Defining microglial states and nomenclature: a roadmap to 2030

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], Ettiene 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], Ginhoux Florent[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], Michael Heneka[60], Frank L. Heppner[61], Soyon Hong[62], Steffen Jung[63], Helmut Kettenmann[64-65], Jonathan Kipnis[66], Ryuta Koyama[67], Greg Lemke[68], Marina Lynch[69], Ania Majewska[70], Marzia Malcangio[71], Tarja Malm[72], Renzo Mancuso[73-74], Michela Matteoli[75], Barry McColl[76], Veronique E. Miron[77-78], Anna Victoria Molofsky[79], Eva Mracsko[80], Agnes Nadjar[81], Jonas J. Neher[82-83], Urte Neniskyte[84-85], Harald Neumann[86], Mami Noda[87], Bo Peng[88], Francesca Peri[89], V. Hugh Perry[90-91], Phillip G. Popovich[92], Josef Priller[93-95], Davide Ragozzino[96-97], Richard M. Ransohoff[98], Michael W. Salter[99-100], Anne Schaefer[101-102], Dorothy P. Schafer[103], Michal Schwartz[104], Mikael Simons[105], Wolfgang J. Streit[106], Tuan Leng Tay[107-111], Alexei Verkhratsky[112-114], Rommy von Bernhardi[115], Hiroaki Wake[116], Valerie Wittamer[117-118], Susanne A. Wolf[119], Long-Jun Wu[120], Tony Wyss-Coray[121].

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

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

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

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

79. *** University of California, San Francisco, USA
80. *** Roche Innovation Center, Basel, Switzerland
81. Neurocentre Magendie, University of Bordeaux, Bordeaux, France
82. Jonas J. Neher. German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany
83. Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
84 Urte Neniskyte. VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center, Vilnius University, Vilnius, Lithuania
85. Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
86. Institute of Reconstructive Neurobiology, Medical Faculty and University Hospital of Bonn, University of Bonn, Bonn, Germany
87. *** Laboratory of Pathophysiology, Kyushu University, Fukuoka, Japan
88. Department of Neurosurgery, Huashan Hospital, Institute for Translational Brain Research, State Key Laboratory of Medical Neurobiology, MOE Frontiers Center for Brain Science, Fudan University, Shanghai, China.
89. Department of Molecular Life Sciences, University of Zurich, Zurich, Switzerland
90. UK Dementia Research Institute, University College London, London, United Kingdom
91. School of Biological Sciences, University of Southampton, Southampton, United Kingdom
92. *** Ohio State College of Medicine, Ohio State University, Columbus, USA
93. Department of Psychiatry & Psychotherapy, School of Medicine, Technical University of Munich, Munich, Germany
94. Charité - Universitätsmedizin Berlin and DZNE, Berlin, Germany
95. University of Edinburgh and UK DRI, Edinburgh, United Kingdom
96. Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy
97. Santa Lucia Foundation (IRCCS Fondazione Santa Lucia), Rome, Italy
98. Third Rock Ventures, Boston, USA
99. Hospital for Sick Children, Toronto, Canada
100. University of Toronto, Toronto, Canada
101. Nash Family Department of Neuroscience, Center for Glial Biology, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, USA
102. Max Planck Institute for Biology of Ageing, Koeln, Germany
103. Department of Neurobiology, Brudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, Worcester, USA
104. Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel
105. Institute of Neuronal Cell Biology, Technical University Munich, German Center for Neurodegenerative Diseases, Munich, Germany

106. Department of Neuroscience, University of Florda College of Medicine, Gainesville, USA
107. Faculty of Biology, University of Freiburg, Freiburg, Germany
108. BrainLinks-BrainTools Centre, University of Freiburg, Freiburg, Germany
109. Freiburg Institute of Advanced Studies, University of Freiburg, Freiburg, Germany
110. Department of Biology, Boston University, Boston, USA
111. Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, USA
112. Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom
113. Achucarro Basque Center for Neuroscience, Ikerbasque, Basque Foundation for Science, Bilbao, Spain
114. Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED, Leioa, Spain
115. Faculty of Health Sciences, Universidad San Sebastian, Santiago, Chile
116. *** Department of Anatomy and Molecular Cell Biology, Graduate School of Medicine, Nagoya University, Nagoya, Japan
117. Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles (ULB), Brussels, Belgium
118. ULB Institute of Neuroscience (UNI), Université Libre de Bruxelles (ULB), Brussels, Belgium
119. CharitéUniversitätsmedizin, Experimental Ophthalmology and Neuroimmunology, Berlin, Germany
120. *** Department of Neurology and Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA
121. *** Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford University, Stanford, USA

*corresponding authors

Abstract

Microglial research has been constrained by a rolling series of dichotomic categories such as "resting *versus* activated" or "M1 *versus* M2", and more recently "homeostatic *versus* DAM" (disease-associated microglia). This Manichean perspective of good or bad microglia is in sharp contrast with the wide repertoire of microglial functions, exerted from development to aging and diseases that have been brought to light in recent years. New designations continuously arising in the attempt to describe different microglial states from transcriptomics or other readouts may thus easily lead to the misleading, although unintentional, coupling of categories and functions. This issue may hamper information retrieval from public databases, as description of certain microglial states is confined to specific newly-coined terms. To address these issues, we report the conclusions from a discussion group of multidisciplinary experts in microglial research and discuss our current understanding of microglial states as a dynamic concept. These states result from the intersection of intrinsic genetic determinants and the specific context they inhabit. These states are further determined by layers of epigenetic, transcriptional, proteomic, metabolomic, and morphological complexity that ultimately result in specific cell functions. In this white paper, we provide a series of recommendations on the use of microglial nomenclature for researchers, reviewers and editors, including neophytes. We discuss gaps in knowledge and future challenges to tackle, providing a conceptual framework to understand microglial states.

Abbreviations

AD – Alzheimer's disease
ARM – activated response microglia
ATM – axon tract-associated microglia
BAM – border-associated macrophage
CAM – CNS-associated macrophages
CNS – central nervous system
CSF – cerebrospinal fluid
CSF1R – colony stimulating factor receptor microglia
DAM – disease-associated microglia
HAM – human AD microglia
iPSC – induced pluripotent stem cells
IRM – interferon-responsive microglia
ISF – interstitial fluid
LDAM – lipid-droplet-accumulating microglia in aging mice and humans
MGnD – microglial neurodegenerative phenotype
MIMS – microglia inflamed in multiple sclerosis

MS – multiple sclerosis

PAM – proliferative-region-associated microglia

ROS – reactive oxygen species

scRNASeq – single-cell RNA sequencing

WAM – white matter-associated microglia

Names, names, names

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

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

To examine and address these issues, we assembled a team of international experts who have made major contributions to microglial research, inclusive of various groups, and balancing gender, geographical distribution, and seniority. Authors from the fields of neuroscience, neurobiology, immunology, neuroimmunology, and neuropathology, both from academia and industry, discussed their perspectives on the use of microglial nomenclature. A questionnaire (**Supplementary Data**) was created to collect all the authors' opinions on several nomenclature issues and the importance of directly addressing microglial function. The responses to the questionnaire and an online meeting held in June 2021 were used as a backbone to develop this white paper.

Here, we first describe our current knowledge about the identity of microglia and discuss best practices for how to define and study microglial state dynamics. We then summarize "classical" microglial nomenclatures, highlighting some of the key discoveries that led to the above classifications and their limitations. We intentionally focus on citing studies related to the nomenclature rather than providing a comprehensive review of the history of microglial

research, as it has been done elsewhere[7,8]. We discuss the overall limitations and, finally, we conclude with recommendations for the proper usage of microglial nomenclature as research evolves, and provide a conceptual framework for discussing microglia, as well as our perspectives on the future questions, gaps in knowledge, and challenges to tackle as a field.

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

The origin and identity of microglia was for many years a matter of debate. In the dim and distant past, Ramón y Cajal´s disciple, Pío del Río-Hortega suggested these cells were of mesodermal origin[9]. However, over time, their ectodermal origin was also proposed[10], sparking controversy until the 1980s. The mesodermal origin took solid hold later on with the advance of technical approaches revealing more similarities than differences with the functions and features of macrophages. In 1999, microglia were reported to appear in the brain rudiment as early as embryonic day E8 in mice, and proposed to originate from yolk sac progenitors[11]. The recent combination of fate mapping studies and transplantation approaches has finally resolved this debate, revealing key aspects of microglial identity and plasticity. It is now known that microglia originate from a pool of Myb-independent macrophages produced during primitive hematopoiesis in the yolk sac, which start invading the neuroepithelium from E8.5 in mice[12-14].

One key signaling pathway for microglial development and maintenance is the CSF-1R (colony stimulating factor receptor). Ligands of CSF-1R that sustain this pathway include two cytokines with different origin and primary sequence, but similar tridimensional structure and binding to CSF-1R: IL-34, which is produced by neurons, and CSF-1, which is secreted by neurons, astrocytes, and microglia themselves[15]. 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 CSF-1R antagonists[16,17] or diphtheria toxin[17]. This process, termed “microglial repopulation” or “microglial self-renewal”[18-20] 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[21-24], peripheral blood cells[23,25], stem cell- or iPSC-derived peripheral blood cells[26], across various experimental or pathological conditions[26-28].

Our current definition is that microglia are yolk sac-derived, long-living cells that persist into adulthood, and self-renew within the CNS parenchyma without any contribution of bone marrow-derived cells in the steady-state. Microglia have been implicated in many critical CNS functions, notably through their motility, release of soluble factors, and capacity for phagocytosis (See **Figure 1** for a schematic overview). Microglia can be identified by the

expression of core transcriptional factors such as SALL1[29,] cytoplasmic markers such as ionized calcium-binding adapter molecule 1 (IBA1), and surface markers such as the purinergic receptor P2YR12, and transmembrane protein 119 (TMEM119). (See **Table 1** for details on **Microglial Markers**).

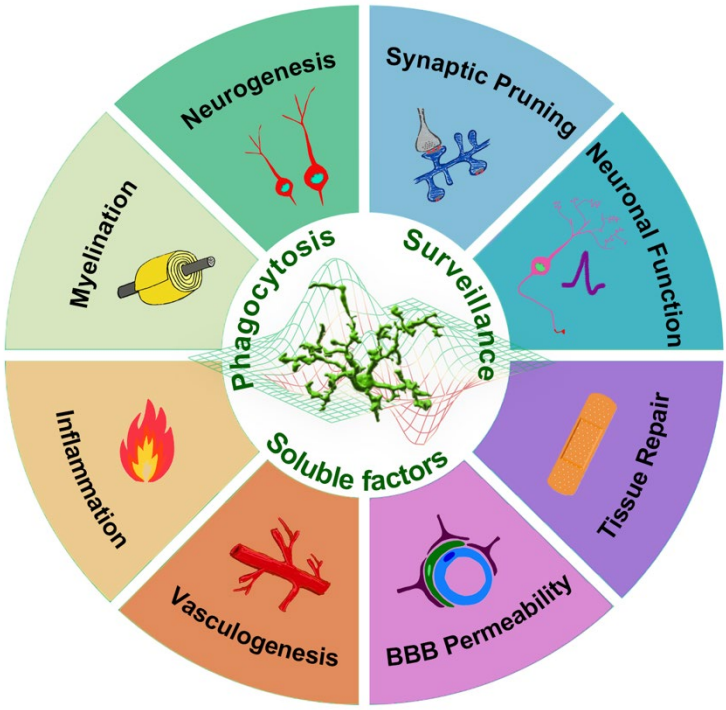

Figure 1. Microglial functions: *Phagocytosis, surveillance and capacity for releasing soluble factors (inner circle) are core properties through which microglia contribute to key biological functions (outer circle).*

Nonetheless, microglia share many features with other brain macrophage populations, the CNS border-associated macrophages (BAMs), which reside in the perivascular space, leptomeninges, and choroid plexus[30,31], and with brain macrophages derived from circulating monocytes that can extravasate, especially when the integrity of the blood-brain barrier is compromised (**Figure 2**). For a long time, the lack of tools to discriminate between these populations hindered studies aimed at defining microglia phenotypically and functionally. The last decade has witnessed an exponential increase in genetic tools and technical advances that have moved the field forward considerably. Transcriptomic approaches, in particular, have significantly contributed to defining a unique microglial signature as compared to other CNS cells and myeloid cells[32-36]. The combined efforts across several laboratories have led to the identification of key genes highly enriched in microglia that represent their core transcriptional identity and are commonly recognized as “microglial markers” (**Table 1. Microglial markers**), although they alone do not define the microglial identity. Based on these markers, newly

generated genetic tools (such as Cx3cr1^{CreERT2}, P2ry12^{CreERT2}, Tmem119^{CreERT2} and Hexb^{CreERT2} mouse lines) are available that allow for a more specific manipulation or visualization of microglia, although they could also target other populations, including BAMs and other glial cells[37-42]. Most recently, a new binary transgenic model relying on coexpression of Sall1 and Cx3cr1 has been introduced that specifically targets microglia[43]. However, caution must be observed because these markers do not imply *bona fide* microglia, as they can be expressed by cells originating from monocytes or iPSCs. These latter cells should be more accurately described as monocyte-derived microglia-like or iPSC-derived microglia-like cells.

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

While core markers of identity are incredibly useful to define microglia as a cell type, taken individually they are not sufficient to inform about the microglial "state". This "state" depends on the physiological conditions in which microglia are found at any given space and time. The microglial state is, thus, highly complex and dynamic and it is produced by the unique combination of the cell´s epigenome, transcriptome, and proteome in that specific context, resulting in a defined morphological and/or functional outcome (**Figure 2**).

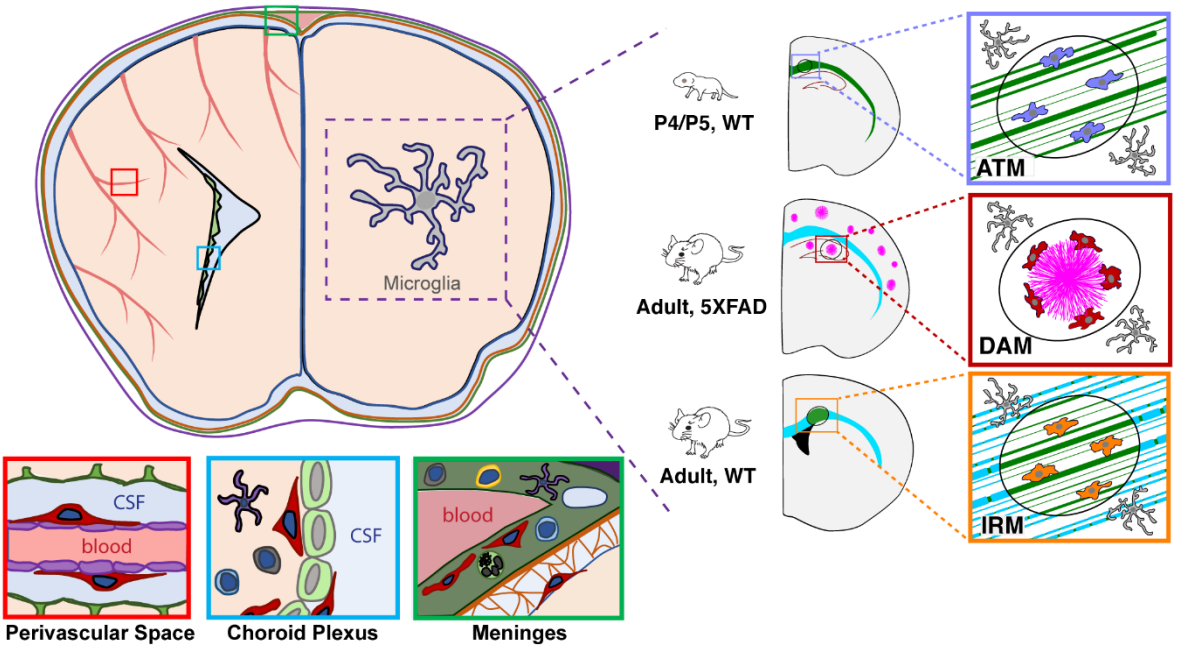

Figure 2. Brain macrophages and microglial states. *Brain parenchyma-resident microglia compose part of a heterogeneous landscape of macrophages in central nervous system-associated tissues. These include macrophages residing in the pial and dural meninges, the perivascular space of the brain, and the choroid plexus (collectively referred to as border associated macrophages, or BAMs). Under homeostatic conditions, microglia are phenotypically and transcriptionally distinct from BAMs. Across development and in various*

neurodegenerative diseases, microglia assume additional transcriptional "states" that may underlie unique, context-specific functions. Some prominent examples of these states include: axon tract-associated microglia (ATM), which emerge in the white matter tracts of the corpus callosum and cerebellum in early postnatal life; disease-associate microglia (DAM), which are elicited in response to various neurodegenerative conditions including models of Alzheimer's Disease (e.g. 5xFAD transgenic mice); and injury responsive microglia (IRM), which have been observed in demyelinated white matter lesions. The right part of this figure was adapted from [52].

Microglia are anything but static, as they are exceptionally responsive to the alterations in their environment. In the mature, healthy CNS microglial cell bodies are largely sessile but their processes are constantly moving and scanning the brain parenchyma. Their functions adapt to their particular 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.

"Cells are residents of a vast "landscape" of possible states, over which they travel during development and in disease".
(C.H. Waddington)[44]

Single-cell technologies have helped to not only locate cells on this landscape, but also to provide new insight into the molecular mechanisms that shape the landscape and regulate specific cell states. Multi-omics and integrative analyses of gene and protein expression have provided unprecedented insight into the microglial states present in a given context (e.g., development, adult, 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[45]; MGnD (microglial neurodegenerative phenotype) documented across several disease models[46]; ARM (activated response microglia) and IRM (interferon-responsive microglia) in an AD pathology mouse model[47]; HAM (human AD microglia)[48]; MIMS (microglia inflamed in multiple sclerosis (MS))[49]; and LDAM (lipid-droplet-accumulating microglia in aging mice and humans)[50]. In the developing brain were described the WAM (white matter-associated microglia)[51]; ATM (axon tract-associated microglia)[52], and PAM (proliferative-region-associated microglia, related to phagocytosis of developing oligodendrocytes), which share some features with the DAM signature[35]. In the developing human CNS, microglia also express some of the DAM/MgND/ARN-like profiles[53]. Nonetheless, their functional implications and relations to one another remain unclear. The ever-growing list of branding

clusters in single-cell RNA sequencing (scRNASeq) experiments could, however, hinder future advance of the field without validation and functional experiments to understand their meaning. Moreover, single-cell technologies possess their own technical limitations, which when layered with isolation artifacts[54-56], can make it difficult to assign state identity across different studies. Another source of complexity comes from evident species differences[57-59], which can further hamper comparisons. New computational tools and approaches enable the alignment and integration of single-cell datasets, providing a powerful way to determine microglial state similarities across contexts[60,61].

A practical limitation of solely defining functional states by their transcriptional signature is that mRNA expression may not directly predict protein levels, and therefore protein signatures obtained by methods such as single-cell mass cytometry may better represent cell states[62,63]. Furthermore, mRNA expression or protein expression alone do not predict microglial function, as implied in many studies. There are many methods allowing the classification of microglia based on their constituent states, such as gene expression, protein expression, post-translational modifications, as well as morphology and ultrastructure. All these approaches can vary in coverage (e.g., expression of a single cell *versus* whole-transcriptome profiling), which has led to overall confusion and mislabeling in the field. Presumably, each microglial state is associated with unique or specialized functions, although the role of any observed state has so far remained elusive. Thus, it is critical that we begin to define microglial states taking into account their specific context in each species (e.g., mouse *versus* human), sex, space and time (e.g., CNS region and biological age) as well as layers of complexity (e.g., epigenetic, transcriptional, translational, metabolic signatures) that ultimately determine together the cell's phenome and function (**Figure 3**).

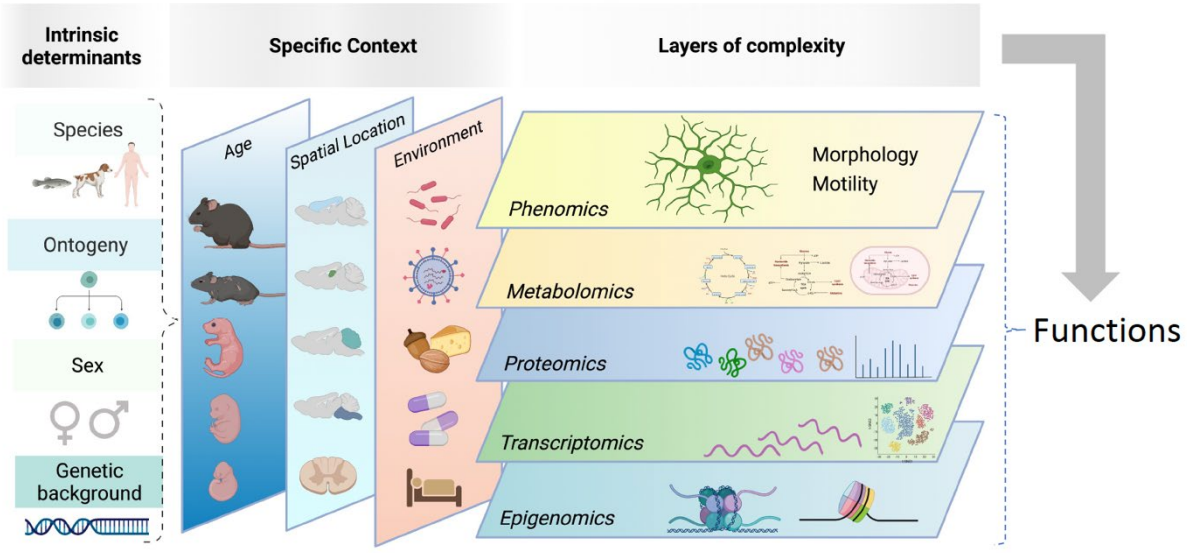

Figure 3. Microglial states defined by their intrinsic and extrinsic determinants, spatiotemporal context, and layers of complexity. *Microglial states depend in intrinsic determinants (such as species, ontogeny, sex, or genetic background) as well as the specific context they inhabit, including age, spatial location, and environmental factors (such as nutrition, pathogens, drugs, etc.). All together, these factors impinge microglia on multiple levels (i.e., epigenomic, transcriptomic, proteomic, metabolomics and phenomic), which ultimately determine microglial functions. Created with BioRender.com*

More importantly, one major conceptual limitation of the various 'one-off' microglial acronyms (e.g., DAM, MGnD, PAM, HAM) is that they suggest stable sub-populations or phenotypes of microglia associated with the disease stage, such as neurodegeneration. Intuitively, this classification system is similar to the concept of neuronal cell types, where neurons cluster into distinct subtypes based on their gene expression or neuroanatomy. However, contrary to microglia, neuronal groupings are considered fixed and terminally differentiated[5]. Another example comes from T lymphocytes, which are categorized into CD4 or CD8: two relatively fixed and terminally differentiated (sub)sets or (sub)populations[64]. However, we do not know how temporally or spatially dynamic the microglial states may be, as microglia are remarkably heterogeneous and plastic and, therefore, are probably not permanently 'locked' into a single state. From the evidence available so far, microglial states appear dynamic and plastic, possibly transitory, and strongly dependent on the context.

Microglial heterogeneity in the healthy brain: it all depends on the context

Microglial states result from various instructional cues provided by the CNS environment superimposed on their embryonic origin [57]. These environmental signals, integrated at the level of enhancers, are converted to different context-specific transcriptional outputs. Key modifying factors that lead to microglial heterogeneous states include age, sex, and local CNS signals, in addition to the pathophysiological state of the CNS and overall organism (discussed in the next section). Age, indeed, is a key component for defining the core microglial identity, which goes through several distinct temporal stages (embryonic, perinatal, adult, and aging microglia), each characterized by an enrichment of defined regulatory factors and gene expression profiles[52,65]. After the initial establishment of microglial identity by a network of developmentally programmed and environment-dependent transcription factors[57,65], microglia become extremely heterogeneous in their transcriptome during early postnatal development, as determined by scRNASeq[35,52,66]. In contrast, microglia display a more limited transcriptomic heterogeneity in the adult CNS, where the different microglial scRNASeq clusters fall into a transcriptional continuum instead of representing distinct subsets[35,52,66]. This relatively small

transcriptional differences may, however, lead to relevant functional differences, as exemplified by the functional differences and CSF1 response between hippocampal and cerebellar microglia[67,68].

Sex differences due to sex chromosomes and/or gonadal hormones may also contribute to defining the microglial state. A growing body of evidence shows that male and female microglia differ in their transcriptomic, proteomic, and morphological profiles, across brain colonization, maturation and function, in health and disease[69-72]. Of note, the microglial sex-specific transcriptomic signatures appear to be intrinsically determined, since they are maintained when microglia are transplanted into the brains of mice from the other sex[72]. Sexually differentiated roles of microglia could critically influence a variety of biological processes, in a time-dependent manner, and thus, emerge as key disease modifiers across various pathological conditions with a sexual dimorphism in prevalence and manifestation, as well as response to treatment.

Regardless of the "limited" adult heterogeneity suggested in scRNASeq studies, microglia do differ among CNS areas in terms of their morphology and ultrastructure, transcriptional, proteomic, epigenetic profiles, and functional specialization[63,73,74]. However, local CNS signals are not sufficient to determine microglial identity because macrophages engrafted in the brain parenchyma can acquire microglia-like morphology without reaching a transcriptomic signature identical to host microglia, even after prolonged CNS residence[21,75,76]. Again, nurture and nature collaborate to produce the microglial state. Altogether, these key findings have helped delineate a unique identity profile for ontogeny and gene expression, supporting the idea that microglia are distinct from peripherally-derived macrophages, even when they colonize a similar niche. In addition, these findings suggest that once their core identity is established, microglia use local CNS signals to take on different states.

Microglial states in the diseased CNS

Context-dependent signals vary dramatically during disease progression; they range from dead cells, extracellular debris, and signals resulting from blood-brain barrier disruption and altered function of neurons and other glial cells. Microglia respond to these challenges by changing their molecular profile, morphology and ultrastructure (**Box 1. Morphological responses across species**), as well as motility and function (see **Figure 1** for a schematic representation). Characteristically, the expression of core microglial markers is also altered over the course of disease. Thus, the microglial state defined by these markers might represent a microglial response to stimuli that may be very different between the young/developing and aged/diseased CNS, and across regions. This apparent contradiction

strengthens the fact that determining microglial expression profile is far from attributing any function to microglia, as it may only be suggestive of a potential functional identity, which – with unanimous consensus from all the authors– requires experimental validation using appropriate animal models and mutagenesis.

One microglial state that has received particular focus is the DAM signature, initially identified in a mouse model with mutations within five AD genes (5XFAD)[45] and later detected in other AD mouse models and samples from human AD (reviewed in [77]) and MS patients[49,78], although not to a full extent. Importantly, profiling of human microglial nuclei discovered a tau-associated microglia cluster that had not been identified in mice, reinforcing the idea that more human studies are needed[79]. The shared DAM signature includes downregulation of CX3CR1 and P2Y12R, and upregulation of APOE, AXL, SPP1, and TREM2[77] (**Figure 4**). Many questions remain open regarding the functional significance of the DAM signature. Several studies, in both mouse and human stem cell-differentiated microglia, demonstrated that the transition to a DAM state is dependent on TREM2[45,46,80,81]. However, how the TREM2 receptor drives the DAM transcriptional phenotype is still unclear, although the TREM2-ApoE signaling pathway is necessary for the switch from homeostatic to MGnD-neurodegenerative state[46]. Many questions remain open on TREM2. For instance, is TREM2 a key sensor for amyloid-beta and other AD-related pathology or does its deletion cause developmental defects in microglia that render them unable to change state? Is TREM2 controlling the microglia state by activating their energetic and anabolic metabolism?[82] New bulk and single-cell epigenetic approaches[57,83-88] will help answer these questions and ultimately may provide a means to toggle microglial states at will, enabling the field to finally understand the function of distinct microglial states and their impact in different contexts.

Additionally, many genes of the DAM signature were identified across various contexts. For instance, a common set of markers including (but not limited to) an upregulation of TREM2, APOE, CD11c, CLEC7A and LPL, and downregulation of TGFβ, CSFR1, P2RY12, and TMEM119 has been recently used to denote a subset of microglia that associate with myelinating areas in the developing brain, but also with aging and several models of degenerative diseases, such as AD, amyotrophic lateral sclerosis[89], and MS[45,51,90]. These observations raise the question as to whether the DAM is a signature strictly associated with certain diseases, as the name implies, or perhaps represents a more universal core signature that appears in response to challenges of various natures. Perhaps the most relevant question to be addressed is to which extent microglial states identified in the mouse brain are conserved and functionally relevant in the human brain.

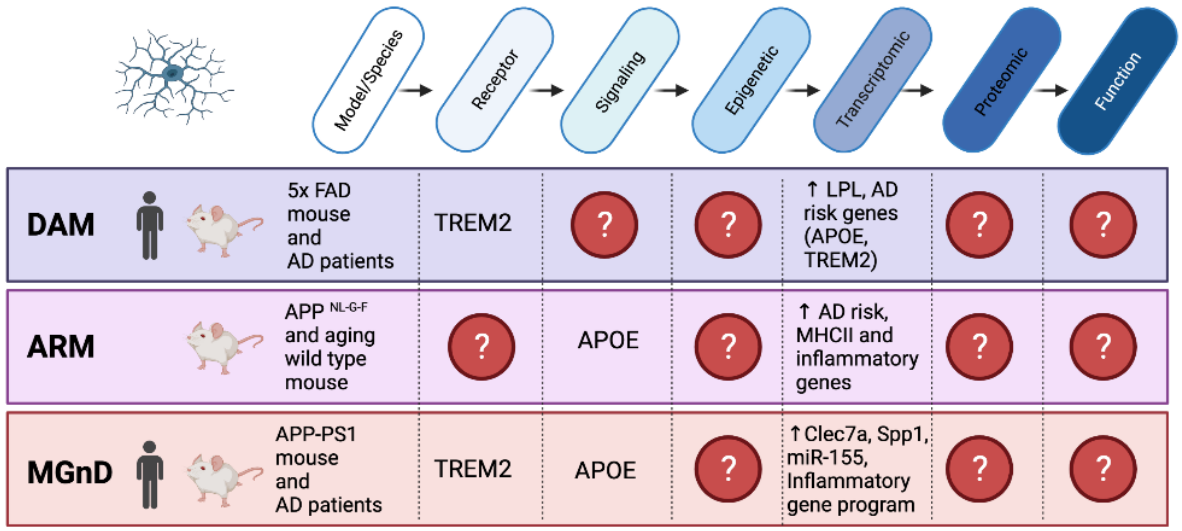

Figure 4: Figure 4: Alzheimer's disease microglia gene signature and regulatory drivers across AD models and patients. *Microglial states that arise in response to neurodegenerative disease include the disease-associated microglia (DAM), microglial neurodegenerative phenotype (MGnD) and activated response microglia (ARM) states, which have distinct and overlapping features. These states have been reported across species and several gaps in knowledge remain to be elucidated. Lipoprotein lipase (LPL), Alzheimer's disease (AD) risk genes including Apolipoprotein E (APOE) and Triggering Receptor Expressed on Myeloid cell 2 (TREM2) were identified as markers of DAM[45]. AD risk genes, genes part of the major histocompatibility complex class 2 (MHC II) and inflammatory genes were identified as markers ARM[47]. C-Type Lectin Domain Containing 7A (Clec7a), Secreted Phosphoprotein 1 (SPP1) and multiple microRNAs, which are known modulators of microglia[91], including miR-155 were identified as markers of MGnD[46].*

Outdated microglial nomenclature

Considering the state-of-the-art, classic microglial nomenclatures are outdated. In this section, we will provide a historical perspective on the terms still used to describe microglia, such as "resting *versus* activated", "M1 *versus* M2", and "homeostatic *versus* DAM". Many in the field fear that these dichotomic, rigid categories which convey the Manichaean idea of good *versus* bad microglia may be halting scientific advancement because using these terms does not address the ultimate question: what are the specific functions of microglia in development, health, aging, and diseases? It is now clear that microglia exist in diverse, dynamic and multi-dimensional states depending on the particular context and local environment (**Figure 5**). We define dimensions as the key variables driving the phenotypic transformations of microglia. A variable is a molecularly distinct signaling pathway regulated at multiple levels (transcriptional,

epigenetic, translational, metabolic) that gives rise to a distinct microglial function or property. Thus, categorizing microglia based on a historical, one-dimensional nomenclature in the absence of any functional data is likely to constrain and stifle future progress and innovation.

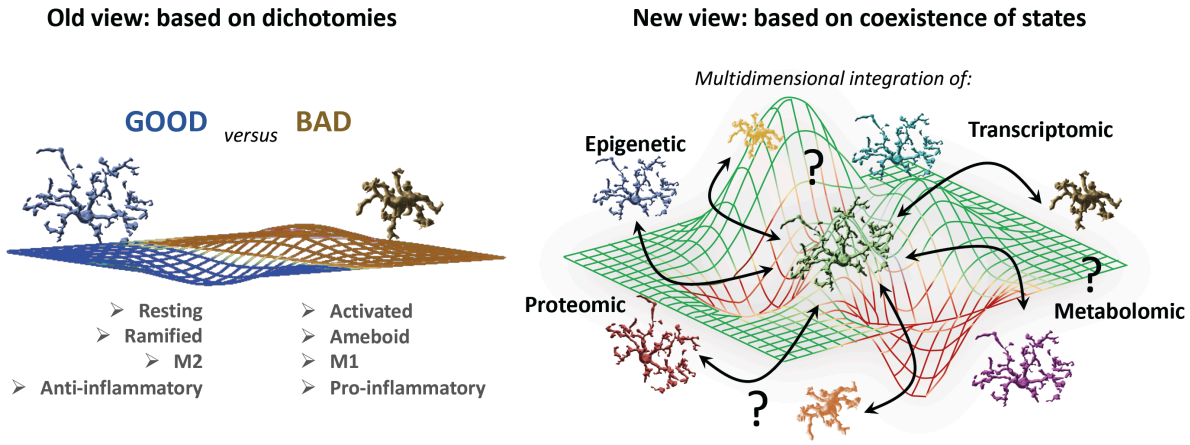

Figure 5. Microglial nomenclatures, past and future. *Microglia have been traditionally framed into dichotomic categories but our current integration of epigenetic, transcriptomic, metabolomics and proteomic data favors a multidimensional integration of coexisting states.*

The development of specific silver staining techniques in 1919 allowed Río-Hortega to clearly identify microglia and study their response to experimental manipulations[7,92]. Early on, Río-Hortega appreciated the striking morphological transformation of microglia following brain damage, but it was in the mid-1970s that the terms "resting" and "activated" microglia first appeared in the literature. These terms were used to describe cells with affinity for silver staining that were found in physiological ("resting") *versus* pathological ("activated") conditions (**Box 2. Resting *versus* activated microglia**). This nomenclature consolidated in the 1980s and became widely used during the 1990s[93], in parallel with the development and use of histochemical and immunohistochemical techniques, such as lectin staining and antibodies against the complement receptor CR3[7]. These techniques and nomenclature were together pivotal in determining that "resting" microglia were unrelated to astrocytes, as some studies had wrongly concluded[94], and that "reactive" microglia shared many characteristics with the blood-borne monocytes[10].

In the early 2000s, tremendous insight came with the development of transgenic mouse lines expressing fluorescent reporters in microglia, such as the CX3CR1-GFP mice[95]. This model system allowed imaging of microglia in a non-invasive manner, in the intact living mouse brain by two-photon *in vivo* imaging. Two seminal papers by Davalos[96] and Nimmerjahn[97] revealed that microglia are far from resting in the healthy brain. The extraordinary motility of microglial

processes that constantly extend, retract, and survey the brain parenchyma inspired a new field of research devoted to uncovering their roles under normal physiological conditions. These and other findings refuted the idea and nomenclature of "resting" microglia and instead prompted recategorization and naming of microglia as "surveillant" or "surveilling" microglia[96-98]. Following a challenge, i.e., a laser injury to the brain, microglial processes rapidly surround the injury site[96]. The primary chemoattractant is extracellular ATP released from the damaged tissue, which regulates microglial branch dynamics and mediates a rapid microglial response towards injury, but other purinergic and non-purinergic signaling also regulate microglia process motility under physiological conditions[96,99-101]. Thus, these microglia are anything but quiescent –they are constantly assessing and responding to signals within the brain parenchyma. Together, these and other multiphoton *in vivo* imaging data put into serious doubt the concept of "activated" microglia, which suggests a unique form of response, as in fact microglia are constantly responding (in different ways depending on the particular context) to the changes in their microenvironment, even under normal physiological conditions. Nonetheless, "resting" and "activated" microglia are names still widely used that should be discontinued.

Another –now problematic– terminology emerged in the early 2000s from immunologists classifying macrophages based on findings obtained in *in vitro* models: "M1", the classical activation, considered pro-inflammatory and neurotoxic, as well as closely related to the concept of "activated" microglia, and "M2", or alternative activation, considered anti-inflammatory and neuroprotective[102] (**Box 3. M1 *versus* M2 microglia**). The terms became widely adopted in microglial research and the 2010s saw a boom of papers phenotyping macrophages and microglia into "M1" and "M2" based on the expression of markers related to these categories, used to indirectly assume a detrimental ("M1") or beneficial ("M2") role[103]. In many cases, editors and reviewers have asked authors to comply with this nomenclature. However, it soon became evident that macrophage responses are more complex than simply "M1" and "M2"[104]. In the case of microglia, the advent of single cell technologies provided clear evidence that microglia in the living brain do not polarize to either of these categories[105], despite the continued use of M1 and M2 in the literature.

In the last few years, the emergence of single cell -omics and transcriptomic methods has led to new microglial categorizations into "homeostatic"[34] and DAM[45]. While this nomenclature seems preferred by many, it is not without limitations. The open questions related to the DAM signature are discussed in the above section. In addition, the term "homeostatic" refers to the condition or state of CNS tissue under physiological conditions and strictly relates to the specific context assessed in space and time; it does not necessarily correspond to a unique

molecular profile because even without any perturbation, microglia display diverse morphological and functional states, as they constantly receive and respond to signals in the CNS microenvironment. This continual sensing results in multiple transcriptional signatures from development until aging, depending on the specific local needs or challenges of the brain at each developmental stage[106]. Every organ system has mechanisms in place to dynamically maintain homeostasis that are essential for normal function and physiology, and these homeostatic mechanisms are often highly active in disease states, when tissues are attempting to restore balance in a disrupted system. In addition, a less responsive microglial cell, which in other contexts would be considered more "homeostatic", might be less effective at responding to damage or pathological cues in aging or disease contexts. These microglial cells might lack the ability to rapidly respond to a challenge (i.e., removing toxic amyloid, infected, damaged or degenerating neurons), leading to CNS dysfunction and disease progression. For example, TREM2 knockout microglia have been described as 'locked in a homeostatic state' as they are less responsive to challenges (such as amyloid) and do not adopt a transcriptional DAM signature in disease contexts[80,107]. From this example, it becomes evident that the term "homeostatic" is not informative if not well-defined and placed in the context of function.

Recommendations: DOs and DON'Ts

With all these evident problems in the current nomenclature, it is clear that a systematic, careful naming approach would greatly benefit microglial biology. When analyzing the responses of our co-authors, there was more consensus than disagreement in most of the issues at stake, quite unexpectedly. The vast majority of correspondents agree that the current nomenclature has severe limitations, and a more useful conceptual framework is needed to properly understand microglial states. This framework should be revisited every five to ten years by an international panel of experts as new discoveries are made. There is also a broad agreement that microglial responses should be framed in a multidimensional space, and should not be simplified as dichotomic good *versus* bad (**Figure 5**). Another point of strong agreement: abandon M1/M2 nomenclature once and for all and generally avoid using the vague term 'neuroinflammation'. Most agree that inflammation is not always detrimental but, instead, represents an adaptive response to damage that can sometimes get out of control, (**Box 4**). Quite importantly, a vast majority of authors support the use of "markers" (genes or proteins) to identify cell populations, but not as a readout of cell functions, which need to be addressed directly.

Nonetheless, there were a few points that are still under hot debate. The term "resting" microglia is strongly avoided by some authors, whereas others acknowledge that they still use

it even with its limitations, for lack of a better term. “Homeostatic” has more acceptance, although it is recognized that it is based on a very particular gene signature not shared by microglia across all physiological conditions, such as development, and that several homeostatic states likely exist. Thus, the term ‘homeostatic’ should always be accompanied by an accurate description of the context. To acknowledge this fact and account for different types of homeostasis, another term that was recently introduced is “ground” microglia[108]. The term “DAM”, on the other hand, is highly polarized. A large number of authors consider that a core set of transcripts in this signature is common to several pathological conditions and some physiological processes, including the development of white matter, whereas an equal number of authors state that there is not enough evidence for “DAM” to be a universal signature of microglial response to damage. Finally, the extent to which microglia are unique or similar to other brain associated or tissue macrophages is evolving with new data and profiling methods: most agree that due to their lineage they are similar to some extent but have unique functions adapted to the CNS environment.

Based on the collective opinions of authors we provide below a series of recommendations for researchers, reviewers, and editors:

Classic Nomenclature

- Consider that, both in the healthy and pathological CNS, microglia are highly dynamic and plastic cells that display multivariate morphological/ultrastructural, transcriptional, and functional states
- Describe microglia using as many as possible layers of complexity: morphology, motility, -omics, and function, always placing them into a species and spatiotemporal context (**Figure 3**).
- Refer to microglia in basal conditions as “homeostatic”, or “ground” microglia, considering the limitations discussed above (i.e., that these terms refer to microglia under physiological conditions and not to the function of microglia). Use the term “surveillant/surveilling” to refer to microglia that is engaged in the surveillance function, but not as a synonym of microglia in physiological conditions.
- Refer to microglia in your experimental condition as “reactive to” or “responding to” while describing the particular signals they respond to (i.e., the context).
- Disregard simplistic, dichotomic categorizations.
- Do not use the terms “resting”, “activated”, or “M1/M2” to refer to microglia.
- When using the term “DAM”, do not use it as a universal term applicable to all diseases. The jury is still out to test whether its full or core signature is common to all or a subset of pathologies, particularly in the human brain.

Introducing new terminology

- Until a consensus is reached about true subtype/s of microglia, with defined ontogeny, physical niches, functions, and transcriptional profiles (whether permanent or transient), use the term "state" rather than "subpopulation". The term "cluster" should be used to refer to groups of transcriptionally similar cells in scRNASeq experiments.
- Use combinations of gene or protein "markers" to identify cell populations but be aware that their expression is plastic and may change over time and under different experimental conditions. Fate mapping approaches will be very useful to track individual microglial cells and assess changes in their state over time[109,110].
- Refrain from rebranding microglia with each new -omics (particularly single-cell RNA sequencing) study. Instead, describe transcriptional signatures: sets or modules of expressed genes that can be compared with other studies[77,111].
- If new terminology needs to be introduced, follow FAIR principles: Findable, Accessible, Interoperable, and Reusable (https://neuronline.sfn.org/professional-development/data-sharing-principles-to-promote-open-science). An example of naming cell lines following these principles can be found here[112].

Microglial markers and function

- Use integrative methodological approaches that allow probing of microglia using different levels of analysis.
- Follow consensus guidelines when using methodologies such as scRNASeq[113], RTqPCR[114], or digital PCR[115].
- Do not use morphology or gene/protein expression as a substitute for directly assessing cell function. Morphology and expression can be used to generate hypotheses about function that need to be specifically tested.

Future questions and challenges

From words to action: A key challenge in the field is to match microglial morphological, ultrastructural, transcriptomic, proteomic, metabolomics and emerging lipidomic changes with functional responses (**Figure 1**). In the current single-cell era, an overwhelming wealth of data has been generated, profiling the expression of millions of microglia in different organisms, at different ages, across diverse brain regions. Yet, such 'omics' identities are not necessarily linked to functional states, and often they lack spatial resolution. Additionally, many important microglial markers are sensome genes, whose expression and activity at the microglial membrane may be more indicative of their functional state than transcription profile. Transcriptional analysis may benefit from ribosome profiling by RiboSeq[116] and from gene-

747 trap insertion profiling by TRAPSeq[117]. Proteomic approaches combined with *in situ* studies
748 would provide better information in this respect, bridging the gap between expression and
749 function. Further integration of complementary approaches, such as spatial transcriptomics,
750 imaging mass cytometry, and correlative or conjugate electron microscopy in combination with
751 other single-cell approaches, will provide a more comprehensive characterization of microglia.
752 Ultimately, functional studies are indispensable to understand the multiple roles of microglia
753 within specific spatiotemporal contexts of health and disease.
754
755 *How are microglial states coordinated?*
756 Even as we acquire more data about microglial states there are still key unanswered
757 questions. Are microglial states plastic and reversible? What is the relationship between cell
758 state and microglial function? These varied single-cell characterizations ultimately need to be
759 linked to particular cellular functions, to become relevant to development, health, and
760 diseases. How do these states come about? How do signals from the CNS environment get
761 integrated in microglia to produce specific states?
762
763 *How similar are peripherally-derived macrophages and microglia?* A burning question that
764 surely requires further investigation is related to the identity and function of microglia *versus*
765 other brain macrophages. Although recent studies have provided evidence for an intrinsic
766 unique core signature of microglia, their functional resemblances and differences remain
767 undetermined. For instance, could engrafted parenchymal macrophages functionally replace
768 the resident microglia, despite having a different molecular identity?
769
770 *The devil is in the details*: Another major caveat is that microglia are incredibly reactive cells
771 and evidence indicates that artifacts are often introduced during sample processing for a
772 variety of methodologies, such as RNA profiling, immunohistochemistry, FACS, *in vivo*
773 imaging, and so on. Hence, we may be missing or confounding important pieces of information
774 because we unintentionally introduce changes in the parameters we are trying to measure. In
775 addition, these artifacts are likely to generate variability across laboratories using different
776 protocols. A future challenge is to promote reproducibility of data across laboratories, by
777 coordinating a shared database of protocols curated under the STAR methods guidelines.
778
779 *Diversity as a source of richness*: How many different microglial states can be identified? And
780 how many different types of homeostatic microglia exist? How do microglia navigate among
781 these states and types? Are they related through a transcriptional continuum, or perhaps as a
782 hub-and-spoke set of states, as has been proposed for macrophages[4]? Embryonic and aged
783 microglia, as well as microglia in the diseased brain, also show higher diversity. How dynamic

784 are these states? And how spatially defined are they? Future research will need to address
785 these important questions.
786
787 *Male versus female microglia:* Sex differences have been reported to affect the brain
788 colonization, maturation, structure, transcriptomic, proteomic, and functional profiles of
789 microglia, in a time-dependent manner. To what extent these differences may regulate the
790 susceptibility to neurological diseases remains a fascinating question that urgently awaits
791 answers. Investigating the molecular and cellular mechanisms underlying sex-mediated
792 differences in microglial states would advance our understanding of microglial implication in
793 diseases with clear sex-related differences in their prevalence, symptoms, and progression,
794 as well as response to treatments.
795
796 *Relevance to humans:* It will be imperative to study developmental and functional differences
797 between human and mouse microglia. To date, most of the studies on microglia were
798 conducted in rodents and a direct comparison among particular brain regions is still missing.
799 Whether microglial states identified in mice also exist in humans is still under debate.
800 Translating and validating these findings across species is critical and will help prevent failure
801 of clinical trials that stem from animal models. In addition, most human microglial studies have
802 been performed in Caucasians and only recently data from African American individuals are
803 available[118].
804
805 *Grammar quandary:* “Microglia” as a population is a plural noun in English but a singular noun
806 in Latin-derived languages, which occasionally causes confusion. In English texts, microglial
807 cells should always be referred to in the plural form unless referring to an individual cell. For
808 example, “microglia are brain cells” but “this microglia is adjacent to a neuron”.
809
810 *Towards a unified nomenclature:* The ultimate conclusion of this white paper is that the
811 community has not yet reached an agreement on what defines microglial identity compared to
812 other cell types; nor consensus on the number, dynamic nature, or definition of microglial
813 states. The community advocates for creating harmonized, curated databases and guidelines
814 for introducing novel terminology; to follow STAR methods; to share data as early as possible
815 and to reassess advances in defining microglial state every five to ten years. Until such
816 consensus is reached, the community urges all microglial studies to carefully define the
817 context within which the study was performed to inform its readers, offer clarity instead of
818 confusion, and thereby contribute to a more thorough understanding of the many facets of
819 microglial biology

Box 1. Microglial morphological responses across species

Microglia display a profusion of morphologies that have fascinated microglial researchers since the early days of Río-Hortega. Many were tempted to equate morphology with function. Indeed, ramified microglia were traditionally called “resting”, although we now know that ramified microglia play many functions in physiological conditions. In contrast, “reactive” microglia (rounder cell body, with fewer and shorter processes) were called “activated” and equated with an inflammatory response. Only recently, however, a mechanistic link between microglial reduced branching and increased release of the inflammatory cytokine interleukin 1β was reported[101]. Activation of P2YR12 by tissue damage signals inhibits the tonically active potassium THIK-1 channel, leading both to decreased microglial ramifications and activation of the inflammasome, the machinery that processes IL-1β precursors into their mature form[101]. Another morphology associated with functional changes is “ameboid” microglia, which were thought to be more “phagocytic”, but it is clear now that ramified microglia also execute phagocytosis through terminal or ‘en passant’ branches[119,120], while in diseases such as epilepsy ameboid microglia can display reduced phagocytosis[121]. Therefore, morphological changes should not be interpreted in functional terms but, rather, taken as a suggestion prompting to investigate their functional implications further. Nonetheless, it is still open to discussion whether this mechanism operates in human pathology, and whether microglial reactivity and inflammation are universally linked.

Studies in *post-mortem* brain samples revealed that human and mouse microglia can adopt similar morphological states. Ramified, “primed” (larger cell body, ramified processes), “reactive” (ameboid, few ramified processes), and “ameboid” (less than two unramified processes) were described in middle-aged individuals[122]. In addition, “rod-shaped” microglia (elongated cell body, polarized processes) were found to become more abundant with aging, in human *post-mortem* brain samples[123]. Similarly, “dystrophic” microglia, with apparently fragmented (but still intact at the ultrastructural level) processes were also found in the aged human brain[124]. These different morphological types were previously described in mouse and rat (reviewed in [125]). Nevertheless, a more sensitive quantitative assessment via a computational pipeline involving cluster analysis revealed differences in microglial morphology between mouse and human, with distinct clusters found to be unique to each species[126]. Subsequently, a high-throughput comparative density and morphology analysis revealed a generally conserved evolutionary pattern, with some intriguing differences observed between leech, zebrafish, axolotl, turtle, chicken, gecko, snake, bearded dragon, bat, boar, sheep, whale, hamster, rat, mouse, marmoset, macaque, and human, and across brain regions between mouse and human[58]. While detailed comparative ultrastructural analyses of microglia between species are currently lacking, the state of “dark microglia”, which is defined using

electron microscopy by their markers of cellular stress in contexts of aging and disease, were found to be conserved across mouse, rat, and human[127]. Future studies will show whether these varied morphologies correlate with the transcriptional and proteomic profile, and what they imply for the cell´s function. At the molecular level, recent single-cell transcriptome analyses further revealed that human microglia show multiple clusters that indicate a greater heterogeneity than in other mammalian species such as the mouse[58,66].

Box 2. Resting *versus* activated microglia

As shown by a PubMed search with microglia in all fields, there were only few papers published on the topic before the 1990s, and then a steady increase until the beginning of our century, followed by an exponential growth[128]. There is a first inflexion point in 2005, with the seminal discovery using non-invasive two-photon *in vivo* imaging that microglia, which used to be called “resting” or “quiescent” in the healthy brain, are extremely dynamic, continuously surveying the parenchyma with their highly motile processes[96,97]. The development of non-invasive methods was a necessary condition for our understanding of the roles of microglia in the healthy brain (reviewed in [129]). In 2005, microglia were examined for the first time in the intact brain of living animals, in a non-invasive manner through the skull, using two-photon *in vivo* imaging of CX3CR1-GFP mice in which microglia are fluorescently labeled [96,97]. As a result,, microglia are now considered to be the most dynamic cells of the healthy mature brain[129]. This seminal discovery prompted to rename quiescent or resting microglia as surveying[97,98] or surveillant (from the verb to survey)[130] microglia, and also lead to propose the concept that microglia are never-resting[131]. Instead, microglia do not switch from “resting” to “activated” in response to trauma, injury, infection, disease, and other challenges. Rather, microglia are continuously active and react to the stage of life, CNS region, species, sex, and context of health or disease by adopting different states and performing different functions.

Box 3. M1 *versus* M2 microglia

It was previously thought that reactive microglia and macrophages existed in two non-exclusive states: M1, which resembles a pro-inflammatory, pathological, and classical reactive phenotype; and M2, which represents an anti-inflammatory, restorative/reparative phenotype. These responses were related to those of T helper lymphocytes (Th1 and Th2) based on their *in vitro* activation by specific immune stimuli that activated differential metabolic programs and changes in cytokine expression[103]. An associated term is “M0” microglia, which describes their phenotype when cultured in the presence of TGFβ (transforming growth factor beta) and CSF-1 to mimic *in vivo* counterparts[34]. It is important to note, however, that this over-simplified classification, which arose from *in vitro* cell culture studies, often failed to translate to *in vivo* conditions. In several contexts, microglia were also shown to co-express M1 and M2 markers,

894 highlighting their irrelevance at providing insights into microglial states. The M1 and M2
895 categorization was thus rejected by the field[105]. Instead, microglia are now known to adopt
896 various states, in which they perform specialized functions, highlighting an important need for
897 more nuanced tools to investigate microglial function (reviewed in [132]). Microglia are
898 heterogeneous: they comprise different subtypes that can be differentially recruited, and each
899 adopt different phenotypes depending on the context of health or disease[127].
900
901 From an historical perspective, M1 cells were thought to be stimulated by interferon-γ and
902 lipopolysaccharide (LPS) or tumor necrosis factor alpha and express mRNA for inducible nitric
903 oxide synthase and CD86 (a co-stimulatory molecule). These cells were believed to
904 upregulate phagocytic activity, secrete pro-inflammatory cytokines (e.g., IL-1β, IL-12), and
905 generate reactive oxygen species (ROS). M1 cell functions, which include increased motility,
906 ROS and other proinflammatory mediator production, and membrane turnover, were thought
907 to require the following metabolic activities: glycolysis, pentose phosphate pathway activity,
908 nicotinamide adenine dinucleotide phosphate production, and fatty acid synthesis. M2 cells,
909 on the other hand, were thought to be stimulated by IL-4 or IL-13 and express mRNA for
910 arginase and CD206. These cells were believed to secrete anti- inflammatory cytokines (e.g.,
911 IL-10) and growth factors, as well as stimulate stem cell production and differentiation. M2
912 cells were considered to need continual stimulation to increase the transcription of repair
913 genes and produce growth factors, which requires energy production utilizing oxidative
914 phosphorylation (and amino acid/fatty acid oxidation)[132-134].
915
916 **Box 4. The polysemic nature of "neuroinflammation"**
917 Polysemic means that a word can have several possible meanings. A most conflictive and
918 polysemic term in the microglia field is "neuroinflammation", as its definition varies dramatically
919 among authors, as has been discussed before[135] and in agreement with our survey. Below
920 are provided representative definitions:
921 a. Neuroinflammation is inflammation of neural tissue particularly mediated by glial cells.
922 b. Neuroinflammation is strictly limited to MS and stroke when leukocytes enter CNS.
923 c. Neuroinflammation is whatever happens when CNS homeostasis is disturbed.
924 d. Neuroinflammation is a mixed cellular response to brain infection or damage involving
925 innate and adaptive responses of resident brain cells and circulating immune cells.
926 e. The term neuroinflammation is too unclear and unprecise and should be avoided.
927
928 Whatever the exact definition, it is generally acknowledged by the authors that protection
929 against tissue damage (i.e., 'inflammation') encompasses, in the CNS, a highly complex set
930 of local responses, and equally complex interactions with circulating immune cells or with

immune cells residing in brain-blood and brain-cerebrospinal fluid interfaces. It is also acknowledged that each and every inflammatory response may adopt adaptive or maladaptive courses with distinct impacts on pathology progression.

Our main recommendation is to liberate neuroinflammation from microglia and microglia from neuroinflammation. We caution researchers about misusing stereotypes regarding when, where, and how neuroinflammation takes place, including the notion that microglial changes always represent neuroinflammation. In addition, the negative connotation of neuroinflammation as something to be avoided at all costs should be dispelled once and for all. Rather, we encourage the use of modest and precise terms to describe specific phenomena such as microglial reaction; astrocytic reaction; loss of barrier integrity and loss of nutrient transport function at the BBB; infiltration of the brain with neutrophils and eosinophils. Finally, in the same way that there is no such thing as “hepatoinflammation”, “dermoinflammation”, “gastroinflammation” or “pulmoinflammation” –perhaps we do not need “neuroinflammation”; “inflammation” may suffice. Therefore, when the term neuroinflammation is encountered in the literature, it must be understood that it is highly general, imprecise and polysemic. In the best case, the use of “neuroinflammation” simply stresses that inflammation associated with nervous system follows unique rules that need to be fully discerned experimentally and not simply extrapolated from observations in non-nervous tissue.

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Marker	Specificity	Labeled states	Staining patterns	Main applications	Ref.
F4/80 (EMR1)	Macrophages including microglia	Homeostatic and disease-associated. Expressed in rodents, but presence not yet confirmed in human.	Does not provide a detailed cellular visualization, especially in homeostatic conditions, due to its low basal expression. Staining can localize to the plasma membrane or diffuse in the cytoplasm	Brightfield or fluorescence analysis of microglial density, distribution, and categorization into morphological states	32,136
CX3CR1	Macrophages including microglia	Homeostatic 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.	45,46,95,137, 138
IBA1	Macrophages including microglia	Homeostatic and disease-associated. Downregulated in some contexts (e.g., obesity and aging) and by some pathological states (e.g., disease-associated microglia, dark microglia). Used to study microglia in early embryonic and postnatal development. Conserved across several species including human.	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.	139,140 45,58,141-145

MerTK	Macrophages including microglia	Homeostatic 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.	146-149
OX-42 (CD11b/CD18 or Mac-1)	Macrophages including microglia	Homeostatic 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.	150 138,151-154
P2YR12	Largely microglia-specific (not expressed by monocytes), but state-dependent	Homeostatic marker. Strongly downregulated in disease-associated and reactive states (but upregulated in *status epilepticus*). Used to study microglia in early postnatal development. Conserved across several species including human.	Visualization of microglial cell body and processes. Staining can localize to the plasma membrane or diffuse throughout the cytoplasm and can be more profuse than Iba1 depending on staining conditions.	Brightfield or fluorescence analysis of microglial density, distribution, and morphology. Ultrastructural studies.	155-158
TMEM119	Largely microglia-specific, but state-dependent	Homeostatic and disease-associated, but downregulated on reactive microglia in some	Partial visualization of microglial cell bodies and diffuse staining of their processes preventing a	Brightfield or fluorescence analysis of microglial density, distribution.	159-163

		contexts (e.g., traumatic brain injury and ischemia, multiple sclerosis). Developmentally regulated. Conserved across species including human.	complete morphological visualization.	Morphological analysis or categorization into morphological states possible in combination with IBA1.	
TREM2	Macrophages including microglia, state-dependent	Microglial subsets in early postnatal development, aging, and disease conditions (e.g., microglia involved in synaptic pruning or associated with amyloid plaques in AD pathology). Shown to label monocytes or neurons instead of microglia in human.	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. Ultrastructural studies of pathological states downregulating IBA1.	138,147,161,164,165

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

Acknowledgements: The authors are deeply grateful to Richard Ransohoff, Monica Carson and Elena Galea, who contributed with 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 Lasse Dissing-Olesen and Alec Walker (who conceived Figure 2), Martine Therrien and Yvanka de Soysa (who conceived Figure 4), and Sol Beccari (who conceived Figure 5). 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 2022: Ifoeluwa Awogbindin, Elisa Gonçalves de Andrade, Fernando Gonzalez Ibanez, Parsa 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 Synapsis Foundation - Alzheimer Research Switzerland ARS, 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 (https://www.ciencia.gob.es/) 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; http://www.euskadi.eus/basque-government/department-education/) 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.

References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

22 Priller, J. *et al.* Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nat Med* **7**, 1356-1361, doi:10.1038/nm1201-1356 (2001).
23 Xu, Z. *et al.* Efficient Strategies for Microglia Replacement in the Central Nervous System. *Cell Rep* **32**, 108041, doi:10.1016/j.celrep.2020.108041 (2020).
24 Xu, Z., Zhou, X., Peng, B. & Rao, Y. Microglia replacement by bone marrow transplantation (Mr BMT) in the central nervous system of adult mice. *STAR Protoc* **2**, 100666, doi:10.1016/j.xpro.2021.100666 (2021).
25 Xu, Z., Rao, Y. & Peng, B. Protocol for microglia replacement by peripheral blood (Mr PB). *STAR Protoc* **2**, 100613, doi:10.1016/j.xpro.2021.100613 (2021).
26 Xu, R. *et al.* Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain. *Nat Commun* **11**, 1577, doi:10.1038/s41467-020-15411-9 (2020).
27 Hasselmann, J. *et al.* Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. *Neuron* **103**, 1016-1033 e1010, doi:10.1016/j.neuron.2019.07.002 (2019).
28 Mancuso, R. *et al.* Stem-cell-derived human microglia transplanted in mouse brain to study human disease. *Nat Neurosci* **22**, 2111-2116, doi:10.1038/s41593-019-0525-x (2019).
29 Buttgereit, A. *et al.* Sall1 is a transcriptional regulator defining microglia identity and function. *Nat Immunol* **17**, 1397-1406, doi:10.1038/ni.3585 (2016).
30 Goldmann, T. *et al.* Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nat Immunol* **17**, 797-805, doi:10.1038/ni.3423 (2016).
31 Van Hove, H. *et al.* A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat Neurosci* **22**, 1021-1035, doi:10.1038/s41593-019-0393-4 (2019).
32 Gautier, E. L. *et al.* Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* **13**, 1118-1128, doi:10.1038/ni.2419 (2012).
33 Zhang, Y. *et al.* An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* **34**, 11929-11947, doi:10.1523/JNEUROSCI.1860-14.2014 (2014).
34 Butovsky, O. *et al.* Identification of a unique TGF-beta-dependent molecular and functional signature in microglia. *Nat Neurosci* **17**, 131-143, doi:10.1038/nn.3599 (2014).
35 Li, Q. *et al.* Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. *Neuron* **101**, 207-223 e210, doi:10.1016/j.neuron.2018.12.006 (2019).
36 Utz, S. G. *et al.* Early Fate Defines Microglia and Non-parenchymal Brain Macrophage Development. *Cell* **181**, 557-573 e518, doi:10.1016/j.cell.2020.03.021 (2020).
37 Kaiser, T. & Feng, G. Tmem119-EGFP and Tmem119-CreERT2 Transgenic Mice for Labeling and Manipulating Microglia. *eNeuro* **6**, doi:10.1523/ENEURO.0448-18.2019 (2019).
38 Chappell-Maor, L. *et al.* Comparative analysis of CreER transgenic mice for the study of brain macrophages: A case study. *Eur J Immunol* **50**, 353-362, doi:10.1002/eji.201948342 (2020).
39 McKinsey, G. L. *et al.* A new genetic strategy for targeting microglia in development and disease. *Elife* **9**, doi:10.7554/eLife.54590 (2020).
40 Masuda, T. *et al.* Novel Hexb-based tools for studying microglia in the CNS. *Nat Immunol* **21**, 802-815, doi:10.1038/s41590-020-0707-4 (2020).
41 Parkhurst, C. N. *et al.* Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* **155**, 1596-1609, doi:10.1016/j.cell.2013.11.030 (2013).

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

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

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

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

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

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

48 Srinivasan, K. *et al.* Alzheimer's Patient Microglia Exhibit Enhanced Aging and Unique Transcriptional Activation. *Cell Rep* **31**, 107843, doi:10.1016/j.celrep.2020.107843 (2020).

49 Absinta, M. *et al.* A lymphocyte-microglia-astrocyte axis in chronic active multiple sclerosis. *Nature* **597**, 709-714, doi:10.1038/s41586-021-03892-7 (2021).

50 Marschallinger, J. *et al.* Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nat Neurosci* **23**, 194-208, doi:10.1038/s41593-019-0566-1 (2020).

51 Safaiyan, S. *et al.* White matter aging drives microglial diversity. *Neuron* **109**, 1100-1117 e1110, doi:10.1016/j.neuron.2021.01.027 (2021).

52 Hammond, T. R. *et al.* Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* **50**, 253-271 e256, doi:10.1016/j.immuni.2018.11.004 (2019).

53 Kracht, L. *et al.* Human fetal microglia acquire homeostatic immune-sensing properties early in development. *Science* **369**, 530-537, doi:10.1126/science.aba5906 (2020).

54 Wu, Y. E., Pan, L., Zuo, Y., Li, X. & Hong, W. Detecting Activated Cell Populations Using Single-Cell RNA-Seq. *Neuron* **96**, 313-329 e316, doi:10.1016/j.neuron.2017.09.026 (2017).

55 Marsh, S. E. *et al.* Single cell sequencing reveals glial specific responses to tssue processing & enzymatic dissociation in mice and humans. *BioRxiv* (2021).

56 Mattei, D. *et al.* Enzymatic Dissociation Induces Transcriptional and Proteotype Bias in Brain Cell Populations. *Int J Mol Sci* **21**, doi:10.3390/ijms21217944 (2020).

57 Gosselin, D. *et al.* An environment-dependent transcriptional network specifies human microglia identity. *Science* **356**, doi:10.1126/science.aal3222 (2017).

58 Geirsdottir, L. *et al.* Cross-Species Single-Cell Analysis Reveals Divergence of the Primate Microglia Program. *Cell* **179**, 1609-1622 e1616, doi:10.1016/j.cell.2019.11.010 (2019).

59 Kolodziejczyk, A. A., Kim, J. K., Svensson, V., Marioni, J. C. & Teichmann, S. A. The technology and biology of single-cell RNA sequencing. *Mol Cell* **58**, 610-620, doi:10.1016/j.molcel.2015.04.005 (2015).

60 Welch, J. D. *et al.* Single-Cell Multi-omic Integration Compares and Contrasts Features of Brain Cell Identity. *Cell* **177**, 1873-1887 e1817, doi:10.1016/j.cell.2019.05.006 (2019).

61 Stuart, T. *et al.* Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902 e1821, doi:10.1016/j.cell.2019.05.031 (2019).

62 Ajami, B. *et al.* Single-cell mass cytometry reveals distinct populations of brain myeloid cells in mouse neuroinflammation and neurodegeneration models. *Nat Neurosci* **21**, 541-551, doi:10.1038/s41593-018-0100-x (2018).
63 Bottcher, C. *et al.* Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci* **22**, 78-90, doi:10.1038/s41593-018-0290-2 (2019).
64 Anderson, D. A., 3rd, Dutertre, C. A., Ginhoux, F. & Murphy, K. M. Genetic models of human and mouse dendritic cell development and function. *Nat Rev Immunol* **21**, 101-115, doi:10.1038/s41577-020-00413-x (2021).
65 Matcovitch-Natan, O. *et al.* Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **353**, aad8670, doi:10.1126/science.aad8670 (2016).
66 Masuda, T. *et al.* Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* **566**, 388-392, doi:10.1038/s41586-019-0924-x (2019).
67 Grabert, K. *et al.* Microglial brain region-dependent diversity and selective regional sensitivities to aging. *Nat Neurosci* **19**, 504-516, doi:10.1038/nn.4222 (2016).
68 Kana, V. *et al.* CSF-1 controls cerebellar microglia and is required for motor function and social interaction. *J Exp Med* **216**, 2265-2281, doi:10.1084/jem.20182037 (2019).
69 Hanamsagar, R. *et al.* Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia* **65**, 1504-1520, doi:10.1002/glia.23176 (2017).
70 Guneykaya, D. *et al.* Transcriptional and Translational Differences of Microglia from Male and Female Brains. *Cell Rep* **24**, 2773-2783 e2776, doi:10.1016/j.celrep.2018.08.001 (2018).
71 Thion, M. S. *et al.* Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell* **172**, 500-516 e516, doi:10.1016/j.cell.2017.11.042 (2018).
72 Villa, A. *et al.* Sex-Specific Features of Microglia from Adult Mice. *Cell Rep* **23**, 3501-3511, doi:10.1016/j.celrep.2018.05.048 (2018).
73 De Biase, L. M. *et al.* Local Cues Establish and Maintain Region-Specific Phenotypes of Basal Ganglia Microglia. *Neuron* **95**, 341-356 e346, doi:10.1016/j.neuron.2017.06.020 (2017).
74 Ayata, P. *et al.* Epigenetic regulation of brain region-specific microglia clearance activity. *Nat Neurosci* **21**, 1049-1060, doi:10.1038/s41593-018-0192-3 (2018).
75 Bennett, F. C. *et al.* A Combination of Ontogeny and CNS Environment Establishes Microglial Identity. *Neuron* **98**, 1170-1183 e1178, doi:10.1016/j.neuron.2018.05.014 (2018).
76 Shemer, A. *et al.* Engrafted parenchymal brain macrophages differ from microglia in transcriptome, chromatin landscape and response to challenge. *Nat Commun* **9**, 5206, doi:10.1038/s41467-018-07548-5 (2018).
77 Chen, Y. & Colonna, M. Microglia in Alzheimer's disease at single-cell level. Are there common patterns in humans and mice? *J Exp Med* **218**, doi:10.1084/jem.20202717 (2021).
78 Zrzavy, T. *et al.* Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain* **140**, 1900-1913, doi:10.1093/brain/awx113 (2017).
79 Gerrits, E. *et al.* Distinct amyloid-beta and tau-associated microglia profiles in Alzheimer's disease. *Acta Neuropathol* **141**, 681-696, doi:10.1007/s00401-021-02263-w (2021).
80 McQuade, A. *et al.* Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. *Nat Commun* **11**, 5370, doi:10.1038/s41467-020-19227-5 (2020).
81 Zhou, Y. *et al.* Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med* **26**, 131-142, doi:10.1038/s41591-019-0695-9 (2020).
82 Ulland, T. K. *et al.* TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. *Cell* **170**, 649-663 e613, doi:10.1016/j.cell.2017.07.023 (2017).

83 Ma, S. *et al.* Chromatin Potential Identified by Shared Single-Cell Profiling of RNA and Chromatin. *Cell* **183**, 1103-1116 e1120, doi:10.1016/j.cell.2020.09.056 (2020).

84 Buenrostro, J. D., Wu, B., Chang, H. Y. & Greenleaf, W. J. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol* **109**, 21 29 21-21 29 29, doi:10.1002/0471142727.mb2129s109 (2015).

85 van Galen, P. *et al.* A Multiplexed System for Quantitative Comparisons of Chromatin Landscapes. *Mol Cell* **61**, 170-180, doi:10.1016/j.molcel.2015.11.003 (2016).

86 Bartosovic, M., Kabbe, M. & Castelo-Branco, G. Single-cell CUT&Tag profiles histone modifications and transcription factors in complex tissues. *Nat Biotechnol*, doi:10.1038/s41587-021-00869-9 (2021).

87 Schaafsma, W. *et al.* Long-lasting pro-inflammatory suppression of microglia by LPS-preconditioning is mediated by RelB-dependent epigenetic silencing. *Brain Behav Immun* **48**, 205-221, doi:10.1016/j.bbi.2015.03.013 (2015).

88 Wendeln, A. C. *et al.* Innate immune memory in the brain shapes neurological disease hallmarks. *Nature* **556**, 332-338, doi:10.1038/s41586-018-0023-4 (2018).

89 Chiu, I. M. *et al.* A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep* **4**, 385-401, doi:10.1016/j.celrep.2013.06.018 (2013).

90 Sobue, A. *et al.* Microglial gene signature reveals loss of homeostatic microglia associated with neurodegeneration of Alzheimer's disease. *Acta Neuropathol Commun* **9**, 1, doi:10.1186/s40478-020-01099-x (2021).

91 Brites, D. Regulatory function of microRNAs in microglia. *Glia* **68**, 1631-1642, doi:10.1002/glia.23846 (2020).

92 Sierra, A. *et al.* The "Big-Bang" for modern glial biology: Translation and comments on Pio del Rio-Hortega 1919 series of papers on microglia. *Glia* **64**, 1801-1840, doi:10.1002/glia.23046 (2016).

93 Streit, W. J., Graeber, M. B. & Kreutzberg, G. W. Functional plasticity of microglia: a review. *Glia* **1**, 301-307, doi:10.1002/glia.440010502 (1988).

94 Kitamura, T., Miyake, T. & Fujita, S. Genesis of resting microglia in the gray matter of mouse hippocampus. *J Comp Neurol* **226**, 421-433, doi:10.1002/cne.902260310 (1984).

95 Jung, S. *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* **20**, 4106-4114, doi:10.1128/mcb.20.11.4106-4114.2000 (2000).

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

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

98 Hanisch, U. K. & Kettenmann, H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* **10**, 1387-1394, doi:10.1038/nn1997 (2007).

99 Eyo, U. B. *et al.* Modulation of microglial process convergence toward neuronal dendrites by extracellular calcium. *J Neurosci* **35**, 2417-2422, doi:10.1523/JNEUROSCI.3279-14.2015 (2015).

100 Dissing-Olesen, L. *et al.* Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. *J Neurosci* **34**, 10511-10527, doi:10.1523/JNEUROSCI.0405-14.2014 (2014).

101 Madry, C. *et al.* Microglial Ramification, Surveillance, and Interleukin-1beta Release Are Regulated by the Two-Pore Domain K(+) Channel THIK-1. *Neuron* **97**, 299-312 e296, doi:10.1016/j.neuron.2017.12.002 (2018).

102 Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E. & Heuschling, P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *J Neuroimmunol* **210**, 3-12, doi:10.1016/j.jneuroim.2009.02.003 (2009).

103 Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J. & Hill, A. M. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* **164**, 6166-6173, doi:10.4049/jimmunol.164.12.6166 (2000).

104 Martinez, F. O. & Gordon, S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* **6**, 13, doi:10.12703/P6-13 (2014).

105 Ransohoff, R. M. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci* **19**, 987-991, doi:10.1038/nn.4338 (2016).

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

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

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

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

110 Jordao, M. J. C. *et al.* Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science* **363**, doi:10.1126/science.aat7554 (2019).

111 Olah, M. *et al.* Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Commun* **11**, 6129, doi:10.1038/s41467-020-19737-2 (2020).

112 Kurtz, A. *et al.* A Standard Nomenclature for Referencing and Authentication of Pluripotent Stem Cells. *Stem Cell Reports* **10**, 1-6, doi:10.1016/j.stemcr.2017.12.002 (2018).

113 Luecken, M. D. & Theis, F. J. Current best practices in single-cell RNA-seq analysis: a tutorial. *Mol Syst Biol* **15**, e8746, doi:10.15252/msb.20188746 (2019).

114 Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**, 611-622, doi:10.1373/clinchem.2008.112797 (2009).

115 d, M. G. & Huggett, J. F. The Digital MIQE Guidelines Update: Minimum Information for Publication of Quantitative Digital PCR Experiments for 2020. *Clin Chem* **66**, 1012-1029, doi:10.1093/clinchem/hvaa125 (2020).

116 Ingolia, N. T., Brar, G. A., Rouskin, S., McGeachy, A. M. & Weissman, J. S. The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. *Nat Protoc* **7**, 1534-1550, doi:10.1038/nprot.2012.086 (2012).

117 Mayor-Ruiz, C., Dominguez, O. & Fernandez-Capetillo, O. Trap(Seq): An RNA Sequencing-Based Pipeline for the Identification of Gene-Trap Insertions in Mammalian Cells. *J Mol Biol* **429**, 2780-2789, doi:10.1016/j.jmb.2017.07.020 (2017).

118 Kunkle, B. W. *et al.* Novel Alzheimer Disease Risk Loci and Pathways in African American Individuals Using the African Genome Resources Panel: A Meta-analysis. *JAMA Neurol* **78**, 102-113, doi:10.1001/jamaneurol.2020.3536 (2021).

119 Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483-495, doi:10.1016/j.stem.2010.08.014 (2010).

120 VanRyzin, J. W. *et al.* Microglial Phagocytosis of Newborn Cells Is Induced by Endocannabinoids and Sculpts Sex Differences in Juvenile Rat Social Play. *Neuron* **102**, 435-449 e436, doi:10.1016/j.neuron.2019.02.006 (2019).

121 Abiega, O. *et al.* Neuronal Hyperactivity Disturbs ATP Microgradients, Impairs Microglial Motility, and Reduces Phagocytic Receptor Expression Triggering Apoptosis/Microglial Phagocytosis Uncoupling. *PLoS Biol* **14**, e1002466, doi:10.1371/journal.pbio.1002466 (2016).

122 Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G. & Mechawar, N. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior

cingulate white matter of depressed suicides. *Brain Behav Immun* **42**, 50-59, doi:10.1016/j.bbi.2014.05.007 (2014).
123 Bachstetter, A. D. *et al.* Rod-shaped microglia morphology is associated with aging in 2 human autopsy series. *Neurobiol Aging* **52**, 98-105, doi:10.1016/j.neurobiolaging.2016.12.028 (2017).
124 Streit, W. J., Sammons, N. W., Kuhns, A. J. & Sparks, D. L. Dystrophic microglia in the aging human brain. *Glia* **45**, 208-212, doi:10.1002/glia.10319 (2004).
125 Savage, J. C., Carrier, M. & Tremblay, M. E. Morphology of Microglia Across Contexts of Health and Disease. *Methods Mol Biol* **2034**, 13-26, doi:10.1007/978-1-4939-9658-2_2 (2019).
126 Salamanca, L. *et al.* MIC-MAC: An automated pipeline for high-throughput characterization and classification of three-dimensional microglia morphologies in mouse and human postmortem brain samples. *Glia* **67**, 1496-1509, doi:10.1002/glia.23623 (2019).
127 Stratoulias, V., Venero, J. L., Tremblay, M. E. & Joseph, B. Microglial subtypes: diversity within the microglial community. *EMBO J* **38**, e101997, doi:10.15252/embj.2019101997 (2019).
128 Tremblay, M. E., Lecours, C., Samson, L., Sanchez-Zafra, V. & Sierra, A. From the Cajal alumni Achucarro and Rio-Hortega to the rediscovery of never-resting microglia. *Front Neuroanat* **9**, 45, doi:10.3389/fnana.2015.00045 (2015).
129 Tremblay, M. E. The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies. *Neuron Glia Biol* **7**, 67-76, doi:10.1017/S1740925X12000038 (2011).
130 Tremblay, M. E., Madore, C., Bordeleau, M., Tian, L. & Verkhratsky, A. Neuropathobiology of COVID-19: The Role for Glia. *Front Cell Neurosci* **14**, 592214, doi:10.3389/fncel.2020.592214 (2020).
131 Sierra, A., Tremblay, M. E. & Wake, H. Never-resting microglia: physiological roles in the healthy brain and pathological implications. *Front Cell Neurosci* **8**, 240, doi:10.3389/fncel.2014.00240 (2014).
132 Devanney, N. A., Stewart, A. N. & Gensel, J. C. Microglia and macrophage metabolism in CNS injury and disease: The role of immunometabolism in neurodegeneration and neurotrauma. *Exp Neurol* **329**, 113310, doi:10.1016/j.expneurol.2020.113310 (2020).
133 David, S. & Kroner, A. Repertoire of microglial and macrophage responses after spinal cord injury. *Nat Rev Neurosci* **12**, 388-399, doi:10.1038/nrn3053 (2011).
134 Tang, Y. & Le, W. Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases. *Mol Neurobiol* **53**, 1181-1194, doi:10.1007/s12035-014-9070-5 (2016).
135 Graeber, M. B. Changing face of microglia. *Science* **330**, 783-788, doi:10.1126/science.1190929 (2010).
136 Lawson, L. J., Perry, V. H., Dri, P. & Gordon, S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151-170, doi:10.1016/0306-4522(90)90229-w (1990).
137 Wolf, Y., Yona, S., Kim, K. W. & Jung, S. Microglia, seen from the CX3CR1 angle. *Front Cell Neurosci* **7**, 26, doi:10.3389/fncel.2013.00026 (2013).
138 Bisht, K. *et al.* Dark microglia: A new phenotype predominantly associated with pathological states. *Glia* **64**, 826-839, doi:10.1002/glia.22966 (2016).
139 Imai, Y., Ibata, I., Ito, D., Ohsawa, K. & Kohsaka, S. A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. *Biochem Biophys Res Commun* **224**, 855-862, doi:10.1006/bbrc.1996.1112 (1996).
140 Ito, D. *et al.* Microglia-specific localisation of a novel calcium binding protein, Iba1. *Brain Res Mol Brain Res* **57**, 1-9, doi:10.1016/s0169-328x(98)00040-0 (1998).
141 Shapiro, L. A., Perez, Z. D., Foresti, M. L., Arisi, G. M. & Ribak, C. E. Morphological and ultrastructural features of Iba1-immunolabeled microglial cells in the hippocampal dentate gyrus. *Brain Res* **1266**, 29-36, doi:10.1016/j.brainres.2009.02.031 (2009).

142 Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S. & Nabekura, J. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci* **29**, 3974-3980, doi:10.1523/JNEUROSCI.4363-08.2009 (2009).
143 Tremblay, M. E., Lowery, R. L. & Majewska, A. K. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol* **8**, e1000527, doi:10.1371/journal.pbio.1000527 (2010).
144 Tischer, J. *et al.* Inhomogeneous distribution of Iba-1 characterizes microglial pathology in Alzheimer's disease. *Glia* **64**, 1562-1572, doi:10.1002/glia.23024 (2016).
145 Lier, J. *et al.* Loss of IBA1-Expression in brains from individuals with obesity and hepatic dysfunction. *Brain Res* **1710**, 220-229, doi:10.1016/j.brainres.2019.01.006 (2019).
146 Fourgeaud, L. *et al.* TAM receptors regulate multiple features of microglial physiology. *Nature* **532**, 240-244, doi:10.1038/nature17630 (2016).
147 Savage, J. C. *et al.* Nuclear receptors license phagocytosis by trem2+ myeloid cells in mouse models of Alzheimer's disease. *J Neurosci* **35**, 6532-6543, doi:10.1523/JNEUROSCI.4586-14.2015 (2015).
148 Healy, L. M. *et al.* MerTK Is a Functional Regulator of Myelin Phagocytosis by Human Myeloid Cells. *J Immunol* **196**, 3375-3384, doi:10.4049/jimmunol.1502562 (2016).
149 Huang, Y. *et al.* Microglia use TAM receptors to detect and engulf amyloid beta plaques. *Nat Immunol* **22**, 586-594, doi:10.1038/s41590-021-00913-5 (2021).
150 Robinson, A. P., White, T. M. & Mason, D. W. Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC OX-41 and MRC OX-42, the latter recognizing complement receptor type 3. *Immunology* **57**, 239-247 (1986).
151 Milligan, C. E., Cunningham, T. J. & Levitt, P. Differential immunochemical markers reveal the normal distribution of brain macrophages and microglia in the developing rat brain. *J Comp Neurol* **314**, 125-135, doi:10.1002/cne.903140112 (1991).
152 McKay, S. M., Brooks, D. J., Hu, P. & McLachlan, E. M. Distinct types of microglial activation in white and grey matter of rat lumbosacral cord after mid-thoracic spinal transection. *J Neuropathol Exp Neurol* **66**, 698-710, doi:10.1097/nen.0b013e3181256b32 (2007).
153 Blackbeard, J. *et al.* Quantification of the rat spinal microglial response to peripheral nerve injury as revealed by immunohistochemical image analysis and flow cytometry. *J Neurosci Methods* **164**, 207-217, doi:10.1016/j.jneumeth.2007.04.013 (2007).
154 Marshall, S. A. *et al.* Microglial activation is not equivalent to neuroinflammation in alcohol-induced neurodegeneration: The importance of microglia phenotype. *Neurobiol Dis* **54**, 239-251, doi:10.1016/j.nbd.2012.12.016 (2013).
155 Avignone, E., Ulmann, L., Levavasseur, F., Rassendren, F. & Audinat, E. Status epilepticus induces a particular microglial activation state characterized by enhanced purinergic signaling. *J Neurosci* **28**, 9133-9144, doi:10.1523/JNEUROSCI.1820-08.2008 (2008).
156 Peng, J. *et al.* Microglial P2Y12 receptor regulates ventral hippocampal CA1 neuronal excitability and innate fear in mice. *Mol Brain* **12**, 71, doi:10.1186/s13041-019-0492-x (2019).
157 Haynes, S. E. *et al.* The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat Neurosci* **9**, 1512-1519, doi:10.1038/nn1805 (2006).
158 Sipe, G. O. *et al.* Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nat Commun* **7**, 10905, doi:10.1038/ncomms10905 (2016).
159 Kanamoto, T. *et al.* Isolation and characterization of a novel plasma membrane protein, osteoblast induction factor (obif), associated with osteoblast differentiation. *BMC Dev Biol* **9**, 70, doi:10.1186/1471-213X-9-70 (2009).
160 Bennett, M. L. *et al.* New tools for studying microglia in the mouse and human CNS. *Proc Natl Acad Sci U S A* **113**, E1738-1746, doi:10.1073/pnas.1525528113 (2016).
161 Satoh, J. *et al.* TMEM119 marks a subset of microglia in the human brain. *Neuropathology* **36**, 39-49, doi:10.1111/neup.12235 (2016).

162 van Wageningen, T. A. *et al.* Regulation of microglial TMEM119 and P2RY12 immunoreactivity in multiple sclerosis white and grey matter lesions is dependent on their inflammatory environment. *Acta Neuropathol Commun* **7**, 206, doi:10.1186/s40478-019-0850-z (2019).
163 Gonzalez Ibanez, F. *et al.* Immunofluorescence Staining Using IBA1 and TMEM119 for Microglial Density, Morphology and Peripheral Myeloid Cell Infiltration Analysis in Mouse Brain. *J Vis Exp*, doi:10.3791/60510 (2019).
164 Chertoff, M., Shrivastava, K., Gonzalez, B., Acarin, L. & Gimenez-Llort, L. Differential modulation of TREM2 protein during postnatal brain development in mice. *PLoS One* **8**, e72083, doi:10.1371/journal.pone.0072083 (2013).
165 Fahrenhold, M. *et al.* TREM2 expression in the human brain: a marker of monocyte recruitment? *Brain Pathol* **28**, 595-602, doi:10.1111/bpa.12564 (2018).