CD11b/c	Macrophages including microglia	Homeostatic conditions and disease-associated. Used to study microglia in early postnatal development. Conserved across species including human.	Visualization of microglial cell body and processes. Low basal expression in adult microglia. Staining is mainly restricted to the plasma membrane.	Brightfield or fluorescence analysis of microglial density, distribution, and morphology Ultrastructural studies of subsets downregulating IBA1.	191 180,192-195
	P2RY12	Largely microglia-specific (not expressed by monocytes),	Homeostatic marker. Strongly downregulated in disease-	Visualization of microglial cell body and processes. Staining can localize to the	Brightfield or fluorescence analysis of microglial density, distribution, and morphology.	117,196-198

		but state-dependent	associated and reactive states (but upregulated in *status epilepticus*). Used to study microglia in early postnatal development. Conserved across several species including human.	plasma membrane or diffuse throughout the cytoplasm and can be more profuse than IBA1 depending on staining conditions.	Ultrastructural studies.	
	TMEM119	Largely microglia-specific, but state-dependent	Homeostatic conditions and disease-associated, but downregulated on reactive microglia in some contexts (e.g., traumatic brain injury and ischemia, MS). Developmentally regulated. Conserved across species including human.	Partial visualization of microglial cell bodies and diffuse staining of their processes preventing a complete morphological visualization.	Brightfield or fluorescence analysis of microglial density, distribution. Morphological analysis or categorization into morphological states possible in combination with IBA1.	199-203
	TREM2	Macrophages including microglia, state-dependent	Microglial subsets in early postnatal development, aging, and disease conditions (e.g., microglia involved in synaptic	Visualization of microglial cell body and processes. Staining diffuses throughout the cytoplasm.	Brightfield or fluorescence analysis of microglial density, distribution, and categorization into morphological states.	180,188,201,204,205

			pruning or associated with amyloid plaques in AD pathology). Shown to label monocytes or neurons instead of microglia in human.		Ultrastructural studies of pathological states downregulating IBA1.	
Mouse lines	CX3CR1-GFP	Macrophages including microglia	Homeostatic conditions and disease-associated, but downregulated in DAM, MGnD, dark microglia, and other pathological states.	Visualization of microglial cell body and processes. Fluorescence diffuses throughout the cytoplasm. Bright enough for two-photon in vivo imaging. A limitation is that the heterozygous mice used for in vivo imaging are partially deficient in fractalkine signaling, with possible outcomes on the brain and behavior[206]. The homozygous mice are knockout for CX3CR1 and used to study the outcomes of fractalkine receptor deficiency.	Two-photon in vivo imaging or fluorescence analysis of microglial density, distribution, dynamics, interactions with other parenchymal elements, and categorization into morphological states. Ultrastructural studies using staining against GFP.	[55,56,178,180,185,207]

	Iba1-EGFP	Macrophages including microglia	Homeostatic conditions and disease-associated. Downregulated in some contexts (e.g., obesity and aging) and in some pathological states (e.g., DAM, dark microglia). Used to study microglia in early embryonic and postnatal development. Conserved across several species including human.	Visualization of microglial cell body and processes. Fluorescence diffuses throughout the cytoplasm. Less bright than fluorescence in CX3CR1-GFP mice, but generally sufficient for two-photon in vivo imaging of cell body and proximal processes. These mice are not partially deficient in IBA1 in their heterozygous state, which is a main advantage.	Two-photon in vivo imaging or fluorescence analysis of microglial density, distribution, dynamics, interactions with other parenchymal elements, and categorization into morphological states. Ultrastructural studies using staining against GFP.	180,184,208
	Fms-EGFP or CSF1R-EGFP; CSF1R-Fusion Red	Macrophages including microglia. CSF1R is expressed by most microglia.	Homeostatic conditions and disease-associated, but considered to be downregulated in DAM and other pathological states.	Fluorescence is less bright than in CX3CR1-GFP mice, and generally sufficient for two-photon in vivo imaging. It also allows for fluorescence-activated cell sorting and fluorescence imaging when combined with immunostaining.	Fluorescence-activated cell sorting and fluorescence analysis of microglial density, distribution, dynamics, interactions with other parenchymal elements, and categorization into morphological states when combined with immunostaining.	34,162,209

				These mice are not partially deficient in CSF1R in their heterozygous state, which is a main advantage.		
	HEXB-TdTomato	Largely overlaps with IBA1 staining but restricted to microglia. Does not label CAMs and other border-associated macrophage populations.	Expression appears stable in homeostatic conditions and disease-associated states. The labeled microglia are also depleted by CSF1R inhibition.	Visualization of microglial cell body and processes. Fluorescence diffuses throughout the cytoplasm. Bright enough for two-photon in vivo imaging. A limitation is that the heterozygous mice used for in vivo imaging are partially deficient in HEXB. However, their microglial gene expression patterns do not appear affected.	Two-photon in vivo imaging or fluorescence analysis of microglial density, distribution, dynamics, interactions with other parenchymal elements, and categorization into morphological states.	[38]

Table 1. Main antibody markers and mouse lines used to visualize microglia in rodents and humans from early embryonic development to adulthood and aging. Other proteins expressed by microglia but whose specificity is not confirmed include APOE, CLEC7A, ITGAX, and LPL.

Acknowledgements: The authors are deeply grateful to Richard Ransohoff, Monica Carson and Elena Galea, who contributed the section on neuroinflammation. We would also like to express our gratitude for our lab members, who contributed with fruitful discussions. We are particularly grateful to Sol Beccari (who conceived Figure 1), Lasse Dissing-Olesen, Alec Walker, Martine Therrien and Yvanka de Soysa. We are very thankful for the technical support of Diane Hirshon during the preparation of the manuscript. We are grateful for the help of all the student hosts who contributed to the virtual workshop held in June 2021: Ifoeluwa Awogbindin, Elisa Gonçalves de Andrade, Fernando Gonzalez Ibanez, Mohammadparsa Khakpour, Torin Halvorson, Victor Lau, Sophia Loewen, Chloe McKee, Jared VanderZwaag, Haley Vecchiarelli (Tremblay lab); An Buckinx, Anne-Claire Compagnion, Fanny Martineau (Paolicelli lab); Sol Beccari, Alice Louail and Noelia Rodriguez-Iglesias (Sierra lab); Martine Therrien, Yvanka DeSoysa and Anna Kane (Stevens lab). Finally, we are grateful for the input we received from young trainees during the EMBO 2021 Workshop on Microglia and for our lab members who helped with the organization. We would like to thank the creativity of Sophie Robinson, who proposed the term “homeodynamic” to refer to the dynamic nature of microglia, although its similar pronunciation with the current term haemodynamic prevented us from recommending its use in this paper.

This work was supported by grants from the Dementia Research Switzerland – Synapsis Foundation, Swiss National Science Foundation (SNSF 310030_197940) and European Research Council (ERC StGrant REMIND 804949) to RCP; the Spanish Ministry of Science and Innovation Competitiveness MCIN/AEI/10.13039/501100011033 and FEDER “A way to make Europe” (RTI2018-099267-B-I00 and RYC-2013-12817), a Tatiana Foundation Award (P-048-FTPGB 2018), and a Basque Government Department of Education project (PIBA 2020_1_0030) to AS; Cure Alzheimer's Fund and Alzheimer's Association to BS; the Canadian Institutes of Health Research (Foundation Grant 341846, Project Grant 461831) and Natural Sciences and Engineering Research Council of Canada (Discovery Grant RGPIN-2014-05308) to MET. MET is a Tier II Canada Research Chair in *Neurobiology of Aging and Cognition.* This work was also funded by DFG CRC/TRR167 “NeuroMac” to IA, JP, MP, SJ. Australian Research Council support for project DP150104472 to MBG is gratefully acknowledged.

References

1 Stafleu, F. A. Linnaeus and the Linnaeans: The Spreading of their Ideas in Systematic Botany, 1735-1789 (1971), p. 80. . (1971).

2 Charmaz, K. The power of names. *Journal of Contemporary Ethnography* **35**, 396-399 (2006).

3 Guilliams, M. *et al.* Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* **14**, 571-578, doi:10.1038/nri3712 (2014).

4 Murray, P. J. *et al.* Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* **41**, 14-20, doi:10.1016/j.immuni.2014.06.008 (2014).

5 Yuste, R. *et al.* A community-based transcriptomics classification and nomenclature of neocortical cell types. *Nat Neurosci* **23**, 1456-1468, doi:10.1038/s41593-020-0685-8 (2020).

6 Escartin, C. *et al.* Reactive astrocyte nomenclature, definitions, and future directions. *Nat Neurosci* **24**, 312-325, doi:10.1038/s41593-020-00783-4 (2021).

7 Sierra, A., Paolicelli, R. C. & Kettenmann, H. Cien Anos de Microglia: Milestones in a Century of Microglial Research. *Trends Neurosci* **42**, 778-792, doi:10.1016/j.tins.2019.09.004 (2019).

8 Rezaie, P. & Hanisch, U.-K. in *Microglia in Health and Disease* (eds M.E. Tremblay & A. Sierra) 7-46 (Springer, 2014).

9 Río-Hortega, P. El “tercer elemento” de los centros nerviosos. III. Naturaleza probable de la microglía. *Boletín de la Sociedad Española de Biología* **VIII**, 108-121 (1919).

10 Oehmichen, M. Are resting and/or reactive microglia macrophages? *Immunobiology* **161**, 246-254, doi:10.1016/S0171-2985(82)80080-6 (1982).

11 Alliot, F., Godin, I. & Pessac, B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res* **117**, 145-152, doi:10.1016/s0165-3806(99)00113-3 (1999).

12 Xu, J. *et al.* Temporal-Spatial Resolution Fate Mapping Reveals Distinct Origins for Embryonic and Adult Microglia in Zebrafish. *Dev Cell* **34**, 632-641, doi:10.1016/j.devcel.2015.08.018 (2015).

13 Ferrero, G. *et al.* Embryonic Microglia Derive from Primitive Macrophages and Are Replaced by cmyb-Dependent Definitive Microglia in Zebrafish. *Cell Rep* **24**, 130-141, doi:10.1016/j.celrep.2018.05.066 (2018).

14 Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* **330**, 841-845, doi:10.1126/science.1194637 (2010).

15 Schulz, C. *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86-90, doi:10.1126/science.1219179 (2012).

16 Kierdorf, K. *et al.* Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* **16**, 273-280, doi:10.1038/nn.3318 (2013).

17 Stremmel, C. *et al.* Yolk sac macrophage progenitors traffic to the embryo during defined stages of development. *Nat Commun* **9**, 75, doi:10.1038/s41467-017-02492-2 (2018).

18 Andjelkovic, A. V., Nikolic, B., Pachter, J. S. & Zecevic, N. Macrophages/microglial cells in human central nervous system during development: an immunohistochemical study. *Brain Res* **814**, 13-25, doi:10.1016/s0006-8993(98)00830-0 (1998).

19 Chitu, V., Gokhan, S., Nandi, S., Mehler, M. F. & Stanley, E. R. Emerging Roles for CSF-1 Receptor and its Ligands in the Nervous System. *Trends Neurosci* **39**, 378-393, doi:10.1016/j.tins.2016.03.005 (2016).

20 Easley-Neal, C., Foreman, O., Sharma, N., Zarrin, A. A. & Weimer, R. M. CSF1R Ligands IL-34 and CSF1 Are Differentially Required for Microglia Development and Maintenance in White and Gray Matter Brain Regions. *Front Immunol* **10**, 2199, doi:10.3389/fimmu.2019.02199 (2019).

21 Ajami, B., Bennett, J. L., Krieger, C., Tetzlaff, W. & Rossi, F. M. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci* **10**, 1538-1543, doi:10.1038/nn2014 (2007).

22 Bruttger, J. *et al.* Genetic Cell Ablation Reveals Clusters of Local Self-Renewing Microglia in the Mammalian Central Nervous System. *Immunity* **43**, 92-106, doi:10.1016/j.immuni.2015.06.012 (2015).

23 Huang, Y. *et al.* Dual extra-retinal origins of microglia in the model of retinal microglia repopulation. *Cell Discov* **4**, 9, doi:10.1038/s41421-018-0011-8 (2018).

24 Huang, Y. *et al.* Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion. *Nat Neurosci* **21**, 530-540, doi:10.1038/s41593-018-0090-8 (2018).

25 Zhan, L. *et al.* Proximal recolonization by self-renewing microglia re-establishes microglial homeostasis in the adult mouse brain. *PLoS Biol* **17**, e3000134, doi:10.1371/journal.pbio.3000134 (2019).

26 Cronk, J. C. *et al.* Peripherally derived macrophages can engraft the brain independent of irradiation and maintain an identity distinct from microglia. *J Exp Med* **215**, 1627-1647, doi:10.1084/jem.20180247 (2018).

27 Priller, J. *et al.* Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nat Med* **7**, 1356-1361, doi:10.1038/nm1201-1356 (2001).

28 Xu, Z. *et al.* Efficient Strategies for Microglia Replacement in the Central Nervous System. *Cell Rep* **32**, 108041, doi:10.1016/j.celrep.2020.108041 (2020).

29 Xu, Z., Zhou, X., Peng, B. & Rao, Y. Microglia replacement by bone marrow transplantation (Mr BMT) in the central nervous system of adult mice. *STAR Protoc* **2**, 100666, doi:10.1016/j.xpro.2021.100666 (2021).

30 Xu, Z., Rao, Y. & Peng, B. Protocol for microglia replacement by peripheral blood (Mr PB). *STAR Protoc* **2**, 100613, doi:10.1016/j.xpro.2021.100613 (2021).

31 Xu, R. *et al.* Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain. *Nat Commun* **11**, 1577, doi:10.1038/s41467-020-15411-9 (2020).

32 Hasselmann, J. *et al.* Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. *Neuron* **103**, 1016-1033 e1010, doi:10.1016/j.neuron.2019.07.002 (2019).

33 Mancuso, R. *et al.* Stem-cell-derived human microglia transplanted in mouse brain to study human disease. *Nat Neurosci* **22**, 2111-2116, doi:10.1038/s41593-019-0525-x (2019).

34 Grabert, K. *et al.* A Transgenic Line That Reports CSF1R Protein Expression Provides a Definitive Marker for the Mouse Mononuclear Phagocyte System. *J Immunol* **205**, 3154-3166, doi:10.4049/jimmunol.2000835 (2020).

35 Kaiser, T. & Feng, G. Tmem119-EGFP and Tmem119-CreERT2 Transgenic Mice for Labeling and Manipulating Microglia. *eNeuro* **6**, doi:10.1523/ENEURO.0448-18.2019 (2019).

36 Chappell-Maor, L. *et al.* Comparative analysis of CreER transgenic mice for the study of brain macrophages: A case study. *Eur J Immunol* **50**, 353-362, doi:10.1002/eji.201948342 (2020).

37 McKinsey, G. L. *et al.* A new genetic strategy for targeting microglia in development and disease. *Elife* **9**, doi:10.7554/eLife.54590 (2020).

38 Masuda, T. *et al.* Novel Hexb-based tools for studying microglia in the CNS. *Nat Immunol* **21**, 802-815, doi:10.1038/s41590-020-0707-4 (2020).

39 Parkhurst, C. N. *et al.* Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* **155**, 1596-1609, doi:10.1016/j.cell.2013.11.030 (2013).

40 Yona, S. *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79-91, doi:10.1016/j.immuni.2012.12.001 (2013).

41 Kim, J. S. *et al.* A Binary Cre Transgenic Approach Dissects Microglia and CNS Border-Associated Macrophages. *Immunity* **54**, 176-190 e177, doi:10.1016/j.immuni.2020.11.007 (2021).
42 Goldmann, T. *et al.* Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat Immunol* **17**, 797-805, doi:10.1038/ni.3423 (2016).
43 Van Hove, H. *et al.* A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat Neurosci* **22**, 1021-1035, doi:10.1038/s41593-019-0393-4 (2019).
44 Masuda, T. *et al.* Specification of CNS macrophage subsets occurs postnatally in defined niches. *Nature* **604**, 740-748, doi:10.1038/s41586-022-04596-2 (2022).
45 Paolicelli, R. C. & Ferretti, M. T. Function and Dysfunction of Microglia during Brain Development: Consequences for Synapses and Neural Circuits. *Front Synaptic Neurosci* **9**, 9, doi:10.3389/fnsyn.2017.00009 (2017).
46 Green, K. N., Crapser, J. D. & Hohsfield, L. A. To Kill a Microglia: A Case for CSF1R Inhibitors. *Trends Immunol* **41**, 771-784, doi:10.1016/j.it.2020.07.001 (2020).
47 Chitu, V., Gokhan, S. & Stanley, E. R. Modeling CSF-1 receptor deficiency diseases - how close are we? *FEBS J*, doi:10.1111/febs.16085 (2021).
48 Oosterhof, N. *et al.* Homozygous Mutations in CSF1R Cause a Pediatric-Onset Leukoencephalopathy and Can Result in Congenital Absence of Microglia. *Am J Hum Genet* **104**, 936-947, doi:10.1016/j.ajhg.2019.03.010 (2019).
49 Guo, L. *et al.* Bi-allelic CSF1R Mutations Cause Skeletal Dysplasia of Dysosteosclerosis-Pyle Disease Spectrum and Degenerative Encephalopathy with Brain Malformation. *Am J Hum Genet* **104**, 925-935, doi:10.1016/j.ajhg.2019.03.004 (2019).
50 Rojo, R. *et al.* Deletion of a Csf1r enhancer selectively impacts CSF1R expression and development of tissue macrophage populations. *Nat Commun* **10**, 3215, doi:10.1038/s41467-019-11053-8 (2019).
51 Kiani Shabestari, S. *et al.* Absence of microglia promotes diverse pathologies and early lethality in Alzheimer's disease mice. *Cell Rep* **39**, 110961, doi:10.1016/j.celrep.2022.110961 (2022).
52 Konishi, H. *et al.* Astrocytic phagocytosis is a compensatory mechanism for microglial dysfunction. *EMBO J* **39**, e104464, doi:10.15252/embj.2020104464 (2020).
53 Hickman, S. E. *et al.* The microglial sensome revealed by direct RNA sequencing. *Nat Neurosci* **16**, 1896-1905, doi:10.1038/nn.3554 (2013).
54 Hume, D. A., Perry, V. H. & Gordon, S. Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *J Cell Biol* **97**, 253-257, doi:10.1083/jcb.97.1.253 (1983).
55 Davalos, D. *et al.* ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* **8**, 752-758, doi:10.1038/nn1472 (2005).
56 Nimmerjahn, A., Kirchhoff, F. & Helmchen, F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* **308**, 1314-1318, doi:10.1126/science.1110647 (2005).
57 Wang, J., Zhang, K., Xu, L. & Wang, E. Quantifying the Waddington landscape and biological paths for development and differentiation. *Proc Natl Acad Sci U S A* **108**, 8257-8262, doi:10.1073/pnas.1017017108 (2011).
58 Keren-Shaul, H. *et al.* A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* **169**, 1276-1290 e1217, doi:10.1016/j.cell.2017.05.018 (2017).
59 Krasemann, S. *et al.* The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* **47**, 566-581 e569, doi:10.1016/j.immuni.2017.08.008 (2017).
60 Sala Frigerio, C. *et al.* The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Abeta Plaques. *Cell Rep* **27**, 1293-1306 e1296, doi:10.1016/j.celrep.2019.03.099 (2019).

61 Srinivasan, K. *et al.* Alzheimer's Patient Microglia Exhibit Enhanced Aging and Unique Transcriptional Activation. *Cell Rep* **31**, 107843, doi:10.1016/j.celrep.2020.107843 (2020).
62 Absinta, M. *et al.* A lymphocyte-microglia-astrocyte axis in chronic active multiple sclerosis. *Nature* **597**, 709-714, doi:10.1038/s41586-021-03892-7 (2021).
63 Marschallinger, J. *et al.* Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nat Neurosci* **23**, 194-208, doi:10.1038/s41593-019-0566-1 (2020).
64 De Andrade Costa, A. *et al.* RNA sequence analysis reveals ITGAL/CD11A as a stromal regulator of murine low-grade glioma growth. *Neuro Oncol* **24**, 14-26, doi:10.1093/neuonc/noab130 (2022).
65 Francesco Limone *et al.* Single-nucleus sequencing reveals enriched expression of genetic risk factors sensitises Motor Neurons to degeneration in ALS. *bioRxiv* (2021).
66 Smajic, S. *et al.* Single-cell sequencing of human midbrain reveals glial activation and a Parkinson-specific neuronal state. *Brain* **145**, 964-978, doi:10.1093/brain/awab446 (2022).
67 Safaiyan, S. *et al.* White matter aging drives microglial diversity. *Neuron* **109**, 1100-1117 e1110, doi:10.1016/j.neuron.2021.01.027 (2021).
68 Hammond, T. R. *et al.* Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* **50**, 253-271 e256, doi:10.1016/j.immuni.2018.11.004 (2019).
69 Li, Q. *et al.* Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. *Neuron* **101**, 207-223 e210, doi:10.1016/j.neuron.2018.12.006 (2019).
70 Kracht, L. *et al.* Human fetal microglia acquire homeostatic immune-sensing properties early in development. *Science* **369**, 530-537, doi:10.1126/science.aba5906 (2020).
71 Wu, Y. E., Pan, L., Zuo, Y., Li, X. & Hong, W. Detecting Activated Cell Populations Using Single-Cell RNA-Seq. *Neuron* **96**, 313-329 e316, doi:10.1016/j.neuron.2017.09.026 (2017).
72 Marsh, S. E. *et al.* Single cell sequencing reveals glial specific responses to tssue processing & enzymatic dissociation in mice and humans. *BioRxiv* (2021).
73 Mattei, D. *et al.* Enzymatic Dissociation Induces Transcriptional and Proteotype Bias in Brain Cell Populations. *Int J Mol Sci* **21**, doi:10.3390/ijms21217944 (2020).
74 Summers, K. M., Bush, S. J. & Hume, D. A. Network analysis of transcriptomic diversity amongst resident tissue macrophages and dendritic cells in the mouse mononuclear phagocyte system. *PLoS Biol* **18**, e3000859, doi:10.1371/journal.pbio.3000859 (2020).
75 Gosselin, D. *et al.* An environment-dependent transcriptional network specifies human microglia identity. *Science* **356**, doi:10.1126/science.aal3222 (2017).
76 Geirsdottir, L. *et al.* Cross-Species Single-Cell Analysis Reveals Divergence of the Primate Microglia Program. *Cell* **179**, 1609-1622 e1616, doi:10.1016/j.cell.2019.11.010 (2019).
77 Kolodziejczyk, A. A., Kim, J. K., Svensson, V., Marioni, J. C. & Teichmann, S. A. The technology and biology of single-cell RNA sequencing. *Mol Cell* **58**, 610-620, doi:10.1016/j.molcel.2015.04.005 (2015).
78 Welch, J. D. *et al.* Single-Cell Multi-omic Integration Compares and Contrasts Features of Brain Cell Identity. *Cell* **177**, 1873-1887 e1817, doi:10.1016/j.cell.2019.05.006 (2019).
79 Stuart, T. *et al.* Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902 e1821, doi:10.1016/j.cell.2019.05.031 (2019).
80 Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J. & Smith, V. A. Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci Rep* **5**, 10775, doi:10.1038/srep10775 (2015).

81 Fernandez-Zapata, C., Leman, J. K. H., Priller, J. & Bottcher, C. The use and limitations of single-cell mass cytometry for studying human microglia function. *Brain Pathol* **30**, 1178-1191, doi:10.1111/bpa.12909 (2020).
82 Ajami, B. *et al.* Single-cell mass cytometry reveals distinct populations of brain myeloid cells in mouse neuroinflammation and neurodegeneration models. *Nat Neurosci* **21**, 541-551, doi:10.1038/s41593-018-0100-x (2018).
83 Bottcher, C. *et al.* Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci* **22**, 78-90, doi:10.1038/s41593-018-0290-2 (2019).
84 Tay, T. L. *et al.* A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat Neurosci* **20**, 793-803, doi:10.1038/nn.4547 (2017).
85 McQuade, A. *et al.* Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. *Nat Commun* **11**, 5370, doi:10.1038/s41467-020-19227-5 (2020).
86 Mazaheri, F. *et al.* TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. *EMBO Rep* **18**, 1186-1198, doi:10.15252/embr.201743922 (2017).
87 Erny, D. *et al.* Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* **18**, 965-977, doi:10.1038/nn.4030 (2015).
88 Thion, M. S. *et al.* Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell* **172**, 500-516 e516, doi:10.1016/j.cell.2017.11.042 (2018).
89 Chatterjee, J. *et al.* Asthma reduces glioma formation by T cell decorin-mediated inhibition of microglia. *Nat Commun* **12**, 7122, doi:10.1038/s41467-021-27455-6 (2021).
90 Matcovitch-Natan, O. *et al.* Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **353**, aad8670, doi:10.1126/science.aad8670 (2016).
91 Masuda, T. *et al.* Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* **566**, 388-392, doi:10.1038/s41586-019-0924-x (2019).
92 Grabert, K. *et al.* Microglial brain region-dependent diversity and selective regional sensitivities to aging. *Nat Neurosci* **19**, 504-516, doi:10.1038/nn.4222 (2016).
93 Kana, V. *et al.* CSF-1 controls cerebellar microglia and is required for motor function and social interaction. *J Exp Med* **216**, 2265-2281, doi:10.1084/jem.20182037 (2019).
94 Hanamsagar, R. *et al.* Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia* **65**, 1504-1520, doi:10.1002/glia.23176 (2017).
95 Guneykaya, D. *et al.* Transcriptional and Translational Differences of Microglia from Male and Female Brains. *Cell Rep* **24**, 2773-2783 e2776, doi:10.1016/j.celrep.2018.08.001 (2018).
96 Villa, A. *et al.* Sex-Specific Features of Microglia from Adult Mice. *Cell Rep* **23**, 3501-3511, doi:10.1016/j.celrep.2018.05.048 (2018).
97 Lynch, M. A. Exploring Sex-Related Differences in Microglia May Be a Game-Changer in Precision Medicine. *Front Aging Neurosci* **14**, 868448, doi:10.3389/fnagi.2022.868448 (2022).
98 Halievski, K., Ghazisaeidi, S. & Salter, M. W. Sex-Dependent Mechanisms of Chronic Pain: A Focus on Microglia and P2X4R. *J Pharmacol Exp Ther* **375**, 202-209, doi:10.1124/jpet.120.265017 (2020).
99 Han, J., Fan, Y., Zhou, K., Blomgren, K. & Harris, R. A. Uncovering sex differences of rodent microglia. *J Neuroinflammation* **18**, 74, doi:10.1186/s12974-021-02124-z (2021).
100 De Biase, L. M. *et al.* Local Cues Establish and Maintain Region-Specific Phenotypes of Basal Ganglia Microglia. *Neuron* **95**, 341-356 e346, doi:10.1016/j.neuron.2017.06.020 (2017).
101 Ayata, P. *et al.* Epigenetic regulation of brain region-specific microglia clearance activity. *Nat Neurosci* **21**, 1049-1060, doi:10.1038/s41593-018-0192-3 (2018).

102 Bennett, F. C. *et al.* A Combination of Ontogeny and CNS Environment Establishes Microglial Identity. *Neuron* **98**, 1170-1183 e1178, doi:10.1016/j.neuron.2018.05.014 (2018).

103 Shemer, A. *et al.* Engrafted parenchymal brain macrophages differ from microglia in transcriptome, chromatin landscape and response to challenge. *Nat Commun* **9**, 5206, doi:10.1038/s41467-018-07548-5 (2018).

104 Abdel-Haq, R., Schlachetzki, J. C. M., Glass, C. K. & Mazmanian, S. K. Microbiome-microglia connections via the gut-brain axis. *J Exp Med* **216**, 41-59, doi:10.1084/jem.20180794 (2019).

105 Erny, D. *et al.* Microbiota-derived acetate enables the metabolic fitness of the brain innate immune system during health and disease. *Cell Metab* **33**, 2260-2276 e2267, doi:10.1016/j.cmet.2021.10.010 (2021).

106 Dantzer, R. Cytokine, sickness behavior, and depression. *Immunol Allergy Clin North Am* **29**, 247-264, doi:10.1016/j.iac.2009.02.002 (2009).

107 Shemer, A. *et al.* Interleukin-10 Prevents Pathological Microglia Hyperactivation following Peripheral Endotoxin Challenge. *Immunity* **53**, 1033-1049 e1037, doi:10.1016/j.immuni.2020.09.018 (2020).

108 Sousa, C. *et al.* Single-cell transcriptomics reveals distinct inflammation-induced microglia signatures. *EMBO Rep* **19**, doi:10.15252/embr.201846171 (2018).

109 Cunningham, C., Wilcockson, D. C., Campion, S., Lunnon, K. & Perry, V. H. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci* **25**, 9275-9284, doi:10.1523/JNEUROSCI.2614-05.2005 (2005).

110 Louveau, A., Harris, T. H. & Kipnis, J. Revisiting the Mechanisms of CNS Immune Privilege. *Trends Immunol* **36**, 569-577, doi:10.1016/j.it.2015.08.006 (2015).

111 Pasciuto, E. *et al.* Microglia Require CD4 T Cells to Complete the Fetal-to-Adult Transition. *Cell* **182**, 625-640 e624, doi:10.1016/j.cell.2020.06.026 (2020).

112 Dong, Y. & Yong, V. W. When encephalitogenic T cells collaborate with microglia in multiple sclerosis. *Nat Rev Neurol* **15**, 704-717, doi:10.1038/s41582-019-0253-6 (2019).

113 Beers, D. R., Henkel, J. S., Zhao, W., Wang, J. & Appel, S. H. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc Natl Acad Sci U S A* **105**, 15558-15563, doi:10.1073/pnas.0807419105 (2008).

114 Mittal, K. *et al.* CD4 T Cells Induce A Subset of MHCII-Expressing Microglia that Attenuates Alzheimer Pathology. *iScience* **16**, 298-311, doi:10.1016/j.isci.2019.05.039 (2019).

115 Di Liberto, G. *et al.* Neurons under T Cell Attack Coordinate Phagocyte-Mediated Synaptic Stripping. *Cell* **175**, 458-471 e419, doi:10.1016/j.cell.2018.07.049 (2018).

116 Chen, Y. & Colonna, M. Microglia in Alzheimer's disease at single-cell level. Are there common patterns in humans and mice? *J Exp Med* **218**, doi:10.1084/jem.20202717 (2021).

117 Avignone, E., Ulmann, L., Levavasseur, F., Rassendren, F. & Audinat, E. Status epilepticus induces a particular microglial activation state characterized by enhanced purinergic signaling. *J Neurosci* **28**, 9133-9144, doi:10.1523/JNEUROSCI.1820-08.2008 (2008).

118 Zrzavy, T. *et al.* Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* **140**, 1900-1913, doi:10.1093/brain/awx113 (2017).

119 Gerrits, E. *et al.* Distinct amyloid-beta and tau-associated microglia profiles in Alzheimer's disease. *Acta Neuropathol* **141**, 681-696, doi:10.1007/s00401-021-02263-w (2021).

120 Silvin, A. *et al.* Dual ontogeny of disease-associated microglia and disease inflammatory macrophages in aging and neurodegeneration. *Immunity* **55**, 1448-1465 e1446, doi:10.1016/j.immuni.2022.07.004 (2022).

121 Zhou, Y. *et al.* Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med* **26**, 131-142, doi:10.1038/s41591-019-0695-9 (2020).
122 Ulland, T. K. *et al.* TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. *Cell* **170**, 649-663 e613, doi:10.1016/j.cell.2017.07.023 (2017).
123 Xiang, X. *et al.* Microglial activation states drive glucose uptake and FDG-PET alterations in neurodegenerative diseases. *Sci Transl Med* **13**, eabe5640, doi:10.1126/scitranslmed.abe5640 (2021).
124 Ma, S. *et al.* Chromatin Potential Identified by Shared Single-Cell Profiling of RNA and Chromatin. *Cell* **183**, 1103-1116 e1120, doi:10.1016/j.cell.2020.09.056 (2020).
125 Buenrostro, J. D., Wu, B., Chang, H. Y. & Greenleaf, W. J. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol* **109**, 21 29 21-21 29 29, doi:10.1002/0471142727.mb2129s109 (2015).
126 van Galen, P. *et al.* A Multiplexed System for Quantitative Comparisons of Chromatin Landscapes. *Mol Cell* **61**, 170-180, doi:10.1016/j.molcel.2015.11.003 (2016).
127 Bartosovic, M., Kabbe, M. & Castelo-Branco, G. Single-cell CUT&Tag profiles histone modifications and transcription factors in complex tissues. *Nat Biotechnol*, doi:10.1038/s41587-021-00869-9 (2021).
128 Schaafsma, W. *et al.* Long-lasting pro-inflammatory suppression of microglia by LPS-preconditioning is mediated by RelB-dependent epigenetic silencing. *Brain Behav Immun* **48**, 205-221, doi:10.1016/j.bbi.2015.03.013 (2015).
129 Wendeln, A. C. *et al.* Innate immune memory in the brain shapes neurological disease hallmarks. *Nature* **556**, 332-338, doi:10.1038/s41586-018-0023-4 (2018).
130 Chiu, I. M. *et al.* A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep* **4**, 385-401, doi:10.1016/j.celrep.2013.06.018 (2013).
131 Sobue, A. *et al.* Microglial gene signature reveals loss of homeostatic microglia associated with neurodegeneration of Alzheimer's disease. *Acta Neuropathol Commun* **9**, 1, doi:10.1186/s40478-020-01099-x (2021).
132 Jordao, M. J. C. *et al.* Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science* **363**, doi:10.1126/science.aat7554 (2019).
133 Olah, M. *et al.* Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Commun* **11**, 6129, doi:10.1038/s41467-020-19737-2 (2020).
134 Kurtz, A. *et al.* A Standard Nomenclature for Referencing and Authentication of Pluripotent Stem Cells. *Stem Cell Reports* **10**, 1-6, doi:10.1016/j.stemcr.2017.12.002 (2018).
135 Luecken, M. D. & Theis, F. J. Current best practices in single-cell RNA-seq analysis: a tutorial. *Mol Syst Biol* **15**, e8746, doi:10.15252/msb.20188746 (2019).
136 Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**, 611-622, doi:10.1373/clinchem.2008.112797 (2009).
137 d, M. G. & Huggett, J. F. The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. *Clin Chem* **66**, 1012-1029, doi:10.1093/clinchem/hvaa125 (2020).
138 Ingolia, N. T., Brar, G. A., Rouskin, S., McGeachy, A. M. & Weissman, J. S. The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. *Nat Protoc* **7**, 1534-1550, doi:10.1038/nprot.2012.086 (2012).
139 Mayor-Ruiz, C., Dominguez, O. & Fernandez-Capetillo, O. Trap(Seq): An RNA Sequencing-Based Pipeline for the Identification of Gene-Trap Insertions in Mammalian Cells. *J Mol Biol* **429**, 2780-2789, doi:10.1016/j.jmb.2017.07.020 (2017).
140 Rautenstrauch, P., Vlot, A. H. C., Saran, S. & Ohler, U. Intricacies of single-cell multi-omics data integration. *Trends Genet* **38**, 128-139, doi:10.1016/j.tig.2021.08.012 (2022).

141 Paczkowska, M. *et al.* Integrative pathway enrichment analysis of multivariate omics data. *Nat Commun* **11**, 735, doi:10.1038/s41467-019-13983-9 (2020).
142 Reel, P. S., Reel, S., Pearson, E., Trucco, E. & Jefferson, E. Using machine learning approaches for multi-omics data analysis: A review. *Biotechnol Adv* **49**, 107739, doi:10.1016/j.biotechadv.2021.107739 (2021).
143 Kunkle, B. W. *et al.* Novel Alzheimer Disease Risk Loci and Pathways in African American Individuals Using the African Genome Resources Panel: A Meta-analysis. *JAMA Neurol* **78**, 102-113, doi:10.1001/jamaneurol.2020.3536 (2021).
144 Río-Hortega, P. d. R. Histogenesis and normal evolution: exodus and regional distribution of microglia. *Memorias de la Real Sociedad Española de Historia Natural* **11:213-268** (1921).
145 Sierra, A. *et al.* The "Big-Bang" for modern glial biology: Translation and comments on Pio del Rio-Hortega 1919 series of papers on microglia. *Glia* **64**, 1801-1840, doi:10.1002/glia.23046 (2016).
146 Streit, W. J., Graeber, M. B. & Kreutzberg, G. W. Functional plasticity of microglia: a review. *Glia* **1**, 301-307, doi:10.1002/glia.440010502 (1988).
147 Acarin, L., Vela, J. M., Gonzalez, B. & Castellano, B. Demonstration of poly-N-acetyl lactosamine residues in ameboid and ramified microglial cells in rat brain by tomato lectin binding. *J Histochem Cytochem* **42**, 1033-1041, doi:10.1177/42.8.8027523 (1994).
148 Castellano, B. *et al.* A double staining technique for simultaneous demonstration of astrocytes and microglia in brain sections and astroglial cell cultures. *J Histochem Cytochem* **39**, 561-568, doi:10.1177/39.5.1707903 (1991).
149 Kitamura, T., Miyake, T. & Fujita, S. Genesis of resting microglia in the gray matter of mouse hippocampus. *J Comp Neurol* **226**, 421-433, doi:10.1002/cne.902260310 (1984).
150 Tremblay, M. E., Lecours, C., Samson, L., Sanchez-Zafra, V. & Sierra, A. From the Cajal alumni Achucarro and Rio-Hortega to the rediscovery of never-resting microglia. *Front Neuroanat* **9**, 45, doi:10.3389/fnana.2015.00045 (2015).
151 Tremblay, M. E. The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies. *Neuron Glia Biol* **7**, 67-76, doi:10.1017/S1740925X12000038 (2011).
152 Hanisch, U. K. & Kettenmann, H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* **10**, 1387-1394, doi:10.1038/nn1997 (2007).
153 Tremblay, M. E., Madore, C., Bordeleau, M., Tian, L. & Verkhratsky, A. Neuropathobiology of COVID-19: The Role for Glia. *Front Cell Neurosci* **14**, 592214, doi:10.3389/fncel.2020.592214 (2020).
154 Sierra, A., Tremblay, M. E. & Wake, H. Never-resting microglia: physiological roles in the healthy brain and pathological implications. *Front Cell Neurosci* **8**, 240, doi:10.3389/fncel.2014.00240 (2014).
155 Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E. & Heuschling, P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *J Neuroimmunol* **210**, 3-12, doi:10.1016/j.jneuroim.2009.02.003 (2009).
156 Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J. & Hill, A. M. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* **164**, 6166-6173, doi:10.4049/jimmunol.164.12.6166 (2000).
157 Butovsky, O. *et al.* Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nat Neurosci* **17**, 131-143, doi:10.1038/nn.3599 (2014).
158 Martinez, F. O. & Gordon, S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* **6**, 13, doi:10.12703/P6-13 (2014).
159 Ransohoff, R. M. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci* **19**, 987-991, doi:10.1038/nn.4338 (2016).

160 Devanney, N. A., Stewart, A. N. & Gensel, J. C. Microglia and macrophage metabolism in CNS injury and disease: The role of immunometabolism in neurodegeneration and neurotrauma. *Exp Neurol* **329**, 113310, doi:10.1016/j.expneurol.2020.113310 (2020).
161 Madry, C. *et al.* Microglial Ramification, Surveillance, and Interleukin-1beta Release Are Regulated by the Two-Pore Domain K(+) Channel THIK-1. *Neuron* **97**, 299-312 e296, doi:10.1016/j.neuron.2017.12.002 (2018).
162 Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483-495, doi:10.1016/j.stem.2010.08.014 (2010).
163 VanRyzin, J. W. *et al.* Microglial Phagocytosis of Newborn Cells Is Induced by Endocannabinoids and Sculpts Sex Differences in Juvenile Rat Social Play. *Neuron* **102**, 435-449 e436, doi:10.1016/j.neuron.2019.02.006 (2019).
164 Abiega, O. *et al.* Neuronal Hyperactivity Disturbs ATP Microgradients, Impairs Microglial Motility, and Reduces Phagocytic Receptor Expression Triggering Apoptosis/Microglial Phagocytosis Uncoupling. *PLoS Biol* **14**, e1002466, doi:10.1371/journal.pbio.1002466 (2016).
165 Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G. & Mechawar, N. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. *Brain Behav Immun* **42**, 50-59, doi:10.1016/j.bbi.2014.05.007 (2014).
166 Bachstetter, A. D. *et al.* Rod-shaped microglia morphology is associated with aging in 2 human autopsy series. *Neurobiol Aging* **52**, 98-105, doi:10.1016/j.neurobiolaging.2016.12.028 (2017).
167 Streit, W. J., Sammons, N. W., Kuhns, A. J. & Sparks, D. L. Dystrophic microglia in the aging human brain. *Glia* **45**, 208-212, doi:10.1002/glia.10319 (2004).
168 Tischer, J. *et al.* Inhomogeneous distribution of Iba-1 characterizes microglial pathology in Alzheimer's disease. *Glia* **64**, 1562-1572, doi:10.1002/glia.23024 (2016).
169 Savage, J. C., Carrier, M. & Tremblay, M. E. Morphology of Microglia Across Contexts of Health and Disease. *Methods Mol Biol* **2034**, 13-26, doi:10.1007/978-1-4939-9658-2_2 (2019).
170 Salamanca, L. *et al.* MIC-MAC: An automated pipeline for high-throughput characterization and classification of three-dimensional microglia morphologies in mouse and human postmortem brain samples. *Glia* **67**, 1496-1509, doi:10.1002/glia.23623 (2019).
171 Stratoulias, V., Venero, J. L., Tremblay, M. E. & Joseph, B. Microglial subtypes: diversity within the microglial community. *EMBO J* **38**, e101997, doi:10.15252/embj.2019101997 (2019).
172 St-Pierre, M. K. *et al.* Ultrastructural characterization of dark microglia during aging in a mouse model of Alzheimer's disease pathology and in human post-mortem brain samples. *J Neuroinflammation* **19**, 235, doi:10.1186/s12974-022-02595-8 (2022).
173 Colombo, G. *et al.* Microglial MorphOMICs unravel region- and sex-dependent morphological phenotypes from postnatal development to degeneration. *bioRxiv*, 2021.2011.2030.470610, doi:10.1101/2021.11.30.470610 (2021).
174 Graeber, M. B. Changing face of microglia. *Science* **330**, 783-788, doi:10.1126/science.1190929 (2010).
175 Lawson, L. J., Perry, V. H., Dri, P. & Gordon, S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151-170, doi:10.1016/0306-4522(90)90229-w (1990).
176 Gautier, E. L. *et al.* Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* **13**, 1118-1128, doi:10.1038/ni.2419 (2012).
177 Waddell, L. A. *et al.* ADGRE1 (EMR1, F4/80) Is a Rapidly-Evolving Gene Expressed in Mammalian Monocyte-Macrophages. *Front Immunol* **9**, 2246, doi:10.3389/fimmu.2018.02246 (2018).

178 Jung, S. *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* **20**, 4106-4114, doi:10.1128/mcb.20.11.4106-4114.2000 (2000).
179 Wolf, Y., Yona, S., Kim, K. W. & Jung, S. Microglia, seen from the CX3CR1 angle. *Front Cell Neurosci* **7**, 26, doi:10.3389/fncel.2013.00026 (2013).
180 Bisht, K. *et al.* Dark microglia: A new phenotype predominantly associated with pathological states. *Glia* **64**, 826-839, doi:10.1002/glia.22966 (2016).
181 Imai, Y., Ibata, I., Ito, D., Ohsawa, K. & Kohsaka, S. A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. *Biochem Biophys Res Commun* **224**, 855-862, doi:10.1006/bbrc.1996.1112 (1996).
182 Ito, D. *et al.* Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res* **57**, 1-9, doi:10.1016/s0169-328x(98)00040-0 (1998).
183 Shapiro, L. A., Perez, Z. D., Foresti, M. L., Arisi, G. M. & Ribak, C. E. Morphological and ultrastructural features of Iba1-immunolabeled microglial cells in the hippocampal dentate gyrus. *Brain Res* **1266**, 29-36, doi:10.1016/j.brainres.2009.02.031 (2009).
184 Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S. & Nabekura, J. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci* **29**, 3974-3980, doi:10.1523/JNEUROSCI.4363-08.2009 (2009).
185 Tremblay, M. E., Lowery, R. L. & Majewska, A. K. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol* **8**, e1000527, doi:10.1371/journal.pbio.1000527 (2010).
186 Lier, J. *et al.* Loss of IBA1-Expression in brains from individuals with obesity and hepatic dysfunction. *Brain Res* **1710**, 220-229, doi:10.1016/j.brainres.2019.01.006 (2019).
187 Fourgeaud, L. *et al.* TAM receptors regulate multiple features of microglial physiology. *Nature* **532**, 240-244, doi:10.1038/nature17630 (2016).
188 Savage, J. C. *et al.* Nuclear receptors license phagocytosis by trem2+ myeloid cells in mouse models of Alzheimer's disease. *J Neurosci* **35**, 6532-6543, doi:10.1523/JNEUROSCI.4586-14.2015 (2015).
189 Healy, L. M. *et al.* MerTK Is a Functional Regulator of Myelin Phagocytosis by Human Myeloid Cells. *J Immunol* **196**, 3375-3384, doi:10.4049/jimmunol.1502562 (2016).
190 Huang, Y. *et al.* Microglia use TAM receptors to detect and engulf amyloid beta plaques. *Nat Immunol* **22**, 586-594, doi:10.1038/s41590-021-00913-5 (2021).
191 Robinson, A. P., White, T. M. & Mason, D. W. Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology* **57**, 239-247 (1986).
192 Milligan, C. E., Cunningham, T. J. & Levitt, P. Differential immunochemical markers reveal the normal distribution of brain macrophages and microglia in the developing rat brain. *J Comp Neurol* **314**, 125-135, doi:10.1002/cne.903140112 (1991).
193 McKay, S. M., Brooks, D. J., Hu, P. & McLachlan, E. M. Distinct types of microglial activation in white and grey matter of rat lumbosacral cord after mid-thoracic spinal transection. *J Neuropathol Exp Neurol* **66**, 698-710, doi:10.1097/nen.0b013e3181256b32 (2007).
194 Blackbeard, J. *et al.* Quantification of the rat spinal microglial response to peripheral nerve injury as revealed by immunohistochemical image analysis and flow cytometry. *J Neurosci Methods* **164**, 207-217, doi:10.1016/j.jneumeth.2007.04.013 (2007).
195 Marshall, S. A. *et al.* Microglial activation is not equivalent to neuroinflammation in alcohol-induced neurodegeneration: The importance of microglia phenotype. *Neurobiol Dis* **54**, 239-251, doi:10.1016/j.nbd.2012.12.016 (2013).
196 Peng, J. *et al.* Microglial P2Y12 receptor regulates ventral hippocampal CA1 neuronal excitability and innate fear in mice. *Mol Brain* **12**, 71, doi:10.1186/s13041-019-0492-x (2019).

197 Haynes, S. E. *et al.* The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat Neurosci* **9**, 1512-1519, doi:10.1038/nn1805 (2006).
198 Sipe, G. O. *et al.* Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nat Commun* **7**, 10905, doi:10.1038/ncomms10905 (2016).
199 Kanamoto, T. *et al.* Isolation and characterization of a novel plasma membrane protein, osteoblast induction factor (obif), associated with osteoblast differentiation. *BMC Dev Biol* **9**, 70, doi:10.1186/1471-213X-9-70 (2009).
200 Bennett, M. L. *et al.* New tools for studying microglia in the mouse and human CNS. *Proc Natl Acad Sci U S A* **113**, E1738-1746, doi:10.1073/pnas.1525528113 (2016).
201 Satoh, J. *et al.* TMEM119 marks a subset of microglia in the human brain. *Neuropathology* **36**, 39-49, doi:10.1111/neup.12235 (2016).
202 van Wageningen, T. A. *et al.* Regulation of microglial TMEM119 and P2RY12 immunoreactivity in multiple sclerosis white and grey matter lesions is dependent on their inflammatory environment. *Acta Neuropathol Commun* **7**, 206, doi:10.1186/s40478-019-0850-z (2019).
203 Gonzalez Ibanez, F. *et al.* Immunofluorescence Staining Using IBA1 and TMEM119 for Microglial Density, Morphology and Peripheral Myeloid Cell Infiltration Analysis in Mouse Brain. *J Vis Exp*, doi:10.3791/60510 (2019).
204 Chertoff, M., Shrivastava, K., Gonzalez, B., Acarin, L. & Gimenez-Llort, L. Differential modulation of TREM2 protein during postnatal brain development in mice. *PLoS One* **8**, e72083, doi:10.1371/journal.pone.0072083 (2013).
205 Fahrenhold, M. *et al.* TREM2 expression in the human brain: a marker of monocyte recruitment? *Brain Pathol* **28**, 595-602, doi:10.1111/bpa.12564 (2018).
206 Rogers, J. T. *et al.* CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J Neurosci* **31**, 16241-16250, doi:10.1523/JNEUROSCI.3667-11.2011 (2011).
207 Paolicelli, R. C., Bisht, K. & Tremblay, M. E. Fractalkine regulation of microglial physiology and consequences on the brain and behavior. *Front Cell Neurosci* **8**, 129, doi:10.3389/fncel.2014.00129 (2014).
208 Hirasawa, T. *et al.* Visualization of microglia in living tissues using Iba1-EGFP transgenic mice. *J Neurosci Res* **81**, 357-362, doi:10.1002/jnr.20480 (2005).
209 Sasmono, R. T. *et al.* A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood* **101**, 1155-1163, doi:10.1182/blood-2002-02-0569 (2003).