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

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

74 32. Department of Pathology and Immunology, Washington University School of Medicine in
75 St. Louis, St. Louis, USA
76 33. James and Lillian Martin Centre for Stem Cell Research, Sir William Dunn School of
77 Pathology, University of Oxford, Oxford, United Kingdom
78 34. School of Biochemistry & Immunology, Trinity Biomedical Sciences Institute, Trinity
79 College, Dublin, Republic of Ireland
80 35. Trinity College Institute of Neuroscience, Trinity College, Dublin, Republic of Ireland
81 36. Department of Neurosciences, Lerner Research Institute, Cleveland Clinic, Cleveland,
82 USA
83 37. Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Case
84 Western Reserve University, Cleveland, USA
85 38. Center for Translational & Computational Neuroimmunology, Department of Neurology,
86 Columbia University Irving Medical Center, New York, USA
87 39. Taub Institute for Research on Alzheimer's disease and the Aging Brain, Columbia
88 University Irving Medical Center, New York, USA
89 40. UK Dementia Research Institute at University College London, London, United Kingdom
90 41. Vlaams Instituut voor Biotechnologie at Katholieke Universiteit Leuven, Leuven, Belgium
91 42. "Momentum" Laboratory of Neuroimmunology, Institute of Experimental Medicine,
92 Budapest, Hungary
93 43. Department of Biomedical Sciences of Cells & Systems, section Molecular Neurobiology,
94 University of Groningen, Groningen, The Netherlands
95 44. University Medical Center Groningen, Groningen, The Netherlands
96 45. Department of Neuroscience, Center for Brain Immunology and Glia, University of Virginia
97 School of Medicine, Charlottesville, USA
98 46. Institut de Neurociències and Departament de Bioquímica, Unitat de Bioquímica,
99 Universitat Autònoma de Barcelona, Barcelona, Spain
100 47. ICREA, Barcelona, Spain
101 48. Institut de Biologie de l'ENS (IBENS), Département de biologie, École normale supérieure,
102 CNRS, INSERM, Paris, France
103 49. College de France, Paris, France
104 50. Singapore Immunology Network (SIgN), Agency for Science, Technology and Research
105 (A*STAR), Singapore
106 51. University of California San Diego School of Medicine, La Jolla, USA
107 52. Institute for Stroke and Dementia Research, Ludwig Maximillian's University of Munich,
108 Munich, Germany
109 53. School of Biological Sciences, University of Southampton, Southampton General Hospital,
110 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

185 102. University of Edinburgh, Centre for Inflammation Research, Edinburgh, United Kingdom
186 103. Department of Psychiatry & Psychotherapy, School of Medicine, Technical University of
187 Munich, Munich, Germany
188 104. Charité - Universitätsmedizin Berlin and DZNE, Berlin, Germany
189 105. University of Edinburgh and UK DRI, Edinburgh, United Kingdom
190 106. Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg,
191 Germany
192 107. Center for Basics in NeuroModulation (NeuroModulBasics), Faculty of Medicine,
193 University of Freiburg, Freiburg, Germany
194 108. Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Freiburg,
195 Germany
196 109. Department of Physiology and Pharmacology, Sapienza University of Rome, Rome,
197 Italy
198 110. Santa Lucia Foundation (IRCCS Fondazione Santa Lucia), Rome, Italy
199 111. Third Rock Ventures, Boston, USA
200 112. Hospital for Sick Children, Toronto, Canada
201 113. University of Toronto, Toronto, Canada
202 114. Nash Family Department of Neuroscience, Center for Glial Biology, Friedman Brain
203 Institute, Icahn School of Medicine at Mount Sinai, New York, USA
204 115. Max Planck Institute for Biology of Ageing, Koeln, Germany
205 116. Department of Neurobiology, Brudnick Neuropsychiatric Research Institute, University of
206 Massachusetts Medical School, Worcester, USA
207 117. Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel
208 118. Institute of Neuronal Cell Biology, Technical University Munich, German Center for
209 Neurodegenerative Diseases, Munich, Germany
210 119. Galvin Life Science Center, University of Notre Dame, Indianapolis, USA
211 120. Department of Neuroscience, University of Florida, Gainesville, USA
212 121. Faculty of Biology, University of Freiburg, Freiburg, Germany
213 122. BrainLinks-BrainTools Centre, University of Freiburg, Freiburg, Germany
214 123. Freiburg Institute of Advanced Studies, University of Freiburg, Freiburg, Germany
215 124. Department of Biology, Boston University, Boston, USA
216 125. Department of Anatomy and Neurobiology, Boston University School of Medicine,
217 Boston, USA
218 126. Picower Institute for Learning and Memory, Massachusetts Institute of Technology,
219 Cambridge, MA, USA
220 127. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology,
221 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

282 **Names, names, names**
283
284 *"If the names are unknown knowledge of the things also perishes."*[1]
285 *(Carolus Linnaeus)*
286
287 And yet, we humans instinctively tend to name things and use that name to define their
288 properties. Biologists are no exception: from the time of 18th century father of taxonomy
289 Carolus Linnaeus, the main purpose of biology has been categorizing the natural world as a
290 way of understanding it. Naming species and grouping them together into taxa served to define
291 evolutionary relationships; even today taxonomy and phylogeny are closely interrelated. But
292 we must never forget that nomenclatures and categories are artificial constructs and biology
293 is seldom black and white, but rather an extended continuum of greys. While giving names is
294 natural and useful, we need to be aware that categorization constrains our thinking by forcing
295 us to fit our observations into established classes. As sociologists say, "categorization spawns
296 expectations"[2]. This semantic issue has already been acknowledged by immunologists
297 because, in fact, the given names have connotations that often imply a specific function[3]. In
298 this paper, we extend similar initiatives on macrophages[4], dendritic cells[3], interneurons[5], and
299 astrocytes[6] to discuss the widespread problems associated with categorization of microglia
300 using outdated terms such as "resting *versus* activated" (**Box 1**) or "M1 *versus* M2" (**Box 2**).
301
302 Dichotomic, rigid categories convey a dualistic idea of good *versus* bad microglia and may
303 actually impede scientific advancement. Widely used terms, such as "neuroinflammation" as
304 a synonym of microglial reactivity (**Box 3**) and naming a panoply of presumed microglial
305 populations and assumed functions arising from single-cell transcriptomics, are misleading
306 and increasingly problematic, especially to those entering the field of glial biology and
307 neuroimmunology. This nomenclature does not address the important question: what are the
308 specific functions of microglia in the contexts of development, health, aging, and disease? It
309 is now clear that microglia exist in diverse, dynamic, and multi-dimensional states depending
310 on the context including local environment (**Figure 1**). We define dimensions as the key
311 variables driving the phenotypic transformations of microglia. These variables are molecularly
312 distinct signaling pathways regulated at multiple levels (e.g., transcriptional, epigenetic,
313 translational, metabolic) that each give rise to distinct microglial functions or properties. In this
314 manner, categorizing microglia based on a historical, one-dimensional nomenclature in the
315 absence of functional data will constrain and stifle future progress and innovation.
316
317 To examine and address these issues, we assembled a team of international experts who
318 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

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

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

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

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

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

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

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

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

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

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

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

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

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

503 ## Microglial heterogeneity: it all depends on the context

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

521

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

537

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

540 microglia differ in their transcriptomic, proteomic, and morphological profiles, across brain
541 colonization, maturation and function, in health and disease[88,94-96]. Of note, the microglial sex-
542 specific transcriptomic signatures appear to be intrinsically determined, being maintained
543 when microglia are transplanted into the brains of mice from the other sex[96]. Sexually
544 differentiated roles of microglia could critically influence a variety of biological processes, in a
545 time-dependent manner, and thus, emerge as key disease modifiers across various
546 pathological conditions with sexual dimorphism in prevalence, manifestation, and response to
547 treatment[97]. A well characterized example for sex-specific divergence is the purinergic
548 receptor P2X4R, identified as the male-biased microglial mediator of chronic pain[98]. Sex
549 differences in sexually dimorphic responses in physiology and pathology likely arise from a
550 combination of Y chromosome-specific genes, sex hormones, neuronal circuit-related factors
551 and epigenetic mechanisms[99].
552
553 Regardless of the reduced heterogeneity in the mature adult (compared to embryonic) CNS
554 [7,68,90], microglia do differ among CNS areas in terms of their morphology and ultrastructure,
555 transcriptional, proteomic, epigenetic profiles, and functional specialization, suggesting that
556 microglial states are modulated by local cues[83,100,101]. However, local CNS signals are not
557 sufficient to determine microglial identity because macrophages engrafted in the brain
558 parenchyma can acquire a microglia-like morphology without reaching a transcriptomic
559 signature identical to host microglia, even after prolonged CNS residence[26,102,103], supporting
560 the idea that microglia are distinct from peripherally-derived macrophages, even when they
561 colonize a similar niche. In addition, these findings suggest that once their identity is
562 established, microglia assume different functional states in response to local CNS signals.
563 Therefore, both the developmental genetic programs and CNS environment (nature and
564 nurture) collaborate to dynamically determine microglial functional states.
565
566 Microglia not only respond to local cues within the brain, but they also receive continuous
567 inputs from the periphery, including signals from the gastrointestinal tract[104]. In this context,
568 the role of the host microbiota is gaining momentum in controlling microglial maturation and
569 function in the CNS[88], with growing evidence that microbiota-derived short-chain fatty acids
570 represent major mediators of the gut-brain axis[87,105]. Another example of cross-talk between
571 microglia and the periphery is the so called "sickness behavior", as a result of the central
572 response to peripherally released cytokines produced by peripheral immune cells and tissue
573 resident macrophages detecting specific pathogen-associated molecular patterns
574 (PAMPs)[106]. This complex and coordinated response, in which the functional role of microglia
575 remains poorly understood, gives rise to adaptive behavioral strategies, including lethargy.

576 Acute systemic inflammation, nevertheless, was extensively shown to impact on
577 microglia[107,108] and induce a microglial state associated with robust IL-1β production[109].
578
579 The concept of the brain as an immune privileged organ has been challenged and definitely
580 revisited in recent years. Indeed, peripherally produced cytokines and immune cells access
581 the CNS and patrol the perivascular space in disease but also in health thus, playing important
582 roles in coordinating central and peripheral immune responses[110]. It was also suggested that
583 microglia require resident CD4+ T cells in the healthy developing brain for proper maturation
584 and complete fetal-to-adult transition[111]. Microglia and T cell cross-talk was shown to help
585 maintain homeostasis in the CNS, with dysfunctional regulation occurring in diseases, such
586 as MS[112], ALS[113], AD[114], and encephalitis[115]. It will be important to continue investigating the
587 influence of the peripheral immune system including B cells, NKs and other cells on microglial
588 states and function in both health and disease.
589
590 **Microglial states in the diseased CNS**
591 Microglia are keen responders and critical players in numerous neurodevelopmental,
592 neurological, and neurodegenerative conditions, as thoroughly reviewed elsewhere. Altered
593 microglial states have been described in the diseased human brain and across various animal
594 models of disease pathology based on morphology and gene expression signature. In
595 addition, these states also differ depending on the timing (i.e., disease stage), genetic
596 background, and local environment. Context-dependent signals vary dramatically during
597 disease progression; they range from apoptotic cells, extracellular debris, toxic proteins (i.e.,
598 amyloid, α-synuclein), and signals resulting from blood-brain barrier disruption and altered
599 function of neurons and other glial cells. Microglia respond to these challenges by changing
600 their molecular profile, morphology and ultrastructure (**Box 3**), as well as motility and function.
601
602 The expression of core microglial markers is also altered over the course of disease, including
603 downregulation of the "homeostatic" microglial signature. A prototypical example is P2RY12,
604 one of the most widely used markers to discriminate microglia from other macrophages, with
605 its reduced expression being one of the salient features of the microglial response to AD
606 pathology and other disease conditions[116], as shown in several mouse models of disease
607 (**Figure 4**). The apparent contradiction that core markers do not have a steady expression, as
608 could perhaps be expected, is likely reflecting the functions those proteins have and how they
609 change in the diseased brain. For instance, P2RY12 upregulation in epilepsy may relate to
610 microglial sensing ATP and nucleotides released during seizures[117]. This seeming paradox
611 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

755 *Grammar Quandary:*

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

760

761 **Future questions and challenges**

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

771

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

781

782 *How are microglial states coordinated?*

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

791

792 *How similar are peripherally-derived macrophages and microglia?* A burning question that
793 surely requires further investigation is related to the identity and function of microglia *versus*
794 other brain macrophages. Although recent studies have provided evidence for an intrinsic
795 unique core signature of microglia, their functional resemblances and differences remain
796 undetermined. For instance, could engrafted parenchymal macrophages functionally replace
797 the resident microglia, despite having a different molecular identity, and could they serve as
798 therapeutic vectors?
799
800 *The devil is in the details*: Another major caveat is that microglia are incredibly reactive cells
801 and evidence indicates that artifacts are often introduced during sample processing for a
802 variety of methodologies, such as RNA profiling, immunohistochemistry, FACS, *in vivo*
803 imaging, and so on. Hence, we may be missing or confounding important pieces of information
804 because we unintentionally introduce changes in the parameters we are trying to measure. In
805 addition, these artifacts are likely to generate variability across laboratories using different
806 protocols. A future challenge is to increase reproducibility of data across laboratories, by
807 coordinating a shared database of protocols and analysis pipelines curated using STAR
808 methods guidelines. In addition, in the current single-cell multi-omics era, the challenges in
809 big data analysis are exponentially growing[140]. Statistical methods (including multivariate
810 statistics)[141] and artificial intelligence-based data mining approaches (such as machine
811 learning)[142] will have to be introduced, to uniformly process and integrate large datasets, as
812 well as extract the biological relevance of the findings.
813
814 *Diversity as a source of richness*: Many transcriptional states have been reported during
815 embryonic development, aging, and disease. How many different microglial states can be
816 identified? Within the homeostatic microglia, how many states exist? How do microglia
817 navigate among their many states? Are they related through a transcriptional continuum, or
818 perhaps as a hub-and-spoke set of states, as has been proposed for macrophages[4]? How
819 dynamic are these states? And how spatially defined are they? Future research will need to
820 address these important questions.
821
822 *Male versus female microglia:* Sex differences have been reported to affect the brain
823 colonization, maturation, structure, transcriptomic, proteomic, and functional profiles of
824 microglia, in a time-dependent manner. To what extent these differences may regulate the
825 susceptibility to neurological diseases remains a fascinating question that urgently awaits
826 answers. Investigating the molecular and cellular mechanisms underlying sex-mediated
827 differences in microglial states would advance our understanding of microglial implication in

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

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

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

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

865 **Box 1. Resting *versus* activated microglia**
866 The development of specific silver staining techniques in 1919 allowed Río-Hortega to clearly
867 identify microglia and study their response to experimental manipulations[7,145]. Early on, Río-
868 Hortega appreciated the striking morphological transformation of microglia following brain
869 damage, but it was in the mid-1970s that the terms "resting" and "activated" microglia first
870 appeared in the literature. These terms were used to morphologically describe cells with
871 affinity for silver staining that were observed in physiological ("resting") *versus* pathological
872 ("activated") conditions. This nomenclature consolidated in the 1980s and became widely
873 used during the 1990s[146], in parallel with the development and use of histochemical and
874 immunohistochemical techniques, such as lectin staining[147], detection of phosphatases and
875 phosphorylases[148], and antibodies against the complement receptor CR3[7]. These techniques
876 and nomenclature were pivotal in determining that "resting" microglia were unrelated to
877 astrocytes, as some studies had wrongly concluded[149], and that "reactive" microglia shared
878 many characteristics with the blood-borne monocytes[10].
879
880 As shown by a PubMed search with microglia in all fields, there were only few papers
881 published on the topic before the 1990s, and then a steady increase until the beginning of our
882 century, followed by an exponential growth[150]. There is a first inflexion point in 2005, with the
883 seminal discovery using non-invasive two-photon *in vivo* imaging that microglia are extremely
884 dynamic in the absence of pathological challenge, continuously surveying the parenchyma
885 with their highly motile processes[55,56]. The development of non-invasive methods was
886 necessary for our understanding of microglial roles in the healthy brain (reviewed in[151]). In
887 2005, microglial extreme dynamism in the intact brain was examined for the first time, through
888 the skull of CX3CR1-GFP mice in which microglia are fluorescently labeled[55,56]. As a result,
889 microglia are now considered to be the most dynamic cells of the healthy mature brain[151]. This
890 seminal discovery prompted to rename quiescent or resting microglia as surveying[56,152] or
891 surveillant (from the verb to survey)[153] microglia, and also led to propose the concept that
892 microglia are never-resting[154]. Together, these and other *in vivo* two-photon imaging data put
893 into serious doubt the concept of "activated" microglia, which suggests a unique form of
894 response, as in fact microglia are always active, constantly responding (in different ways
895 depending on the context) to the changes in their CNS environment, even under normal
896 physiological conditions. Therefore, microglia do not switch from "resting" to "activated" in
897 response to trauma, injury, infection, disease, and other challenges. Rather, microglia are
898 continuously active and react to the stage of life, CNS region, species, sex, and context of
899 health or disease by adopting different states and performing different functions. Thus,
900 although still widely used, "resting" and "activated microglia" are labels that should be
901 discontinued.

902 **Box 2. M1 *versus* M2 microglia**
903 Another terminology emerged in the early 2000s from immunologists classifying macrophages
904 based on findings obtained using *in vitro* models: "M1", the classical activation, considered
905 pro-inflammatory and neurotoxic, as well as closely related to the concept of "activated"
906 microglia, and "M2", or alternative activation, considered anti-inflammatory and
907 neuroprotective[155]. These responses were related to those of T helper lymphocytes (Th1 and
908 Th2) based on their *in vitro* activation by specific immune stimuli that activated differential
909 metabolic programs and changes in cytokine expression[156]. An associated term is "M0"
910 microglia, which describes their state when cultured in the presence of TGFβ (transforming
911 growth factor beta) and CSF-1 to mimic *in vivo* counterparts[157]. The terms became widely
912 adopted in microglial research and the 2010s saw a boom of papers phenotyping
913 macrophages and microglia into "M1" and "M2" based on the expression of markers related to
914 these categories, used to indirectly assume a detrimental ("M1") or beneficial ("M2") microglial
915 role[156]. In many cases, editors and reviewers have asked authors to comply with this
916 nomenclature. However, it soon became evident that macrophage responses are more
917 complex than simply "M1" and "M2"[158]. In the case of microglia, the advent of single cell
918 technologies provided clear evidence that microglia in the living brain do not polarize to either
919 of these categories, often co-expressing M1 and M2 markers[159], despite the continued use of
920 M1 and M2 in the literature. We thus recommend to strictly avoid M1 and M2 labels and use
921 more nuanced tools to investigate microglial function (reviewed in[160]).
922
923 **Box 3. Microglial morphological responses across species**
924 Microglial cells display a profusion of morphologies that have fascinated researchers since the
925 early days of Río-Hortega. Many were tempted to equate morphology with function. Ramified
926 microglia were traditionally associated with the "resting" state, although we now know that
927 ramified microglia actively play many functions during normal physiological conditions. In
928 contrast, "reactive" microglia (rounder cell body, generally with fewer and shorter processes)
929 were called "activated" and equated with an inflammatory response. Only recently, however,
930 a mechanistic link between microglial reduced branching and increased release of the
931 inflammatory cytokine interleukin 1β was reported[161]. Activation of P2YR12 by tissue damage
932 signals potentiates the tonically active potassium THIK-1 channel, expressed in microglia,
933 leading both to decreased microglial ramifications and activation of the inflammasome
934 machinery processing IL-1β precursors into their mature form[161]. Another morphology
935 associated with functional changes is "ameboid" microglia, which were thought to be more
936 "phagocytic", but it is clear now that ramified microglia execute phagocytosis through their
937 terminal or 'en passant' branches notably during adult neurogenesis[162,163], while in disease
938 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

976 “neuroinflammation” is encountered in the literature, the reader must be aware that it means
977 different things depending on the context.
978 While the term “neuroinflammation” is widely used in the field as a synonym of microglial
979 “activation”[174], its definition also varies dramatically among authors, according to our survey.
980 Below are representative definitions which are currently used by the authors:
981
982 a. Neuroinflammation is inflammation of neural tissue particularly mediated by glial cells.
983 b. Neuroinflammation is strictly limited to conditions in which leukocytes enter CNS, e.g., in
984 stroke and MS.
985 c. Neuroinflammation is a mixed cellular response to brain infection or damage involving innate
986 and adaptive responses of resident brain cells and circulating immune cells.
987 d. The term neuroinflammation is too unclear and imprecise and should be avoided.
988
989 Considering that different definitions are used across authors, our main recommendation for
990 the field is to liberate neuroinflammation from microglia and microglia from neuroinflammation,
991 and to use both terms rigorously. The consensus among authors is four-fold. First, protection
992 against tissue damage and extreme departures from homeostasis as well as repair (i.e.,
993 ‘inflammation’) encompasses, in the CNS, a highly complex set of local responses, and equally
994 complex interactions with circulating immune cells or with immune cells residing in brain-blood
995 and brain-cerebrospinal fluid interphases. In other words, ‘neuroinflammation’ is not a
996 substitute for ‘microglial reaction’. Second, there are numerous transcriptional states of
997 microglia, astrocytes and oligodendrocytes. The functional outcomes of cells undergoing
998 these transcriptional states remain incompletely understood. Furthermore, it is uncertain which
999 transcriptional states are transient or represent durable cell fate choices. It is also unknown
1000 whether changes in states during diseases are ‘inflammatory’ or dedicated to maintaining
1001 microglial homeostatic functions. Taking these considerations together, one should exercise
1002 extreme caution in simplifying these phenomena as ‘neuroinflammation’, as at least some of
1003 these phenomena may represent alternative homeostatic or non-inflammatory reactive states.
1004 Third, it is not appropriate to imply that neuroinflammation is invariably deleterious. Rather, it
1005 should be recognized that each inflammatory response may exert adaptive or maladaptive
1006 effects, contingent on context. To be more specific, research is necessary to explore functions
1007 and distinct actions of cytokine-enriched microglia secretomes beyond binary
1008 characterizations such as ‘pro-’ and ‘anti-inflammatory’. Fourth, with regards to nomenclature,
1009 we recommend the use of modest and precise terms to describe specific phenomena such
1010 as: microglial reaction; astrocytic reaction; molecules involved; loss of barrier function at the
1011 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

1015 **TABLES AND FIGURE LEGENDS:**

1016 **Figure 1. Microglial nomenclatures, past and future.** Microglia have been traditionally
1017 framed into dichotomic categories but our current integration of epigenetic, transcriptomic,
1018 metabolomic and proteomic data favors a multidimensional integration of coexisting states.
1019

1020 **Figure 2. Microglial core properties and functions:** Phagocytosis, surveillance and
1021 capacity for releasing soluble factors (inner circle) are core properties through which microglia
1022 contribute to key biological functions (outer circle). Created with BioRender.com.
1023

1024 **Figure 3. Microglial identity and states.** The identity of microglia, compared to other CNS-
1025 associated macrophages in the perivascular space, choroid plexus and leptomeninges, is
1026 established early on from yolk sac-derived progenitors. Once they colonize the brain
1027 parenchyma and differentiate, they can adopt multiple states depending on the particular
1028 spatio-temporal context, as shown in more detail in **Figure 5**. Created with BioRender.com.
1029

1030 **Figure 4. Microglial transcriptomic signatures.** Recent scRNA-Seq studies have identified
1031 many microglial transcriptional signatures including but not limited to PAM and ATM in
1032 development; DAM, MgnD, ARM, MIMS in disease models of AD, MS, ALS and PD; and
1033 WAM, LDAM, HAM in aging, both in mice and human. The key upregulated (red) and
1034 downregulated (blue) genes in each signature are indicated. Created with BioRender.com.
1035

1036 **Figure 5. Microglial states defined by their intrinsic and extrinsic determinants,**
1037 **spatiotemporal context, and layers of complexity.** Microglial states depend on intrinsic
1038 determinants (such as species, ontogeny, sex, or genetic background) as well as the specific
1039 context they inhabit, including age, spatial location, and environmental factors (such as
1040 nutrition, microbiota, pathogens, drugs, etc.). All together, these factors impinge on microglia
1041 at multiple levels (i.e., epigenomic, transcriptomic, proteomic, metabolomics, ultrastructural
1042 and phenomic), which ultimately determine microglial functions. Created with BioRender.com
1043
1044

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

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

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

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

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

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

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

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

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

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

1082 **References**

1083 1 Stafleu, F. A. Linnaeus and the Linnaeans: The Spreading of their Ideas in Systematic
1084 Botany, 1735-1789 (1971), p. 80. . (1971).
1085 2 Charmaz, K. The power of names. *Journal of Contemporary Ethnography* **35**, 396-399
1086 (2006).
1087 3 Guilliams, M. *et al.* Dendritic cells, monocytes and macrophages: a unified
1088 nomenclature based on ontogeny. *Nat Rev Immunol* **14**, 571-578, doi:10.1038/nri3712
1089 (2014).
1090 4 Murray, P. J. *et al.* Macrophage activation and polarization: nomenclature and
1091 experimental guidelines. *Immunity* **41**, 14-20, doi:10.1016/j.immuni.2014.06.008
1092 (2014).
1093 5 Yuste, R. *et al.* A community-based transcriptomics classification and nomenclature of
1094 neocortical cell types. *Nat Neurosci* **23**, 1456-1468, doi:10.1038/s41593-020-0685-8
1095 (2020).
1096 6 Escartin, C. *et al.* Reactive astrocyte nomenclature, definitions, and future directions.
1097 *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
1099 Century of Microglial Research. *Trends Neurosci* **42**, 778-792,
1100 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
1102 & A. Sierra) 7-46 (Springer, 2014).
1103 9 Río-Hortega, P. El "tercer elemento" de los centros nerviosos. III. Naturaleza probable
1104 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*
1106 **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
1108 yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res* **117**, 145-152,
1109 doi:10.1016/s0165-3806(99)00113-3 (1999).
1110 12 Xu, J. *et al.* Temporal-Spatial Resolution Fate Mapping Reveals Distinct Origins for
1111 Embryonic and Adult Microglia in Zebrafish. *Dev Cell* **34**, 632-641,
1112 doi:10.1016/j.devcel.2015.08.018 (2015).
1113 13 Ferrero, G. *et al.* Embryonic Microglia Derive from Primitive Macrophages and Are
1114 Replaced by cmyb-Dependent Definitive Microglia in Zebrafish. *Cell Rep* **24**, 130-141,
1115 doi:10.1016/j.celrep.2018.05.066 (2018).
1116 14 Ginhoux, F. *et al.* Fate mapping analysis reveals that adult microglia derive from
1117 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
1119 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-
1121 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
1123 defined stages of development. *Nat Commun* **9**, 75, doi:10.1038/s41467-017-02492-2
1124 (2018).
1125 18 Andjelkovic, A. V., Nikolic, B., Pachter, J. S. & Zecevic, N. Macrophages/microglial
1126 cells in human central nervous system during development: an immunohistochemical
1127 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
1129 CSF-1 Receptor and its Ligands in the Nervous System. *Trends Neurosci* **39**, 378-393,
1130 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
1132 Ligands IL-34 and CSF1 Are Differentially Required for Microglia Development and
1133 Maintenance in White and Gray Matter Brain Regions. *Front Immunol* **10**, 2199,
1134 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).

1352 102 Bennett, F. C. *et al.* A Combination of Ontogeny and CNS Environment Establishes
1353 Microglial Identity. *Neuron* **98**, 1170-1183 e1178, doi:10.1016/j.neuron.2018.05.014
1354 (2018).
1355 103 Shemer, A. *et al.* Engrafted parenchymal brain macrophages differ from microglia in
1356 transcriptome, chromatin landscape and response to challenge. *Nat Commun* **9**, 5206,
1357 doi:10.1038/s41467-018-07548-5 (2018).
1358 104 Abdel-Haq, R., Schlachetzki, J. C. M., Glass, C. K. & Mazmanian, S. K. Microbiome-
1359 microglia connections via the gut-brain axis. *J Exp Med* **216**, 41-59,
1360 doi:10.1084/jem.20180794 (2019).
1361 105 Erny, D. *et al.* Microbiota-derived acetate enables the metabolic fitness of the brain
1362 innate immune system during health and disease. *Cell Metab* **33**, 2260-2276 e2267,
1363 doi:10.1016/j.cmet.2021.10.010 (2021).
1364 106 Dantzer, R. Cytokine, sickness behavior, and depression. *Immunol Allergy Clin North
1365 Am* **29**, 247-264, doi:10.1016/j.iac.2009.02.002 (2009).
1366 107 Shemer, A. *et al.* Interleukin-10 Prevents Pathological Microglia Hyperactivation
1367 following Peripheral Endotoxin Challenge. *Immunity* **53**, 1033-1049 e1037,
1368 doi:10.1016/j.immuni.2020.09.018 (2020).
1369 108 Sousa, C. *et al.* Single-cell transcriptomics reveals distinct inflammation-induced
1370 microglia signatures. *EMBO Rep* **19**, doi:10.15252/embr.201846171 (2018).
1371 109 Cunningham, C., Wilcockson, D. C., Campion, S., Lunnon, K. & Perry, V. H. Central
1372 and systemic endotoxin challenges exacerbate the local inflammatory response and
1373 increase neuronal death during chronic neurodegeneration. *J Neurosci* **25**, 9275-9284,
1374 doi:10.1523/JNEUROSCI.2614-05.2005 (2005).
1375 110 Louveau, A., Harris, T. H. & Kipnis, J. Revisiting the Mechanisms of CNS Immune
1376 Privilege. *Trends Immunol* **36**, 569-577, doi:10.1016/j.it.2015.08.006 (2015).
1377 111 Pasciuto, E. *et al.* Microglia Require CD4 T Cells to Complete the Fetal-to-Adult
1378 Transition. *Cell* **182**, 625-640 e624, doi:10.1016/j.cell.2020.06.026 (2020).
1379 112 Dong, Y. & Yong, V. W. When encephalitogenic T cells collaborate with microglia in
1380 multiple sclerosis. *Nat Rev Neurol* **15**, 704-717, doi:10.1038/s41582-019-0253-6
1381 (2019).
1382 113 Beers, D. R., Henkel, J. S., Zhao, W., Wang, J. & Appel, S. H. CD4+ T cells support
1383 glial neuroprotection, slow disease progression, and modify glial morphology in an
1384 animal model of inherited ALS. *Proc Natl Acad Sci U S A* **105**, 15558-15563,
1385 doi:10.1073/pnas.0807419105 (2008).
1386 114 Mittal, K. *et al.* CD4 T Cells Induce A Subset of MHCII-Expressing Microglia that
1387 Attenuates Alzheimer Pathology. *iScience* **16**, 298-311, doi:10.1016/j.isci.2019.05.039
1388 (2019).
1389 115 Di Liberto, G. *et al.* Neurons under T Cell Attack Coordinate Phagocyte-Mediated
1390 Synaptic Stripping. *Cell* **175**, 458-471 e419, doi:10.1016/j.cell.2018.07.049 (2018).
1391 116 Chen, Y. & Colonna, M. Microglia in Alzheimer's disease at single-cell level. Are there
1392 common patterns in humans and mice? *J Exp Med* **218**, doi:10.1084/jem.20202717
1393 (2021).
1394 117 Avignone, E., Ulmann, L., Levavasseur, F., Rassendren, F. & Audinat, E. Status
1395 epilepticus induces a particular microglial activation state characterized by enhanced
1396 purinergic signaling. *J Neurosci* **28**, 9133-9144, doi:10.1523/JNEUROSCI.1820-
1397 08.2008 (2008).
1398 118 Zrzavy, T. *et al.* Loss of 'homeostatic' microglia and patterns of their activation in active
1399 multiple sclerosis. *Brain* **140**, 1900-1913, doi:10.1093/brain/awx113 (2017).
1400 119 Gerrits, E. *et al.* Distinct amyloid-beta and tau-associated microglia profiles in
1401 Alzheimer's disease. *Acta Neuropathol* **141**, 681-696, doi:10.1007/s00401-021-02263-
1402 w (2021).
1403 120 Silvin, A. *et al.* Dual ontogeny of disease-associated microglia and disease
1404 inflammatory macrophages in aging and neurodegeneration. *Immunity* **55**, 1448-1465
1405 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