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.

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

111   54. Unidad de Histología Medica, Depto. Biología Celular, Fisiología e Inmunología and

112   Instituto de Neurociencias, Universidad Autónoma de Barcelona, Barcelona, Spain

113   55. Sir William Dunn School of Pathology, Oxford, United Kingdom

114   56. Ken Parker Brain Tumour Research Laboratories, Brain and Mind Centre, Faculty of

115   Medicine and Health, The University of Sydney, Camperdown, Australia

116   57. Lydia Becker Institute of Immunology and Inflammation, Geoffrey Jefferson Brain

117   Research Centre, Division of Infection, Immunity & Respiratory Medicine, Faculty of Biology,

118   Medicine and Health, The University of Manchester, Manchester, United Kingdom

119   58. Université Paris Cité, Inserm, NeuroDiderot, F-75019 Paris, France

120   59. Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland

121   60. Department of Neurology, Washington University School of Medicine, St. Louis MO USA

122   61. Division of Metabolic Biochemistry, Faculty of Medicine, Biomedical Center (BMC),

123   Ludwig-Maximilians-Universität Munchen, Munich, Germany

124   62. German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

125   63. Munich Cluster for Systems Neurology (SyNergy); Munich, Germany

126   64. Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belvaux,

127   Luxembourg

128   65. Department of Neuropathology, Charité - Universitätsmedizin Berlin, Berlin, Germany

129   66. UK Dementia Research Institute at University College London, London, United Kingdom

130   67. Mater Research Institute-University of Queensland, Brisbane, Australia

131   68. Department of Immunology and Regenerative Biology, Weizmann Institute of Science,

132   Rehovot, Israel

133   69. Max-Delbrück Center for Molecular Medicine, Berlin, Germany

134   70. Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen,

135   China

136   71. Center for Brain Immunology and Glia (BIG), Department of Pathology and Immunology,

137   Washington University in St. Louis, St. Louis, MO, USA

138   72. Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The

139   University of Tokyo, Tokyo, Japan

140   73. MNL-L, The Salk Institute for Biological Studies, La Jolla, USA

141   74. Trinity College Institute of Neuroscience, Trinity College, Dublin, Republic of Ireland

142   75. Department of Neuroscience, University of Rochester, Rochester, New York, USA

143   76. Wolfson Centre for Age-Related Diseases, Institute of Psychiatry, Psychology and

144   Neuroscience, King's College London, London, United Kingdom

145   77. University of Eastern Finland, Kuopio, Finland

146   78. Microglia and Inflammation in Neurological Disorders (MIND) Lab, VIB Center for

147   Molecular Neurology, VIB, Antwerp, Belgium

148   79. Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

149   80. Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical

150   Sciences, Kyushu University, Japan

151   81. Humanitas University, Department of Biomedical Sciences, Milan, Italy

152   82. UK Dementia Research Institute, Centre for Discovery Brain Sciences, University of

153   Edinburgh, Edinburgh BioQuarter, Edinburgh, United Kingdom

154   83. MRC Centre for Reproductive Health, The Queen's Medical Research Institute, Edinburgh

155   BioQuarter, Edinburgh, United Kingdom

156   84. UK Dementia Research Institute at the University of Edinburgh, Edinburgh BioQuarter,

157   Edinburgh, United Kingdom

158   85. University of California, San Francisco, USA

159   86. Department of Neurology and Neurological Sciences, Stanford University School of

160   Medicine, Stanford University, Stanford, USA

161   87. Roche Innovation Center, Basel, Switzerland

162   88. Neurocentre Magendie, University of Bordeaux, Bordeaux, France

163   89. Institut Universitaire de France (IUF), France

164   90. German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany

165   91. Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University

166   of Tübingen, Tübingen, Germany

167   92. VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center,

168   Vilnius University, Vilnius, Lithuania

169   93. Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania

170   94. Institute of Reconstructive Neurobiology, Medical Faculty and University Hospital of Bonn,

171   University of Bonn, Bonn, Germany

172   95. Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu

173   University, Fukuoka, Japan

174   96. Institute of Mitochondrial Biology and Medicine of Xi'an Jiaotong University School of Life

175   Science and Technology, Xi'an, China

176   97. Department of Neurosurgery, Huashan Hospital, Institute for Translational Brain

177   Research, State Key Laboratory of Medical Neurobiology, MOE Frontiers Center for Brain

178   Science, Fudan University, Shanghai, China

179   98. Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland

180   99. UK Dementia Research Institute, University College London, London, United Kingdom

181   100. School of Biological Sciences, University of Southampton, Southampton, United

182   Kingdom

183   101 Department of Neuroscience, College of Medicine, The Ohio State University, Columbus,

184   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

222 128. Faculty of Biology, Medicine and Health, The University of Manchester, Manchester,
223 United Kingdom

224 129. Faculty of Medicine and Science, Universidad San Sebastian, Santiago, Chile

225 130. Department of Anatomy and Molecular Cell Biology, Graduate School of Medicine,
226 Nagoya University, Nagoya, Japan

227 131. Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM),
228 Université Libre de Bruxelles (ULB), Brussels, Belgium

229 132. ULB Institute of Neuroscience (UNI), Université Libre de Bruxelles (ULB), Brussels,
230 Belgium

231 133. Charité Universitätsmedizin, Experimental Ophthalmology and Neuroimmunology,
232 Berlin, Germany

233 134. Department of Neurology and Department of Immunology, Mayo Clinic, Rochester,
234 Minnesota, USA

235
236
237
238
239 *Co-corresponding authors:
240 rosachiara.paolicelli@unil.ch (R.C.P.)
241 amanda.sierra@ehu.eus (A.S.)
242 beth.stevens@childrens.harvard.edu (B.S.)
243 evetremblay@uvic.ca (M.E.T.)
244

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

319 balancing gender, geographical distribution, and seniority. Authors from the fields of
320 neuroscience, neurobiology, immunology, neuroimmunology, oncology, and neuropathology,
321 both from academia and industry, discussed their perspectives on the current and future
322 challenges in defining microglial states and nomenclature. A questionnaire (**Supplementary**
323 **Data**) was created to collect all the authors' opinions on several nomenclature issues and the
324 importance of directly addressing microglial function. The responses to the questionnaire, an
325 online meeting held in June 2021 and an open session held at the EMBO meeting Microglia
326 2021 were used as a backbone to develop this paper.
327
328 Herein, we summarize our current knowledge about the identity of microglia and discuss best
329 practices for how to define and study microglial state dynamics. We then outline "classical"
330 microglial nomenclatures, highlighting some of the key discoveries that led to the above
331 classifications and their limitations. We intentionally focus on citing studies related to the
332 nomenclature, rather than providing a comprehensive review of the history of microglial
333 research, as it has been done elsewhere[7,8]. We discuss the overall limitations and conclude
334 with recommendations for the proper usage of microglial nomenclature as research evolves,
335 provide a conceptual framework for discussing microglia, and offer perspectives on the future
336 questions, gaps in knowledge, and challenges to tackle as a field.
337
338 **Microglial identity: what we mean about when we talk about microglia**
339 The origin and identity of microglia was for many years a matter of debate. In the dim and
340 distant past, Ramón y Cajal´s disciple, Pío del Río-Hortega suggested that these cells were
341 of mesodermal origin[9]. However, over time, an ectodermal origin was also proposed[10],
342 sparking controversy until the 1980s. The mesodermal origin took solid hold later with the
343 advance of technical approaches revealing more similarities than differences with the
344 functions and features of macrophages. In 1999, microglia were reported to appear in the
345 brain rudiment as early as embryonic day E8 in mice, and proposed to originate from yolk sac
346 progenitors[11]. The recent combination of fate mapping studies and transplantation approaches
347 this debate, revealing key aspects of microglial identity and plasticity. In mice, unlike other
348 model organisms such as zebrafish[12,13], microglia are now considered to originate from a pool
349 of macrophages produced during primitive hematopoiesis in the yolk sac, which start invading
350 the neuroepithelium at E8.5[14-17]. In humans, microglial precursors invade the brain primordium
351 around 4.5 to 5.5 gestational weeks[18].
352
353 One key signaling pathway critical for microglial development and maintenance is the CSF1R
354 (colony stimulating factor receptor). Ligands of CSF1R that sustain this pathway include two
355 cytokines with different origins and primary sequences, but similar tridimensional structures

356   and binding to CSF1R: IL-34 and CSF1[19]. IL34 is produced by neurons, while CSF1 is
357   secreted primarily by oligodendrocytes and astrocytes. Accordingly, the two ligands have
358   distinct and non-overlapping functions in the establishment and maintenance of microglia
359   within the grey and white matter[20]. Microglia have the capacity for self-renewal in certain
360   contexts, allowing them to repopulate the central nervous system (CNS) within one week of
361   depletion, even when more than 99% of microglia are ablated with CSF1R antagonists[21,22] or
362   diphtheria toxin[22]. This process, termed "microglial repopulation" or "microglial self-renewal"[23-
363   25] is different from "microglia replacement" which, in contrast, occurs when endogenous
364   microglia are replaced by exogenous cells that can include bone marrow-derived myeloid
365   cells[26-29], peripheral blood cells[28,30], stem cell- or iPSC-derived peripheral blood cells[31], across
366   various experimental or pathological conditions[31-33].

367

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

371

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

387

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

393   microglia. These cells should be more accurately described as monocyte-derived microglia-

394   like or iPSC-derived microglia-like cells (iMGL cells).

395

396   As resident macrophages of the brain parenchyma, microglia participate in many critical CNS

397   functions ranging from glio-, vasculo- and neurogenesis to synaptic and myelination, through

398   their process motility, release of soluble factors, and capacity for phagocytosis (**Figure 2**).

399   These functions have been revealed using several constitutive and inducible knock-out

400   models for microglial-specific genes[45] and by microglial-depletion paradigms in animal

401   models[46], particularly rodents and zebrafish.

402

403   The key role of microglia in maintaining CNS health is also supported by the severe phenotype

404   displayed by patients lacking microglia due to loss-of-function CSFR1 mutations.

405   Heterozygous mutations, particularly in the kinase domain of *CSF1R* are associated with

406   ALSP (adult-onset leukoencephalopathy with axonal spheroids and pigmented glia,

407   OMIM:221820) characterized by reduced microglial numbers and white matter atrophy that

408   result in progressive cognitive and motor impairment, dementia, and early death[47].

409   Additionally, bi-allelic mutations are reported to cause complete absence of microglia with

410   developmental brain malformation, hydrocephalus, bony lesions, and early death[48,49]. This

411   phenotype, however, seems in apparent contradiction with the reported absence of gross

412   neurological abnormalities at birth observed in mice with genomic deletion of FIRE, an intra-

413   intronic super enhancer in the *Csfr1*gene enhancer region, whose brains lack microglia[50],

414   though more nuanced analyses are needed. Nonetheless, FIRE mice have premature lethality

415   and increased amyloid pathology as early as 5 months of age[51]. The source of discrepancy

416   between the developmental impact of CSFR1 mutations in humans and mice is not yet fully

417   understood. One possibility is that microglial developmental functions are partly redundant,

418   modified by other environmental factors, or compensated in their absence by other cell types,

419   such as astrocytes[52]. It will be important to determine how microglia communicate with other

420   glial cells and immune cell populations to support CNS maturation and function in the future.

421

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

423   Core markers of cellular identity are useful to identify microglia, but are not necessarily

424   informative about the functional "state" of microglia, which depends on the context (i.e., the

425   physiological conditions in which microglia are found at any given CNS region and time).

426   Microglia have a complex "sensome"[53], a series of surface receptors that allow them to detect

427   changes in their environment. Microglial states are thus dynamic, and the outcome of the cell´s

428   epigenome, transcriptome, proteome, and metabolome yields discrete morphological,

429   ultrastructural and/or functional outputs (**Figure 3**). Microglia are anything but static, as they

430   are exceptionally responsive to alterations in their local environment. In the mature healthy
431   CNS, the distribution of microglia is largely uniform and generally regular with little overlap
432   between adjacent territories[54]. The cell bodies are largely sessile, but their processes are
433   constantly moving and scanning the brain parenchyma[55,56]. Microglial functions adapt to their
434   location and reciprocal interactions with nearby cells and structures. Their morphology,
435   ultrastructure and molecular profile are similarly dynamic and plastic, resulting in many
436   different cell states. As Conrad H. Waddington, founding father of systems biology, eloquently
437   described: "*Cells are residents of a vast 'landscape' of possible states, over which they travel*
438   *during development and in disease".[57]*
439
440   Single-cell technologies, multi-omics and integrative analyses of gene and protein expression
441   have helped to not only locate cells on this landscape, but also provide new insight into the
442   molecular mechanisms that shape the landscape and regulate specific cell states in a given
443   context (e.g., development, adult, disease or injury model, etc.). Many diverse and context-
444   dependent microglial states have been observed across species and models. Some examples
445   of these states are the DAM (disease-associated microglia), originally associated with
446   Alzheimer´s disease (AD) pathology models [58]; MGnD (microglial neurodegenerative
447   phenotype) documented across several disease models[59]; ARM (activated response
448   microglia) and IRM (interferon-responsive microglia) in an AD pathology mouse model[60]; HAM
449   (human AD microglia)[61]; MIMS (microglia inflamed in multiple sclerosis (MS))[62]; and LDAM
450   (lipid-droplet-accumulating microglia in aging mice and humans)[63], brain tumors (glioma-
451   associated microglia, GAM)[64], amyotrophic lateral sclerosis (ALS)-associated signature[65] and
452   Parkinson's disease (PD)-microglial signature[66]. In the developing and aging brain the WAM
453   (white matter-associated microglia)[67]; ATM (axon tract-associated microglia)[68], and PAM
454   (proliferative-region-associated microglia, related to phagocytosis of developing
455   oligodendrocytes)[69], may share some features with the core DAM signature. In the developing
456   human CNS, microglia also express some of the DAM/MGnD/ARM-like profiles[70].
457
458   While gene expression signatures indicate biological pathways, the functional implications of
459   these states and relationship to one another remain unclear. In fact, the ever-growing list of
460   branding clusters in single-cell RNA sequencing (scRNASeq) experiments and use of
461   acronyms is not consistent across research groups and could hinder future advance of the
462   field without validation and functional experiments to understand their meaning. Moreover,
463   transcriptomic signatures depend on tissue dissection and gating strategies that can lead to
464   isolation artifacts[71-74], which, when layered with the technical limitations of single-cell
465   sequencing, can make it difficult to assign state identity across different studies. Another
466   source of complexity comes from evident interspecies differences[75-77], which can further

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

470

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

488

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

502

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

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

616

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

628

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

640

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

649 challenges and may differ between the young/developing *versus* aged/diseased CNS, and
650 across distinct regions. Most likely, the same states that are beneficial in certain contexts may
651 be detrimental in others, strictly depending on the complex interactions between microglia and
652 their surrounding environment. One of the most relevant questions to be addressed is to which
653 extent microglial states identified in the mouse brain are conserved and functionally relevant
654 in the human brain.
655
656 **Nomenclature troubles**
657 Our current understanding of the plasticity of microglial states is at odds with the simplistic
658 scenario established using outdated microglial nomenclature (resting *versus* activated and M1
659 *versus* M2, **Boxes 1 and 2**). Thus, a systematic, careful naming approach would greatly
660 benefit microglial biology. As a first step to guide the field regarding the use of nomenclature,
661 we generated a questionnaire (**Supplemental Data**) and collected the responses from the co-
662 authors.
663
664 Surprisingly, there was more consensus than disagreement that the current nomenclature has
665 severe limitations, and a more useful conceptual framework is needed to properly understand
666 microglial states. There is also agreement that this framework is a first important step to guide
667 the field and should be revisited every five to ten years by an international panel of experts as
668 new discoveries are made. There is also a broad agreement that microglial responses should
669 be framed in a multidimensional space, and should not be simplified as dichotomic good
670 *versus* bad (**Figure 1**). Another point of strong agreement: abandon M1/M2 (and similar)
671 nomenclature once and for all and generally avoid using the vague term 'neuroinflammation'.
672 Most agree that inflammation is not always detrimental but, instead, represents an adaptive
673 response to damage that can sometimes get out of control (**Box 4**). Quite importantly, a vast
674 majority of authors support the use of "markers" (genes or proteins) to identify cell populations,
675 but not as a readout of cell functions, which need to be addressed directly.
676
677 Nonetheless, there were a few points that are still under intense debate. The term "resting"
678 microglia is strongly avoided by some authors, whereas others acknowledge that they still use
679 it even with its limitations, for lack of a better term. "Homeostatic" has more acceptance,
680 although it is recognized that it is based on a very particular gene signature not shared by
681 microglia across all physiological contexts, such as embryonic and postnatal development,
682 and that several homeostatic states likely exist. Thus, the term 'homeostatic' should always
683 be accompanied by an accurate description of the context.
684

685 The opinion on use of the term "DAM", on the other hand, is highly polarized. Many authors
686 consider that a core set of transcripts in this signature is common to several pathological
687 conditions and some physiological processes, including the development of white matter,
688 whereas an equal number of authors state there is not enough evidence for "DAM" to be a
689 universal signature of microglial response to damage. Finally, the extent to which microglia
690 are unique or similar to other brain associated or tissue macrophages is evolving with new
691 data and profiling methods: most agree that due to their lineage, microglia are to some extent
692 similar to other macrophages but have unique functions resulting from their longer residence
693 in the CNS environment.
694

695 **Recommendations: DOs and DON'Ts**
696 Based on the collective opinions from the authors, we provide a series of recommendations
697 for researchers, reviewers, and editors. As the field has not yet reached a consensus on
698 several nomenclature topics, including the appropriate use of descriptors for microglial states,
699 it is premature to provide clearer recommendations. Nevertheless, we aim to raise awareness
700 on these issues and stimulate the launch of further initiatives that will guide the field and allow
701 to develop more specific guidelines.
702

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

720 • Describe profiles of cytokine expression, considering that microglial complexity cannot
721 be reduced to oversimplified and polarized "pro-inflammatory" *versus* "anti-inflammatory"
722 categories. Similarly, do not use M1 *versus* M2 classification.
723 • When using the term "DAM", do not use it as a universal term applicable to all diseases,
724 models or challenges. The jury is still out to test whether its full or core signature is common
725 to all or a subset of pathologies, particularly in the human brain.
726
727 *Introducing New Terminology*
728 • Until a consensus is reached about true subtype/s of microglia, with defined ontogeny,
729 physical niches, functions, and transcriptional profiles (whether permanent or transient), use
730 the term "state" rather than "subpopulation.
731 • Use combinations of gene or protein "markers" to identify putative supopulations but
732 be aware that their expression is plastic and may change over time and under different
733 experimental conditions. Use fate mapping approaches with lineage tracing to track individual
734 microglial cells and assess possible intrinsic differences as well as changes in their state over
735 time[84,132].
736 • In scRNASeq studies, describe the transcriptional signatures (sets or modules of
737 expressed genes) that can be compared with other studies[116,133] To describe groups of
738 transcriptionally similar cells in terms of signature, use the term "cluster".
739 • Avoid the use of acronyms wherever possible, and only use these once multiple
740 laboratories have defined a stable state with a clearly defined functional role.
741 • If new terminology needs to be introduced, follow FAIR principles: Findable,
742 Accessible, Interoperable, and Reusable (https://neuronline.sfn.org/professional-
743 development/data-sharing-principles-to-promote-open-science). An example of naming cell
744 lines following these principles can be found here[134].
745
746 *Microglial Markers and Function*
747 • Use integrative methodological approaches that allow probing of microglia using
748 different levels of analysis (**Figure 5**).
749 • Follow updated consensus guidelines when using methodologies such as
750 scRNASeq[135], RTqPCR[136], or digital PCR[137].
751 • Do not use morphology or gene/protein expression as a substitute for directly
752 assessing cell function. Morphology and expression can be used to generate hypotheses
753 about function that need to be specifically tested.
754

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

828   diseases with clear sex-related differences in their prevalence, symptoms, and progression,

829   as well as response to treatments.

830

831   *Relevance to humans:* It will be imperative to study developmental and functional differences

832   between human and animal model microglia. To date, most of the studies on microglia were

833   conducted in mice and a direct comparison among brain regions is still missing. Whether

834   microglial states identified in mice also exist in humans is still under debate. Translating and

835   validating these findings across species is critical and will help prevent failure of clinical trials

836   that stem from animal model limitations. In addition, most human microglial studies were

837   performed in Caucasians and only recently data from other groups, such as African American

838   individuals, are becoming available[143].

839

840   *Towards a unified nomenclature:* The conclusion of this paper is that the community has not

841   yet reached an agreement on what defines microglial identity compared to other cell types;

842   nor consensus on the number, dynamic nature, or definition of microglial states. The

843   community advocates for creating harmonized, curated databases and guidelines for

844   introducing novel terminology; to follow STAR methods; and share data as early as possible.

845   Until such consensus is reached, the community urges all microglial studies to present data

846   with all their layers of complexity and carefully define the context examined to offer clarity

847   instead of confusion, thereby contributing to a more thorough understanding of the many

848   facets of microglial biology. To establish new guidelines for microglial states and nomenclature

849   we call for a community-based approach, whereby the issues and progress are discussed

850   openly in workshops and meetings, with input from diverse researchers across fields and

851   career stages. A useful model to look after are the 10 Human Leukocyte Differentiation Antigen

852   workshops that have taken place since 1982, in charge of renaming CD (cluster of

853   differentiation) antigens (https://www.sinobiological.com/research/cd-antigens/hlda1). We

854   lastly advocate for the creation of an international panel/committee of experts in charge of

855   overseeing the guidelines and establishing a specific roadmap to write a white paper in the

856   nearest future.

857

858   We would like to conclude with the words of Río-Hortega, who sarcastically identified the

859   problems of microglial nomenclature already 100 years ago: "If we were fond of introducing

860   new nomenclature to describe microglia, as many modern histologists are, who think that

861   enriching nomenclature resolves problems, we would find for microglia names that would

862   indicate their origin, or morphology, or function, in addition to classify all the shapes that

863   acquire when moving and evolving - resulting in the same absurdity that occurs in some

864   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].

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

944

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

971

972 **Box 4. Microglia and the term "neuroinflammation"**
973 There is a long historical literature stating that inflammation is an important part of recovery
974 from infection, injury, and disease, and it is the lack of resolution of this inflammatory response
975 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

1012   inflammation associated with the CNS follows unique rules that need to be fully discerned

1013   experimentally and not simply extrapolated from observations in non-nervous tissue.

1014

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.
Anti bodi es	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

			plasma membrane or diffuse throughout the cytoplasm and can be more profuse than IBA1 depending on staining conditions.	Ultrastructural studies.	
	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.			
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]

1045

1046 **Table 1. Main antibody markers and mouse lines used to visualize microglia in rodents**

1047 **and humans from early embryonic development to adulthood and aging.** Other proteins

1048 expressed by microglia but whose specificity is not confirmed include APOE, CLEC7A, ITGAX,

1049 and LPL.

1050

References

1083   1   Stafleu, F. A. Linnaeus and the Linnaeans: The Spreading of their Ideas in Systematic Botany, 1735-1789 (1971), p. 80. . (1971).

1085   2   Charmaz, K. The power of names. *Journal of Contemporary Ethnography* **35**, 396-399 (2006).

1087   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).

1090   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).

1093   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).

1096   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).

1098   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).

1101   8   Rezaie, P. & Hanisch, U.-K. in *Microglia in Health and Disease* (eds M.E. Tremblay & A. Sierra) 7-46 (Springer, 2014).

1103   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).

1105   10   Oehmichen, M. Are resting and/or reactive microglia macrophages? *Immunobiology* **161**, 246-254, doi:10.1016/S0171-2985(82)80080-6 (1982).

1107   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).

1110   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).

1113   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).

1116   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).

1118   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).

1120   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).

1122   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).

1125   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).

1128   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).

1131   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).

1135 21 Ajami, B., Bennett, J. L., Krieger, C., Tetzlaff, W. & Rossi, F. M. Local self-renewal can
1136 sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci*
1137 **10**, 1538-1543, doi:10.1038/nn2014 (2007).
1138 22 Bruttger, J. *et al.* Genetic Cell Ablation Reveals Clusters of Local Self-Renewing
1139 Microglia in the Mammalian Central Nervous System. *Immunity* **43**, 92-106,
1140 doi:10.1016/j.immuni.2015.06.012 (2015).
1141 23 Huang, Y. *et al.* Dual extra-retinal origins of microglia in the model of retinal microglia
1142 repopulation. *Cell Discov* **4**, 9, doi:10.1038/s41421-018-0011-8 (2018).
1143 24 Huang, Y. *et al.* Repopulated microglia are solely derived from the proliferation of
1144 residual microglia after acute depletion. *Nat Neurosci* **21**, 530-540,
1145 doi:10.1038/s41593-018-0090-8 (2018).
1146 25 Zhan, L. *et al.* Proximal recolonization by self-renewing microglia re-establishes
1147 microglial homeostasis in the adult mouse brain. *PLoS Biol* **17**, e3000134,
1148 doi:10.1371/journal.pbio.3000134 (2019).
1149 26 Cronk, J. C. *et al.* Peripherally derived macrophages can engraft the brain independent
1150 of irradiation and maintain an identity distinct from microglia. *J Exp Med* **215**, 1627-
1151 1647, doi:10.1084/jem.20180247 (2018).
1152 27 Priller, J. *et al.* Targeting gene-modified hematopoietic cells to the central nervous
1153 system: use of green fluorescent protein uncovers microglial engraftment. *Nat Med* **7**,
1154 1356-1361, doi:10.1038/nm1201-1356 (2001).
1155 28 Xu, Z. *et al.* Efficient Strategies for Microglia Replacement in the Central Nervous
1156 System. *Cell Rep* **32**, 108041, doi:10.1016/j.celrep.2020.108041 (2020).
1157 29 Xu, Z., Zhou, X., Peng, B. & Rao, Y. Microglia replacement by bone marrow
1158 transplantation (Mr BMT) in the central nervous system of adult mice. *STAR Protoc* **2**,
1159 100666, doi:10.1016/j.xpro.2021.100666 (2021).
1160 30 Xu, Z., Rao, Y. & Peng, B. Protocol for microglia replacement by peripheral blood (Mr
1161 PB). *STAR Protoc* **2**, 100613, doi:10.1016/j.xpro.2021.100613 (2021).
1162 31 Xu, R. *et al.* Human iPSC-derived mature microglia retain their identity and functionally
1163 integrate in the chimeric mouse brain. *Nat Commun* **11**, 1577, doi:10.1038/s41467-
1164 020-15411-9 (2020).
1165 32 Hasselmann, J. *et al.* Development of a Chimeric Model to Study and Manipulate
1166 Human Microglia In Vivo. *Neuron* **103**, 1016-1033 e1010,
1167 doi:10.1016/j.neuron.2019.07.002 (2019).
1168 33 Mancuso, R. *et al.* Stem-cell-derived human microglia transplanted in mouse brain to
1169 study human disease. *Nat Neurosci* **22**, 2111-2116, doi:10.1038/s41593-019-0525-x
1170 (2019).
1171 34 Grabert, K. *et al.* A Transgenic Line That Reports CSF1R Protein Expression Provides
1172 a Definitive Marker for the Mouse Mononuclear Phagocyte System. *J Immunol* **205**,
1173 3154-3166, doi:10.4049/jimmunol.2000835 (2020).
1174 35 Kaiser, T. & Feng, G. Tmem119-EGFP and Tmem119-CreERT2 Transgenic Mice for
1175 Labeling and Manipulating Microglia. *eNeuro* **6**, doi:10.1523/ENEURO.0448-18.2019
1176 (2019).
1177 36 Chappell-Maor, L. *et al.* Comparative analysis of CreER transgenic mice for the study
1178 of brain macrophages: A case study. *Eur J Immunol* **50**, 353-362,
1179 doi:10.1002/eji.201948342 (2020).
1180 37 McKinsey, G. L. *et al.* A new genetic strategy for targeting microglia in development
1181 and disease. *Elife* **9**, doi:10.7554/eLife.54590 (2020).
1182 38 Masuda, T. *et al.* Novel Hexb-based tools for studying microglia in the CNS. *Nat*
1183 *Immunol* **21**, 802-815, doi:10.1038/s41590-020-0707-4 (2020).
1184 39 Parkhurst, C. N. *et al.* Microglia promote learning-dependent synapse formation
1185 through brain-derived neurotrophic factor. *Cell* **155**, 1596-1609,
1186 doi:10.1016/j.cell.2013.11.030 (2013).
1187 40 Yona, S. *et al.* Fate mapping reveals origins and dynamics of monocytes and tissue
1188 macrophages under homeostasis. *Immunity* **38**, 79-91,
1189 doi:10.1016/j.immuni.2012.12.001 (2013).

1190 41 Kim, J. S. *et al.* A Binary Cre Transgenic Approach Dissects Microglia and CNS
1191 Border-Associated Macrophages. *Immunity* **54**, 176-190 e177,
1192 doi:10.1016/j.immuni.2020.11.007 (2021).

1193 42 Goldmann, T. *et al.* Origin, fate and dynamics of macrophages at central nervous
1194 system interfaces. *Nat Immunol* **17**, 797-805, doi:10.1038/ni.3423 (2016).

1195 43 Van Hove, H. *et al.* A single-cell atlas of mouse brain macrophages reveals unique
1196 transcriptional identities shaped by ontogeny and tissue environment. *Nat Neurosci*
1197 **22**, 1021-1035, doi:10.1038/s41593-019-0393-4 (2019).

1198 44 Masuda, T. *et al.* Specification of CNS macrophage subsets occurs postnatally in
1199 defined niches. *Nature* **604**, 740-748, doi:10.1038/s41586-022-04596-2 (2022).

1200 45 Paolicelli, R. C. & Ferretti, M. T. Function and Dysfunction of Microglia during Brain
1201 Development: Consequences for Synapses and Neural Circuits. *Front Synaptic*
1202 *Neurosci* **9**, 9, doi:10.3389/fnsyn.2017.00009 (2017).

1203 46 Green, K. N., Crapser, J. D. & Hohsfield, L. A. To Kill a Microglia: A Case for CSF1R
1204 Inhibitors. *Trends Immunol* **41**, 771-784, doi:10.1016/j.it.2020.07.001 (2020).

1205 47 Chitu, V., Gokhan, S. & Stanley, E. R. Modeling CSF-1 receptor deficiency diseases -
1206 how close are we? *FEBS J*, doi:10.1111/febs.16085 (2021).

1207 48 Oosterhof, N. *et al.* Homozygous Mutations in CSF1R Cause a Pediatric-Onset
1208 Leukoencephalopathy and Can Result in Congenital Absence of Microglia. *Am J Hum*
1209 *Genet* **104**, 936-947, doi:10.1016/j.ajhg.2019.03.010 (2019).

1210 49 Guo, L. *et al.* Bi-allelic CSF1R Mutations Cause Skeletal Dysplasia of
1211 Dysosteosclerosis-Pyle Disease Spectrum and Degenerative Encephalopathy with
1212 Brain Malformation. *Am J Hum Genet* **104**, 925-935, doi:10.1016/j.ajhg.2019.03.004
1213 (2019).

1214 50 Rojo, R. *et al.* Deletion of a Csf1r enhancer selectively impacts CSF1R expression and
1215 development of tissue macrophage populations. *Nat Commun* **10**, 3215,
1216 doi:10.1038/s41467-019-11053-8 (2019).

1217 51 Kiani Shabestari, S. *et al.* Absence of microglia promotes diverse pathologies and early
1218 lethality in Alzheimer's disease mice. *Cell Rep* **39**, 110961,
1219 doi:10.1016/j.celrep.2022.110961 (2022).

1220 52 Konishi, H. *et al.* Astrocytic phagocytosis is a compensatory mechanism for microglial
1221 dysfunction. *EMBO J* **39**, e104464, doi:10.15252/embj.2020104464 (2020).

1222 53 Hickman, S. E. *et al.* The microglial sensome revealed by direct RNA sequencing. *Nat*
1223 *Neurosci* **16**, 1896-1905, doi:10.1038/nn.3554 (2013).

1224 54 Hume, D. A., Perry, V. H. & Gordon, S. Immunohistochemical localization of a
1225 macrophage-specific antigen in developing mouse retina: phagocytosis of dying
1226 neurons and differentiation of microglial cells to form a regular array in the plexiform
1227 layers. *J Cell Biol* **97**, 253-257, doi:10.1083/jcb.97.1.253 (1983).

1228 55 Davalos, D. *et al.* ATP mediates rapid microglial response to local brain injury in vivo.
1229 *Nat Neurosci* **8**, 752-758, doi:10.1038/nn1472 (2005).

1230 56 Nimmerjahn, A., Kirchhoff, F. & Helmchen, F. Resting microglial cells are highly
1231 dynamic surveillants of brain parenchyma in vivo. *Science* **308**, 1314-1318,
1232 doi:10.1126/science.1110647 (2005).

1233 57 Wang, J., Zhang, K., Xu, L. & Wang, E. Quantifying the Waddington landscape and
1234 biological paths for development and differentiation. *Proc Natl Acad Sci U S A* **108**,
1235 8257-8262, doi:10.1073/pnas.1017017108 (2011).

1236 58 Keren-Shaul, H. *et al.* A Unique Microglia Type Associated with Restricting
1237 Development of Alzheimer's Disease. *Cell* **169**, 1276-1290 e1217,
1238 doi:10.1016/j.cell.2017.05.018 (2017).

1239 59 Krasemann, S. *et al.* The TREM2-APOE Pathway Drives the Transcriptional
1240 Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* **47**,
1241 566-581 e569, doi:10.1016/j.immuni.2017.08.008 (2017).

1242 60 Sala Frigerio, C. *et al.* The Major Risk Factors for Alzheimer's Disease: Age, Sex, and
1243 Genes Modulate the Microglia Response to Abeta Plaques. *Cell Rep* **27**, 1293-1306
1244 e1296, doi:10.1016/j.celrep.2019.03.099 (2019).

1245 61 Srinivasan, K. *et al.* Alzheimer's Patient Microglia Exhibit Enhanced Aging and Unique
1246   Transcriptional Activation. *Cell Rep* **31**, 107843, doi:10.1016/j.celrep.2020.107843
1247   (2020).
1248 62 Absinta, M. *et al.* A lymphocyte-microglia-astrocyte axis in chronic active multiple
1249   sclerosis. *Nature* **597**, 709-714, doi:10.1038/s41586-021-03892-7 (2021).
1250 63 Marschallinger, J. *et al.* Lipid-droplet-accumulating microglia represent a dysfunctional
1251   and proinflammatory state in the aging brain. *Nat Neurosci* **23**, 194-208,
1252   doi:10.1038/s41593-019-0566-1 (2020).
1253 64 De Andrade Costa, A. *et al.* RNA sequence analysis reveals ITGAL/CD11A as a
1254   stromal regulator of murine low-grade glioma growth. *Neuro Oncol* **24**, 14-26,
1255   doi:10.1093/neuonc/noab130 (2022).
1256 65 Francesco Limone *et al.* Single-nucleus sequencing reveals enriched expression of
1257   genetic risk factors sensitises Motor Neurons to degeneration in ALS. *bioRxiv* (2021).
1258 66 Smajic, S. *et al.* Single-cell sequencing of human midbrain reveals glial activation and
1259   a Parkinson-specific neuronal state. *Brain* **145**, 964-978, doi:10.1093/brain/awab446
1260   (2022).
1261 67 Safaiyan, S. *et al.* White matter aging drives microglial diversity. *Neuron* **109**, 1100-
1262   1117 e1110, doi:10.1016/j.neuron.2021.01.027 (2021).
1263 68 Hammond, T. R. *et al.* Single-Cell RNA Sequencing of Microglia throughout the Mouse
1264   Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* **50**,
1265   253-271 e256, doi:10.1016/j.immuni.2018.11.004 (2019).
1266 69 Li, Q. *et al.* Developmental Heterogeneity of Microglia and Brain Myeloid Cells
1267   Revealed by Deep Single-Cell RNA Sequencing. *Neuron* **101**, 207-223 e210,
1268   doi:10.1016/j.neuron.2018.12.006 (2019).
1269 70 Kracht, L. *et al.* Human fetal microglia acquire homeostatic immune-sensing properties
1270   early in development. *Science* **369**, 530-537, doi:10.1126/science.aba5906 (2020).
1271 71 Wu, Y. E., Pan, L., Zuo, Y., Li, X. & Hong, W. Detecting Activated Cell Populations
1272   Using Single-Cell RNA-Seq. *Neuron* **96**, 313-329 e316,
1273   doi:10.1016/j.neuron.2017.09.026 (2017).
1274 72 Marsh, S. E. *et al.* Single cell sequencing reveals glial specific responses to tssue
1275   processing & enzymatic dissociation in mice and humans. *BioRxiv* (2021).
1276 73 Mattei, D. *et al.* Enzymatic Dissociation Induces Transcriptional and Proteotype Bias
1277   in Brain Cell Populations. *Int J Mol Sci* **21**, doi:10.3390/ijms21217944 (2020).
1278 74 Summers, K. M., Bush, S. J. & Hume, D. A. Network analysis of transcriptomic diversity
1279   amongst resident tissue macrophages and dendritic cells in the mouse mononuclear
1280   phagocyte system. *PLoS Biol* **18**, e3000859, doi:10.1371/journal.pbio.3000859
1281   (2020).
1282 75 Gosselin, D. *et al.* An environment-dependent transcriptional network specifies human
1283   microglia identity. *Science* **356**, doi:10.1126/science.aal3222 (2017).
1284 76 Geirsdottir, L. *et al.* Cross-Species Single-Cell Analysis Reveals Divergence of the
1285   Primate Microglia Program. *Cell* **179**, 1609-1622 e1616,
1286   doi:10.1016/j.cell.2019.11.010 (2019).
1287 77 Kolodziejczyk, A. A., Kim, J. K., Svensson, V., Marioni, J. C. & Teichmann, S. A. The
1288   technology and biology of single-cell RNA sequencing. *Mol Cell* **58**, 610-620,
1289   doi:10.1016/j.molcel.2015.04.005 (2015).
1290 78 Welch, J. D. *et al.* Single-Cell Multi-omic Integration Compares and Contrasts Features
1291   of Brain Cell Identity. *Cell* **177**, 1873-1887 e1817, doi:10.1016/j.cell.2019.05.006
1292   (2019).
1293 79 Stuart, T. *et al.* Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902
1294   e1821, doi:10.1016/j.cell.2019.05.031 (2019).
1295 80 Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J. & Smith, V. A. Relationship
1296   between differentially expressed mRNA and mRNA-protein correlations in a xenograft
1297   model system. *Sci Rep* **5**, 10775, doi:10.1038/srep10775 (2015).

1298   81   Fernandez-Zapata, C., Leman, J. K. H., Priller, J. & Bottcher, C. The use and
1299   limitations of single-cell mass cytometry for studying human microglia function. *Brain*
1300   *Pathol* **30**, 1178-1191, doi:10.1111/bpa.12909 (2020).

1301   82   Ajami, B. *et al.* Single-cell mass cytometry reveals distinct populations of brain myeloid
1302   cells in mouse neuroinflammation and neurodegeneration models. *Nat Neurosci* **21**,
1303   541-551, doi:10.1038/s41593-018-0100-x (2018).

1304   83   Bottcher, C. *et al.* Human microglia regional heterogeneity and phenotypes determined
1305   by multiplexed single-cell mass cytometry. *Nat Neurosci* **22**, 78-90,
1306   doi:10.1038/s41593-018-0290-2 (2019).

1307   84   Tay, T. L. *et al.* A new fate mapping system reveals context-dependent random or
1308   clonal expansion of microglia. *Nat Neurosci* **20**, 793-803, doi:10.1038/nn.4547 (2017).

1309   85   McQuade, A. *et al.* Gene expression and functional deficits underlie TREM2-knockout
1310   microglia responses in human models of Alzheimer's disease. *Nat Commun* **11**, 5370,
1311   doi:10.1038/s41467-020-19227-5 (2020).

1312   86   Mazaheri, F. *et al.* TREM2 deficiency impairs chemotaxis and microglial responses to
1313   neuronal injury. *EMBO Rep* **18**, 1186-1198, doi:10.15252/embr.201743922 (2017).

1314   87   Erny, D. *et al.* Host microbiota constantly control maturation and function of microglia
1315   in the CNS. *Nat Neurosci* **18**, 965-977, doi:10.1038/nn.4030 (2015).

1316   88   Thion, M. S. *et al.* Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific
1317   Manner. *Cell* **172**, 500-516 e516, doi:10.1016/j.cell.2017.11.042 (2018).

1318   89   Chatterjee, J. *et al.* Asthma reduces glioma formation by T cell decorin-mediated
1319   inhibition of microglia. *Nat Commun* **12**, 7122, doi:10.1038/s41467-021-27455-6
1320   (2021).

1321   90   Matcovitch-Natan, O. *et al.* Microglia development follows a stepwise program to
1322   regulate brain homeostasis. *Science* **353**, aad8670, doi:10.1126/science.aad8670
1323   (2016).

1324   91   Masuda, T. *et al.* Spatial and temporal heterogeneity of mouse and human microglia
1325   at single-cell resolution. *Nature* **566**, 388-392, doi:10.1038/s41586-019-0924-x (2019).

1326   92   Grabert, K. *et al.* Microglial brain region-dependent diversity and selective regional
1327   sensitivities to aging. *Nat Neurosci* **19**, 504-516, doi:10.1038/nn.4222 (2016).

1328   93   Kana, V. *et al.* CSF-1 controls cerebellar microglia and is required for motor function
1329   and social interaction. *J Exp Med* **216**, 2265-2281, doi:10.1084/jem.20182037 (2019).

1330   94   Hanamsagar, R. *et al.* Generation of a microglial developmental index in mice and in
1331   humans reveals a sex difference in maturation and immune reactivity. *Glia* **65**, 1504-
1332   1520, doi:10.1002/glia.23176 (2017).

1333   95   Guneykaya, D. *et al.* Transcriptional and Translational Differences of Microglia from
1334   Male and Female Brains. *Cell Rep* **24**, 2773-2783 e2776,
1335   doi:10.1016/j.celrep.2018.08.001 (2018).

1336   96   Villa, A. *et al.* Sex-Specific Features of Microglia from Adult Mice. *Cell Rep* **23**, 3501-
1337   3511, doi:10.1016/j.celrep.2018.05.048 (2018).

1338   97   Lynch, M. A. Exploring Sex-Related Differences in Microglia May Be a Game-Changer
1339   in Precision Medicine. *Front Aging Neurosci* **14**, 868448,
1340   doi:10.3389/fnagi.2022.868448 (2022).

1341   98   Halievski, K., Ghazisaeidi, S. & Salter, M. W. Sex-Dependent Mechanisms of Chronic
1342   Pain: A Focus on Microglia and P2X4R. *J Pharmacol Exp Ther* **375**, 202-209,
1343   doi:10.1124/jpet.120.265017 (2020).

1344   99   Han, J., Fan, Y., Zhou, K., Blomgren, K. & Harris, R. A. Uncovering sex differences of
1345   rodent microglia. *J Neuroinflammation* **18**, 74, doi:10.1186/s12974-021-02124-z
1346   (2021).

1347   100   De Biase, L. M. *et al.* Local Cues Establish and Maintain Region-Specific Phenotypes
1348   of Basal Ganglia Microglia. *Neuron* **95**, 341-356 e346,
1349   doi:10.1016/j.neuron.2017.06.020 (2017).

1350   101   Ayata, P. *et al.* Epigenetic regulation of brain region-specific microglia clearance
1351   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).

1406 121 Zhou, Y. *et al.* Human and mouse single-nucleus transcriptomics reveal TREM2-
1407 dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat*
1408 *Med* **26**, 131-142, doi:10.1038/s41591-019-0695-9 (2020).
1409 122 Ulland, T. K. *et al.* TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's
1410 Disease. *Cell* **170**, 649-663 e613, doi:10.1016/j.cell.2017.07.023 (2017).
1411 123 Xiang, X. *et al.* Microglial activation states drive glucose uptake and FDG-PET
1412 alterations in neurodegenerative diseases. *Sci Transl Med* **13**, eabe5640,
1413 doi:10.1126/scitranslmed.abe5640 (2021).
1414 124 Ma, S. *et al.* Chromatin Potential Identified by Shared Single-Cell Profiling of RNA and
1415 Chromatin. *Cell* **183**, 1103-1116 e1120, doi:10.1016/j.cell.2020.09.056 (2020).
1416 125 Buenrostro, J. D., Wu, B., Chang, H. Y. & Greenleaf, W. J. ATAC-seq: A Method for
1417 Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol* **109**, 21 29 21-
1418 21 29 29, doi:10.1002/0471142727.mb2129s109 (2015).
1419 126 van Galen, P. *et al.* A Multiplexed System for Quantitative Comparisons of Chromatin
1420 Landscapes. *Mol Cell* **61**, 170-180, doi:10.1016/j.molcel.2015.11.003 (2016).
1421 127 Bartosovic, M., Kabbe, M. & Castelo-Branco, G. Single-cell CUT&Tag profiles histone
1422 modifications and transcription factors in complex tissues. *Nat Biotechnol*,
1423 doi:10.1038/s41587-021-00869-9 (2021).
1424 128 Schaafsma, W. *et al.* Long-lasting pro-inflammatory suppression of microglia by LPS-
1425 preconditioning is mediated by RelB-dependent epigenetic silencing. *Brain Behav*
1426 *Immun* **48**, 205-221, doi:10.1016/j.bbi.2015.03.013 (2015).
1427 129 Wendeln, A. C. *et al.* Innate immune memory in the brain shapes neurological disease
1428 hallmarks. *Nature* **556**, 332-338, doi:10.1038/s41586-018-0023-4 (2018).
1429 130 Chiu, I. M. *et al.* A neurodegeneration-specific gene-expression signature of acutely
1430 isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep* **4**, 385-
1431 401, doi:10.1016/j.celrep.2013.06.018 (2013).
1432 131 Sobue, A. *et al.* Microglial gene signature reveals loss of homeostatic microglia
1433 associated with neurodegeneration of Alzheimer's disease. *Acta Neuropathol*
1434 *Commun* **9**, 1, doi:10.1186/s40478-020-01099-x (2021).
1435 132 Jordao, M. J. C. *et al.* Single-cell profiling identifies myeloid cell subsets with distinct
1436 fates during neuroinflammation. *Science* **363**, doi:10.1126/science.aat7554 (2019).
1437 133 Olah, M. *et al.* Single cell RNA sequencing of human microglia uncovers a subset
1438 associated with Alzheimer's disease. *Nat Commun* **11**, 6129, doi:10.1038/s41467-020-
1439 19737-2 (2020).
1440 134 Kurtz, A. *et al.* A Standard Nomenclature for Referencing and Authentication of
1441 Pluripotent Stem Cells. *Stem Cell Reports* **10**, 1-6, doi:10.1016/j.stemcr.2017.12.002
1442 (2018).
1443 135 Luecken, M. D. & Theis, F. J. Current best practices in single-cell RNA-seq analysis:
1444 a tutorial. *Mol Syst Biol* **15**, e8746, doi:10.15252/msb.20188746 (2019).
1445 136 Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of
1446 quantitative real-time PCR experiments. *Clin Chem* **55**, 611-622,
1447 doi:10.1373/clinchem.2008.112797 (2009).
1448 137 d, M. G. & Huggett, J. F. The Digital MIQE Guidelines Update: Minimum Information
1449 for Publication of Quantitative Digital PCR Experiments for 2020. *Clin Chem* **66**, 1012-
1450 1029, doi:10.1093/clinchem/hvaa125 (2020).
1451 138 Ingolia, N. T., Brar, G. A., Rouskin, S., McGeachy, A. M. & Weissman, J. S. The
1452 ribosome profiling strategy for monitoring translation in vivo by deep sequencing of
1453 ribosome-protected mRNA fragments. *Nat Protoc* **7**, 1534-1550,
1454 doi:10.1038/nprot.2012.086 (2012).
1455 139 Mayor-Ruiz, C., Dominguez, O. & Fernandez-Capetillo, O. Trap(Seq): An RNA
1456 Sequencing-Based Pipeline for the Identification of Gene-Trap Insertions in
1457 Mammalian Cells. *J Mol Biol* **429**, 2780-2789, doi:10.1016/j.jmb.2017.07.020 (2017).
1458 140 Rautenstrauch, P., Vlot, A. H. C., Saran, S. & Ohler, U. Intricacies of single-cell multi-
1459 omics data integration. *Trends Genet* **38**, 128-139, doi:10.1016/j.tig.2021.08.012
1460 (2022).

1461 141 Paczkowska, M. *et al.* Integrative pathway enrichment analysis of multivariate omics
1462   data. *Nat Commun* **11**, 735, doi:10.1038/s41467-019-13983-9 (2020).
1463 142 Reel, P. S., Reel, S., Pearson, E., Trucco, E. & Jefferson, E. Using machine learning
1464   approaches for multi-omics data analysis: A review. *Biotechnol Adv* **49**, 107739,
1465   doi:10.1016/j.biotechadv.2021.107739 (2021).
1466 143 Kunkle, B. W. *et al.* Novel Alzheimer Disease Risk Loci and Pathways in African
1467   American Individuals Using the African Genome Resources Panel: A Meta-analysis.
1468   *JAMA Neurol* **78**, 102-113, doi:10.1001/jamaneurol.2020.3536 (2021).
1469 144 Río-Hortega, P. d. R. Histogenesis and normal evolution: exodus and regional
1470   distribution of microglia. *Memorias de la Real Sociedad Española de Historia Natural*
1471   **11:213-268** (1921).
1472 145 Sierra, A. *et al.* The "Big-Bang" for modern glial biology: Translation and comments on
1473   Pio del Rio-Hortega 1919 series of papers on microglia. *Glia* **64**, 1801-1840,
1474   doi:10.1002/glia.23046 (2016).
1475 146 Streit, W. J., Graeber, M. B. & Kreutzberg, G. W. Functional plasticity of microglia: a
1476   review. *Glia* **1**, 301-307, doi:10.1002/glia.440010502 (1988).
1477 147 Acarin, L., Vela, J. M., Gonzalez, B. & Castellano, B. Demonstration of poly-N-acetyl
1478   lactosamine residues in ameboid and ramified microglial cells in rat brain by tomato
1479   lectin binding. *J Histochem Cytochem* **42**, 1033-1041, doi:10.1177/42.8.8027523
1480   (1994).
1481 148 Castellano, B. *et al.* A double staining technique for simultaneous demonstration of
1482   astrocytes and microglia in brain sections and astroglial cell cultures. *J Histochem
1483   Cytochem* **39**, 561-568, doi:10.1177/39.5.1707903 (1991).
1484 149 Kitamura, T., Miyake, T. & Fujita, S. Genesis of resting microglia in the gray matter of
1485   mouse hippocampus. *J Comp Neurol* **226**, 421-433, doi:10.1002/cne.902260310
1486   (1984).
1487 150 Tremblay, M. E., Lecours, C., Samson, L., Sanchez-Zafra, V. & Sierra, A. From the
1488   Cajal alumni Achucarro and Rio-Hortega to the rediscovery of never-resting microglia.
1489   *Front Neuroanat* **9**, 45, doi:10.3389/fnana.2015.00045 (2015).
1490 151 Tremblay, M. E. The role of microglia at synapses in the healthy CNS: novel insights
1491   from recent imaging studies. *Neuron Glia Biol* **7**, 67-76,
1492   doi:10.1017/S1740925X12000038 (2011).
1493 152 Hanisch, U. K. & Kettenmann, H. Microglia: active sensor and versatile effector cells
1494   in the normal and pathologic brain. *Nat Neurosci* **10**, 1387-1394, doi:10.1038/nn1997
1495   (2007).
1496 153 Tremblay, M. E., Madore, C., Bordeleau, M., Tian, L. & Verkhratsky, A.
1497   Neuropathobiology of COVID-19: The Role for Glia. *Front Cell Neurosci* **14**, 592214,
1498   doi:10.3389/fncel.2020.592214 (2020).
1499 154 Sierra, A., Tremblay, M. E. & Wake, H. Never-resting microglia: physiological roles in
1500   the healthy brain and pathological implications. *Front Cell Neurosci* **8**, 240,
1501   doi:10.3389/fncel.2014.00240 (2014).
1502 155 Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E. & Heuschling, P.
1503   Characterization of the microglial phenotype under specific pro-inflammatory and anti-
1504   inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *J
1505   Neuroimmunol* **210**, 3-12, doi:10.1016/j.jneuroim.2009.02.003 (2009).
1506 156 Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J. & Hill, A. M. M-1/M-2 macrophages
1507   and the Th1/Th2 paradigm. *J Immunol* **164**, 6166-6173,
1508   doi:10.4049/jimmunol.164.12.6166 (2000).
1509 157 Butovsky, O. *et al.* Identification of a unique TGF-beta-dependent molecular and
1510   functional signature in microglia. *Nat Neurosci* **17**, 131-143, doi:10.1038/nn.3599
1511   (2014).
1512 158 Martinez, F. O. & Gordon, S. The M1 and M2 paradigm of macrophage activation: time
1513   for reassessment. *F1000Prime Rep* **6**, 13, doi:10.12703/P6-13 (2014).
1514 159 Ransohoff, R. M. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci*
1515   **19**, 987-991, doi:10.1038/nn.4338 (2016).

1516 160 Devanney, N. A., Stewart, A. N. & Gensel, J. C. Microglia and macrophage metabolism
1517   in CNS injury and disease: The role of immunometabolism in neurodegeneration and
1518   neurotrauma. *Exp Neurol* **329**, 113310, doi:10.1016/j.expneurol.2020.113310 (2020).
1519 161 Madry, C. *et al.* Microglial Ramification, Surveillance, and Interleukin-1beta Release
1520   Are Regulated by the Two-Pore Domain K(+) Channel THIK-1. *Neuron* **97**, 299-312
1521   e296, doi:10.1016/j.neuron.2017.12.002 (2018).
1522 162 Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-
1523   coupled phagocytosis. *Cell Stem Cell* **7**, 483-495, doi:10.1016/j.stem.2010.08.014
1524   (2010).
1525 163 VanRyzin, J. W. *et al.* Microglial Phagocytosis of Newborn Cells Is Induced by
1526   Endocannabinoids and Sculpts Sex Differences in Juvenile Rat Social Play. *Neuron*
1527   **102**, 435-449 e436, doi:10.1016/j.neuron.2019.02.006 (2019).
1528 164 Abiega, O. *et al.* Neuronal Hyperactivity Disturbs ATP Microgradients, Impairs
1529   Microglial Motility, and Reduces Phagocytic Receptor Expression Triggering
1530   Apoptosis/Microglial Phagocytosis Uncoupling. *PLoS Biol* **14**, e1002466,
1531   doi:10.1371/journal.pbio.1002466 (2016).
1532 165 Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G. & Mechawar, N. Evidence
1533   for increased microglial priming and macrophage recruitment in the dorsal anterior
1534   cingulate white matter of depressed suicides. *Brain Behav Immun* **42**, 50-59,
1535   doi:10.1016/j.bbi.2014.05.007 (2014).
1536 166 Bachstetter, A. D. *et al.* Rod-shaped microglia morphology is associated with aging in
1537   2 human autopsy series. *Neurobiol Aging* **52**, 98-105,
1538   doi:10.1016/j.neurobiolaging.2016.12.028 (2017).
1539 167 Streit, W. J., Sammons, N. W., Kuhns, A. J. & Sparks, D. L. Dystrophic microglia in the
1540   aging human brain. *Glia* **45**, 208-212, doi:10.1002/glia.10319 (2004).
1541 168 Tischer, J. *et al.* Inhomogeneous distribution of Iba-1 characterizes microglial
1542   pathology in Alzheimer's disease. *Glia* **64**, 1562-1572, doi:10.1002/glia.23024 (2016).
1543 169 Savage, J. C., Carrier, M. & Tremblay, M. E. Morphology of Microglia Across Contexts
1544   of Health and Disease. *Methods Mol Biol* **2034**, 13-26, doi:10.1007/978-1-4939-9658-
1545   2_2 (2019).
1546 170 Salamanca, L. *et al.* MIC-MAC: An automated pipeline for high-throughput
1547   characterization and classification of three-dimensional microglia morphologies in
1548   mouse and human postmortem brain samples. *Glia* **67**, 1496-1509,
1549   doi:10.1002/glia.23623 (2019).
1550 171 Stratoulias, V., Venero, J. L., Tremblay, M. E. & Joseph, B. Microglial subtypes:
1551   diversity within the microglial community. *EMBO J* **38**, e101997,
1552   doi:10.15252/embj.2019101997 (2019).
1553 172 St-Pierre, M. K. *et al.* Ultrastructural characterization of dark microglia during aging in
1554   a mouse model of Alzheimer's disease pathology and in human post-mortem brain
1555   samples. *J Neuroinflammation* **19**, 235, doi:10.1186/s12974-022-02595-8 (2022).
1556 173 Colombo, G. *et al.* Microglial MorphOMICs unravel region- and sex-dependent
1557   morphological phenotypes from postnatal development to degeneration. *bioRxiv*,
1558   2021.2011.2030.470610, doi:10.1101/2021.11.30.470610 (2021).
1559 174 Graeber, M. B. Changing face of microglia. *Science* **330**, 783-788,
1560   doi:10.1126/science.1190929 (2010).
1561 175 Lawson, L. J., Perry, V. H., Dri, P. & Gordon, S. Heterogeneity in the distribution and
1562   morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151-170,
1563   doi:10.1016/0306-4522(90)90229-w (1990).
1564 176 Gautier, E. L. *et al.* Gene-expression profiles and transcriptional regulatory pathways
1565   that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* **13**,
1566   1118-1128, doi:10.1038/ni.2419 (2012).
1567 177 Waddell, L. A. *et al.* ADGRE1 (EMR1, F4/80) Is a Rapidly-Evolving Gene Expressed
1568   in Mammalian Monocyte-Macrophages. *Front Immunol* **9**, 2246,
1569   doi:10.3389/fimmu.2018.02246 (2018).

1570	178	Jung, S. *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion
1571		and green fluorescent protein reporter gene insertion. *Mol Cell Biol* **20**, 4106-4114,
1572		doi:10.1128/mcb.20.11.4106-4114.2000 (2000).
1573	179	Wolf, Y., Yona, S., Kim, K. W. & Jung, S. Microglia, seen from the CX3CR1 angle.
1574		*Front Cell Neurosci* **7**, 26, doi:10.3389/fncel.2013.00026 (2013).
1575	180	Bisht, K. *et al.* Dark microglia: A new phenotype predominantly associated with
1576		pathological states. *Glia* **64**, 826-839, doi:10.1002/glia.22966 (2016).
1577	181	Imai, Y., Ibata, I., Ito, D., Ohsawa, K. & Kohsaka, S. A novel gene iba1 in the major
1578		histocompatibility complex class III region encoding an EF hand protein expressed in
1579		a monocytic lineage. *Biochem Biophys Res Commun* **224**, 855-862,
1580		doi:10.1006/bbrc.1996.1112 (1996).
1581	182	Ito, D. *et al.* Microglia-specific localisation of a novel calcium binding protein, Iba1.
1582		*Brain Res Mol Brain Res* **57**, 1-9, doi:10.1016/s0169-328x(98)00040-0 (1998).
1583	183	Shapiro, L. A., Perez, Z. D., Foresti, M. L., Arisi, G. M. & Ribak, C. E. Morphological
1584		and ultrastructural features of Iba1-immunolabeled microglial cells in the hippocampal
1585		dentate gyrus. *Brain Res* **1266**, 29-36, doi:10.1016/j.brainres.2009.02.031 (2009).
1586	184	Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S. & Nabekura, J. Resting microglia
1587		directly monitor the functional state of synapses in vivo and determine the fate of
1588		ischemic terminals. *J Neurosci* **29**, 3974-3980, doi:10.1523/JNEUROSCI.4363-
1589		08.2009 (2009).
1590	185	Tremblay, M. E., Lowery, R. L. & Majewska, A. K. Microglial interactions with synapses
1591		are modulated by visual experience. *PLoS Biol* **8**, e1000527,
1592		doi:10.1371/journal.pbio.1000527 (2010).
1593	186	Lier, J. *et al.* Loss of IBA1-Expression in brains from individuals with obesity and
1594		hepatic dysfunction. *Brain Res* **1710**, 220-229, doi:10.1016/j.brainres.2019.01.006
1595		(2019).
1596	187	Fourgeaud, L. *et al.* TAM receptors regulate multiple features of microglial physiology.
1597		*Nature* **532**, 240-244, doi:10.1038/nature17630 (2016).
1598	188	Savage, J. C. *et al.* Nuclear receptors license phagocytosis by trem2+ myeloid cells in
1599		mouse models of Alzheimer's disease. *J Neurosci* **35**, 6532-6543,
1600		doi:10.1523/JNEUROSCI.4586-14.2015 (2015).
1601	189	Healy, L. M. *et al.* MerTK Is a Functional Regulator of Myelin Phagocytosis by Human
1602		Myeloid Cells. *J Immunol* **196**, 3375-3384, doi:10.4049/jimmunol.1502562 (2016).
1603	190	Huang, Y. *et al.* Microglia use TAM receptors to detect and engulf amyloid beta
1604		plaques. *Nat Immunol* **22**, 586-594, doi:10.1038/s41590-021-00913-5 (2021).
1605	191	Robinson, A. P., White, T. M. & Mason, D. W. Macrophage heterogeneity in the rat as
1606		delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter
1607		recognizing complement receptor type 3. *Immunology* **57**, 239-247 (1986).
1608	192	Milligan, C. E., Cunningham, T. J. & Levitt, P. Differential immunochemical markers
1609		reveal the normal distribution of brain macrophages and microglia in the developing
1610		rat brain. *J Comp Neurol* **314**, 125-135, doi:10.1002/cne.903140112 (1991).
1611	193	McKay, S. M., Brooks, D. J., Hu, P. & McLachlan, E. M. Distinct types of microglial
1612		activation in white and grey matter of rat lumbosacral cord after mid-thoracic spinal
1613		transection. *J Neuropathol Exp Neurol* **66**, 698-710,
1614		doi:10.1097/nen.0b013e3181256b32 (2007).
1615	194	Blackbeard, J. *et al.* Quantification of the rat spinal microglial response to peripheral
1616		nerve injury as revealed by immunohistochemical image analysis and flow cytometry.
1617		*J Neurosci Methods* **164**, 207-217, doi:10.1016/j.jneumeth.2007.04.013 (2007).
1618	195	Marshall, S. A. *et al.* Microglial activation is not equivalent to neuroinflammation in
1619		alcohol-induced neurodegeneration: The importance of microglia phenotype.
1620		*Neurobiol Dis* **54**, 239-251, doi:10.1016/j.nbd.2012.12.016 (2013).
1621	196	Peng, J. *et al.* Microglial P2Y12 receptor regulates ventral hippocampal CA1 neuronal
1622		excitability and innate fear in mice. *Mol Brain* **12**, 71, doi:10.1186/s13041-019-0492-x
1623		(2019).

1624	197	Haynes, S. E. *et al.* The P2Y12 receptor regulates microglial activation by extracellular
1625		nucleotides. *Nat Neurosci* **9**, 1512-1519, doi:10.1038/nn1805 (2006).
1626	198	Sipe, G. O. *et al.* Microglial P2Y12 is necessary for synaptic plasticity in mouse visual
1627		cortex. *Nat Commun* **7**, 10905, doi:10.1038/ncomms10905 (2016).
1628	199	Kanamoto, T. *et al.* Isolation and characterization of a novel plasma membrane protein,
1629		osteoblast induction factor (obif), associated with osteoblast differentiation. *BMC Dev*
1630		*Biol* **9**, 70, doi:10.1186/1471-213X-9-70 (2009).
1631	200	Bennett, M. L. *et al.* New tools for studying microglia in the mouse and human CNS.
1632		*Proc Natl Acad Sci U S A* **113**, E1738-1746, doi:10.1073/pnas.1525528113 (2016).
1633	201	Satoh, J. *et al.* TMEM119 marks a subset of microglia in the human brain.
1634		*Neuropathology* **36**, 39-49, doi:10.1111/neup.12235 (2016).
1635	202	van Wageningen, T. A. *et al.* Regulation of microglial TMEM119 and P2RY12
1636		immunoreactivity in multiple sclerosis white and grey matter lesions is dependent on
1637		their inflammatory environment. *Acta Neuropathol Commun* **7**, 206,
1638		doi:10.1186/s40478-019-0850-z (2019).
1639	203	Gonzalez Ibanez, F. *et al.* Immunofluorescence Staining Using IBA1 and TMEM119
1640		for Microglial Density, Morphology and Peripheral Myeloid Cell Infiltration Analysis in
1641		Mouse Brain. *J Vis Exp*, doi:10.3791/60510 (2019).
1642	204	Chertoff, M., Shrivastava, K., Gonzalez, B., Acarin, L. & Gimenez-Llort, L. Differential
1643		modulation of TREM2 protein during postnatal brain development in mice. *PLoS One*
1644		**8**, e72083, doi:10.1371/journal.pone.0072083 (2013).
1645	205	Fahrenhold, M. *et al.* TREM2 expression in the human brain: a marker of monocyte
1646		recruitment? *Brain Pathol* **28**, 595-602, doi:10.1111/bpa.12564 (2018).
1647	206	Rogers, J. T. *et al.* CX3CR1 deficiency leads to impairment of hippocampal cognitive
1648		function and synaptic plasticity. *J Neurosci* **31**, 16241-16250,
1649		doi:10.1523/JNEUROSCI.3667-11.2011 (2011).
1650	207	Paolicelli, R. C., Bisht, K. & Tremblay, M. E. Fractalkine regulation of microglial
1651		physiology and consequences on the brain and behavior. *Front Cell Neurosci* **8**, 129,
1652		doi:10.3389/fncel.2014.00129 (2014).
1653	208	Hirasawa, T. *et al.* Visualization of microglia in living tissues using Iba1-EGFP
1654		transgenic mice. *J Neurosci Res* **81**, 357-362, doi:10.1002/jnr.20480 (2005).
1655	209	Sasmono, R. T. *et al.* A macrophage colony-stimulating factor receptor-green
1656		fluorescent protein transgene is expressed throughout the mononuclear phagocyte
1657		system of the mouse. *Blood* **101**, 1155-1163, doi:10.1182/blood-2002-02-0569 (2003).
1658		