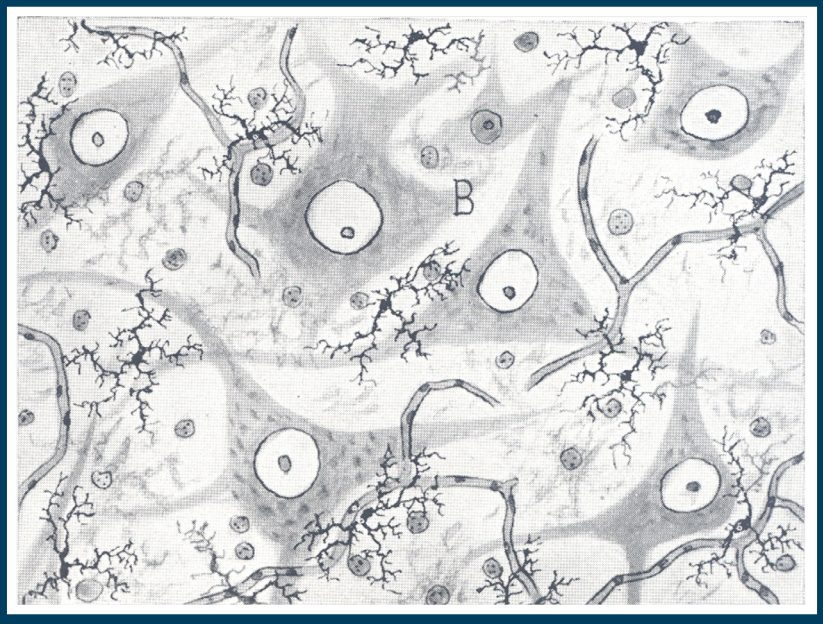
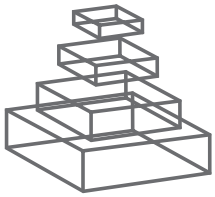
frontiers
RESEARCH TOPICS



NEVER-RESTING MICROGLIA: PHYSIOLOGICAL ROLES IN THE HEALTHY BRAIN AND PATHOLOGICAL IMPLICATIONS

Topic Editors
Amanda Sierra, Marie-Ève Tremblay and Hiroaki Wake

frontiers in
CELLULAR NEUROSCIENCE



frontiers

ISSN 1664-8714
ISBN 978-2-88919-369-1
DOI 10.3389/978-2-88919-369-1

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.
All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

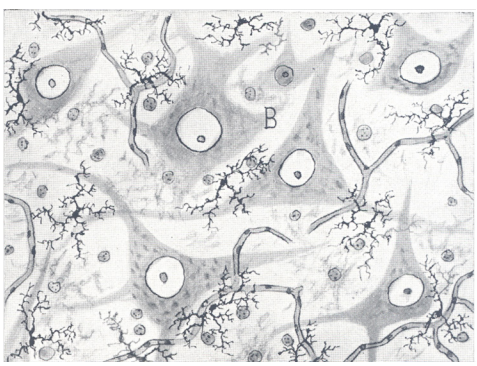
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!
Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

NEVER-RESTING MICROGLIA: PHYSIOLOGICAL ROLES IN THE HEALTHY BRAIN AND PATHOLOGICAL IMPLICATIONS

Topic Editors:
Amanda Sierra, University of the Basque Country EHU/UPV, Spain
Marie-Ève Tremblay, Centre de recherche du CHU de Québec, Université Laval, Canada
Hiroaki Wake, National Institute for Basic Biology, NINS, Japan



Pio del Rio Hortega, Memorias de la Real Sociedad Española de Historia Natural, Tomo XI, Mem VI, Lam XIII. (1921). Reprinted with permission.

Microglia are largely known as the major orchestrators of the brain inflammatory response. As such, they have been traditionally studied in various contexts of disease, where their activation has been assumed to induce a wide range of detrimental effects. In the last few years, a series of discoveries have challenged the current view of microglia, showing their active and positive contribution to normal brain function. This Research Topic reviewed the novel physiological roles of microglia in the developing, mature and aging brain, under non-pathological conditions. In particular, this Research Topic discussed the cellular and molecular mechanisms by which microglia contribute to the formation, pruning and plasticity of synapses; the regulation of adult neurogenesis as well as hippocampal learning and memory; among other important roles. Because these novel findings defy our understanding of microglial function in health as much as in disease, this Research Topic also summarized the current view of microglial nomenclature, phenotypes, origin and differentiation, and contribution to various brain pathologies. Additionally, novel imaging approaches and molecular tools to study microglia in their non-activated state have been discussed. In conclusion, this Research Topic seeked to emphasize how the current research in neuroscience is challenged by never-resting microglia.

Table of Contents

frontiers in
CELLULAR NEUROSCIENCE

EDITORIAL
published: 15 August 2014
doi: 10.3389/fncel.2014.00240

Never-resting microglia: physiological roles in the healthy brain and pathological implications

***Amanda Sierra*[1], *Marie-Ève Tremblay*[2]* *and Hiroaki Wake*[3]**

[1] *Ikerbasque Foundation, University of the Basque Country EHU/UPV, Bilbao, Spain*
[2] *Axe Neurosciences, Centre de Recherche du CHU de Québec, Université Laval, Québec, Canada*
[3] *Division of Brain Circuits, National Institute for Basic Biology, NINS, Okazaki, Japan*
**Correspondence: tremblay0202@gmail.com*

Edited and reviewed by:
Egidio D'Angelo, University of Pavia, Italy

Keywords: microglia, health, disease, phenotype, neuroprotection, phagocytosis, synapses, neurogenesis

Microglia are the resident macrophages of the central nervous system (CNS), largely known as the major orchestrators of the immune response. As such, they have been traditionally studied in various contexts of trauma, injury and disease, where their phenotypic transformation (or "activation") has been assumed to induce a wide range of mostly detrimental effects. In the last few years, however, a series of discoveries have started to unravel their active and positive contribution to normal CNS function.

Within this perspective, our research topic *Never-resting microglia: physiological roles in the healthy brain and pathological implications* reviews the emerging roles of microglia in the healthy CNS across development, adulthood, and normal aging. The cellular and molecular mechanisms underlying these new roles are particularly covered, such as microglial phagocytosis and dynamic interactions with synapses, as much as their functional consequences for the regulation of neuronal circuit maturation and refinement, activity, and plasticity, including adult hippocampal neurogenesis.

Since these findings provide a better understanding of microglial function in health as much as in disease, our research topic also summarizes the current view about microglial origin, homeostasis, diversity, phenotypic transformation, neurotoxicity, relationships with macrophages from the periphery, and contribution to normal CNS aging and various pathological conditions. Additionally, novel methodological approaches and molecular tools to study microglia in their normally prevailing state are discussed.

In particular, Ginhoux et al. (2013) review the history of microglial cells and discuss the latest advances in our understanding of their origin, differentiation, and homeostasis, thus providing new insights into their roles in health and disease.

Wolf et al. (2013) summarize the recent efforts to exploit CX3CR1 promoter activity for the visualization and genetic manipulation of microglia, in order to probe their functional contributions in the CNS, and the resulting insights into the role of CX3CR1.

Karperien et al. (2013) review current trends and methods of fractal analysis, used for quantitating changes in microglial morphology and differentiate subtle differences amongst ramified cells, while focusing on box counting analysis, including lacunarity and multifractal analysis.

Hanisch (2013) discuss the diversity in microglial cells protein expression, housekeeping functions, and reactive phenotypes, which could result from differences in lineage commitment and microenvironment, or stochastic variation.

Kierdorf and Prinz (2013) summarize current knowledge of the intrinsic (e.g., Runx-1, Irf8, Pu.1) and extrinsic factors (e.g., CD200, CX3CR1, TREM2) which regulate the transition from a surveying microglial phenotype to an activated stage.

Hellwig et al. (2013) critically reconsider the term microglial neurotoxicity and discuss experimental problems around microglial biology (e.g., *in vitro* preparations and transgenic strategies) which often have led to the conclusion that microglia are neurotoxic cells.

London et al. (2013) discuss the functional heterogeneity and relationships between microglia and bone-marrow derived macrophages, their contribution to CNS plasticity and repair, and the lessons derived from other populations of tissue-resident macrophages.

Sierra et al. (2013) summarize the current state of the literature regarding the role of microglial phagocytosis in maintaining tissue homeostasis in health as in disease, and the underlying molecular mechanisms including find-me, eat-me, and digest-me signals.

Miyamoto et al. (2013) focus on the interactions between microglia and synapses, reviewing the cellular and molecular mechanisms mediating their contacts, and their possible implications in the fine tuning of neural circuits during normal physiological conditions.

Domercq et al. (2013) summarize the relevant data regarding the role of neurotransmitter receptors in microglial physiology and pathology, with an emphasis on purinergic and glutamate receptors which modulate microglial physiology in various manners.

Béchade et al. (2013) discuss the role of microglia in the control of neuronal activity, describing how their dysfunction is responsible for the alteration of neuronal activity in pathological situations, and how microglia can be considered as partners of neurotransmission in the healthy brain.

Gemma and Bachstetter (2013) review the role of microglia in hippocampal neurogenesis during normal physiological conditions, with an emphasis on microglial phagocytosis, release of trophic factors, and the involvement of CX3CR1.

Belarbi and Rosi (2013) summarize the current knowledge on how the production, distribution, and recruitment of new neurons into behaviorally relevant neural networks are modified in the inflamed hippocampus.

Laslty, Wong (2013) explore the hypothesis that age-related changes in microglia could be implicated in the pathogenic mechanisms of age-related neurodegenerative diseases, discussing the possible underlying cellular mechanisms, as well as "rejuvenative" measures and strategies.

In conclusion, this special issue seeks to emphasize how the current research in neuroscience is being challenged by never-resting microglia.

REFERENCES

Béchade, C., Cantaut-Belarif, Y., and Bessis, A. (2013). Microglial control of neuronal activity. *Front. Cell. Neurosci.* 7:32. doi: 10.3389/fncel.2013.00032

Belarbi, K., and Rosi, S. (2013). Modulation of adult-born neurons in the inflamed hippocampus. *Front. Cell. Neurosci.* 7:145. doi: 10.3389/fncel.2013.00145

Domercq, M., Vazquez-Villoldo, N., and Matute, C. (2013). Neurotransmitter signaling in the pathophysiology of microglia. *Front. Cell. Neurosci.* 7:49. doi: 10.3389/fncel.2013.00049

Gemma, C., and Bachstetter, A. D. (2013). The role of microglia in adult hippocampal neurogenesis. *Front. Cell. Neurosci.* 7:229. doi: 10.3389/fncel.2013.00229

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., and Huber, T. (2013). Origin and differentiation of microglia. *Front. Cell. Neurosci.* 7:45. doi: 10.3389/fncel.2013.00045

Hanisch, U. K. (2013). Functional diversity of microglia—how heterogeneous are they to begin with? *Front. Cell. Neurosci.* 7:65. doi: 10.3389/fncel.2013.00065

Hellwig, S., Heinrich, A., and Biber, K. (2013). The brain's best friend: microglial neurotoxicity revisited. *Front. Cell. Neurosci.* 7:71. doi: 10.3389/fncel.2013.00071

Karperien, A., Ahammer, H., and Jelinek, H. F. (2013). Quantitating the subtleties of microglial morphology with fractal analysis. *Front. Cell. Neurosci.* 7:3. doi: 10.3389/fncel.2013.00003

Kierdorf, K., and Prinz, M. (2013). Factors regulating microglia activation. *Front. Cell. Neurosci.* 7:44. doi: 10.3389/fncel.2013.00044

London, A., Cohen, M., and Schwartz, M. (2013). Microglia and monocyte-derived macrophages: functionally distinct populations that act in concert in CNS plasticity and repair. *Front. Cell. Neurosci.* 7:34. doi: 10.3389/fncel.2013.00034

Miyamoto, A., Wake, H., Moorhouse, A. J., and Nabekura, J. (2013). Microglia and synapse interactions: fine tuning neural circuits and candidate molecules. *Front. Cell. Neurosci.* 7:70. doi: 10.3389/fncel.2013.00070

Sierra, A., Abiega, O., Shahraz, A., and Neumann, H. (2013). Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. *Front. Cell. Neurosci.* 7:6. doi: 10.3389/fncel.2013.00006

Wolf, Y., Yona, S., Kim, K. W., and Jung, S. (2013). Microglia, seen from the CX3CR1 angle. *Front. Cell. Neurosci.* 7:26. doi: 10.3389/fncel.2013.00026

Wong, W. T. (2013). Microglial aging in the healthy CNS: phenotypes, drivers, and rejuvenation. *Front. Cell. Neurosci.* 7:22. doi: 10.3389/fncel.2013.00022

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 July 2014; accepted: 29 July 2014; published online: 15 August 2014.
Citation: Sierra A, Tremblay M-È and Wake H (2014) Never-resting microglia: physiological roles in the healthy brain and pathological implications. Front. Cell. Neurosci. ***8****:240. doi: 10.3389/fncel.2014.00240*
This article was submitted to the journal Frontiers in Cellular Neuroscience.

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 17 April 2013
doi: 10.3389/fncel.2013.00045

Origin and differentiation of microglia

Florent Ginhoux[1], Shawn Lim[2], Guillaume Hoeffel[1], Donovan Low[1] and Tara Huber[2,3]*

[1] Singapore Immunology Network, Agency for Science, Technology, and Research, Singapore
[2] Genome Institute Singapore, Agency for Science, Technology, and Research, Singapore
[3] Department of Biological Science, National University of Singapore, Singapore

Edited by:
Amanda Sierra, University of the Basque Country EHU/UPV, Spain

Reviewed by:
Payam Rezaie, The Open University, UK
Miguel A. Cuadros, Universidad de Granada Spain, Spain

****Correspondence:***
Florent Ginhoux, Singapore Immunology Network, 8A Biomedical Grove, Immunos Building, Level 4, Singapore, 138648.
e-mail: florent_ginhoux@immunol.a-star.edu.sg

Microglia are the resident macrophage population of the central nervous system (CNS). Adequate microglial function is crucial for a healthy CNS. Microglia are not only the first immune sentinels of infection, contributing to both innate and adaptive immune responses locally, but are also involved in the maintenance of brain homeostasis. Emerging data are showing new and fundamental roles for microglia in the control of neuronal proliferation and differentiation, as well as in the formation of synaptic connections. While microglia have been studied for decades, a long history of experimental misinterpretation meant that their true origins remained debated. However, recent studies on microglial origin indicate that these cells in fact arise early during development from progenitors in the embryonic yolk sac (YS) that seed the brain rudiment and, remarkably, appear to persist there into adulthood. Here, we review the history of microglial cells and discuss the latest advances in our understanding of their origin, differentiation, and homeostasis, which provides new insights into their roles in health and disease.

Keywords: microglia, macrophage, central nervous system, origin, yolk sac

INTRODUCTION

Microglia are the resident mononuclear phagocytes of the central nervous system (CNS), belonging to the glial system of non-neuronal cells that support and protect neuronal functions. Microglia are broadly distributed throughout the brain and the spinal cord (Lawson et al., 1990), and account for 5–20% of the total glial cell population within the CNS parenchyma (Perry, 1998). Adequate and appropriate microglial function is crucial for the homeostasis of the CNS in both health and disease (Perry et al., 2010).

There are two main functional aspects of microglia: immune defense and CNS maintenance. As immune cells, they act as sentinels, detecting the first signs of pathogenic invasion or tissue damage in this delicate immune-privileged site that is actively protected by the brain blood barrier (Daneman, 2012). Under the inflammatory conditions of an active immune response however, microglia must also moderate the potential damage to the CNS and support tissue repair and remodeling. Perhaps unsurprisingly, dysregulated microglial activation and microglia-induced inflammation is observed in virtually all brain pathologies; emerging evidence suggests that microglia exert direct effects on neurons, contributing to disease progression (Perry et al., 2010; Kettenmann et al., 2011; Kingwell, 2012).

In recent years there has been an increasing appreciation of the importance of microglia for normal CNS function. In addition to their immune functions, emerging data are showing new and fundamental roles for microglia in the control of neuronal proliferation and differentiation as well as in the formation of synaptic connections (Graeber, 2010; Hughes, 2012). In the steady state, microglial cells constantly survey their local microenvironment, extending their motile processes to make transient contact with neuronal synapses, contributing to the modification and the elimination of synaptic structures (Tremblay et al., 2010). Microglia also contribute to the remodeling of post-natal neural circuits as they have been recently shown to play a role in synaptic pruning during post-natal development in mice (Paolicelli et al., 2011).

Thus, microglia occupy a central position in the defense and maintenance of the CNS and so are attracting interest as potential therapeutic targets in neurological disorders and recovery from brain injury. However, in order to exploit the abilities of the microglial population, we must first understand their origins and homeostasis before attempting to manipulate their functions. In this review we will present the latest advances in our knowledge on the origin of microglia, revisit early studies in light of recent developments and highlight some of the most relevant strategies to generate microglia for therapeutic approaches of neurological disorders.

HISTORICAL PERSPECTIVES ON THE NATURE OF MICROGLIA

Defining the origin of microglia has been an elusive goal for generations of researchers and a longstanding issue of debate. Multiple schools of thought have emerged. The first description of the cells came from the work of Franz Nissl in the late nineteenth century, who described rod cells ("Stabchenzellen") as reactive glial elements with migratory, phagocytic and proliferative potential. In the late nineteenth century, W. Ford Robertson introduced the term "mesoglia" to describe mesoderm-derived phagocytic elements in the nervous system that had origins distinct from those of neurons and neuroglia. Neuroglia were first described by Virchow, in 1856, who named them "nevernkitt" meaning nerve-glue, later translated as "neuroglia," though in fact they corresponded to the macroglial population, which comprises astrocytes and oligodendrocytes (Rio-Hortega, 1939). While this

idea had merit, in fact, Robertson's mesoglia similarly turned out to correspond mainly to oligodendrocytes. Santiago Ramon y Cajal later renamed the same cells the "third element of the nervous system" to further differentiate them from neurons and neuroglia, and stated that they were of probable mesodermal origin. This "third element" concept was refined further in 1919 by del Rio-Hortega, a student of Ramon y Cajal, who made the distinction between various cell types within the cells of the "third element" based on morphological and functional differences. Del Rio-Hortega introduced the term "microglial cell" to describe the non-neuronal, non-astrocytic third element as distinct from neurectodermal oligodendroglia or oligodendrocytes (Rio-Hortega, 1939) (For historic review see Rezaie and Male, 2002).

Although both W. Ford Robertson and Santiago Ramon y Cajal suspected a mesodermal origin of what were to become known as "microglial cells" (their mesoglia/third element of the nervous system), it was commonly held at the time that all glial cells were of neuro-ectodermal origin. Further dissecting the heterogeneity of the mesoglia, del Rio-Hortega was the first to introduce the term "microglia" to discriminate true mesodermal elements from oligodendrocytes, which were previously considered a component of the mesoglia. Del Rio-Hortega exploited silver staining techniques to describe the two types of cells as differing in origin, distribution, form, and function: the major population, called oligodendroglia, that lacked phagocytic activity, and the minor population of ramified resting cells. This minor population was then clearly defined as the "third element of the CNS" with a mesodermal origin, containing phagocytic corpuscles and with migratory and phagocytic activity (Rio-Hortega, 1932).

Despite del Rio-Hortega's seminal work, his theories were largely overlooked and have only recently come back to the forefront of scientific thinking (Rezaie and Male, 2002). At the time, there was much support for the belief that microglia shared a neuro-ectodermal origin with the other glial cells. Several studies supported this belief well into the twentieth century, including reports from Fujita who proposed a common, matrix-derived progenitor for microglia, astrocytes, and oligodendrocytes (Fujita and Kitamura, 1975). The work of Kitamura was similarly interpreted to indicate that microglia, as well as astrocytes, originated from neuro-ectodermal-derived glioblasts (Kitamura et al., 1984). As late as the 1990's, new studies continued to emerge that seemed to show a common origin of astrocytes and microglia; Hao reported that cultures of either murine embryonic neuroepithelial cells or astrocytes could differentiate *in vitro* to give microglial-like cells, an idea which was supported by Fedoroff's work showing that clonal cultures of disaggregated neopallial cells from newborn mice gave rise to mixed microglial-astroglial cells (Hao et al., 1991; Fedoroff et al., 1997). In addition, data showing that donor bone marrow cells failed to contribute to the adult microglial population in either newborn (De Groot et al., 1992) or adult rodents (Matsumoto and Fujiwara, 1987) was interpreted to mean that the majority of microglial cells were of local neuro-ectodermal origin. However, this interpretation was soon updated in response to the finding that microglia (in contrast to other blood leucocyte populations) are highly radio-resistant. In 1993, Lassmann and co-workers were the first to demonstrate that resident microglia in rats are a very stable cell pool, in contrast to meningeal and perivascular macrophages, which in adult animals are only exceptionally replaced by circulating blood cells, even after recovery from severe brain inflammation (Lassmann et al., 1993). Such observations were later confirmed in mice by Priller, where the majority (85–95%) of microglial cells remained of host origin up to 15 weeks after bone marrow transplantation (Priller et al., 2001). The importance of the observation that newborn microglia are not replaced by donor bone marrow-derived cells (De Groot et al., 1992) will be discussed later, as it has the significant implication that the adult microglial population can be maintained solely by local radio-resistant precursors which are present in the brain prior to birth.

Other hypotheses on the origin of microglia included their derivation from the pericytes associated with blood vessels (Mori and Leblond, 1969; Baron and Gallego, 1972) or from the subependyma adjacent to the lateral ventricles (Lewis, 1968).

At the same time, a second school of thought was developing which paralleled del Rio-Hortega's original hypothesis. His conviction of the mesodermal origin of microglia was supported both by studies coupling light/electron microscopy and immunohistochemistry, which recognized typical morphological features of macrophages in the various stages of microglial development (Murabe and Sano, 1982), and by the demonstration that microglial cells reacted positively to antisera recognizing monocyte/macrophage antigens (Hume et al., 1983; Murabe and Sano, 1983). Despite the emerging evidence of the relationship of microglia to macrophages, other reports led to variable interpretations due to a lack of homology between monocytes and mature microglia in the expression of certain antigens, complicating the issue (Oehmichen et al., 1979; Wood et al., 1979). Nevertheless, the data showing phenotypic homologies between monocytes/macrophages and microglia were eventually validated by immunohistochemical studies that reported the specific expression of macrophage markers, including F4/80, Fc receptor and CD11b in mouse microglia (Perry et al., 1985), as well as FcGRI, and CD11b in their human counterparts (Akiyama and McGeer, 1990). Finally, a pivotal genetic study revealed that mice lacking PU.1, a crucial transcription factor for myeloid cells, were also devoid of microglia (McKercher et al., 1996; Beers et al., 2006). This unequivocally established the myeloid nature of microglia and simultaneously suggested that these cells might be ontogenetically related to macrophages.

THE ORIGIN(S) OF MURINE MICROGLIA

Although there is a consensus about the myeloid origin of microglia, much controversy remains regarding the precise nature of microglial progenitors. Initial studies described the presence of microglial cells during early development, suggesting that microglia arise from embryonic progenitors. While del Rio-Hortega proposed that microglia originate from meningeal macrophages penetrating the brain during embryonic development, many authors including del Rio-Hortega himself, claimed that brain parenchymal microglia could also be derived from blood monocytes. Monocytes are indeed recruited to the neonatal and adult brain, in the latter case most often under inflammatory

conditions, where they can differentiate into microglia-like cells. This knowledge long supported the prevailing viewpoint that circulating blood monocytes represent microglial progenitors, replacing those seeding the brain during embryonic development. In fact, until recently, the most consensual hypothesis was that embryonic and peri-natal hematopoietic waves of microglial recruitment and differentiation occurred in the CNS (Chan et al., 2007). However, we now know that the situation is somewhat different. Here, we will describe and discuss the recent advances in understanding of the origin of microglia, and will also revisit the data from earlier studies in light of these developments.

EARLY DEVELOPMENT

In addition to having first described microglia, del Rio-Hortega also proposed that they might initially arise in the early stages of development from mesodermal cells of the pia mater, the innermost layer of the meninges (the membranes surrounding the brain and spinal cord). From his work on embryonic brains, he reported the "migration of embryonic corpuscles from the pia into the nerve centers" with morphological similarity to lymphocytes (Rio-Hortega, 1939). However, del Rio-Hortega also proposed that "microglia may eventually arise from other related elements, chiefly the blood mononuclears" based on the similarities in morphology and phagocytic activities of the microglia and monocytes (Rio-Hortega, 1939), thereby founding the "origin of microglia" controversy.

The immunohistochemical study conducted by Perry et al., more precisely described this phenomenon using macrophage markers such as F4/80. They concluded that as early as embryonic day 16 (E16) of development, macrophage-like cells that had extravasated into the brain parenchyma were localized in "hot spots," from where they subsequently invaded the brain and differentiated through a series of transitional forms to finally become ramified microglia (Perry et al., 1985). Other studies later detected dispersed F4/80 expressing macrophages distributed within loose connective tissue surrounding the neuroectoderm in E12 rat embryos (Morris et al., 1991). Also in the rat, amoeboid microglial cells expressing monocytic markers are present as early as E12 in the neuro-epithelium (Wang et al., 1996). Interestingly, such embryonic cells were proposed to be microglial progenitors not only due to the similarities in phenotype and morphology, but because of their potent proliferative response to mitogenic stimulation *in vitro* (Alliot et al., 1991).

Similarly, in human fetuses, microglia-like cells with a range of morphologies can be detected from as early as 3 weeks of estimated gestational age (EGA) (Hutchins et al., 1990). However, it appears that maturation of the microglial compartment is ongoing throughout the majority of gestation: colonization of the spinal cord begins at around 9 weeks, the major influx and distribution of microglia commences at about 16 weeks, and ramified microglial forms take up to 22 weeks to become widely distributed within the intermediate zone (Rezaie and Male, 1999; Rezaie et al., 2005). It is only close to term, at 35 weeks, that well-differentiated microglial populations can be detected (Esiri et al., 1991) (for review Rezaie, 2003 and Verney et al., 2010).

Altogether, these seminal studies strongly suggested that microglia derive from embryonic hematopoietic precursors that seed the CNS prior to birth and, more importantly, before the onset of bone marrow hematopoiesis. However, the exact tissue origin and developmental cell lineage of precursors that migrate to the CNS to give rise to the "first" endogenous wave of microglia remained unknown and a topic of debate until recently.

MOUSE AND HUMAN EMBRYONIC HEMATOPOIESIS

A major challenge in defining the embryonic microglial precursor was the complication of the dual source of blood cell formation during embryogenesis. Two major hematopoietic sites contribute to this process: the extra-embryonic yolk sac (YS) and the fetal liver (Tavian and Peault, 2005; Orkin and Zon, 2008). In mice, primitive hematopoiesis initiates in the YS around E7.0, shortly after the onset of gastrulation, leading mainly to the production of erythrocytes and macrophages (Moore and Metcalf, 1970; Palis et al., 1999; Bertrand et al., 2005). Primitive macrophages first appear in the blood islands of the mouse YS on the ninth day of gestation and their pattern of differentiation is unique in the sense that they do not go through a monocytic intermediate stage, as seen in adult macrophages (Takahashi et al., 1989). YS-derived primitive macrophages will spread into the embryo proper through the blood after the circulatory system has been fully established (from E8.5 to E10) (McGrath et al., 2003) and migrate to various tissues, including the brain. Once in the tissues, they differentiate into so-called "fetal macrophage populations" even before the onset of monocyte production by the fetal liver (Naito et al., 1990). These fetal macrophages have high proliferative potential, not only in the YS where they are produced but also in the tissues that they colonize (Takahashi et al., 1989; Sorokin et al., 1992; Naito et al., 1996; Lichanska and Hume, 2000). After E8.5, with the determination of the intra-embryonic mesoderm toward the hematopoietic lineage, a new wave of hematopoietic progenitors is generated within the embryo proper, first in the para-aortic splanchnopleura (P-Sp) region and then in the aorta, gonads, and mesonephros (AGM) region (Godin et al., 1993; Medvinsky et al., 1993). The hematopoietic stem cells generated within the AGM will lead to the establishment of definitive hematopoiesis (Orkin and Zon, 2008). Around E10.5, YS- and AGM-derived hematopoietic progenitors colonize the fetal liver (Kumaravelu et al., 2002), which serves as the major hematopoietic organ after E11.5, generating all hematopoietic lineages, including monocytes (Naito et al., 1990). A recent study highlighted further differences between primitive and definitive hematopoiesis, showing that the latter relies on the transcription factor Myb, while YS-derived macrophages are Myb-independent. This further underlines the fact that YS-derived macrophages constitute an independent lineage, distinct from the progeny of definitive hematopoietic stem cells (Schulz et al., 2012).

Human hematopoiesis also begins in the YS around day 19 of the EGA, and YS-derived stem cells are similarly limited/committed to myelo-erythroid development. Hematopoiesis then moves transitorily to the fetal liver around 4–5 weeks EGA, before being definitively established in the BM approximately at 10.5 weeks EGA (Tavian and Peault, 2005).

THE YOLK SAC HYPOTHESIS OF MICROGLIAL ORIGIN

Ashwell was the first to report the presence of round and amoeboid microglial cells in the fetal mouse cerebellum (Ashwell, 1990) and then in rat forebrain (Ashwell and Waite, 1991) as early as E11.0. Sorokin soon after detected macrophage-like cells and their precursors in blood vessels and the embryonic mesenchyme in rat embryos from 10.5, and noted that the developing brain was the first organ to be colonized (Sorokin et al., 1992). Interestingly, cells with the capacity to differentiate into microglia-like cells *in vitro* (expressing Mac-1, Mac-3, F4/80 and Fc antigens, with a macrophage-like morphology and ultrastructure) can be detected in the developing neuro-epithelium at days E8.5/E9.0, suggesting that in mice, the earliest developmental stage at which seeding of cells with myeloid features occurs in the brain is at E8.5/E9.0 (Alliot et al., 1991). Later reports confirmed the presence at similar stages of amoeboid cells expressing macrophage (Alliot et al., 1999; Ginhoux et al., 2010) and microglial markers (Chan et al., 2007; Mizutani et al., 2012) in both the cephalic mesenchyme and the neuro-epithelium, in accordance with the idea that the YS contributes to microglial genesis.

However, the evidence for a YS origin of such microglial progenitors was, at first, mixed. Initially, data from one of the aforementioned *in vitro* studies were interpreted to support the hypothesis that these macrophage-like cells that will give rise to microglia originated from the neuro-ectoderm (Hao et al., 1991). Takashi and Naito drew a different conclusion after they described the first emergence of immature macrophages within blood islands of embryonic YS at fetal day 9 in both mouse (Takahashi et al., 1989) and rat (Takahashi and Naito, 1993). Following the establishment of fetal blood circulation, these cells colonize the embryonic tissues, including the brain rudiment. By [3H]-thymidine autoradiography, YS macrophages were shown to possess high proliferative potential, which suggested that these fetal macrophages were in fact primitive macrophages from the YS (Takahashi and Naito, 1993). Alliot also clearly and convincingly proposed that such cells were true microglial progenitors of YS origin, as, at that stage, the YS is the only hematopoietic site in the embryo. This group then conclusively documented the presence of potential microglial progenitors in the YS and then the brain rudiment, with their numbers increasing dramatically from E9.0/E9.5 until around 2 weeks after birth (Alliot et al., 1999).

A similar pattern of events is likely observed in humans, where, from 4.5 weeks gestation, amoeboid microglial cells (characterized by the expression of Iba1, CD68, CD45, and MHC-II) enter the cerebral wall from the ventricular lumen and the leptomeninges (Rezaie et al., 2005; Monier et al., 2007). In the YS and mesenchyme at 4–6 weeks after fertilization, two populations of cells with a dendritic morphology could be distinguished: a majority that expressed monocyte/macrophage-associated markers but no detectable HLA-DR antigen, while the minority constitutively expressed MHC class II (HLA-DR and -DP) but no monocyte/macrophage-associated markers (Janossy et al., 1986). The emergence of this heterogeneity preceded the formation of both thymus and bone marrow, suggesting the independent development of these macrophage populations (for review Verney et al., 2010).

Interestingly, the YS derivation of microglia appears to be conserved across diverse species as shown in the zebrafish (Herbomel et al., 2001), and in avians (Cuadros and Navascues, 1998). However, what differs in mice is the requirement for a functional blood circulation for the spreading of YS macrophages in the embryo proper. In zebrafish, this colonization appears independent of the blood circulation as YS macrophages first directly invade the whole cephalic mesenchyme, and from there invade epithelial tissues including the brain, while other macrophages enter the blood circulation (Herbomel et al., 1999). Similarly, using chick-quail blood chimeras, Kurz showed that in avians, YS macrophages do not penetrate through the wall of embryonic CNS vessels, but rather from the pial surface (Kurz et al., 2001). In contrast, in mouse embryos, there is a clear requirement for the circulatory system as E9.5–E10.5 *Ncx-1*$^{-/-}$ embryos, which lack a heartbeat and therefore have no functional blood circulation due to a defect in the sodium calcium exchanger 1 (Koushik et al., 2001), also lack microglial progenitors (Ginhoux et al., 2010), as well as other fetal macrophages. However, this defect does not affect YS hematopoiesis as *Ncx-1*$^{-/-}$ embryos have similar numbers of YS macrophages as their control, normal phenotype littermates (Ginhoux et al., 2010). Whether murine YS macrophages enter via the blood circulation directly in the brain parenchyma or first enter in the cephalic mesenchyme and then migrate to the neuro-epithelium (Chan et al., 2007) remains to be clearly defined.

The overall conclusion of these studies in rodents, humans, and other species is that microglia derive from the YS macrophages that seed the brain rudiment during early fetal development (**Figure 1**). However, these reports could not exclude the possibility that others progenitors could supersede the YS contribution. In fact, some data that will be discussed below, continued to emerge that suggested a requirement for the contribution of blood-borne cells to both generate the post-natal microglial compartment, and to maintain it into adulthood.

THE EARLY POST-NATAL CONTRIBUTION OF MONOCYTES TO THE MICROGLIAL POPULATION

Shortly after birth in rodents, the microglial population expands dramatically (Alliot et al., 1999; Tambuyzer et al., 2009), leading to the suggestion that the proliferation of embryonic microglial cells alone could not account for the steep rise in numbers and that there must be a fresh influx of cells from another compartment. As initially suggested by del Rio-Hortega, blood monocytes were believed to invade the CNS in the perinatal period and give rise to microglia, replacing the embryonic microglial cells.

There was support for this belief from several studies, notably an early report where round, amoeboid, phagocytic cells were seen in rat corpus callosum during the first few days of life and then disappeared coincident with the appearance of ramified microglia. These cells were typical macrophages, but some displayed features of monocytes, while others appeared to be transitional between the two types. The authors of this study concluded that circulating monocytes enter the developing brain to assume the form of ameboid microglia that subsequently evolved to become ramified microglia (Ling, 1976). Subsequent studies gave neonatal rats an intra-peritoneal pulse of [3H]-thymidine to

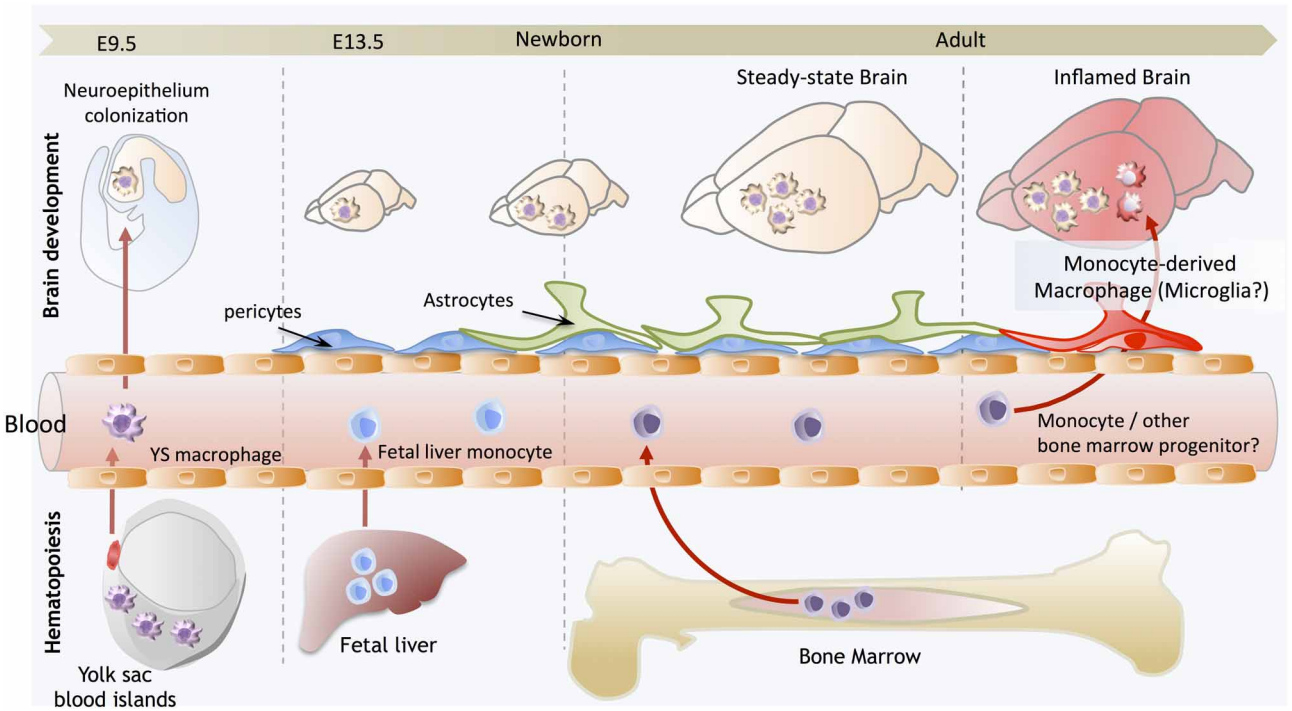


FIGURE 1 | Brain development and microglial homeostasis. Primitive macrophages exit the yolk sac blood islands at the onset of circulation and colonize the neuroepithelium from E9.5 to give rise to microglia. The blood brain barrier starts to form from E13.5 and may isolate the developing brain from the contribution of fetal liver hematopoiesis. Embryonic microglia expand and colonize the whole CNS until adulthood. Importantly, in steady state conditions, embryonically-derived microglia will maintain themselves until adulthood, via local proliferation during late gestation and post-natal development as well as in the injured adult brain in reaction to inflammation. Nevertheless, during certain inflammatory conditions found for example after bone marrow transplantation, the recruitment of monocytes or other bone marrow-derived progenitors can supplement the microglial population to some extent. However, we do not understand yet whether these cells persist and become integrated in the microglial network, or are a temporary addition to the endogenous population.

allow tracking of labeled blood cells by autoradiography. Labeled immature amoeboid cells were detected in the corpus callosum few hours after administration, while the majority of newly-ramified microglia were labeled one week later. These observations implied that labeled microglial cells must therefore have come from the transformation of immature amoeboid cells that acquired the tracer earlier (Imamoto and Leblond, 1978). This possibility was further tested by injecting a suspension of carbon particles into the circulation of rats of various ages to enable tracing of carbon-labeled monocytes, or by direct adoptive transfer of carbon-labeled monocytes. Later on, carbon particles were sequentially found in amoeboid cells of the corpus callosum and then on ramified microglial cells, suggesting again that blood monocytes, after ingesting carbon particles in the circulation or after transfer, migrated to the corpus callosum and differentiated into microglial cells via an amoeboid stage (Ling, 1979; Ling et al., 1980; Leong and Ling, 1992). However, while such data suggest that circulating blood monocytes can enter the CNS right after birth, perhaps in a specific site, it is important to note that such studies were rather qualitative and did not clearly address the exact relative contributions of post-natal monocytes versus embryonic progenitors to adult microglial homeostasis. In fact, the authors had themselves clearly recognized that such events were infrequent (Ling et al., 1980).

Nevertheless, later studies employing the PU.1 knockout (KO) mouse model, that lacks embryonic microglia, demonstrated the capacity of bone marrow-derived cells to contribute to the post-natal microglial population. In this study, neonates received wild-type bone marrow transplants within 24 h of birth, which resulted in *de novo* generation of the full microglial compartment (Beers et al., 2006). Therefore, it must be concluded that, at least under exceptional circumstances such as in the PU.1 KO mouse where endogenous embryonic microglia are completely absent, some bone marrow-derived cells have the capacity to infiltrate the CNS and assume the morphology and phagocytic capacity of microglia.

A CONTRIBUTION OF MONOCYTES TO THE ADULT MICROGLIAL POPULATION IN THE STEADY STATE?

Following the observations that monocytes might be able to contribute to the microglial population immediately after birth, it became implicitly accepted that they could also do so in adults. The idea that monocytes, or any bone marrow-derived cells, might then be able to be engineered and used as a delivery system into the CNS for therapies, the "Trojan Horse" theory, motivated investigators to discover the underlying mechanisms. The main hypothesis became that embryonic microglia disappear and are replaced by post-natal bone marrow-derived cells.

That is not to say that there were not data supporting the hypothesis of a role of monocytes in maintaining the adult microglial population: a seminal study employing [3H]-thymidine incorporation and autoradiography in normal adult mice concluded that cells can be recruited from the circulating monocyte pool through an intact blood-brain barrier (BBB) and rapidly differentiate into resident microglia (Lawson et al., 1992). However, the authors also noted that the resident microglia were proliferating, which suggested that the microglial population might maintain itself through either mechanism, or perhaps both. Other data supporting the idea that adult bone marrow-derived cells can give rise to microglia included the observation that following total bone marrow transplantation, some donor hematopoietic cells differentiated into microglia within the brains of adult mice (Eglitis and Mezey, 1997; Mezey et al., 2000; Simard and Rivest, 2004).

However, these results were in slight disagreement with a previous, and importantly, more quantitative, study which showed that the majority of microglial cells remained of host origin up to 15 weeks after bone marrow transplantation in mice (Priller et al., 2001). Similar results were also initially reported in rats as several investigators concluded that there was little or no contribution of bone marrow-derived cells to the adult microglial pool (Matsumoto and Fujiwara, 1987; Lassmann et al., 1993). In addition, it was shown that while microglia are not bone marrow-derived in adults, the closely-associated meningeal and perivascular macrophages are, perhaps going some way to explaining the confusion. Schelper and Adrian bluntly concluded that "monocytes become macrophages; they do not become microglia," in this case, following CNS lesions (Schelper and Adrian, 1986), while Hickey and Kimura showed that the stable pool of resident microglia is only exceptionally supplemented by hematopoietic cells, even after recovery from severe brain inflammation (Hickey and Kimura, 1988). Similarly, Vallieres reported that many of these cells were in fact perivascular macrophages and that newly-formed parenchymal microglia were found in significant numbers only in the cerebellum and at injury sites (Vallieres and Sawchenko, 2003). Importantly, in humans, taking advantage of sex-mismatched donor bone marrow transplant (male into female) where Y-chromosome specific *in situ* hybridization can be performed to follow the origin of cells, similar results were obtained. The only donor male cells detected corresponded to mononuclear leucocytes within the vessel lumen and infiltrating the perivascular space and parenchyma, and perivascular cells (Unger et al., 1993). In fact, the observation that bone marrow-derived microglia were only found in notable amounts under certain conditions highlighted some significant shortfalls of the earlier studies: while it was successfully shown that monocytes could differentiate into cells that resembled microglia, few had quantified the effect or attempted to define the phenomenon in space and time, or to monitor the persistence of the bone marrow-derived microglia.

A CONTRIBUTION OF MONOCYTES TO THE ADULT MICROGLIAL POPULATION DURING INFLAMMATION?

What became clear was that although the monocyte-to-microglia path may exist in adult brain, it is unlikely to be a significant source for maintaining the microglial population, although this might change during CNS inflammation or disease (Vallieres and Sawchenko, 2003; Ladeby et al., 2005; Mildner et al., 2007). In fact, in response to CNS inflammation and damage, an increase in microglial number is often observed, a phenomenon called reactive microgliosis, which has become a hallmark of many CNS pathologies. However, it remained to be elucidated whether such increases in number rely on local expansion of mature microglia or are achieved by recruitment of blood precursors such as monocytes.

Two recent studies clarified the relative contribution of blood monocytes to microglia in experimental models of CNS pathologies. Both revealed that the irradiation regimen used to prepare recipient animals for bone marrow transplants is necessary for the recruitment and differentiation of monocytes into microglia (Ajami et al., 2007; Mildner et al., 2007). Mildner showed that recipient mice in which the CNS was shielded to protect from the irradiation and its associated inflammation, which induces the release of pro-inflammatory cytokines and chemokines, did not experience a significant invasion of bone marrow-derived cells into the brain, in contrast to the unshielded mice (Mildner et al., 2007). Beyond the irradiation issue, these data also suggest that microglial engraftment from the blood requires pre-conditioning of the CNS that likely disrupts the BBB. Additional clarity came from experiments in parabiotic mice, which have undergone surgery to physically link their circulatory systems, providing a more physiological means to study the turnover of hematopoietic cells for prolonged periods without the need for irradiation (Ajami et al., 2007). Ajami used such mice to show that in contrast to what was observed in irradiated and transplanted mice, there was no microglial progenitor recruitment from the circulation in either denervation or CNS neurodegenerative disease, despite the fact that the mixing of leucocyte populations can reach up to 50% in the blood of both parabionts. In agreement with their findings, we found no contribution of bone marrow-derived cells to CNS microglia up to 12 months after parabiosis (Ginhoux et al., 2010). Such data suggest that maintenance and local expansion of microglia are solely dependent on the self-renewal of CNS-resident cells in these models.

Interestingly, with this parabiotic model, in the context of irradiation of one parabiont, no further contribution from the other parabiont was detected in contradiction with the results of Mildner. However, Ajami further clarified that although irradiation is required for donor cells to engraft, it is not sufficient; another important, but overlooked, requirement is the artificial introduction of a critical number of bone marrow cells into the blood circulation (where they are not normally found) in conjunction with the inflammation of BBB caused by irradiation, a situation found only upon lethal total bone marrow transplantation (Diserbo et al., 2002; Li et al., 2004; Linard et al., 2004). More recently, the same group used a similar approach combining parabiosis and myelo-ablation to show that recruited monocytes do not persist and therefore do not contribute to the resident microglial pool. However, recruited monocytes contribute to the severity of disease in multiple sclerosis and the experimental autoimmune encephalitis mouse model (Ajami et al., 2011). Similarly, in transgenic mouse models of Alzheimer's disease,

irradiation was shown to condition the brain for engraftment of myeloid cells, a phenomenon that does not occur normally during disease progression (Mildner et al., 2011). Interestingly, in this study, perivascular macrophages, rather than microglia and monocytes, modulated β-amyloid deposition in the brains of AD transgenic mice by clearing Aβ in a CCR2-dependent fashion (Mildner et al., 2011). This highlights the distinct and non-redundant roles of microglia, monocyte, and perivascular macrophages in acute injury and autoimmune inflammation (Jung and Schwartz, 2012). Finally, Capotondo recently clarified that the conditioning regimen also contributes to the ablation of endogenous microglia, thereby allowing the local proliferation of invading blood cells (Capotondo et al., 2012). In conclusion, parabiotic mice provided, for the first time, unequivocal evidence that the microglial population during the steady state is able to maintain itself throughout adult life by local renewal, independent of circulating precursors in steady state. Conversely, in transplant models, which are perhaps not so much reflections of normal physiology, a fraction of microglia can arise from adult bone marrow.

As discussed before, we know that adult bone marrow cells can also enter into the CNS and differentiate into microglia in exceptional circumstances: when the endogenous microglial niche is completely vacant, such as in the PU.1 KO (Beers et al., 2006), or experimentally depleted, for example using Gancyclovir in a mouse model expressing the thymidine kinase under the CD11b promoter (Varvel et al., 2012). Importantly, microglial repopulation in the latter study did not require any conditioning regimen such as irradiation, as the microglial pool reconstituted itself after cessation of the Gancyclovir treatment. However, the effect of Gancyclovir on the permeability of the BBB was not evaluated in this model and we do not know if the bone marrow progenitors require additional "help" in order to cross the BBB, perhaps through the presence of as yet undefined inflammatory mediators. In addition, in their experimental setting, the authors could not formally track the origin of the cells that repopulate the microglia and therefore were unable to exclude local repopulation from non-depleted microglial cells. Nevertheless, the fact that adult bone marrow cells can give rise to microglia in the context of hematopoietic cell transplantation with a conditioning regimen open the door for invaluable therapeutic strategies for the correction of CNS conditions in which defects of microglia are implicated. For example, bone marrow transplantation of mouse models for metachromatic leukodystrophy (Biffi et al., 2004), the obsessive compulsive disorder trichotillomania (Chen et al., 2010), and Rett syndrome (Derecki et al., 2012) has been shown to ameliorate disease symptoms.

In conclusion, in these transplant models, which are perhaps not so much reflections of normal physiology, a fraction of microglia are of bone marrow origin. However, during the steady state, monocytes or other bone marrow-derived cells do not enter the CNS and do not significantly contribute to the microglial population.

EVIDENCE FOR THE PERSISTENCE OF THE EMBRYONIC WAVE OF MICROGLIA

What remained unclear in the field, however, was the relative contribution of embryonic and post-natal hematopoietic progenitors to the steady-state microglial population in adults: are the embryonic microglia responsible for maintaining the adult pool or do embryonic and adult microglia in fact have different origins? One of the studies already discussed, from De Groot, had implied that embryonic microglia were the sole contributors to the adult microglial pool. This study observed that donor bone marrow cells failed to contribute to the adult microglial population in a model of newborn transplantation, and concluded therefore that the adult microglial population was totally independent of post-natal bone marrow-derived circulating precursors from birth onward (De Groot et al., 1992). Recently, we revisited their experiments with a more quantitative aim and found that while most circulating leucocytes were of donor origin, the majority of microglia remained of host origin for more than 3 months after transplantation, confirming that post-natal hematopoietic precursors, including monocytes, likely do not contribute to the adult microglial population (Ginhoux et al., 2010).

We also employed a more advanced technique of YS progenitor fate mapping to definitively answer this question. Our fate mapping mouse model expresses a fluorescent protein (eYFP) exclusively in YS progenitors and their progeny, which include YS macrophages. Briefly, this mouse model expresses a tamoxifen-activated *MER-Cre-MER* recombinase gene under the control of one of the endogenous promoters of the runt-related transcription factor 1 (Runx1) locus (Samokhvalov et al., 2007). When crossed with a Cre-reporter mouse strain, recombination can be induced in embryos by a single injection of 4-Hydroxytamoxifen (4′OHT) into pregnant females. Active recombination in these knock-in mice occurs in a short time frame that does not exceed 24 h post-injection and leads to irreversible expression of eYFP in Runx1^{+} cells and their progeny (Samokhvalov et al., 2007).

Although both YS and fetal liver hematopoietic progenitors express Runx1, YS progenitors are the only cells present at E7.5 and so injection of tamoxifen at E7.5 will therefore allow the specific and irreversible tagging of YS progenitors and their progeny but not of fetal liver-derived progeny. In contrast, injection of tamoxifen at later time points (from E8.5) will favor the tagging of AGM-derived hematopoietic progenitors and not the YS progenitors (North et al., 1999; Samokhvalov et al., 2007). We can use this model to accurately ask about the origins of different cell types; for example, in the case of microglia, if they are predominantly derived from YS tagged progenitors, they should express *eYFP* in the adult CNS when 4′OHT is injected at E7.5 and not at E8.5. In contrast, circulating leukocytes, including monocytes that are known to derive from AGM hematopoietic progenitors, will present the opposite profile, expressing eYFP when 4′OHT is injected at E8.5 instead of E7.5. In addition, if the microglial population does predominantly derive from YS progenitors without a significant contribution from fetal liver- or bone marrow-derived hematopoiesis, they should be tagged at a higher level than circulating leukocytes, which derive predominantly from mature hematopoiesis. To test this hypothesis, we injected the mice with tamoxifen at closely spaced time points of gestation and compared the number of *eYFP*-tagged microglia and circulating monocytes in the mice as adults. Strikingly, the relative number of tagged microglia in mice injected at E7.25 was much greater than of

blood monocytes or other circulating leukocytes (Ginhoux et al., 2010).

In contrast, the relative number of tagged microglia in mice injected from E8.0 onwards decreased dramatically to reach undetectable levels as soon as E8.5, while the relative number of *eYFP*$^+$ leukocytes, including monocytes, increased progressively in adult blood. This opposing pattern of recombination in microglia compared to circulating leukocytes strongly supports the idea that the major contribution to microglial numbers comes from YS progenitors, and formally excludes the contribution of definitive hematopoiesis. Altogether these results establish that microglia originate from E7.25 Runx1 YS-derived hematopoietic progenitors, with little, if any, contribution from hematopoietic progenitors arising later in embryonic development. Recent studies confirmed our findings (Schulz et al., 2012; Kierdorf et al., 2013). In particular, the latest study from Kierdorf refined the characterization of the YS precursors that give rise to microglia and identified them as early E8 primitive c-kit$^+$ erythromyeloid YS precursors which develop into CD45$^+$c-kitloCX3CR1$^-$ cells before their maturation and migration into the developing brain as CD45$^+$c-kit-CX3CR1$^+$ cells (Kierdorf et al., 2013).

Altogether, these studies conclusively demonstrated that primitive macrophages are the embryonic source of the steady-state adult microglial population, which was particularly interesting as it implied that microglia not only have a unique functional specialization within the CNS, but also a unique origin, arising from YS progenitors that maintain themselves by proliferating *in situ* throughout adulthood (**Figure 1**). Beyond the case of microglia, it also provided startling evidence for a broader conclusion, that primitive macrophages are the ultimate source of a functional immune compartment that persists throughout adulthood. However, the case of the microglia seems to be unique, as other fetal macrophage populations in the embryo will mostly be replaced by fetal liver-derived monocytes that seed the tissues later and differentiate into macrophages, as we have recently shown in the case of Langerhans cells (Hoeffel et al., 2012). Lack of differentiation of fetal liver-derived monocytes into microglial progenitors could result from their lack of intrinsic differentiation potential or lack of access to the developing brain. Corroborating the latter hypothesis, the BBB in rodents is starting to be established at approximately E13.5, at the time of fetal liver monocyte release into the blood circulation (Daneman et al., 2010), but after YS-derived macrophages start to invade the neuro-epithelium from E9.5 (Ginhoux and Merad, 2010), possibly restricting the access of fetal liver-derived cells to the embryonic brain (**Figure 1**).

MOVING TOWARD HARNESSING MICROGLIA TO IMPROVE HUMAN HEALTH

It is well established that microglia are intimately involved in the pathology of neurological disease. However, efforts to elucidate the specific roles of microglia, their activation phenotypes and how they can be harnessed to ameliorate disease, are hampered by the lack of access to sufficient numbers of cells for comprehensive *in vitro* studies. Isolation of primary rodent microglia is generally achieved either by cell sorting or stepwise cell culture, both of which are time-consuming and generally yield few cells. While of limited use in rodents, this approach is entirely unfeasible for obtaining human microglia.

Although there has been some success producing microglia-like cells from bone marrow stem cells and circulating monocytes, they are perhaps poor models of "true" microglia as these cell populations do not share the embryonic origin of the vast majority of microglia in the homeostatic brain. Furthermore, their relatively advanced states of differentiation also make them unsuitable for asking questions about the intrinsic and extrinsic regulators of microglial development and specification. In contrast, pluripotent stem cells are already widely used in investigations of embryonic development, have undergone directed differentiation into astrocytes and neuronal subtypes of the CNS, and even been used for cellular transplantation therapies for neurodegenerative diseases (Park et al., 2008a; Kiskinis and Eggan, 2010; Wu and Hochedlinger, 2011; Ben-David et al., 2012). The exciting discovery that terminally-differentiated somatic cells can be reprogrammed into an embryonic-like "induced pluripotent stem cell" (iPSC) has also opened up new possibilities for disease modeling and developing patient-specific therapies (Takahashi and Yamanaka, 2006; Okita et al., 2007; Takahashi et al., 2007; Wernig et al., 2007; Yu et al., 2007; Park et al., 2008b).

Both embryonic stem cells (ESCs) and iPSCs have virtually unlimited expansion potential, can be cultured under defined conditions to ensure reproducible and scalable differentiation protocols, and are amenable to genetic manipulation to create tools for deeper functional studies. Large-scale *in vitro* generation of microglia would also allow us to perform high-throughput genetic screens aiming to uncover key transcription factors responsible for specifying the microglial phenotype. Even more attractively, we could potentially differentiate iPSCs from human patients to re-create a "disease in a dish," forming a crucial bridge between animal models (which are often deficient) and pathological disease states in humans. Thus far, efforts to recapitulate neurological disease features *in vitro* from human iPSCs have mainly focused on the afflicted neurons (Dimos et al., 2008; Park et al., 2008b; Lee et al., 2009; Soldner et al., 2009; Marchetto et al., 2010). The development of more complex disease models incorporating multiple cell types, particularly microglia, remains a challenge.

In contrast to the numerous protocols for efficient generation of astrocytes and neurons from pluripotent stem cells (Lee et al., 2000; Zhang et al., 2001; Hu et al., 2010), there is little literature reporting methods for obtaining phenotypically-correct microglia from the same cell sources. An early study on the differentiation of mouse ESCs (mESCs) into CNS cells in retinoic acid-induced embryoid bodies (EBs) showed that incidental cells expressing microglial markers were generated, in addition to neurons and astrocytes (Angelov et al., 1998). This preliminary success was the motivation for subsequent attempts to generate microglia from mESCs via neuronal differentiation strategies (Tsuchiya et al., 2005; Napoli et al., 2009). In brief, mESCs were induced to differentiate as EBs following withdrawal of leukemia inhibitory factor (LIF),

and then progenitors were expanded and further differentiated with neuronal-supportive media and cytokine stimulation. After 21–50 days $CD45^{low}/CD11b^{+}$ putative microglia-like cells were observed in these cultures; they expressed surface markers consistent with primary microglia, responded to classical immune activators such as lipopolysaccharide and interferon-γ, and appeared to survive implantation into the mouse brain. However, the low yields and prolonged culture period required, together with our current understanding of the ontogeny of microglia, suggest that these microglia-like cells perhaps are arising as a side population in the neuro-ectodermal differentiation process. On the other hand, we should not discount the possibility that neural cells present in these cultures might have provided a signaling milieu supportive of genuine microglial maturation. For example, brain- and bone marrow-derived $Mac\text{-}1^{+}$ progenitors co-cultured on a supportive layer of astroglial cells proliferated and matured into microglial-like cells (Alliot et al., 1991). Sievers and colleagues also showed that co-culture with astrocytes induced blood monocytes and spleen macrophages to adopt a ramified morphology akin to microglia (Sievers et al., 1994a,b). In light of our current understanding that adult microglia originate as primitive macrophages from the embryonic YS, a more effective strategy for approaching pluripotent stem cell differentiation may be to attempt to recapitulate YS hematopoiesis *in vitro* (**Figure 2**). In the mouse, hemangioblast precursors migrate from the posterior primitive streak into the YS proper, where they form the blood islands and surrounding endothelial cells (Huber et al., 2004). YS hematopoiesis yields primitive erythroblasts as early as E7.0, followed by definitive erythroblasts and macrophage progenitors between E8.5–9.0 (Palis et al., 1999). However, it has been known for some time that these developmental stages can be closely mirrored in mESC differentiation *in vitro*, specifically in terms of the kinetics of hematopoietic gene expression, as well as the order in which hematopoietic progenitors appear. In two modalities of differentiation, either in a co-culture with a hematopoietic-supportive stromal cell layer such as OP9 cells, or as EBs, mESCs sequentially generate *in vitro* equivalents of the primitive streak, hemangioblast, and YS hematopoietic progenitors (Risau et al., 1988; Wiles and Keller, 1991; Nakano et al., 1996; Ogawa et al., 1999; Kennedy and Keller, 2003; Hirai et al., 2005). These processes in mESCs are controlled by the same molecular regulators as those operative during early hematopoiesis *in vivo*. For example, mouse embryos with targeted gene disruption of the hematopoietic master regulator SCL/Tal-1 have no YS hematopoiesis and fail to develop beyond E9.5 (Robb et al., 1995; Shivdasani et al., 1995); likewise abrogation of SCL expression in both mouse and human ESCs

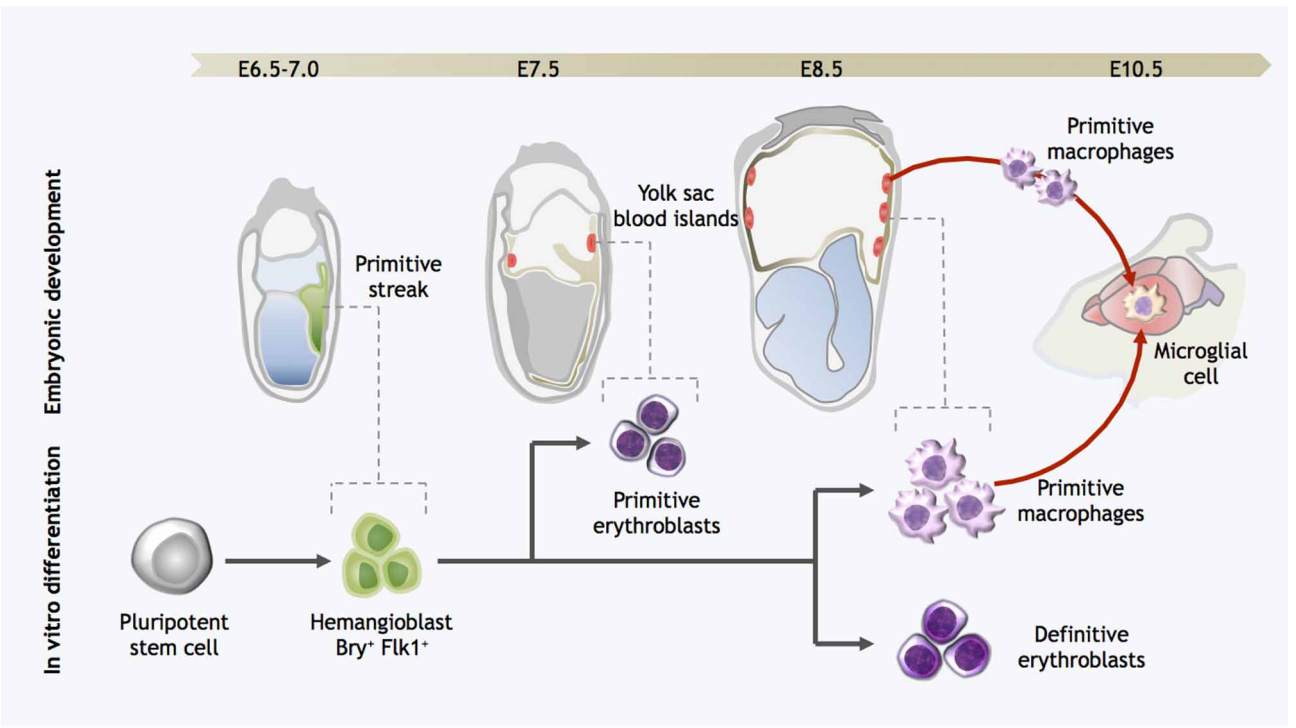


FIGURE 2 | Strategy for directed differentiation of microglial precursors from pluripotent stem cells. Microglial differentiation *in vitro* can be achieved by recapitulating the steps of yolk sac hematopoiesis. Hemangioblast cells arise in the posterior primitive streak and migrate into the yolk sac, giving rise to the blood islands. Primitive erythroblasts are observed from E7.0–7.5, followed by definitive erythroblasts and primitive macrophages at E8.5. At the onset of circulation, primitive macrophages exit the yolk sac and seed the developing brain, forming microglia. Likewise, pluripotent stem cells can be differentiated into $Bry^{+}Flk^{+}$ cells with hemangioblast properties; these cells are then further differentiated into both primitive and definitive eryothroblasts and primitive macrophages. *In vitro*-derived primitive macrophages may be the functional equivalent to primitive microglia in the embryo.

completely represses early hematopoietic specification (D'souza et al., 2005; Real et al., 2012). Similarly, PU.1 KO ESCs could not be differentiated into macrophages (Henkel et al., 1996; Anderson et al., 1998), consistent with the finding that PU.1 KO embryos lack both macrophages and microglia (McKercher et al., 1996).

However, useful the mouse developmental model may be, the paucity of equivalent human *in vivo* models makes it imperative to develop accurate *in vitro* alternatives using human pluripotent stem cells. Classical lineage tracing studies of blood development in human embryos showed that many essential features of YS hematopoiesis are conserved from mice to humans (Migliaccio et al., 1986; Huyhn et al., 1995; Palis and Yoder, 2001). As was the case in the mouse system, human pluripotent stem cells could also be differentiated into cells representative of the early hematopoietic developmental stages, albeit with extended kinetics (Wang et al., 2004; Zambidis et al., 2005; Kennedy et al., 2007), suggesting that hematopoietic cell emergence in the YS might be closely modeled by human ESC differentiation (Zambidis et al., 2005).

Given their direct ontogenetic relationship, we postulate that techniques for directing primitive macrophage fate specification from pluripotent stem cells will also yield microglial precursors. Our proposed approach is to further refine protocols for recapitulating YS hematopoiesis, with the aim of increasing the yield of primitive macrophages and levels of reproducibility. Serum-based protocols introduce intrinsic variability, so we will turn to a defined serum-free and feeder cell-free procedure, using only specific combinations of cytokines to replicate the developmental signals during embryogenesis. The key challenge in this approach will be to screen appropriate combinations of factors as well as the time window for treatment. Candidate primitive macrophage/microglial cells can then be isolated based on surface marker expression, and assayed for microglial-appropriate phenotypes such as response to immune stimulation, morphological analysis and phagocytic ability (Giulian and Baker, 1986; Sedgwick et al., 1991). Eventually, the ability to engraft within the brain, with classic resting microglial morphology will be the true test of successful differentiation.

CONCLUSION

The "origin of murine microglia controversy" is now resolved in steady state conditions and in a few mouse models of CNS pathologies. We have learned that microglia arise from YS macrophages that seed the brain rudiment from the cephalic mesenchyme very early during development, as predicted earlier by the founder of the microglia field, Pio del Rio-Hortega. Importantly, embryonically-derived microglia will maintain themselves until adulthood. While much progress has been made in terms of our understanding of both the origin and importance of microglia, many questions remain unanswered.

As discussed before, this knowledge may have implications for the use of embryonically-derived microglial progenitors in the treatment of brain inflammatory diseases. Moreover, these results have fundamental implications for the understanding of microglial function in CNS development. First, the conservation of primitive macrophages and their YS derivation throughout evolution and across diverse species suggests that microglia play an important physiological role in the development of the CNS. Furthermore, microglial cells are present in all stages of brain development, including the early prenatal stages of neuronal circuit building as well as the post-natal stage of synapse elimination.

An earlier report had highlighted that, in contrast to their broad distribution in the adult brain, embryonic microglia have a strikingly uneven distribution during embryogenesis (Perry et al., 1985). Microglial cells accumulate in hot spots that were initially proposed to be most likely related to the clearance of apoptotic bodies and the remodeling of brain tissues. In light of the recent work which has shown that microglia contribute to the control of synaptic connections, it will be interesting to verify whether such hotspots co-localize with areas crucial for neuronal development. This will indicate that microglia play an important role in development of neuronal circuits of the brain and proposes more questions to be answered regarding the integrated development of the neural and immune systems. Such questions also have tremendous implications beyond the simple biology of microglia. Do defects affecting microglial development have a long-term impact on the functional vulnerability of CNS? And do defects in microglial function perhaps contribute to synaptic abnormalities seen in some neurodevelopmental disorders? In support of such hypotheses, prenatal inflammation, which triggers the activation of microglia, is thought to be a risk factor for the development of neuropsychiatric disorders such as schizophrenia and autism spectrum disorders in the unborn child (Patterson, 2009, 2011).

Importantly, embryonic microglia will maintain themselves until adulthood via local proliferation during late gestation and post-natal development as well as in the injured adult brain in reaction to inflammation. They are unlikely to be replaced by blood-derived monocytes or any bone marrow-derived cells. Nevertheless, during certain inflammatory conditions found for example after bone marrow transplantation or in chronic neurodegenerative diseases such as Multiple Sclerosis and Alzheimer's disease (Simard and Rivest, 2006; Jung and Schwartz, 2012), the recruitment of monocytes or other bone marrow-derived progenitors can supplement the microglial population to some extent, but we do not understand whether these cells persist and become integrated, or are a temporary addition to the endogenous population. The interactive dynamic between embryonic and adult microglial populations requires further study: We need now to understand to what extent the endogenous microglia is replaced, where and how it is done, and if engrafted cells have a selective advantage over the endogenous microglia, how well they "compete" against the endogenous embryonic microglial population and how long they will persist. Finally, we do not know yet precisely if these bone marrow-derived microglia can fulfill the functional roles of the endogenous population.

ACKNOWLEDGMENTS

This work was supported by the Singapore Immunology Network core grant. We thank Dr. L. Robinson for critical review and editing of the manuscript.

REFERENCES

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M., and Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* 14, 1142–1149.

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

Akiyama, H., and McGeer, P. L. (1990). Brain microglia constitutively express beta-2 integrins. *J. Neuroimmunol.* 30, 81–93.

Alliot, F., Godin, I., and Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* 117, 145–152.

Alliot, F., Lecain, E., Grima, B., and Pessac, B. (1991). Microglial progenitors with a high proliferative potential in the embryonic and adult mouse brain. *Proc. Natl. Acad. Sci. U.S.A.* 88, 1541–1545.

Anderson, K. L., Smith, K. A., Conners, K., McKercher, S. R., Maki, R. A., and Torbett, B. E. (1998). Myeloid development is selectively disrupted in PU.1 null mice. *Blood* 91, 3702–3710.

Angelov, D. N., Arnhold, S., Andressen, C., Grabsch, H., Puschmann, M., Hescheler, J., et al. (1998). Temporospatial relationships between macroglia and microglia during *in vitro* differentiation of murine stem cells. *Dev. Neurosci.* 20, 42–51.

Ashwell, K. (1990). Microglia and cell death in the developing mouse cerebellum. *Brain Res. Dev. Brain Res.* 55, 219–230.

Ashwell, K. W., and Waite, P. M. (1991). Cell death in the developing trigeminal nuclear complex of the rat. *Brain Res. Dev. Brain Res.* 63, 291–295.

Baron, M., and Gallego, A. (1972). The relation of the microglia with the pericytes in the cat cerebral cortex. *Z. Zellforsch. Mikrosk. Anat.* 128, 42–57.

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., et al. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16021–16026.

Ben-David, U., Kopper, O., and Benvenisty, N. (2012). Expanding the boundaries of embryonic stem cells. *Cell Stem Cell* 10, 666–677.

Bertrand, J. Y., Jalil, A., Klaine, M., Jung, S., Cumano, A., and Godin, I. (2005). Three pathways to mature macrophages in the early mouse yolk sac. *Blood* 106, 3004–3011.

Biffi, A., De Palma, M., Quattrini, A., Del Carro, U., Amadio, S., Visigalli, I., et al. (2004). Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. *J. Clin. Invest.* 113, 1118–1129.

Capotondo, A., Milazzo, R., Politi, L. S., Quattrini, A., Palini, A., Plati, T., et al. (2012). Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation. *Proc. Natl. Acad. Sci. U.S.A.* 109, 15018–15023.

Chan, W. Y., Kohsaka, S., and Rezaie, P. (2007). The origin and cell lineage of microglia: new concepts. *Brain Res. Rev.* 53, 344–354.

Chen, S. K., Tvrdik, P., Peden, E., Cho, S., Wu, S., Spangrude, G., et al. (2010). Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* 141, 775–785.

Cuadros, M. A., and Navascues, J. (1998). The origin and differentiation of microglial cells during development. *Prog. Neurobiol.* 56, 173–189.

D'souza, S. L., Elefanty, A. G., and Keller, G. (2005). SCL/Tal-1 is essential for hematopoietic commitment of the hemangioblast but not for its development. *Blood* 105, 3862–3870.

Daneman, R. (2012). The blood-brain barrier in health and disease. *Ann. Neurol.* 72, 648–672.

Daneman, R., Zhou, L., Kebede, A. A., and Barres, B. A. (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 468, 562–566.

De Groot, C. J., Huppes, W., Sminia, T., Kraal, G., and Dijkstra, C. D. (1992). Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive in situ hybridization and immunoperoxidase techniques. *Glia* 6, 301–309.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

Dimos, J. T., Rodolfa, K. T., Niakan, K. K., Weisenthal, L. M., Mitsumoto, H., Chung, W., et al. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321, 1218–1221.

Diserbo, M., Agin, A., Lamproglou, I., Mauris, J., Staali, F., Multon, E., et al. (2002). Blood-brain barrier permeability after gamma whole-body irradiation: an *in vivo* microdialysis study. *Can. J. Physiol. Pharmacol.* 80, 670–678.

Eglitis, M. A., and Mezey, E. (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 4080–4085.

Esiri, M. M., Al Izzi, M. S., and Reading, M. C. (1991). Macrophages, microglial cells, and HLA-DR antigens in fetal and infant brain. *J. Clin. Pathol.* 44, 102–106.

Fedoroff, S., Zhai, R., and Novak, J. P. (1997). Microglia and astroglia have a common progenitor cell. *J. Neurosci. Res.* 50, 477–486.

Fujita, S., and Kitamura, T. (1975). Origin of brain macrophages and the nature of the so-called microglia. *Acta Neuropathol. Suppl.* 6, 291–296.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Ginhoux, F., and Merad, M. (2010). Ontogeny and homeostasis of Langerhans cells. *Immunol. Cell Biol.* 88, 387–392.

Giulian, D., and Baker, T. J. (1986). Characterization of ameboid microglia isolated from developing mammalian brain. *J. Neurosci.* 6, 2163–2178.

Godin, I. E., Garcia-Porrero, J. A., Coutinho, A., Dieterlen-Lievre, F., and Marcos, M. A. (1993). Para-aortic splanchnopleura from early mouse embryos contains B1a cell progenitors. *Nature* 364, 67–70.

Graeber, M. B. (2010). Changing face of microglia. *Science* 330, 783–788.

Hao, C., Richardson, A., and Fedoroff, S. (1991). Macrophage-like cells originate from neuroepithelium in culture: characterization and properties of the macrophage-like cells. *Int. J. Dev. Neurosci.* 9, 1–14.

Henkel, G. W., McKercher, S. R., Yamamoto, H., Anderson, K. L., Oshima, R. G., and Maki, R. A. (1996). PU.1 but not ets-2 is essential for macrophage development from embryonic stem cells. *Blood* 88, 2917–2926.

Herbomel, P., Thisse, B., and Thisse, C. (1999). Ontogeny and behaviour of early macrophages in the zebrafish embryo. *Development* 126, 3735–3745.

Herbomel, P., Thisse, B., and Thisse, C. (2001). Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. *Dev. Biol.* 238, 274–288.

Hickey, W. F., and Kimura, H. (1988). Perivascular microglial cells of the CNS are bone marrow-derived and present antigen *in vivo*. *Science* 239, 290–292.

Hirai, H., Samokhvalov, I. M., Fujimoto, T., Nishikawa, S., and Imanishi, J. (2005). Involvement of Runx1 in the down-regulation of fetal liver kinase-1 expression during transition of endothelial cells to hematopoietic cells. *Blood* 106, 1948–1955.

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., et al. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* 209, 1167–1181.

Hu, B. Y., Weick, J. P., Yu, J., Ma, L. X., Zhang, X. Q., Thomson, J. A., et al. (2010). Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4335–4340.

Huber, T. L., Kouskoff, V., Fehling, H. J., Palis, J., and Keller, G. (2004). Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature* 432, 625–630.

Hughes, V. (2012). Microglia: the constant gardeners. *Nature* 485, 570–572.

Hume, D. A., Perry, V. H., and Gordon, S. (1983). Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *J. Cell Biol.* 97, 253–257.

Hutchins, K. D., Dickson, D. W., Rashbaum, W. K., and Lyman, W. D. (1990). Localization of morphologically distinct microglial populations in the developing human fetal brain: implications for ontogeny. *Brain Res. Dev. Brain Res.* 55, 95–102.

Huyhn, A., Dommergues, M., Izac, B., Croisille, L., Katz, A., Vainchenker, W., et al. (1995). Characterization of hematopoietic progenitors from human yolk sacs and embryos. *Blood* 86, 4474–4485.

Imamoto, K., and Leblond, C. P. (1978). Radioautographic investigation of gliogenesis in the corpus callosum of young rats. II. Origin of microglial cells. *J. Comp. Neurol.* 180, 139–163.

Janossy, G., Bofill, M., Poulter, L. W., Rawlings, E., Burford, G. D., Navarrete, C., et al. (1986). Separate ontogeny of two macrophage-like accessory cell populations in the human fetus. *J. Immunol.* 136, 4354–4361.

Jung, S., and Schwartz, M. (2012). Non-identical twins - microglia and monocyte-derived macrophages in acute injury and autoimmune inflammation. *Front. Immunol.* 3:89. doi: 10.3389/fimmu.2012.00089

Kennedy, M., D'souza, S. L., Lynch-Kattman, M., Schwantz, S., and Keller, G. (2007). Development of the hemangioblast defines the onset of hematopoiesis in human ES cell differentiation cultures. *Blood* 109, 2679–2687.

Kennedy, M., and Keller, G. M. (2003). Hematopoietic commitment of ES cells in culture. *Meth. Enzymol.* 365, 39–59.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* 16, 273–280.

Kingwell, K. (2012). Neurodegenerative disease: microglia in early disease stages. *Nat. Rev. Neurol.* 8, 475.

Kiskinis, E., and Eggan, K. (2010). Progress toward the clinical application of patient-specific pluripotent stem cells. *J. Clin. Invest.* 120, 51–59.

Kitamura, T., Miyake, T., and Fujita, S. (1984). Genesis of resting microglia in the gray matter of mouse hippocampus. *J. Comp. Neurol.* 226, 421–433.

Koushik, S. V., Wang, J., Rogers, R., Moskophidis, D., Lambert, N. A., Creazzo, T. L., et al. (2001). Targeted inactivation of the sodium-calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization. *FASEB J.* 15, 1209–1211.

Kumaravelu, P., Hook, L., Morrison, A. M., Ure, J., Zhao, S., Zuyev, S., et al. (2002). Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. *Development* 129, 4891–4899.

Kurz, H., Korn, J., Eggli, P. S., Huang, R., and Christ, B. (2001). Embryonic central nervous system angiogenesis does not involve blood-borne endothelial progenitors. *J. Comp. Neurol.* 436, 263–274.

Ladeby, R., Wirenfeldt, M., Garcia-Ovejero, D., Fenger, C., Dissing-Olesen, L., Dalmau, I., et al. (2005). Microglial cell population dynamics in the injured adult central nervous system. *Brain Res. Brain Res. Rev.* 48, 196–206.

Lassmann, H., Schmied, M., Vass, K., and Hickey, W. F. (1993). Bone marrow derived elements and resident microglia in brain inflammation. *Glia* 7, 19–24.

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170.

Lawson, L. J., Perry, V. H., and Gordon, S. (1992). Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* 48, 405–415.

Lee, G., Papapetrou, E. P., Kim, H., Chambers, S. M., Tomishima, M. J., Fasano, C. A., et al. (2009). Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 461, 402–406.

Lee, S. H., Lumelsky, N., Studer, L., Auerbach, J. M., and McKay, R. D. (2000). Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat. Biotechnol.* 18, 675–679.

Leong, S. K., and Ling, E. A. (1992). Amoeboid and ramified microglia: their interrelationship and response to brain injury. *Glia* 6, 39–47.

Lewis, P. D. (1968). The fate of the subependymal cell in the adult rat brain, with a note on the origin of microglia. *Brain* 91, 721–736.

Li, Y. Q., Chen, P., Jain, V., Reilly, R. M., and Wong, C. S. (2004). Early radiation-induced endothelial cell loss and blood-spinal cord barrier breakdown in the rat spinal cord. *Radiat. Res.* 161, 143–152.

Lichanska, A. M., and Hume, D. A. (2000). Origins and functions of phagocytes in the embryo. *Exp. Hematol.* 28, 601–611.

Linard, C., Marquette, C., Mathieu, J., Pennequin, A., Clarencon, D., and Mathe, D. (2004). Acute induction of inflammatory cytokine expression after gamma-irradiation in the rat: effect of an NF-kappaB inhibitor. *Int. J. Radiat. Oncol. Biol. Phys.* 58, 427–434.

Ling, E. A. (1976). Some aspects of amoeboid microglia in the corpus callosum and neighbouring regions of neonatal rats. *J. Anat.* 121, 29–45.

Ling, E. A. (1979). Transformation of monocytes into amoeboid microglia in the corpus callosum of postnatal rats, as shown by labelling monocytes by carbon particles. *J. Anat.* 128, 847–858.

Ling, E. A., Penney, D., and Leblond, C. P. (1980). Use of carbon labeling to demonstrate the role of blood monocytes as precursors of the 'ameboid cells' present in the corpus callosum of postnatal rats. *J. Comp. Neurol.* 193, 631–657.

Marchetto, M. C., Carromeu, C., Acab, A., Yu, D., Yeo, G. W., Mu, Y., et al. (2010). A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* 143, 527–539.

Matsumoto, Y., and Fujiwara, M. (1987). Absence of donor-type major histocompatibility complex class I antigen-bearing microglia in the rat central nervous system of radiation bone marrow chimeras. *J. Neuroimmunol.* 17, 71–82.

McGrath, K. E., Koniski, A. D., Malik, J., and Palis, J. (2003). Circulation is established in a stepwise pattern in the mammalian embryo. *Blood* 101, 1669–1676.

McKercher, S. R., Torbett, B. E., Anderson, K. L., Henkel, G. W., Vestal, D. J., Baribault, H., et al. (1996). Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J.* 15, 5647–5658.

Medvinsky, A. L., Samoylina, N. L., Muller, A. M., and Dzierzak, E. A. (1993). An early pre-liver intraembryonic source of CFU-S in the developing mouse. *Nature* 364, 64–67.

Mezey, E., Chandross, K. J., Harta, G., Maki, R. A., and McKercher, S. R. (2000). Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow. *Science* 290, 1779–1782.

Migliaccio, G., Migliaccio, A. R., Petti, S., Mavilio, F., Russo, G., Lazzaro, D., et al. (1986). Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac—liver transition. *J. Clin. Invest.* 78, 51–60.

Mildner, A., Schlevogt, B., Kierdorf, K., Bottcher, C., Erny, D., Kummer, M. P., et al. (2011). Distinct and non-redundant roles of microglia and myeloid subsets in mouse models of Alzheimer's disease. *J. Neurosci.* 31, 11159–11171.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., et al. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* 10, 1544–1553.

Mizutani, M., Pino, P. A., Saederup, N., Charo, I. F., Ransohoff, R. M., and Cardona, A. E. (2012). The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. *J. Immunol.* 188, 29–36.

Monier, A., Adle-Biassette, H., Delezoide, A. L., Evrard, P., Gressens, P., and Verney, C. (2007). Entry and distribution of microglial cells in human embryonic and fetal cerebral cortex. *J. Neuropathol. Exp. Neurol.* 66, 372–382.

Moore, M. A., and Metcalf, D. (1970). Ontogeny of the haemopoietic system: yolk sac origin of *in vivo* and *in vitro* colony forming cells in the developing mouse embryo. *Br. J. Haematol.* 18, 279–296.

Mori, S., and Leblond, C. P. (1969). Identification of microglia in light and electron microscopy. *J. Comp. Neurol.* 135, 57–80.

Morris, L., Graham, C. F., and Gordon, S. (1991). Macrophages in haemopoietic and other tissues of the developing mouse detected by the monoclonal antibody F4/80. *Development* 112, 517–526.

Murabe, Y., and Sano, Y. (1982). Morphological studies on neuroglia. VI. Postnatal development of microglial cells. *Cell Tissue Res.* 225, 469–485.

Murabe, Y., and Sano, Y. (1983). Morphological studies on neuroglia. VII. Distribution of "brain macrophages" in brains of neonatal and adult rats, as determined by means of immunohistochemistry. *Cell Tissue Res.* 229, 85–95.

Naito, M., Takahashi, K., and Nishikawa, S. (1990). Development, differentiation, and maturation of macrophages in the fetal mouse liver. *J. Leukoc. Biol.* 48, 27–37.

Naito, M., Umeda, S., Yamamoto, T., Moriyama, H., Umezu, H., Hasegawa, G., et al. (1996). Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. *J. Leukoc. Biol.* 59, 133–138.

Nakano, T., Kodama, H., and Honjo, T. (1996). *In vitro* development of primitive and definitive

erythrocytes from different precursors. *Science* 272, 722–724.
Napoli, I., Kierdorf, K., and Neumann, H. (2009). Microglial precursors derived from mouse embryonic stem cells. *Glia* 57, 1660–1671.
North, T., Gu, T. L., Stacy, T., Wang, Q., Howard, L., Binder, M., et al. (1999). Cbfa2 is required for the formation of intra-aortic hematopoietic clusters. *Development* 126, 2563–2575.
Oehmichen, M., Wietholter, H., Gruninger, H., and Gencic, M. (1979). Features and distribution of intracerebrally injected peritoneal macrophages. *Exp. Pathol. (Jena)* 17, 71–76.
Ogawa, M., Kizumoto, M., Nishikawa, S., Fujimoto, T., Kodama, H., and Nishikawa, S. I. (1999). Expression of alpha4-integrin defines the earliest precursor of hematopoietic cell lineage diverged from endothelial cells. *Blood* 93, 1168–1177.
Okita, K., Ichisaka, T., and Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature* 448, 313–317.
Orkin, S. H., and Zon, L. I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* 132, 631–644.
Palis, J., Robertson, S., Kennedy, M., Wall, C., and Keller, G. (1999). Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development* 126, 5073–5084.
Palis, J., and Yoder, M. C. (2001). Yolk-sac hematopoiesis: the first blood cells of mouse and man. *Exp. Hematol.* 29, 927–936.
Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.
Park, I. H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., et al. (2008a). Disease-specific induced pluripotent stem cells. *Cell* 134, 877–886.
Park, I. H., Zhao, R., West, J. A., Yabuuchi, A., Huo, H., Ince, T. A., et al. (2008b). Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451, 141–146.
Patterson, P. H. (2009). Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behav. Brain Res.* 204, 313–321.
Patterson, P. H. (2011). Maternal infection and immune involvement in autism. *Trends Mol. Med.* 17, 389–394.
Perry, V. H. (1998). A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J. Neuroimmunol.* 90, 113–121.
Perry, V. H., Hume, D. A., and Gordon, S. (1985). Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience* 15, 313–326.
Perry, V. H., Nicoll, J. A., and Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat. Rev. Neurol.* 6, 193–201.
Priller, J., Flugel, A., Wehner, T., Boentert, M., Haas, C. A., Prinz, M., et al. (2001). Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nat. Med.* 7, 1356–1361.
Real, P. J., Ligero, G., Ayllon, V., Ramos-Mejia, V., Bueno, C., Gutierrez-Aranda, I., et al. (2012). SCL/TAL1 regulates hematopoietic specification from human embryonic stem cells. *Mol. Ther.* 20, 1443–1453.
Rezaie, P. (2003). Microglia in the human nervous system during development. *Neuroembryology* 2, 18–31.
Rezaie, P., Dean, A., Male, D., and Ulfig, N. (2005). Microglia in the cerebral wall of the human telencephalon at second trimester. *Cereb. Cortex* 15, 938–949.
Rezaie, P., and Male, D. (1999). Colonisation of the developing human brain and spinal cord by microglia: a review. *Microsc. Res. Tech.* 45, 359–382.
Rezaie, P., and Male, D. (2002). Mesoglia and microglia–a historical review of the concept of mononuclear phagocytes within the central nervous system. *J. Hist. Neurosci.* 11, 325–374.
Rio-Hortega, D. (1932). "Microglia," in *Cytology and Cellular Pathology of the Nervous System*, Vol. 2, ed W. Penfield, (New York, NY: P. B. Hoeber, Inc.), 482–534.
Rio-Hortega, D. (1939). The Microglia. *Lancet* 233, 1023–1026.
Risau, W., Sariola, H., Zerwes, H. G., Sasse, J., Ekblom, P., Kemler, R., et al. (1988). Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. *Development* 102, 471–478.
Robb, L., Lyons, I., Li, R., Hartley, L., Kontgen, F., Harvey, R. P., et al. (1995). Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7075–7079.
Samokhvalov, I. M., Samokhvalova, N. I., and Nishikawa, S. (2007). Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* 446, 1056–1061.
Schelper, R. L., and Adrian, E. K. Jr. (1986). Monocytes become macrophages; they do not become microglia: a light and electron microscopic autoradiographic study using 125-iododeoxyuridine. *J. Neuropathol. Exp. Neurol.* 45, 1–19.
Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336, 86–90.
Sedgwick, J. D., Schwender, S., Imrich, H., Dorries, R., Butcher, G. W., and Ter Meulen, V. (1991). Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* 88, 7438–7442.
Shivdasani, R. A., Mayer, E. L., and Orkin, S. H. (1995). Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature* 373, 432–434.
Sievers, J., Parwaresch, R., and Wottge, H. U. (1994a). Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: morphology. *Glia* 12, 245–258.
Sievers, J., Schmidtmayer, J., and Parwaresch, R. (1994b). Blood monocytes and spleen macrophages differentiate into microglia-like cells when cultured on astrocytes. *Ann. Anat.* 176, 45–51.
Simard, A. R., and Rivest, S. (2004). Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J.* 18, 998–1000.
Simard, A. R., and Rivest, S. (2006). Neuroprotective properties of the innate immune system and bone marrow stem cells in Alzheimer's disease. *Mol. Psychiatry* 11, 327–335.
Soldner, F., Hockemeyer, D., Beard, C., Gao, Q., Bell, G. W., Cook, E. G., et al. (2009). Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 136, 964–977.
Sorokin, S. P., Hoyt, R. F. Jr., Blunt, D. G., and McNelly, N. A. (1992). Macrophage development: II. Early ontogeny of macrophage populations in brain, liver, and lungs of rat embryos as revealed by a lectin marker. *Anat. Rec.* 232, 527–550.
Takahashi, K., and Naito, M. (1993). Development, differentiation, and proliferation of macrophages in the rat yolk sac. *Tissue Cell* 25, 351–362.
Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872.
Takahashi, K., Yamamura, F., and Naito, M. (1989). Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. *J. Leukoc. Biol.* 45, 87–96.
Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676.
Tambuyzer, B. R., Ponsaerts, P., and Nouwen, E. J. (2009). Microglia: gatekeepers of central nervous system immunology. *J. Leukoc. Biol.* 85, 352–370.
Tavian, M., and Peault, B. (2005). Embryonic development of the human hematopoietic system. *Int. J. Dev. Biol.* 49, 243–250.
Tremblay, M. E., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527
Tsuchiya, T., Park, K. C., Toyonaga, S., Yamada, S. M., Nakabayashi, H., Nakai, E., et al. (2005). Characterization of microglia induced from mouse embryonic stem cells and their migration into the brain parenchyma. *J. Neuroimmunol.* 160, 210–218.
Unger, E. R., Sung, J. H., Manivel, J. C., Chenggis, M. L., Blazar, B. R., and Krivit, W. (1993). Male donor-derived cells in the brains of female sex-mismatched bone marrow transplant recipients: a Y-chromosome specific in situ hybridization study. *J. Neuropathol. Exp. Neurol.* 52, 460–470.
Vallieres, L., and Sawchenko, P. E. (2003). Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. *J. Neurosci.* 23, 5197–5207.
Varvel, N. H., Grathwohl, S. A., Baumann, F., Liebig, C., Bosch, A., Brawek, B., et al. (2012). Microglial repopulation model reveals a robust homeostatic process for replacing

CNS myeloid cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 18150–18155.

Verney, C., Monier, A., Fallet-Bianco, C., and Gressens, P. (2010). Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. *J. Anat.* 217, 436–448.

Wang, C. C., Wu, C. H., Shieh, J. Y., Wen, C. Y., and Ling, E. A. (1996). Immunohistochemical study of amoeboid microglial cells in fetal rat brain. *J. Anat.* 189(Pt 3), 567–574.

Wang, L., Li, L., Shojaei, F., Levac, K., Cerdan, C., Menendez, P., et al. (2004). Endothelial and hematopoietic cell fate of human embryonic stem cells originates from primitive endothelium with hemangioblastic properties. *Immunity* 21, 31–41.

Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., et al. (2007). *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448, 318–324.

Wiles, M. V., and Keller, G. (1991). Multiple hematopoietic lineages develop from embryonic stem (ES) cells in culture. *Development* 111, 259–267.

Wood, G. W., Gollahon, K. A., Tilzer, S. A., Vats, T., and Morantz, R. A. (1979). The failure of microglia in normal brain to exhibit mononuclear phagocyte markers. *J. Neuropathol. Exp. Neurol.* 38, 369–376.

Wu, S. M., and Hochedlinger, K. (2011). Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nat. Cell Biol.* 13, 497–505.

Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917–1920.

Zambidis, E. T., Peault, B., Park, T. S., Bunz, F., and Civin, C. I. (2005). Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive, and definitive stages resembling human yolk sac development. *Blood* 106, 860–870.

Zhang, S. C., Wernig, M., Duncan, I. D., Brustle, O., and Thomson, J. A. (2001). *In vitro* differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol.* 19, 1129–1133.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2013; paper pending published: 24 February 2013; accepted: 30 March 2013; published online: 17 April 2013.

Citation: Ginhoux F, Lim S, Hoeffel G, Low D and Huber T (2013) Origin and differentiation of microglia. Front. Cell. Neurosci. 7:45. doi: 10.3389/fncel.2013.00045

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 18 March 2013
doi: 10.3389/fncel.2013.00026

Microglia, seen from the CX_3CR1 angle

Yochai Wolf, Simon Yona†, Ki-Wook Kim† and Steffen Jung*

Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

Edited by:
Amanda Sierra, University of the Basque Country EHU/UPV, Spain

Reviewed by:
Pascal Legendre, Universite Pierre et MArie Curie CNRS INSERM, France
Marie-Eve Tremblay, Université Laval, Canada

***Correspondence:**
Steffen Jung, Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.
e-mail: s.jung@weizmann.ac.il

† Present address:
Simon Yona, University College London, London, UK.
Ki-Wook Kim, Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06510, USA.

Microglial cells in brain and spinal cord are characterized by high expression of the chemokine receptor CX_3CR1. Expression of the sole CX_3CR1 ligand, the membrane-tethered and sheddable chemokine CX_3CL1/fractalkine, is restricted in the brain parenchyma to selected neurons. Here we summarize our current understanding of the physiological role of CX_3CR1 for microglia function and the CX_3C axis in microglial/neuronal crosstalk in homeostasis and under challenge. Moreover, we will discuss the efforts of our laboratory and others to exploit CX_3CR1 promoter activity for the visualization and genetic manipulation of microglia to probe their functional contributions in the central nerve system (CNS) context.

Keywords: microglia, neuropathology, Cre-loxP knock-in mice, CX_3CR1, neuroimmunology

INTRODUCTION

Microglia are members of the mononuclear phagocyte system alongside other macrophages, monocytes and dendritic cells (Geissmann et al., 2010). Sequestered behind the blood brain barrier (BBB) in the unique neuronal/macroglial context, microglia display a gene expression profile that significantly differs from other tissue macrophages (Gautier et al., 2012). Moreover, highlighting its independence the microglia compartment is established well before birth from a primitive hematopoietic wave and subsequently maintains itself throughout adulthood through longevity and limited self-renewal (Alliot et al., 1999; Ginhoux et al., 2010). Microglia share this prenatal establishment with other tissue macrophage populations; however the latter seem less secluded and more promiscuous with respect to the incorporation of monocytic cells derived from the fetal liver or during challenge (Hoeffel et al., 2012; Yona et al., 2013). In contrast, even after a prominent trauma-associated influx of monocytes into the injured central nerve system (CNS), these cells do not seem to permanently seed the brain. Rather, the steady state relying solely on microglia seems to be restored (Ajami et al., 2011). Steady state microglia are distributed throughout the CNS, including brain and spinal cord, although there is evidence for considerable region specific differences in density, phenotype and responsiveness (de Haas et al., 2008). As immune cells, microglia are sensors of injury and pathologic conditions (Hanisch and Kettenmann, 2007). More recent data have furthermore revealed critical microglia contributions during CNS development and brain homeostasis (Tremblay et al., 2011). Much of our knowledge about microglia biology currently relies on data obtained from *in vitro* cultured cells. Under these conditions microglia might however loose much of their uniqueness and turn into prototype macrophages. This calls for the development of experimental systems that will allow the study of microglia in their unique physiological CNS environment.

Microglia are characterized by prominent expression of the chemokine receptor CX_3CR1. According to the current chemokine nomenclature, which is based on the spacing of N-terminal cysteines, the chemokine CX_3CL1/fractalkine and its sole receptor CX_3CR1 constitute their own CX_3C "family" (Bazan et al., 1997; Imai et al., 1997; Pan et al., 1997). CX_3CR1 is a conventional $G\alpha_i$-coupled seven-transmembrane receptor. Its ligand CX_3CL1 differs however from conventional small peptide chemokines by the fact that it is synthesized as a trans-membrane protein with the CX_3C chemokine domain displayed on an extended highly glycosylated, mucin-like stalk (Bazan et al., 1997; Pan et al., 1997) (**Figure 1A**). To date, CX_3CL1 shares this unique membrane anchorage only with one other chemokine, the CXCR6 ligand CXCL16 (Matloubian et al., 2000). Proteolytic cleavage of CX_3CL1 by the disintegrin-like metalloproteinase ADAM10 results in constitutive release of different sized shed CX_3CL1 entities (Hundhausen et al., 2003). Moreover, under inflammatory conditions, CX_3CL1 shedding is also promoted by ADAM17/TACE (Garton et al., 2001; Tsou et al., 2001). Aside from the prominent expression in the mononuclear myeloid compartment (Jung et al., 2000), CX_3CR1 receptor expression has also been reported for an NK cell subset and certain T cell populations (Imai et al., 1997). Expression of the ligand CX_3CL1 outside the CNS has been reported for intestinal epithelium and endothelium, potentially restricted to inflammatory settings (Muehlhoefer et al., 2000; Kim et al., 2011).

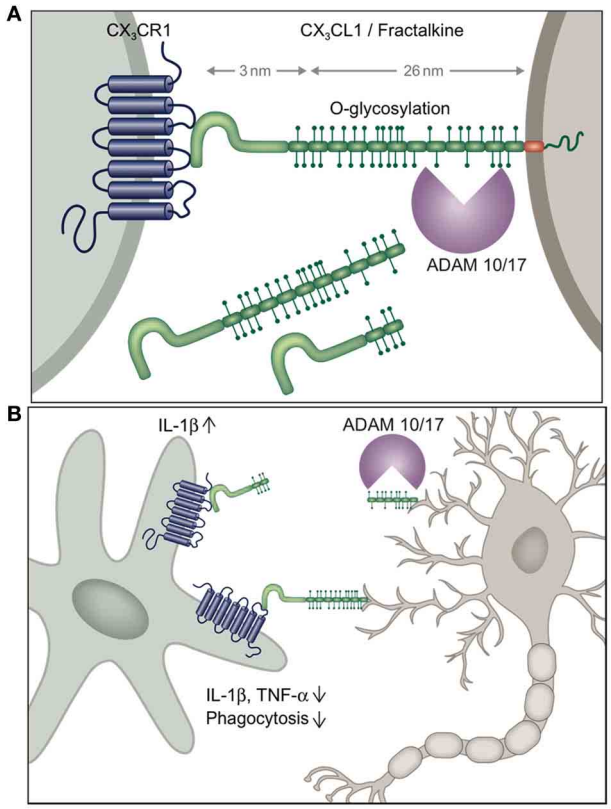


FIGURE 1 | (A) Schematic of CX_3C chemokine family and **(B)** potential scenario of differential outcomes of neuronal shed and membrane-anchored CX_3CL1 engagement by microglia inducing or suppressing microglial IL-1β production, respectively.

Although CX_3CL1 and CX_3CR1 are hence widely distributed throughout the organism, their expression in given tissues is often highly cell type-specific. This is particularly evident in the CNS, where CX_3CR1 expression is restricted to microglia and CX_3CL1 expression is confine to particular neurons (Nishiyori et al., 1998; Hughes et al., 2002; Tarozzo et al., 2003). This is best highlighted in CNS sections of double reporter animals, that combine a CX_3CR1^{gfp} locus (Jung et al., 2000) with a BAC transgene harboring a CX_3CL1-promoter driven gene encoding a red fluorescent cherry reporter (**Figure 2**) (Kim et al., 2011). In $CX_3CL1^{cherry}/CX_3CR1^{gfp}$ mice, $mCherry^+$ neurons are $NeuN^+$ DCX^- mature neurons which are located in spatially specific regions of the brain (**Figure 2**), with restricted expression in the hippocampus, striatum and cortical layer II of the cerebral cortex, as well as dorsal horn neurons in the spinal cord (Kim et al., 2011). The exact nature and function of these CX_3CL1-expressing neurons remains to be deciphered.

SEEING IS BELIEVING—*In situ* VISUALIZATION OF MICROGLIA USING CX_3CR1^{gfp} MICE

Microglial cells were originally identified in 1932 by the Cajal disciple del Rio-Hortega using silver staining and light microscopy. However, unraveling the role of microglia in CNS had to wait for the era of live brain imaging. Two seminal intravital microscopy studies revealed that these presumably static ramified cells are indeed highly dynamic and continuously extend fine highly motile processes that allow them to survey their immediate surrounding (Davalos et al., 2005; Nimmerjahn et al., 2005). Moreover, in response to laser-mediated lesions, microglial cells were found to rapidly respond to ATP released by astrocytes and redirected these processes toward the site of injury (Davalos et al., 2005). These pioneering imaging studies took advantage of CX_3CR1^{gfp} mice (Jung et al., 2000) that harbor a targeted replacement of the CX_3CR1 gene with a cDNA encoding enhanced fluorescent protein and ever since have become a popular research tool of the microglia research community. Specifically, CX_3CR1^{gfp} mice have allowed researchers to interrogate the microglia/neuron interface at unprecedented resolution complementing electron microscopy studies with critical dynamic data (Tremblay et al., 2010). These studies revealed that microglia are in intimate contact with neuronal synapses and that these interactions are affected by visual experience (Tremblay et al., 2010). Moreover, since the insertion of the reporter gene in CX_3CR1^{gfp} mice generates a CX_3CR1 null locus (Jung et al., 2000) (**Figure 3A**), comparison of $CX_3CR1^{gfp/+}$ and $CX_3CR1^{gfp/gfp}$ mice readily allows the probing for phenotypes resulting from CX_3CR1-deficiency using imaging strategies (see below). Of note, a potential confounding problem is the fact that when using CX_3CR1^{gfp} mice for microglia studies, animals are heterozygotes for the chemokine receptor and microglia display due to the haplo-insufficiency considerable less CX_3CR1 surface expression (Jung et al., 2000). However, no microglial phenotype has so far been reported for heterozygote mutant $CX_3CR1^{gfp/+}$ animals, when compared to mice harboring GFP transgene under the macrophage-specific ionized calcium-binding adaptor molecule 1 (Iba1) promoter (Hirasawa et al., 2005).

MICROGLIA AND THE CX_3C AXIS

Analysis of receptor and ligand knock-out mice (Jung et al., 2000; Cook et al., 2001; Combadiere et al., 2003) has revealed a number of phenotypes resulting from the lack of $CX_3CR1/L1$ interactions outside the CNS, including effects on artherogenesis (Liu and Jiang, 2011) and the ability of intestinal macrophages to sense gut lumen content (Niess et al., 2005). The physiological role of the CX_3C axis and in particular mechanistic aspects, such as potential differential functions of the membrane-tethered and shed CX_3CL1 entities, remain incompletely understood.

Of note, the membrane-tethered CX_3CL1 molecule has a short cytoplasmic tail lacking signaling motives. Moreover, CX_3CL1 is not known to associate with signaling competent co-receptors. Hence, it remains unclear whether interactions of microglial CX_3CR1 and CX_3CL1 expressed on the neuronal surface trigger a direct response in neurons. Rather, functional outcomes of CX_3CL1 ligation with CX_3CR1 seem to be restricted to the microglial partner, and $CX_3CR1/L1$ deficiencies seem to impinge indirectly on neurons.

Below we will discuss the impacts of CX_3CR1 and CX_3CL1 deficiencies on microglia functions in CNS development, the maintenance of CNS homeostasis and for the robustness of the organism to handle pathological challenges.

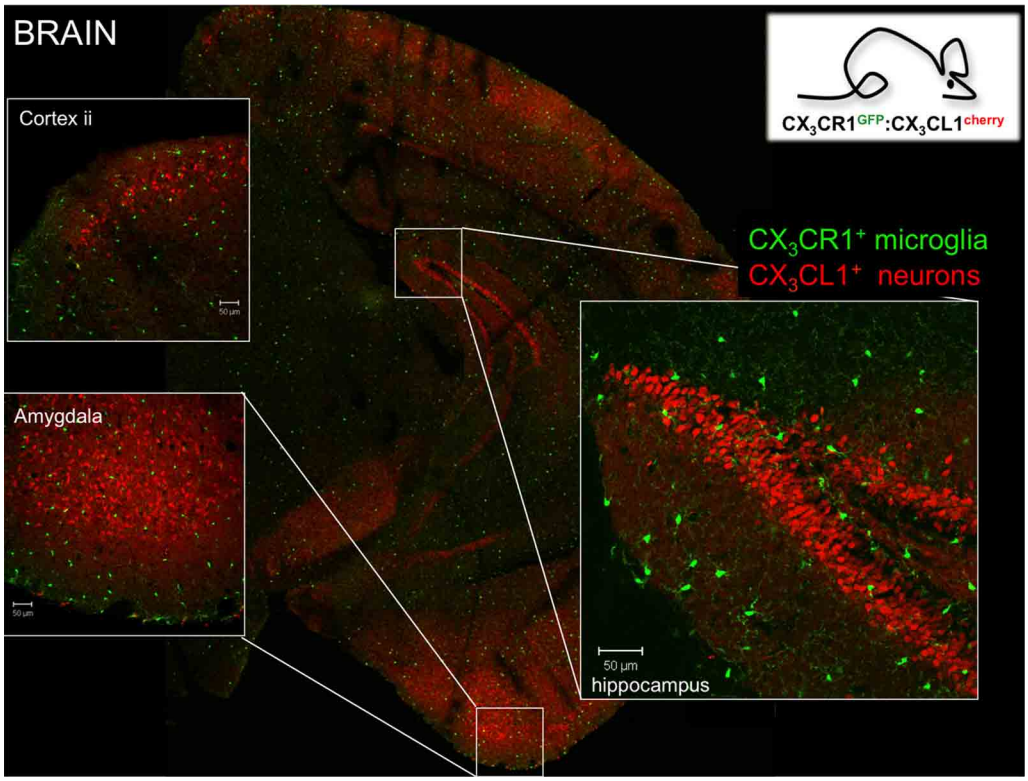


FIGURE 2 | Brain section of CX_3CR1^{gfp}:CX_3CL1^{cherry} double reporter animals (Kim et al., 2011) highlighting CX_3CR1-expressing microglia and subsets of CX_3CL1-expressing neurons in specific brain regions. Note that both reporters are expressed in the cytoplasm and not as fusion proteins. Thus they reflect the respective promoter activity but not the presence of the respective proteins. Hence no co-localization has to be expected.

THE CX_3C AXIS IN CNS DEVELOPMENT AND HOMEOSTASIS

Macrophages are long known to critically contribute to development and the maintenance of homeostasis (Stefater et al., 2011). The housekeeping functions of microglia are however only beginning to be appreciated to their full extent (Tremblay et al., 2011). As efficient phagocytes, microglia have emerged as key players in postnatal synaptic pruning that trim excess connections established in the developing brain for neuronal maturation. More specifically, microglia were shown to engulf presynaptic inputs during the pruning peak of retinal ganglion cells and the engulfment was dependent upon neural activity and the microglia-specific phagocytic signaling pathway involving complement receptor 3 (CR3) (Schafer et al., 2012). Interestingly, this developmental synaptic pruning by microglia seems affected in CX_3CR1-deficient mice, which reportedly display higher number of immature dendritic spines, correlating with transiently reduced microglia densities in their two first postnatal weeks (Paolicelli et al., 2011). In the developing barrel cortex, CX_3CR1-deficient microglia enter the thalamocortical axon cluster to a lesser degree, accompanied by delayed developmental switch of glutamate receptor subunits—a hallmark of barrel cortex development (Hoshiko et al., 2012). Here, microglia are thought to influence the maturation of synapses in a non-phagocytic, and probably soluble factor-mediated mechanism, which is yet to be elucidated. However, it remains to be established, if late maturation is directly mediated by the CX_3C axis, or the effect seen results from impaired entry of the cells to the barrel cortex. Other approaches, such as conditional mutagenesis of microglial CX_3CR1 will be required to distinguish between these two scenarios.

Microglia also have been shown to shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis (Sierra et al., 2010). Again, the CX_3CR1-deficiency seems to interfere with this process, probably as a result of deleterious microglia hyper-activation. Alongside reduced hippocampal neurogenesis, $CX_3CR1^{-/-}$ mice were thus reported to exhibit excessive IL-1β expression and attenuated long term potentiation (LTP) resulting in impaired cognitive functions (Bachstetter et al., 2011; Rogers et al., 2011). However, others reported that $CX_3CR1^{-/-}$ mice have enhanced LTP and perform better in the cognitive tests, but fail to improve these cognitive functions following exposure to enriched environment, a known stimulator of neurogenesis (Maggi et al., 2011). Interestingly, the seemingly contradicting reports share the observation of reduced neuronal precursors in neurogenic niches in the absence of CX_3CR1, which may hint at importance of CX_3CL1 signaling for synaptic plasticity, rather than basal synaptic activity. Further substantiating the immunosuppressive role of the CX_3C axis, antibody-mediated blockade of CX_3CR1 in young adult rats induced IL-1β production and decreased survival and proliferation of neural progenitor cells (Bachstetter et al., 2011). Moreover, treatment with recombinant

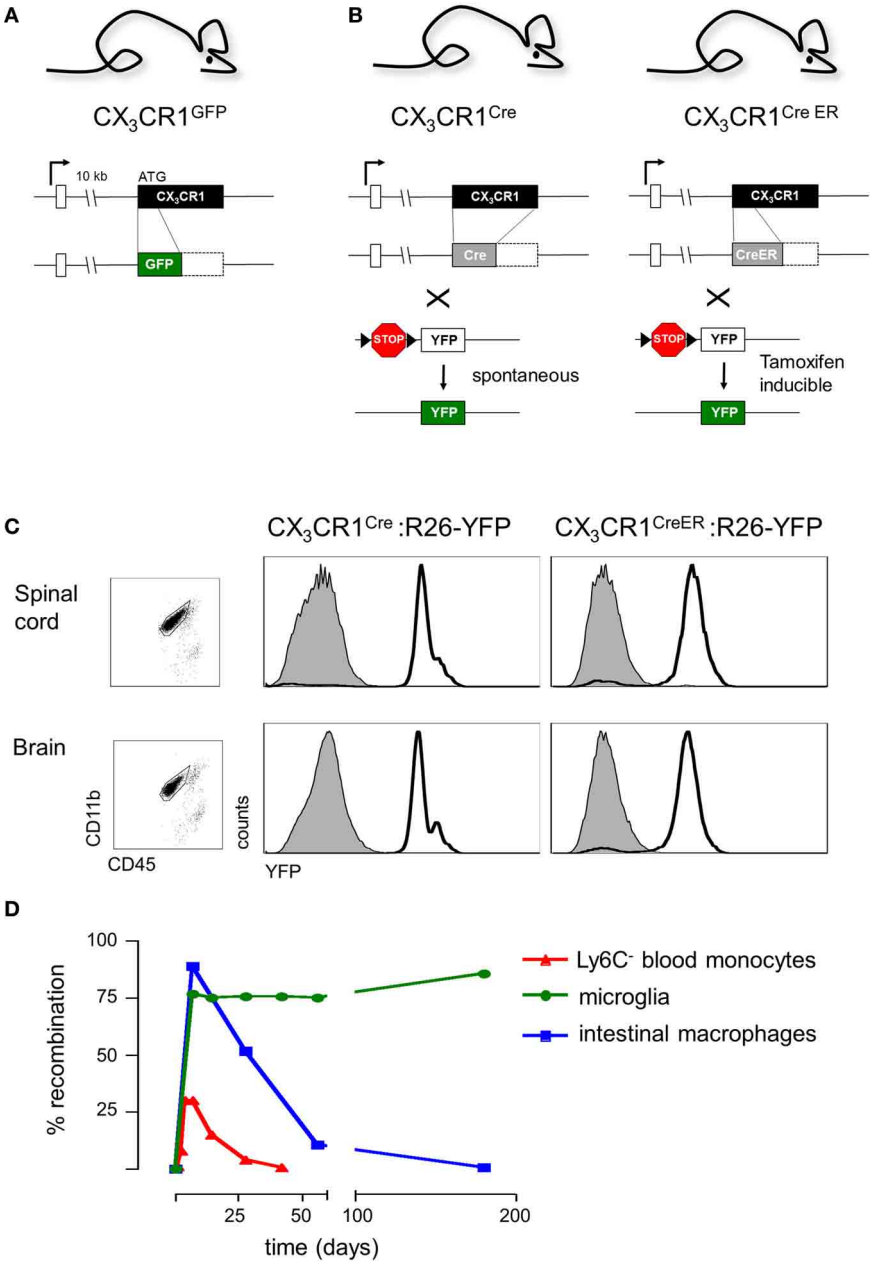


FIGURE 3 | (A) Schematic of modified CX_3CR1 loci of CX_3CR1^{gfp} mice (Jung et al., 2000) and **(B)** CX_3CR1^{Cre} and CX_3CR1^{CreER} mice (Yona et al., 2013); **(C)** Efficient YFP labeling of both spinal cord and brain microglia in CX_3CR1^{CreER} and CX_3CR1^{Cre} mice crossed with R26-YFP reporter mice. CX_3CR1^{CreER}:R26-YFP mice were treated with tamoxifen to induce the rearrangement (Yona et al., 2013); **(D)** Induction but subsequent progressive loss of cells harboring gene rearrangements in peripheral myeloid cells (monocytes, intestinal macrophages) and persistence of genomic modification in microglial cells.

CX_3CL1 was found to reverse the age-related decrease in neurogenesis (Bachstetter et al., 2011).

THE CX_3C AXIS AND PATHOLOGICAL SETTINGS

The CX_3CR1 deficiency also effects neuro-inflammatory and neurodegenerative diseases, as established in murine models of Alzheimer's and Parkinson's disease (AD, PD) and amyothrophic lateral sclerosis (ALS), as well as neuropathological conditions, such as neuropathic pain and cerebral ischemia.

AD is characterized by the presence of extracellular amyloid-β peptide (Aβ) deposits surrounded by activated glia and dystrophic neurites. The CX_3CR1-deficiency has been introduced into several established murine AD models, including the hTAu (Bhaskar et al., 2010), APP/PS1 (Fuhrmann

et al., 2010; Lee et al., 2010), and CRND8 background (Liu et al., 2010). Due to neuronal activation of the p38 MAPK pathway, HTau transgenic $CX_3CR1^{-/-}$ mice exhibit hyperphosphorylation of the tau protein, one of the AD hallmarks (Bhaskar et al., 2010). Again this is probably the result of excessive microglial IL-1β secretion (Bhaskar et al., 2010). Interestingly though, in combination with APP/PS1 transgenes the CX_3CR1 deficiency reduced amyloid plaque aggregation (Lee et al., 2010). Moreover, also the two-photon microscopic comparison of APP:PS1:$taup^{301L}$:Thy1-YFP:$CX_3CR1^{GFP/+}$ and APP:PS1:$taup^{301L}$:Thy1-YFP: $CX_3CR1^{gfp/gfp}$ mice revealed that the CX_3CR1 deficiency was beneficent in that it resulted in diminished microglia-mediated neuronal cell death, as well as attenuated microglia migration velocity (Fuhrmann et al., 2010). This suggests that microglia hyperactivation can have opposing outcomes in different models and potentially AD stages. Crossing the $CX_3CR1^{-/-}$ mice to CRND8 transgenic mice that harbor a gene encoding a mutant human amyloid precursor protein also resulted in reduced Aβ deposits, with the CX_3CR1-deficient microglia phagocytosing amyloid plaques and displaying higher proliferation rates in the plaque regions (Liu et al., 2010). Interestingly, microglia treated *in vitro* with beta-amyloid down-regulate CX_3CR1 with concomitant induction of IL-6 and TNF-α levels (Cho et al., 2011). Moreover, also in AD patients both CX_3CL1 and CX_3CR1 are reportedly down-modulated (Cho et al., 2011). In summary, disruption of the CX_3C axis results in microglia activation that includes beneficial enhanced phagocytosis of amyloid plaques, but is also associated with potentially detrimental secretion of pro-inflammatory cytokines causing neurotoxicity.

In rodent models of LPS-induced neuroinflammation, PD and ALS, CX_3CR1-deficient microglia were found to overexpress IL-1β and display neurotoxic activity (Cardona et al., 2006). Moreover, in the intrastriatal 6-hydroxydopamine (6-OHDA) rat model of PD administration CX_3CL1 prevented dopaminergic neuron death in the substantia nigra (Pabon et al., 2011). The therapeutic potential of CX_3CL1 regimens is further supported by the fact that exogenous CX_3CR1 reduced ischemia-induced cerebral infarct size, neurological deficits, and caspase-3 activation (Cipriani et al., 2011). Interestingly, in this study CX_3CL1-mediated neuroprotection required the adenosine system. However of note, another group reported that lack of CX_3CR1 did not result microglial neurotoxicity, but rather significantly reduces ischemic damage and inflammation, alongside with reduced IL-1β and TNFα expression as well as smaller infarcts (Denes et al., 2008). The reason for these discrepancies remains unclear. Of note, the above-mentioned studies that evoke therapeutic potential of CX_3CL1 administration (Bachstetter et al., 2011; Cipriani et al., 2011; Pabon et al., 2011) suggest that the soluble, shed CX_3CL1 isoform suffices to trigger CX_3CR1 signaling on microglia and suppress microglia activation. In support of this notion, adenovirus-mediated gene therapy that selectively expressed synthetic shed or non-sheddable CX_3CL1 variants was shown to ameliorate neurotoxicity when injected directly into the substantia nigra of $CX_3CL1^{-/-}$ mice that had been subjected to a PD model initiated by MPTP neurotoxin challenge (Morganti et al., 2012).

Challenging the notion of a mere anti-inflammatory role of CX_3CL1-induced CX_3CR1 signaling, deficient CX_3CR1 signaling was reported to promote recovery after mouse spinal cord injury (Donnelly et al., 2011). However, the effects observed in this study might not be directly related to microglia but rather linked to the concomitant impaired recruitment and activation of $Ly6C^-$ blood monocytes (Donnelly et al., 2011), which depend on CX_3CR1 signaling for survival (Landsman et al., 2009). Nevertheless, the authors reported that *in vitro* exposure of wt but not CX_3CR1-deficient microglia induced their expression of IL-6, though not IL-1β (Donnelly et al., 2011).

Microglia residing in the dorsal horn of the spinal cord are critical contributors to nociceptive transmission following peripheral nerve/tissue injury (Milligan and Watkins, 2009). Shed neuronal CX_3CL1 was proposed to be a critical mediator of spinal neuronal-microglial communication in chronic pain. CX_3CL1 cleavage in the dorsal root ganglion (DRG) was reported to depend on microglial release of the lysosomal protease cathepsin S potentially in response to ATP release and P2X7 receptor (Clark et al., 2007, 2010). In keeping with the notion that CX_3CL1 is an ADAM substrate (Garton et al., 2001; Tsou et al., 2001; Hundhausen et al., 2003), CX_3CL1 could also be cleaved by the matrix metalloprotease MMP9 that was found upregulated in injured DRG primary sensory neurons (Kawasaki et al., 2008). Shed CX_3CL1 was proposed to engage microglial CX_3CR1 and trigger via p38 MAPK phosphorylation pain-causing migroglial IL-1β secretion (Zhuang et al., 2007). CX_3CR1-deficient mice reportedly display unimpaired responses to acute thermal and mechanical noxious stimuli, but displayed deficits in inflammatory and neuropathic nociceptive responses in the partial sciatic nerve ligation model (Staniland et al., 2010). The CX_3C axis also was reported to be involved in the IL-1β-induced hyperalgesia observed in animals that have a microglia-restricted reduction of the G protein-coupled receptor kinase 2 (GRK2) (LysM-Cre:$GRK2^{f/+}$ mice) (Willemen et al., 2010).

One issue complicating the exact assessment of the importance of the CX_3C axis for microglia function in pain perception is that the involved anatomic sites include the spinal cord and DRG that are part of the CNS and peripheral nerve system (PNS) and are hence located behind and in front of the BBB, respectively. Spinal cord and DRG are therefore differentially accessible to the influx of monocytes and contribution of monocyte-derived macrophages (see below).

The emerging recurrent theme of most of the studies addressing the role of the CX_3C axis in microglia biology is that constitutively expressed membrane-tethered neuronal CX_3CL1 seems to provide a tonic inhibitory signal to microglia that keeps these cells in a quiescent "sampling" or surveillance mode (Biber et al., 2007) (**Figure 1B**). Conversely, CX_3CR1 and CX_3CL1 deficiencies result in hyper-activated microglia, that depending on the particular setting can be detrimental or beneficial to its environment. As opposed to the homeostatic role of trans-membrane CX_3CL1, under conditions of challenge such as pain-inducing stimuli, CX_3CL1 is shed from the neuronal surface and in this context seems to trigger IL-1β production by microglia. Differential activities of shed or membrane-tethered CX_3CL1

might be related to the recently reported requirement for integrin binding for CX_3CR1 signaling, although both isoforms interacted with integrins (Fujita et al., 2012). Interestingly, shed, presumably neuronal-derived CX_3CL1 becomes detectable in serum, when CX_3CR1 is absent suggesting that CX_3CR1-expressing cells provide a constitutive sink for it (Cardona et al., 2008). Clearly though, further experimentation will be required to study potential differential effects of membrane-tethered and shed CX_3CL1 entities on microglial CX_3CR1 signaling in the *in vivo* context, for instance involving animals manipulated to express exclusively shed CX_3CL1 (Kim et al., 2011).

THE CHALLENGE—MICROGLIA vs. MONOCYTE-DERIVED MACROPHAGES

The high CX_3CR1 expression in microglia and resulting bright green fluorescence of CX_3CR1^{gfp} microglia turned CX_3CR1^{gfp} mice into a valuable tool to probe microglia function. Moreover, since circulating blood monocytes express CX_3CR1, these reporter animals became instrumental to solve a long-standing debate about the origins of microglia cells. Microglia are hematopoietic cells that develop independent of neuroectoderm-derived neurons, astrocytes and oligodendrocytes. However, the relationship microglia have with bone marrow-derived macrophages that originate from blood monocytes had long remained a matter of dispute. Irradiation chimeras were generated to define the hematopoietic stem cell (HSC) origin of microglia. Yet, interpretation of the results obtained was confounded by the facts that first microglia are radio-resistant and thus not replaced by a bone marrow graft, and that second the irradiation compromised the BBB allowing monocyte infiltrates. Revision of these studies taking advantage of CX_3CR1^{gfp} mice to mark either the microglia or the blood compartment and introducing cellular exchange in parabionts and CCR2 dependency as a indication of monocyte-derivation (Mildner et al., 2007; Ajami et al., 2011) have now firmly established that microglia and monocyte-derived brain macrophages are distinct entities. Moreover, these studies were confirmed by fate mapping studies and the demonstration that amoeboid CX_3CR1/GFP^+ cells seed the neuroepithelium of the developing brain well before the emergence of "definitive" hematopoietic precursors, at E10.5–E11.5, and following infiltration proliferate and change their morphology into a more branched phenotype (Ginhoux et al., 2010; Swinnen et al., 2013). As opposed to the macrophages colonizing the surface ectoderm these early microglia lack CCR2 expression, established using CX_3CR1^{gfp}:$CCR2^{rfp}$ double reporter mice (Mizutani et al., 2012). Also the spinal cord was shown to be colonized tissue as early as E10.5, although the route by which the microglia precursors reach the respective tissues remain to be resolved (Rigato et al., 2011). The emerging scheme thus holds that microglia are established from primitive macrophages and subsequently maintain themselves through longevity and limited self-renewal independent from further input from definitive hematopoiesis through the HSC-monocyte axis. This prenatally established, hard-wired resident microglia compartment can be complemented on demand by macrophages that arise from monocytes recruited from the blood circulation as a transient "emergency squad" during injury or challenge (Mildner et al., 2009). Depending on the time and route of their arrival to tissues, monocytes can contribute both pro- and anti-inflammatory (Mildner et al., 2009; Shechter et al., 2009), but seem eventually to be purged from the CNS context after resolution of the inflammation (Ajami et al., 2011).

Monocytic infiltrates pose a unique challenge to the study of *bona fide* microglia, as monocyte-derived macrophages become phenotypically indistinguishable from resident microglia, in particular upon activation of the latter. This is particularly evident in the study of neuro-inflammatory disorders, such as the multiple sclerosis model of experimental autoimmune encephalitis (EAE) that involves the BBB breakage and substantial monocyte recruitment (Mildner et al., 2009). Emerging evidence from mixed BM chimeras and parabionts indicates that functional contributions of monocyte-derived cells differ from that of the microglia (Ajami et al., 2011). However, efforts to address these differential functions so far relied largely on the generation of BM chimeras and the manipulation of BM-derived component. Definitive evidence for microglia functions calls for experimental systems that will allow the exclusive manipulation of microglia in non-irradiated mice.

GENETIC MANIPULATION OF MICROGLIA IN CONTEXT—CX_3CR1^{Cre} AND CX_3CR1^{CreER} ANIMALS

In order to overcome the above constraints of current microglia studies, we decided to exploit the high CX_3CR1 promoter activity of microglial cells for their genetic manipulation. We generated CX_3CR1^{Cre} and CX_3CR1^{CreER} animals (Yona et al., 2013), by targeted insertion of the recombinase genes that mimicked the situation of the CX_3CR1^{gfp} locus previously shown to tightly reflect endogenous CX_3CR1 expression (Jung et al., 2000) (**Figure 3B**). CX_3CR1^{Cre} mice express constitutively active Cre recombinase resulting in the spontaneous non-reversible rearrangement of loxP site-flanked alleles in CX_3CR1-expressing cells. In contrast, the CX_3CR1^{CreER} system comprises a conditional active Cre-ERT2 recombinase that is fused to a mutated ligand-binding domain (LBD) of the human estrogen receptor (ER) (Feil et al., 2009). Two point mutations in the ER-LBD allow binding of the synthetic estrogen antagonist tamoxifen (TAM) and prevents constitutive activation of the CreER by endogenous estradiol (Feil et al., 2009). In the unbound form, the CreER fusion protein resides in the cytoplasm in an inactive complex with heat shock proteins. Upon administration and binding of TAM, the CreER is freed to translocate to the nucleus and mediate the site-specific recombination.

Importantly, CX_3CR1^{Cre} and CX_3CR1^{CreER} animals differ considerably with respect to the cells targeted. In CX_3CR1^{CreER} animals only cells that express CX_3CR1 and hence the CreER transgene will undergo rearrangement at the time of the TAM treatment. In contrast and as best demonstrated in combination with respective reporter mouse strains (Yona et al., 2013), in CX_3CR1^{Cre} animals also cells that are derived from CX_3CR1^+ cells but subsequently silenced CX_3CR1 expression will have recombined the loxP-flanked loci. CX_3CR1^{Cre} animals therefore report on the history of the cell and can be used for fate mapping studies (Yona et al., 2013).

Analysis of CX_3CR1^{Cre} and CX_3CR1^{CreER} mice crossed to animals harboring a floxed YFP reporter gene (Srinivas et al., 2001) established that these systems indeed efficiently target CNS microglia (Yona et al., 2013). As expected, in CX_3CR1^{Cre}: YFP mice, and only in TAM-treated CX_3CR1^{CreER}: YFP mice, more than 95% of both brain and spinal cord microglia were found YFP labeled (**Figure 3C**). Thus, when crossed to mice harboring "floxed" candidate genes, these systems can be utilized to delete or express specific genes in CX_3CR1^+ microglia, either straight from their development onwards, using the constitutive Cre system, or in specific time windows, using the inducible Cre system.

Of note, certain lymphocyte subsets and myeloid cells, other than microglia express CX_3CR1. TAM treatment of CX_3CR1^{CreER} mice results accordingly also in gene rearrangement in these cells (Yona et al., 2013) (**Figure 3D**), including monocytes and peripheral macrophages, i.e., exactly the cells that have confounded the functional analysis of the microglia compartment in the previous studies. However, most peripheral myeloid cells that underwent Cre activation and rearrangements have a limited half-life and are hence continuously replaced by BM-derived cells. Genetic modifications, such as the activation of the YFP reporter gene or the deletion of "floxed" alleles are thus progressively lost with time in these populations. In contrast, the resident microglia pool which self-renews without further input from the BM retains once introduced gene modifications throughout the life of the organism (**Figure 3D**). This feature thus allows generation of animals that harbor specific genetic manipulations restricted to microglia. Of note, there are also other tissue macrophage populations such as Kupffer cells and peritoneal macrophages that are established before birth and then self renew (Schulz et al., 2012; Yona et al., 2013). However, as most of these cells lack CX_3CR1 expression, they are not targeted by the CX_3CR1^{CreER} approach in adulthood (Yona et al., 2013). CX_3CR1^+ intestinal macrophages on the other hand loose their gene modifications, as they are progressively replaced by monocytes (Zigmond et al., 2012) (**Figure 3D**). Taken together, the combination of CX_3CR1^{Cre} and CX_3CR1^{CreER} animals provides a unique tool to probe for the involvement of microglia in CNS development, CNS maintenance and CNS responses to pathological challenges.

CONCLUSION

Microglia are the main representatives of the immune system in the healthy brain as such likely to critically contribute to the brain's resistance to pathological challenges. Moreover, recent data highlight the critical involvement of microglia in CNS development and homeostasis. Given its strategic localization at the neuronal/microglial interface the CX_3C axis is likely to play a prominent role in these activities. Thus accumulating evidence suggest that neuronal CX_3CL1 acts as a critical inhibitory signal retaining microglia in quiescent mode and preventing collateral damage due to microglia hyper-activation. Aside from its biological role, the CX_3CR1 chemokine receptor provides us however also with a unique foothold to study microglia in context using state of the art imaging and gene manipulation approaches. The near future is hence likely to provide valuable insight into contributions of these intriguing cells to brain physiology and might pave the way for the development of microglia manipulation for therapeutic purposes.

ACKNOWLEDGMENTS

This work was supported the Israel Science Foundation (ISF), the Deutsche Forschungsgemeinschaft (DFG) Research Unit (FOR) 1336.

REFERENCES

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M., and Rossi, F. M. V. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* 14, 1142–1149.

Alliot, F., Godin, I., and Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* 117, 145–152.

Bachstetter, A. D., Morganti, J. M., Jernberg, J., Schlunk, A., Mitchell, S. H., Brewster, K. W., et al. (2011). Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol. Aging* 32, 2030–2044.

Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., et al. (1997). A new class of membrane-bound chemokine with a CX_3C motif. *Nature* 385, 640–644.

Bhaskar, K., Konerth, M., Kokiko-Cochran, O. N., Cardona, A., Ransohoff, R. M., and Lamb, B. T. (2010). Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* 68, 19–31.

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci.* 30, 596–602.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., et al. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* 9, 917–924.

Cardona, A. E., Sasse, M. E., Liu, L., Cardona, S. M., Mizutani, M., Savarin, C., et al. (2008). Scavenging roles of chemokine receptors: chemokine receptor deficiency is associated with increased levels of ligand in circulation and tissues. *Blood* 112, 256–263.

Cho, S. H., Sun, B., Zhou, Y., Kauppinen, T. M., Halabisky, B., Wes, P., et al. (2011). CX_3CR1 protein signaling modulates microglial activation and protects against plaque-independent cognitive deficits in a mouse model of Alzheimer disease. *J. Biol. Chem.* 286, 32713–32722.

Cipriani, R., Villa, P., Chece, G., Lauro, C., Paladini, A., Micotti, E., et al. (2011). CX_3CL1 is neuroprotective in permanent focal cerebral ischemia in rodents. *J. Neurosci.* 31, 16327–16335.

Clark, A. K., Staniland, A. A., Marchand, F., Kaan, T. K., McMahon, S. B., and Malcangio, M. (2010). P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. *J. Neurosci.* 30, 573–582.

Clark, A. K., Yip, P. K., Grist, J., Gentry, C., Staniland, A. A., Marchand, F., et al. (2007). Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10655–10660.

Combadiere, C., Potteaux, S., Gao, J. L., Esposito, B., Casanova, S., Lee, E. J., et al. (2003). Decreased atherosclerotic lesion formation in CX_3CR1/apolipoprotein E double knockout mice. *Circulation* 107, 1009–1016.

Cook, D. N., Chen, S. C., Sullivan, L. M., Manfra, D. J., Wiekowski, M. T., Prosser, D. M., et al. (2001). Generation and analysis of mice lacking the chemokine fractalkine. *Mol. Cell. Biol.* 21, 3159–3165.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758.

de Haas, A. H., Boddeke, H. W., and Biber, K. (2008). Region-specific expression of immunoregulatory proteins on microglia in the healthy CNS. *Glia* 56, 888–894.

Denes, A., Ferenczi, S., Halasz, J., Kornyei, Z., and Kovacs, K. J. (2008). Role of CX_3CR1 (fractalkine receptor) in brain damage and inflammation induced

by focal cerebral ischemia in mouse. *J. Cereb. Blood Flow Metab.* 28, 1707–1721.

Donnelly, D. J., Longbrake, E. E., Shawler, T. M., Kigerl, K. A., Lai, W., Tovar, C. A., et al. (2011). Deficient CX_3CR1 signaling promotes recovery after mouse spinal cord injury by limiting the recruitment and activation of Ly6Clo/iNOS+ macrophages. *J. Neurosci.* 31, 9910–9922.

Feil, S., Valtcheva, N., and Feil, R. (2009). Inducible Cre mice. *Methods Mol. Biol.* 530, 343–363.

Fuhrmann, M., Bittner, T., Jung, C. K., Burgold, S., Page, R. M., Mitteregger, G., et al. (2010). Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nat. Neurosci.* 13, 411–413.

Fujita, M., Takada, Y. K., and Takada, Y. (2012). Integrins alphavbeta3 and alpha4beta1 act as coreceptors for fractalkine, and the integrin-binding defective mutant of fractalkine is an antagonist of CX_3CR1. *J. Immunol.* 189, 5809–5819.

Garton, K. J., Gough, P. J., Blobel, C. P., Murphy, G., Greaves, D. R., Dempsey, P. J., et al. (2001). Tumor necrosis factor-alpha-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX_3CL1). *J. Biol. Chem.* 276, 37993–38001.

Gautier, E. L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., et al. (2012). Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 13, 1118–1128.

Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., and Ley, K. (2010). Development of monocytes, macrophages, and dendritic cells. *Science* 327, 656–661.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394.

Hirasawa, T., Ohsawa, K., Imai, Y., Ondo, Y., Akazawa, C., Uchino, S., et al. (2005). Visualization of microglia in living tissues using Iba1-EGFP transgenic mice. *J. Neurosci. Res.* 81, 357–362.

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., et al. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* 209, 1167–1181.

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012). Deficiency of the microglial receptor CX_3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J. Neurosci.* 32, 15106–15111.

Hughes, P. M., Botham, M. S., Frentzel, S., Mir, A., and Perry, V. H. (2002). Expression of fractalkine (CX_3CL1) and its receptor, CX_3CR1, during acute and chronic inflammation in the rodent CNS. *Glia* 37, 314–327.

Hundhausen, C., Misztela, D., Berkhout, T. A., Broadway, N., Saftig, P., Reiss, K., et al. (2003). The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX_3CL1 (fractalkine) and regulates CX_3CL1-mediated cell-cell adhesion. *Blood* 102, 1186–1195.

Imai, T., Hieshima, K., Haskell, C., Baba, M., Nagira, M., Nishimura, M., et al. (1997). Identification and molecular characterization of fractalkine receptor CX_3CR1, which mediates both leukocyte migration and adhesion. *Cell* 91, 521–530.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M. J., Kreutzberg, G. W., Sher, A., et al. (2000). Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol. Cell. Biol.* 20, 4106–4114.

Kawasaki, Y., Xu, Z. Z., Wang, X., Park, J. Y., Zhuang, Z. Y., Tan, P. H., et al. (2008). Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. *Nat. Med.* 14, 331–336.

Kim, K. W., Vallon-Eberhard, A., Zigmond, E., Farache, J., Shezen, E., Shakhar, G., et al. (2011). *In vivo* structure/function and expression analysis of the CX_3C chemokine fractalkine. *Blood* 118, e156–e167.

Landsman, L., Bar-On, L., Zernecke, A., Kim, K. W., Krauthgamer, R., Shagdarsuren, E., et al. (2009). CX_3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood* 113, 963–972.

Lee, S., Varvel, N. H., Konerth, M. E., Xu, G., Cardona, A. E., Ransohoff, R. M., et al. (2010). CX_3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. *Am. J. Pathol.* 177, 2549–2562.

Liu, H., and Jiang, D. (2011). Fractalkine/CX_3CR1 and atherosclerosis. *Clin. Chim. Acta* 412, 1180–1186.

Liu, Z., Condello, C., Schain, A., Harb, R., and Grutzendler, J. (2010). CX_3CR1 in microglia regulates brain amyloid deposition through selective protofibrillar amyloid-beta phagocytosis. *J. Neurosci.* 30, 17091–17101.

Maggi, L., Scianni, M., Branchi, I., D'Andrea, I., Lauro, C., and Limatola, C. (2011). CX(3)CR1 deficiency alters hippocampal-dependent plasticity phenomena blunting the effects of enriched environment. *Front. Cell. Neurosci.* 5:22. doi: 10.3389/fncel.2011.00022

Matloubian, M., David, A., Engel, S., Ryan, J. E., and Cyster, J. G. (2000). A transmembrane CXC chemokine is a ligand for HIV-coreceptor Bonzo. *Nat. Immunol.* 1, 298–304.

Mildner, A., Mack, M., Schmidt, H., Bruck, W., Djukic, M., Zabel, M. D., et al. (2009). CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain* 132, 2487–2500.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U.-K., Mack, M., et al. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* 10, 1544–1553.

Milligan, E. D., and Watkins, L. R. (2009). Pathological and protective roles of glia in chronic pain. *Nat. Rev. Neurosci.* 10, 23–36.

Mizutani, M., Pino, P. A., Saederup, N., Charo, I. F., Ransohoff, R. M., and Cardona, A. E. (2012). The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. *J. Immunol.* 188, 29–36.

Morganti, J. M., Nash, K. R., Grimmig, B. A., Ranjit, S., Small, B., Bickford, P. C., et al. (2012). The soluble isoform of CX_3CL1 is necessary for neuroprotection in a mouse model of Parkinson's disease. *J. Neurosci* 32, 14592–14601.

Muehlhoefer, A., Saubermann, L. J., Gu, X., Luedtke-Heckenkamp, K., Xavier, R., Blumberg, R. S., et al. (2000). Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestinal mucosa. *J. Immunol.* 164, 3368–3376.

Niess, J. H., Brand, S., Gu, X., Landsman, L., Jung, S., McCormick, B. A., et al. (2005). CX_3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 307, 254–258.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318.

Nishiyori, A., Minami, M., Ohtani, Y., Takami, S., Yamamoto, J., Kawaguchi, N., et al. (1998). Localization of fractalkine and CX_3CR1 mRNAs in rat brain: does fractalkine play a role in signaling from neuron to microglia? *FEBS Lett.* 429, 167–172.

Pabon, M. M., Bachstetter, A. D., Hudson, C. E., Gemma, C., and Bickford, P. C. (2011). CX_3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease. *J. Neuroinflammation* 8:9. doi: 10.1186/1742-2094-8-9

Pan, Y., Lloyd, C., Zhou, H., Dolich, S., Deeds, J., Gonzalo, J. A., et al. (1997). Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 387, 611–617.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Rigato, C., Buckinx, R., Le-Corronc, H., Rigo, J. M., and Legendre, P. (2011). Pattern of invasion of the embryonic mouse spinal cord by microglial cells at the time of the onset of functional neuronal networks. *Glia* 59, 675–695.

Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., et al. (2011). CX_3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J. Neurosci.* 31, 16241–16250.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and

hematopoietic stem cells. *Science* 336, 86–90.

Shechter, R., London, A., Varol, C., Raposo, C., Cusimano, M., Yovel, G., et al. (2009). Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. *PLoS Med.* 6:e1000113. doi: 10.1371/journal.pmed.1000113

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Srinivas, S., Watanabe, T., Lin, C. S., William, C. M., Tanabe, Y., Jessell, T. M., et al. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev. Biol.* 1:4. doi: 10.1186/1471-213X-1-4

Staniland, A. A., Clark, A. K., Wodarski, R., Sasso, O., Maione, F., D'Acquisto, F., et al. (2010). Reduced inflammatory and neuropathic pain and decreased spinal microglial response in fractalkine receptor (CX_3CR1) knockout mice. *J. Neurochem.* 114, 1143–1157.

Stefater, J. A. 3rd., Ren, S., Lang, R. A., and Duffield, J. S. (2011). Metchnikoff's policemen: macrophages in development, homeostasis and regeneration. *Trends Mol. Med.* 17, 743–752.

Swinnen, N., Smolders, S., Avila, A., Notelaers, K., Paesen, R., Ameloot, M., et al. (2013). Complex invasion pattern of the cerebral cortex bymicroglial cells during development of the mouse embryo. *Glia* 61, 150–163.

Tarozzo, G., Bortolazzi, S., Crochemore, C., Chen, S. C., Lira, A. S., Abrams, J. S., et al. (2003). Fractalkine protein localization and gene expression in mouse brain. *J. Neurosci. Res.* 73, 81–88.

Tremblay, M. E., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Tremblay, M. E., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069.

Tsou, C. L., Haskell, C. A., and Charo, I. F. (2001). Tumor necrosis factor-alpha-converting enzyme mediates the inducible cleavage of fractalkine. *J. Biol. Chem.* 276, 44622–44626.

Willemen, H. L., Eijkelkamp, N., Wang, H., Dantzer, R., Dorn, G. W. 2nd., Kelley, K. W., et al. (2010). Microglial/macrophage GRK2 determines duration of peripheral IL-1beta-induced hyperalgesia: contribution of spinal cord CX_3CR1, p38 and IL-1 signaling. *Pain* 150, 550–560.

Yona, S., Kim, K. W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 38, 79–91.

Zhuang, Z. Y., Kawasaki, Y., Tan, P. H., Wen, Y. R., Huang, J., and Ji, R. R. (2007). Role of the CX_3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. *Brain Behav. Immun.* 21, 642–651.

Zigmond, E., Varol, C., Farache, J., Elmaliah, E., Satpathy, A. T., Friedlander, G., et al. (2012). Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* 37, 1076–1090.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 December 2012; paper pending published: 12 February 2013; accepted: 28 February 2013; published online: 18 March 2013.

Citation: Wolf Y, Yona S, Kim K-W and Jung S (2013) Microglia, seen from the CX_3CR1 angle. Front. Cell. Neurosci. 7:26. doi: 10.3389/fncel.2013.00026

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 30 January 2013
doi: 10.3389/fncel.2013.00003

Quantitating the subtleties of microglial morphology with fractal analysis

Audrey Karperien[1]*, Helmut Ahammer[2] and Herbert F. Jelinek[1,3]

[1] *Centre for Research in Complex Systems, School of Community Health, Charles Sturt University, Albury, NSW, Australia*
[2] *Institute of Biophysics, Medical University of Graz, Graz, Austria*
[3] *Rural Clinical School, University of New South Wales, Sydney, NSW, Australia*

Edited by:
Marie-Eve Tremblay, Université Laval, Canada

Reviewed by:
Hermann Cuntz, Ernst Strüngmann Institute, Germany
Denis Soulet, Laval University, Canada

****Correspondence:***
Audrey Karperien, Centre for Research in Complex Systems, School of Community Health, Charles Sturt University, Albury, NSW 2640, Australia.
e-mail: akarpe01@postoffice.csu.edu.au

It is well established that microglial form and function are inextricably linked. In recent years, the traditional view that microglial form ranges between "ramified resting" and "activated amoeboid" has been emphasized through advancing imaging techniques that point to microglial form being highly dynamic even within the currently accepted morphological categories. Moreover, microglia adopt meaningful intermediate forms between categories, with considerable crossover in function and varying morphologies as they cycle, migrate, wave, phagocytose, and extend and retract fine and gross processes. From a quantitative perspective, it is problematic to measure such variability using traditional methods, but one way of quantitating such detail is through fractal analysis. The techniques of fractal analysis have been used for quantitating microglial morphology, to categorize gross differences but also to differentiate subtle differences (e.g., amongst ramified cells). Multifractal analysis in particular is one technique of fractal analysis that may be useful for identifying intermediate forms. Here we review current trends and methods of fractal analysis, focusing on box counting analysis, including lacunarity and multifractal analysis, as applied to microglial morphology.

Keywords: microglia, cell shape, image interpretation: computer-assisted, fractals, models: biological, box counting, lacunarity, multifractal analysis

THE FORM-FUNCTION CONNECTION

Microglia, small in size but enormous in significance, occupy a conspicuous space in presumably all nervous systems. They interweave intimately and abundantly with the generally much larger neurons at a ratio that can be said to average one to one in normal adult human tissue, but depends on when and from where in the central nervous system (CNS) the tissue sample is taken. The 3-dimensional space microglia occupy constantly changes as they move their cell processes and migrate, but if caught in stop motion, microglia can be seen to come in an extraordinary variety of intricate and complex morphologies (Dowding et al., 1991; Dowding and Scholes, 1993; Sonetti et al., 1994; Magazine et al., 1996; Dobrenis, 1998; Perry, 1998; Alliot et al., 1999; Bernhardi and Nicholls, 1999; McMenamin, 1999; Streit et al., 1999; Navascues et al., 2000).

As **Figure 1** illustrates, individual microglial cells can cycle reversibly from a simple rounded to a complex branched form. At any point in time they might be found as round to amorphous blobs with any of a variety of intriguing membrane features such as pseudopodia and ruffles. Or at the other extreme, they may adopt a form with a relatively very small soma and long, tortuous primary processes that can be characterized as "spider-like," "jointed," or "thorny" with secondary and tertiary branches endowed with wispy ends or yet further branches (Kreutzberg, 1995; Dailey and Waite, 1999; Ohsawa et al., 2000; Streit, 2000; Bohatschek et al., 2001; Nimmerjahn et al., 2005; Tremblay et al., 2011; Liu et al., 2012).

On the basis of this highly flexible morphology, neuropathologists have fashioned a model linking names for microglial forms to microglial function. In the mature CNS, microglia in their unramified and intermediate forms (moving upward from the bottom of **Figure 1**) are generally considered to be "activated," "reactive," or "intermediate." They are activated for an immunoinflammatory role that includes traveling to sites of injury where they can recruit or activate other cells, proliferate, phagocytose, clear debris, and contribute to healing and cortical reorganization. They may also express a typical immunoinflammatory profile such as upregulated CD68 and major histocompatibility complex II proteins (MHC-II) (Streit and Kreutzberg, 1987). Seen in a dish, on a slide, or in a live organism, microglia thus may appear plump, dragging and heaving corpulent bodies, or perhaps scalloping at the edges. They may be rapidly protruding in and out a few or many stout processes, and may simultaneously be, a little more slowly, winding in their finer arbor. In this role, microglial cell bodies can be elongated lumpy, rod-like or tortuous with swollen branching projections, or microglia can be more radial, spiky figures (Streit, 2000; Soltys et al., 2001; Stence et al., 2001).

In their fully ramified forms (atop **Figure 1**) in normal mature CNS, microglia are actively engaged in essential physiological roles. They are vigilant sentinels and "synaptic partners," watching over and ensuring proper functioning of neurons, providing neurotrophic substances (Nakajima and Shinichi, 2002), acting

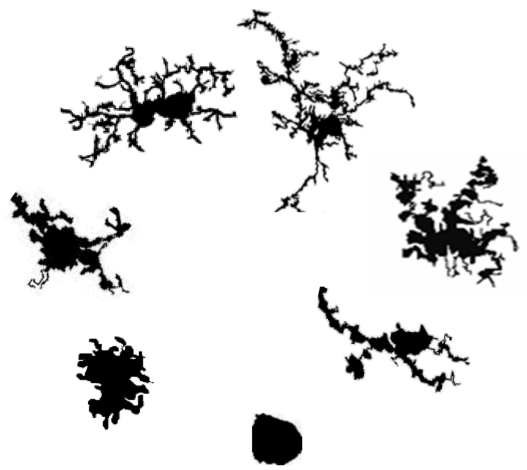


FIGURE 1 | Microglial morphology in adult human CNS. Microglia are morphologically and functionally dynamic cells able to change form from highly ramified to completely lacking processes. The transition can be very rapid or microglia can remain in a form for years (Colton et al., 2000). The forms illustrated here represent snapshots of a transformation that is reversible at every point, with variation within each form shown. The figures are not shown to scale; they are adjusted to compare detail.

on and regulating neurotransmitters and hormones (Garcia-Segura et al., 1996), mediating pain (Watkins et al., 2001; Inoue, 2006) and responses to psychological stress (Hinwood et al., 2012), protecting neurons from damage (Vinet et al., 2012), and responding to changes in the microenvironment (e.g., stretch, depolarization, glycemic status, etc.) (Eder et al., 1998; Lyons and Kettenmann, 1998; Polito et al., 2011; Tremblay et al., 2011; Won et al., 2012).

As they do their multifarious duties, ramified cells change and move in many ways over multiple time scales. Their arbor itself changes as they wriggle and wave, extend and retract fine and gross processes, tend to synapses, migrate, and phagocytose (Pow et al., 1989; Dailey and Waite, 1999; Lee et al., 2008; Marker et al., 2010; Tremblay et al., 2011). Microglia have been observed moving their processes "exuberantly," more at the ends than near the soma, in seemingly random directions but within a volume so that they maintain a consistent basic symmetry and overall arbor size. They might start to move more rapidly and become more polarized as they modify and extend their processes toward a site of injury, and may migrate toward the site while still ramified. Indeed, they can continue to mark their post and send off a replica if they feel a need to tend to something at a distance (Radewicz et al., 2000; Aarum et al., 2003; Nimmerjahn et al., 2005; Lee et al., 2008; Wake et al., 2009; Perego et al., 2011).

Knowledge of this model is a powerful tool in the neuroscientist's toolkit. It suggests that based on visual impressions of local microglial morphology, much can be inferred about what is going on in a particular location. Such visual impressions do in fact inform the decisions of pathologists and researchers (Streit, 2000). The model is especially powerful because microglia play an integral role in the developing and mature CNS, during pathological and normal states, affecting structure, plasticity, and function in virtually all circumstances. Microglia have been found to mediate effects of and respond to a host of substances as diverse as minocycline (Hinwood et al., 2012), ethanol (Crews et al., 2006; Kane et al., 2011; Zhao et al., 2012), nonsteroidal anti-inflammatories (Varvel et al., 2009), opioids (Wen et al., 2011), cannabinoids (Toth et al., 2010), and neuroleptics (Yrjanheikki et al., 1998; Busse et al., 2012), and are increasingly being seen as potential targets for therapeutic intervention and monitoring of events in the nervous system (Billiards et al., 2006; Liu et al., 2012; Pascual et al., 2012).

However powerful this form-function model is, it is also limited. Applying it too generally can gloss over important features of intermediate forms between categories, subtle variation within a category, and the considerable crossover in function amongst cells in different categories. Ramified cells were historically characterized as "resting," for example, from observations of fixed microscope slides, but advancing imaging techniques including thinned-skull, live imaging revealed that ramified microglia are "resting" only from their alternative starring role in emergency response. Indeed, the moniker "never resting microglia" is rising in popularity amongst microgliologists. Microglia were also thought of as unable to respond to compromise without being morphologically activated, but this was another of our assumptions advancing research revealed to be wrong. In sum, it has proven extremely useful in many ways but in others inadequate to infer function from biochemical markers and preconceived morphological categories. So, the model must continue to grow. The investigator who is attempting to unravel brain function, track responses to treatments, and detect pathological changes must remember that the form-function model is a starting point, and be vigilant to the possibility of multifarious factors affecting microglial morphology and function (Radewicz et al., 2000; Aarum et al., 2003; Nimmerjahn et al., 2005; Wake et al., 2009; Perego et al., 2011).

In doing so, the microgliologist might also note that the form-function model is poor at quantitating subtle morphological differences. At what point should one consider a cell to have crossed over from ramified to activated, for instance? In a still photo, how should one decide if a cell is de- or re-ramifying? How should an investigator decide if the subtle difference in branching angle between two cells is part of the puzzle they are trying to solve? Or how should one classify a cell with a very large soma but a single, long, slender, branching process, and what should one assume such a cell is doing based on traditional classification systems? On another level, how should one interpret an area with half of the microglia in one category and the other half in another? These questions are more than academic when it comes to both practice and research. Microglia undergo changes in a host of domains along multiple time scales (e.g., soma size and shape, relative cytoplasmic volume, membrane configuration, receptor distribution, cytoskeletal organization, process length, diameter, and degree of branching, branch configuration and tortuosity). These are challenging to distil into practical metrics using traditional objective

measuring methods, but the model is at a point where it needs to become more quantitative.

One method that may contribute to this need is fractal analysis. Thus, the rest of this review comments on how fractal analysis is helping make models of microglial form and function more quantitative. To help the reader interpret fractal analysis studies of microglia, we provide a brief methodological overview. We also discuss how fractal analysis has been used to quantitate microglial morphology and how it might generate hypotheses about the form-function connection in the future. We focus on one particular type of analysis, *box counting*, which has been used to quantify not only gross morphological differences but also subtle nuances of microglial morphology that may be important for understanding normal and pathological CNS.

FRACTAL ANALYSIS

Fractal analysis is a group of methods for quantifying difficult to describe patterns (Jelinek and Fernandez, 1998). We summarize here only a few of its elements, those necessary to understand the results discussed in this review. Box counting and the box counting dimension are our exemplar for three reasons. First, box counting is exquisitely sensitive to morphological features that are analogous to key structural features of microglia (i.e., branching patterns and contours, respectively analogous to ramified processes and membrane detail) (Losa et al., 1997). Second, it has proven successful for analyzing microglia. And third, box counting software has become increasingly accessible to the neuroscience community (Karperien, 2001a; Baksi and Fidler, 2012). The reader should be aware that alternative fractal methods such as the *dilation method* and *mass radius method* have been used to characterize microglial morphology (Soltys et al., 2001; Orlowski et al., 2003; Varvel et al., 2009), and that others may prove useful (e.g., local connected fractal dimension) (Karperien, 2001a; Losa et al., 2005; Karperien et al., 2008b) but have not yet been tried.

The reader should also be aware that a dearth of data exists on fractal analysis of microglia in general. As computerized methods of image analysis have burgeoned in the last decades, fractal analysis in neuroscience has grown to include many applications ranging from classifying neural cells to assessing diabetic retinopathy (Smith et al., 1996; Fernandez and Jelinek, 2001; Karperien et al., 2008b; Jelinek et al., 2010; Kim et al., 2011). As substantial as this body of literature is, it contains only a very small number of studies reporting on fractal analysis of microglia, many published by our lab using *FracLac for ImageJ*. FracLac is open-source software freely available to the bioscience community through the ImageJ website at the National Institutes of Health. It was developed by our lab to control and automate fractal analysis of microglia and provide complementary measures of cell morphology (Karperien, 2001a; Mancardi et al., 2008; Kam et al., 2009; Sant and Fairgrieve, 2012; Schneider et al., 2012).

FRACTAL DIMENSIONS

At the heart of fractal analysis is the concept of a *fractal dimension* (D_F). A D_F is a number describing how the detail in a pattern changes as the pattern is examined at varying scales. This scaling is generally referred to as *complexity*. The higher the dimension, the more complex the pattern. This is not to say that one would expect to be able to characterize microglia along an infinite spectrum; rather, from a practical perspective, one can generally expect that for 2-dimensional patterns, calculated D_Fs will generally fall between 1 and 2, and for 3-dimensional, between 2 and 3.

Finding a D_F for a structure is similar to zooming in with a microscope to examine tissue at different magnifications, but with an important difference. Normally, as one zooms in, one sees at a finer resolution the more fundamental building blocks of a structure; but for a fractal pattern, with each increase in magnification the observer finds the original structure composed of parts identical to itself, just smaller. This *self-similarity* stands out because it is detailed, as opposed to the uninteresting self-similarity in the curve of a simple circle, for instance, which has a theoretical D_F of 1.0 and would forever be seen to be made up of merely smaller curves. Moreover, the number of new parts within a structure changes consistently with the scale, such that there is a predictable ratio of new parts to scale, which is the mathematical basis of the D_F. As shown in Equations 1 and 2, the D_F is the exponent to which scale (ε) is raised to get the number of new parts (N_ε):

$$N_\varepsilon = \varepsilon^{D_F} \tag{1}$$

$$D_F = \frac{\ln N_\varepsilon}{\ln \varepsilon} \tag{2}$$

Figure 2A illustrates self-similarity in one example of a fractal pattern known as a quadric fractal curve. A practical approximation (D) of a D_F can be estimated from a sample of information based on the limit as scale decreases:

$$D = \lim_{\varepsilon \to 0} \left[\frac{\ln N_\varepsilon}{\ln \varepsilon} \right] \tag{3}$$

D from Equation 3 is usually calculated as the slope over a straight interval of the linear regression line for a sample dataset of N_ε vs. ε.

Relevant to fractal analysis of microglia and other biological phenomena, both theoretical fractals and biological fractal-like patterns (**Figures 2B** and **C**) may have statistical rather than strict self-similarity. Furthermore, although in theory a D_F describes an infinite scaling ratio, as **Figure 2** shows, physically manifested fractal patterns are limited by physical bounds so a D measured for such a pattern is taken to be an approximation of scaling within such bounds (Mandelbrot, 1983).

BOX COUNTING FRACTAL ANALYSIS

The prohibitively tedious task of gathering a sample dataset implied by **Figure 2** is usually approximated by computer software. The inputs to the software are usually 2-dimensional binary patterns (i.e., black and white images), in which pixels can have one of two values, foreground or background; but 3-dimensional data and grayscale images can also be analyzed (Sheets et al., 2010; Ahammer, 2011; Kim et al., 2011).

In order to gather a dataset, box counting software, in essence, lays successively smaller calibre grids over an image, counting the number of boxes containing any foreground pixels to get a proxy

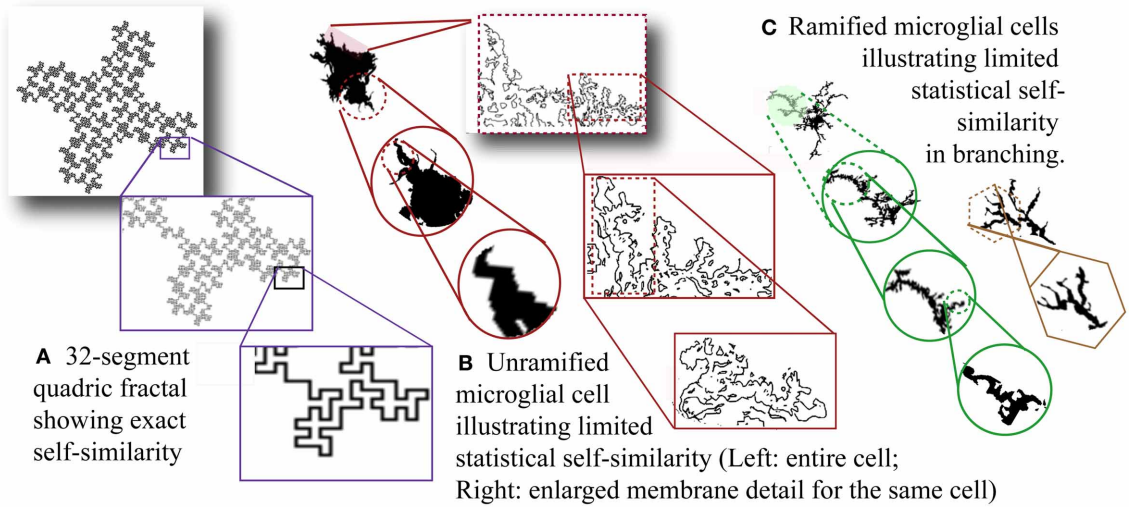


FIGURE 2 | Self-similarity and scale invariance. (A) Thirty-two-segment quadric fractal contour illustrating exact but limited self-similarity. The pattern always resolves into 32 new parts each 1/8 of the previous size so the fractal dimension is ln 32/ln 8 = 1.67. The theoretical pattern is infinitely self-similar, but the image is limited by the smallest possible line size that can be used to draw it. [Image generated with ImageJ (Schneider et al.), fractal generator plugin. http://imagej.nih.gov/ij/plugins/fractal-generator.html. Modified from http://rsb.info.nih.gov/ij/plugins/fraclac/FLHelp/Fractals.htm#32seg.] Silhouettes of **(B)** unramified and **(C)** ramified microglia. [Note that the rightmost figure in **(C)** could be classified as intermediate.] **(B)** Showing membrane detail, and **(C)** showing branching detail, depict scaling that is not exactly self-similar and referred to as scale invariant. Moreover, scaling is only detectable within physical limits and the limits of the methods and media used to reveal morphology including staining and recording methods as well as magnification and resolution (both optical and digital).

for N at each calibre, ε = box size. It may also gather the number of pixels in each box, or in the case of grayscale images, the difference in pixel intensity in each box. After gathering the data, the software calculates the box counting dimension (D_B) according to Equation 4, accounting for box size as related to scale, then using again a regression line.

$$D = \lim_{\varepsilon \to 0} \left[\frac{\ln N_\varepsilon}{\ln \varepsilon^{-1}} \right] \quad (4)$$

Thus, one can think of a D_B found for microglia as the mean rate of change in detail with change in resolution sampled from an image. For more about box counting algorithms and implementations, the reader can see Mandelbrot (1983), Karperien (2001b), or Ristanović et al. (2009).

FRACTAL LITERACY

Before going on, we should address two fundamental "fractal literacy" (Jelinek et al., 2005) issues that can have bearing on how one interprets fractal analysis of microglia. First, a D_F is neither a unique nor a complete descriptor. To elaborate, although one can generate a set of rules to construct a series of related forms of increasing known complexity that looks like what it is and has calculated D_Bs in agreement with the rules that were used to construct the series (e.g., see **Figure 3**), one cannot do this in reverse. Knowing a pattern's D_F or D_B does not tell what the underlying structure looks like, how it was constructed, or how it functions. As a consequence, a box counting algorithm may quantitatively and correctly assess two objects as similar, but the eye may see something very different. As another consequence, a fractal dimension alone cannot describe any feature of microglia. Rather, it is a statistical index of complexity only, a unitless dimension that is entirely independent of traditional measures such as length, area, etc.

Second, box counting neither finds nor confirms the existence of a fundamentally repeating unit; rather, it measures scaling in an image as the averaged dependency between pixel arrangement and box size. The self-similarity in **Figure 2** shown for microglia, for instance, was not detected by fractal analysis but was selected for illustrative purposes. An important implication of this point is that being able to determine a D_B for a pattern does not mean the phenomenon from which it was gleaned is fractal; neither does a phenomenon have to be fractal to be investigated with box counting fractal analysis (Jelinek et al., 2005).

BENCHMARKING

One further point to mention when interpreting fractal analysis studies is that all methods of fractal analysis have limitations. Technical issues specific to box counting, such as effects of grid orientation and calibre, and smoothing the data to find an optimal scaling interval, must be addressed and are generally accounted for within software (Karperien, 2001a; Kam et al., 2009; Sant and Fairgrieve, 2012). In this regard, box counting software is generally validated for any particular analysis using benchmarks having known D_Bs and that are relevant to the images being analyzed (e.g., for microglia, a benchmark would have size, resolution, density of foreground pixels, and pattern features similar to the images being analyzed) (Mandelbrot, 1983; Vicsek, 1992; Karperien, 2001b). Some benchmarks that have been proposed include diffusion limited aggregation and

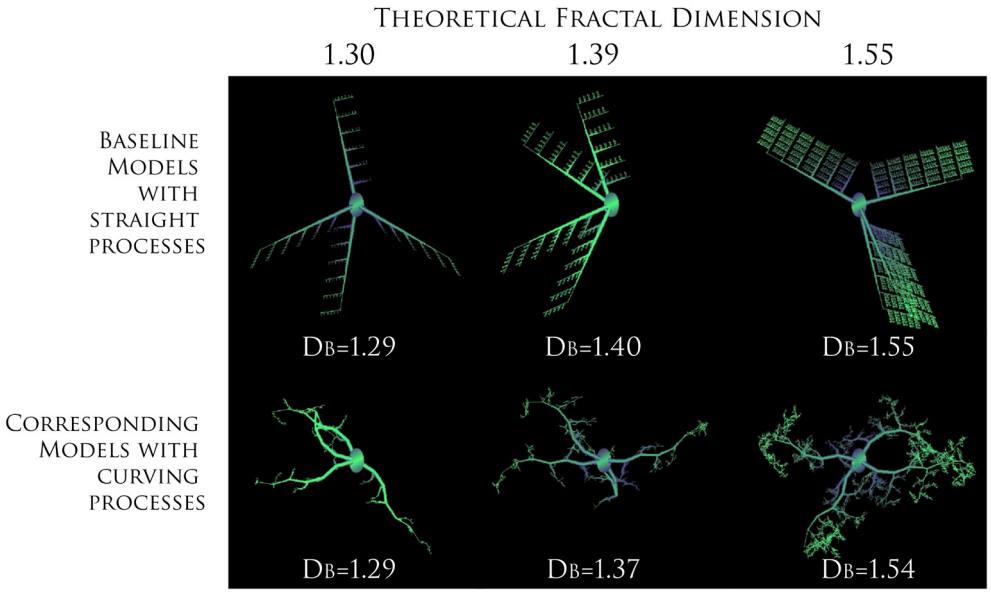


FIGURE 3 | Increasing complexity of branching patterns. Branching structures generated by computer based modeling, according to known rules. At each iteration, the ratio of branch length to parent length was changed to illustrate how branching features influence theoretical D_{Fs} and calculated D_Bs. For both series, the direction a branch was allowed to grow in was random. The only difference between the two series was in the amount of curving allowed in processes, which was greater in the images on the bottom. The values for the D_B are averages for sets of models rather than only the particular model shown for each series. Modeled with MicroMod, free, open-source modeling software (Karperien, 2001c; Jelinek et al., 2002).

various fractal contours (Karperien, 2004; Jelinek et al., 2011, 2013).

SUPPLEMENTARY MEASURES: LACUNARITY AND MULTIFRACTALITY

In addition to fractal dimensions, two other measures obtained from box counting that have been applied to microglia that we will discuss here are lacunarity and multifractality. Both measure scaling of the "mass" or number of pixels per box rather than scaling of the presence of pixels in a box, so if box counting records the number of pixels in each box, these supplementary measures can be calculated as part of the analysis and provide additional features for classification. Whereas the D_B measures self-similarity, lacunarity measures nearly the opposite, heterogeneity—patterns with high lacunarity are inhomogeneous and with low lacunarity are homogeneous or rotationally invariant (see **Figure 4**). There are different ways to define and determine lacunarity; methods that depend on the same data used to calculate a D_F are correlated with it and therefore redundant, but those that do not depend on the same data are complementary (Smith et al., 1996). Lacunarity calculated from box counting (Λ), found as the coefficient of variation in pixel density with scale, is independent of the D_B, and patterns indistinguishable by their D_B are often distinguishable by Λ or vice versa (Karperien, 2004; Jelinek et al., 2011). As will be discussed later, with respect to microglia, lacunarity has been associated with changes in the soma and additional morphological features.

Multifractality on the other hand is relevant to patterns in which a spectrum of fractal dimensions can be identified rather than a single global D_B. The theory and calculations behind multifractal measures are available elsewhere (Chhabra and Jensen, 1989; Smith et al., 1996; Karperien, 2001a).

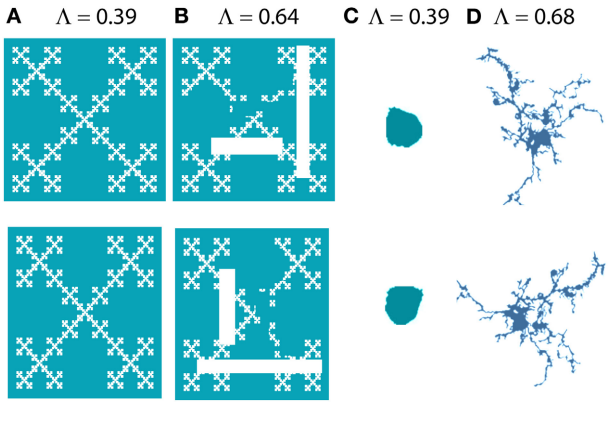


FIGURE 4 | Lacunarity. The images on the bottom row are 90° rotations of the images on the top row. Images **(A)** and **(C)** look similar to their originals when rotated, but **(B)** and **(D)** are more affected by rotation; they are more heterogeneous or less rotationally invariant and this is captured and quantified by Λ [e.g., **(B)** has greater lacunarity than **(A)**]. **(A)** and **(B)** were generated with ImageJ fractal generator plugin http://imagej.nih.gov/ij/plugins/fractal-generator.html; **(C)** and **(D)** are from **Figure 1**.

PATTERN ACQUISITION

For microglia and cells with related morphologies or functions (e.g., neurons, astroglia, dendritic cells, and macrophages), the

features usually looked at by fractal analysis are branching patterns and cytoplasmic membrane configuration, but other features relevant to microglia such as nuclear membrane, intracellular structures, textures, movement patterns, or distributions of cells or groups of cells in time and space are amenable to fractal analysis (Losa et al., 1997, 2005; Baatz et al., 2005). In all cases, the feature being investigated has to be converted to a pattern such as a binary image that fractal analysis software can analyze.

What you see is what you look for

To convert a chosen structural feature into such a pattern, the feature has to first be revealed by some method that can deliver a signal the software can make sense of. For box counting, as we alluded to earlier, the signal pattern is usually input as a still digital image; that input image is usually made from an original digital image. Microglia are not normally visible, so the digital image is constructed from a histological slide or other visualization method via some amplified signal marker. Such markers are specific for different cell parts and can reveal different features of microglial morphology. Electron microscopy, for instance, reveals exquisite intracellular and the finest membrane detail, the peripheral benzodiazepine receptor, used in live imaging studies, tracks events at the outer mitochondrial membrane, cytoskeletal protein markers reveal differently arranged networks of whichever particular filament is being exposed, antibodies to CD68 show binding on primarily lysosomes in the cytoplasm but also on the outer membrane, RCA-1 shows binding over the entire plasma membrane, and scanning electron microscopy shows gross morphological detail (Streit and Kreutzberg, 1987; Kreutzberg, 1995; Banati et al., 1999; Cross and Woodroofe, 2001). As an example, then, electron microscopy would be more suited to grayscale analysis of intracellular texture and lectin binding to binary analysis of ramification.

Another factor influencing the final pattern of the image is the original size of the region of interest. As their name suggests, *micro*glia are typically smaller than most other cells found in the nervous system (i.e., neurons and other glia). A survey of published values suggests that for human microglia observed at all stages of their activation cycle and the human life span, grown in culture or found in tissue samples, nuclei average roughly 5–10 μm in diameter, cell bodies range from 7 to 20 μm in diameter, and cell branches (if present) radiate outward from the cell body so that an entire cell spans, on average, from 30 to 120 μm (de Groot et al., 1992, 2000; Rezaie et al., 1997, 1999, 2002; Andjelkovic et al., 1998; Sheng et al., 1998a; Stoll and Jander, 1999; Radewicz et al., 2000).

With such small subject matter, it is important to consider the level of detail analyzed and issues such as how well the staining, magnification, and resolution preserved information (e.g., terminal branches or fine membrane fringes). Indeed, despite that fractal dimensions are independent of object size, measured D_Bs are not entirely independent of imaging protocols, including staining methods and image size and resolution, which can affect the results of a fractal analysis to varying degrees. In practice, this limits the applicability of comparing fractal dimensions across paradigms. Over and above absolute differences owing to methodology or image size, however, as is discussed in section "One Fundamental Relative Pattern Across Paradigms," when looking at branching and membrane detail at least, relative effects are preserved across many paradigms. More work is still needed to characterize issues related to specific methods of visualization and compare how different types of fractal analysis are affected by different visualization techniques.

Preprocessing

A major challenge in any type of digital image analysis, pattern extraction itself is another element that must be understood to interpret results of fractal analysis studies. Pattern extraction means removing noise—that can mean overall background and can include other cells or structures. For grayscale box counting analysis, this is often less a challenge than for binary image analysis. Extracting a grayscale pattern is usually relatively straightforward, sometimes involving only isolating the relevant part and converting the original image to grayscale in an image processing program, in which case images of individual cells or entire fields with multiple cells can be processed rapidly. Grayscale analysis assumes that the image background is supposed to be processed along with any objects of interest; if not, grayscale pattern extraction becomes more involved.

Rather than grayscale analysis, however, most of the work published on microglia to date has used binary image analysis. Patterns have been extracted by either manual tracing or automated segmenting methods (Soltys et al., 2001; Jelinek et al., 2008). Both approaches incur their own level of bias. As an illustration, staining in unactivated cells may be splotchier than in activated cells owing to upregulation of the marker on activation, so the operator may interpolate in a "connect the dots" fashion; in automated thresholding, only the actual "dots" are rendered as signal. However, in both cases, a person, either a tracer or a programmer, decides what is background and what is foreground, a universal bias affecting any assessment of staining of biological cells. Also, in automated methods, there is a bias away from overlapping cells so that such methods are usually limited to investigations where clustering and overlap are considered part of the signal rather than noise.

The patterns extracted have typically been profiles or contours of single cells or of fields containing multiple cells. If the goal is to identify branching and membrane characteristics, the patterns generally exclude intracellular content; if the goal is to assess the actual pattern of distribution of a marker, then including inner detail can be appropriate. Depending on the feature being investigated, the final pattern can be filled or outlined; filled patterns of the same cell tend to have higher D_Bs. Ramification has been assessed using another method, skeletonizing (e.g., a function in ImageJ), which is especially suited to the dilation method (Orlowski et al., 2003; Soltys et al., 2005). Of note, the dilation method measures different features of the image so produces different values for the fractal dimension compared to box counting unless the image is Euclidean in which case all fractal dimensions are equal (Jelinek et al., 2005; Losa et al., 2005; Karperien et al., 2008a).

Reproducibility

Little has been published on pattern acquisition per se for microglia, but one investigation carried out in our lab compared D_Bs for images of individual microglia obtained using a tracing and a thresholding method (Karperien, 2004). Operator bias within each method was minimizable by having clearly defined rules for the methods, and training operators over a few practice sessions. Although the tracing and thresholding results deviated somewhat from each other for certain images, overall the differences were not statistically significant. That is, the patterns from both methods were not identical, but the results imply that they contained essentially the same information relevant to the D_B. This investigation was very limited, however, and preprocessing is a contentious issue in digital image analysis (Jelinek and Fernandez, 1998; Jelinek et al., 2005).

Another finding of this work was that D_Bs were statistically significantly lower for images obtained from one compared to many focal planes (Karperien, 2004). This may be attributable to the point that the box counting method used to find the D_B from the extracted pattern was intended for 2-dimensional data rather than a sample from 3 dimensions.

Manual tracing methods have the distinct disadvantage of being potentially very time consuming, so have been considered impractical for some types of investigation (Donnelly et al., 2009; Kozlowski and Weimer, 2012). Nonetheless, they are well-suited to investigations of branching patterns and have been used to extract intricate patterns from images of microglia (Soltys et al., 2001; Karperien et al., 2008c).

Semi-automated segmentation methods have also been used (Jelinek et al., 2008; Karperien et al., 2008a); these are quicker, but depend more on suitable starting material. For example, with strong contrast and little overlap of cells an operator can preselect regions of interest, or, alternatively, algorithms have been designed to automatically segment and analyze images of individual microglia or fields of multiple cells without intervention (Karperien, 2001a; Karperien et al., 2008a; Kam et al., 2009). Fully automated segmentation of various shapes is a broad goal in digital image analysis (Kim et al., 2011). Kozlowski and Weimer (2012) have published a promising study in which they demonstrate an automated segmentation method designed specifically for identifying microglia visualized by a variety of protocols, although it is not clear if the method is likely to reveal fine branching patterns; future work is needed to find out if the method extracts patterns suitable for fractal analysis and how generally applicable it is.

Validating the result

Another issue one should be conversant in is how well the patterns typically analyzed represent actual microglia. There can be large gaps in the correspondence between overall visual impressions of cells in their original contexts and the final binary patterns, especially when using automated methods geared to identify only pixels corresponding to staining past a certain threshold. Such discrepancies may reflect losses, gains, or distortions of information, or they may be artefacts of perception. That is, regardless of the pattern extraction method used, the essential procedure is to take 3-dimensional, multiply motile, functional cells, and derive from them still, binary, 2-dimensional contours or silhouettes. In addition to containing information about the cell's actual morphology and orientation in space, the information available in the final image will depend on how the original image was acquired and encoded to digital format as well as how the information was extracted. In the case of microglia, detail lost, gained, or distorted could be in intracellular or nuclear features, soma shape, fine branches, membrane protrusions, information about how a cell cuts out its unique volume within the CNS at the moment the image is taken, etc.

Whereas changes to the information may cause the final pattern to bear little resemblance to the original cell or image, this perceptual disjunction has generally been deemed to be negligible based on conventions that have been widely applied to other biological cells. One is the assumption that the information of interest (e.g., in the branching of processes and the contour of the membrane) is sufficiently preserved at least on average (which means sample size and random selection should be considered in any investigation). Another is the assumption that objects contained in 3D without completely filling 3-dimensional space are adequately represented by projections onto 2-dimensional space (e.g., theoretically, a contour of a tree's branches should hold the same information as the tree) (Takayasu, 1990; Vicsek, 1992; Smith et al., 1996; Losa et al., 1997; Jelinek and Fernandez, 1998; Fernandez and Jelinek, 2001). As discussed in the next section, computer based modeling has been used to test the validity of these assumptions for branching patterns in microglia.

Microglia in silico

Modeling lets us test our understanding of and predict the behavior of biological systems. Work by Jelinek et al. (2002) and others from that laboratory demonstrated that increasing ramification in microglia can be modeled by increasing complexity. Changes in complexity input to models can, in turn, be accurately measured back from binary outlines extracted from *in silico* microglia. Jelinek's team created computer-simulated models from images of microglia in normal and pathological elderly human brain, as well as several other samples from humans and other animals. The models were specified by recursively applied rules derived from measuring microglial features. These included features associated with fractal geometry, such as process length and diameter, branching frequency, and branching angle, as well as other features, including the tortuousness of processes and size and shape of the soma, that were expected to influence the results. The models were generated as several 200-member populations of both idealized models and statistical models designed to emulate microglia in the 3D space they occupy under real circumstances, or at least in observed circumstances (Karperien, 2001c, 2004; Jelinek and Karperien, 2008).

Supporting the conclusion that binary contours from microglia realistically represent underlying scaling features, the average D_Bs of binary contours extracted from the model populations corresponded to the D_Bs that would be expected for theoretical fractal microglia having the same underlying scaling features (see **Figure 5**). Ramified microglia modeled on a population with a mean D_B of 1.423 for instance, had a mean D_B of 1.425. Furthermore, the D_Bs changed as would be expected

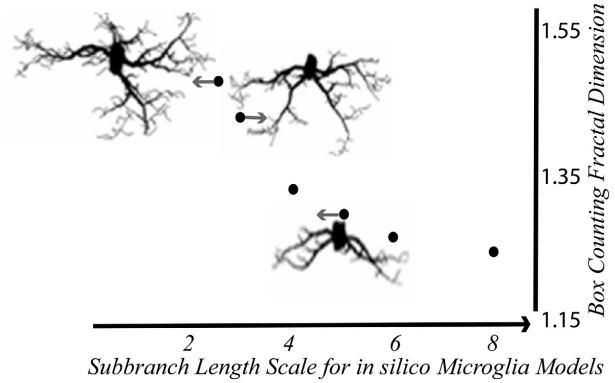


FIGURE 5 | *In silico* microglia illustrate the relationship between the D_B and branching features. The D_B decreased with scale—that is, it increased nonlinearly with the relative length of sub-branches Modified from Karperien (2004); models generated with MicroMod (Karperien, 2001c; Jelinek et al., 2002).

for fractal patterns when the length or diameter of the modeled branches changed, but were not affected when the soma size or elongation changed. The results were robust under the emulations of 3D space, but only within limits (Jelinek et al., 2002; Karperien, 2004; Karperien et al., 2005; Jelinek and Karperien, 2008).

Overall, the *in silico* studies suggest that, in practice, some variation in calculated D_Bs is predictable even for cells in equivalent activation states having essentially the same branching ratios, attributable to the space the microglia occupy, and the orientation they assume at any point in time. The variation not related to scaling is small enough to conclude that binary contours represent microglial morphology for box counting fractal analysis, at least for the particular pattern extraction methods and large sample sizes used. However, a feature of the models that limits the applicability of these results is that the modeller could control the background and the degree of "staining," and the models had very high contrast (Jelinek et al., 2002).

WHAT FRACTAL ANALYSIS HAS TOLD US

The preceding section outlined what fractal analysis is and provided some fundamental information to help the reader understand how box counting analysis is applied and gain insight into interpreting it when applied to microglia in particular. The present section outlines some of the key results that have been obtained for investigations of branching pattern and membrane detail, and discusses issues in understanding how they all fit together and directions they suggest for the future.

ONE FUNDAMENTAL RELATIVE PATTERN ACROSS PARADIGMS

Mostly in agreement with modeling results and fractal theory, the results of fractal analysis studies using box counting and other techniques support the broad idea that the D_F for overall microglial morphology increases with ramification (but, as discussed below, does not necessarily change inversely with activation state) (Soltys et al., 2001, 2005; Orlowski et al., 2003; Jelinek et al., 2008; Karperien et al., 2008c). This was seen for a variety of image sizes, resolutions, staining methods, species, and brain locations. One study in particular compared several paradigms, and found that despite there being differences in the actual values obtained for the D_B, a basic relative relationship was preserved over many protocols, suggesting there is a fundamental pattern transcending many factors (Karperien, 2004).

One investigation (Jelinek et al., 2008) using cultured rodent cells demonstrated the general pattern with the D_B corresponding to responses to graded levels of a stimulus with de-ramifying on one side of a cycle and re-ramifying on the other. The general pattern emerges in graphics when cells with the lowest D_B, approaching theoretical values for simple shapes like circles (i.e., 1.0), are placed at the bottom, and cells with the highest D_B (i.e., the most complex) are placed at the top. This basic pattern was mapped in **Figure 1**. The figure is illustrative rather than strictly accurate in that it uses only a few representative morphologies arranged by their corresponding D_Bs but not necessarily separated by re- vs. de-ramifying or pathological vs. normal status.

The cycle of complexity is not quite as expected

Empirical results add to the fundamental relative pattern that "most activated" means "least ramified," but "least activated" is not necessarily "most ramified." This is because the most complex microglial form seems to be ramified cells that are subtly activated.

Thus, the evidence suggests that the basic pattern is characterized by an initial increase in the D_B when unstimulated ramified cells start responding, followed by a decrease until the roundest forms are reached, then an increase back up to the ramified states. The amount of data available at this point is insufficient to establish normative ranges, but, as a rough guide and taking note that the actual values varied with the investigative protocol, the peak in D_B was typically around 1.42, and rarely above 1.50. The essential cycles illustrated in **Figures 1** and **7** thus can be tentatively understood as cycles going from 1.00 at the bottom to 1.50 at the top, but establishing normative ranges for ramified cells is another challenge for future fractal analysts (Soltys et al., 2001, 2005; Karperien, 2004; Jelinek et al., 2008; Hinwood et al., 2012). Various studies have shown that the cells with the peak values for complexity are both highly ramified and in compromised environments (i.e., ramified cells in uncompromised environments had slightly lower D_Bs). In some studies these were cells with many medium to long, hypertrophied processes. This configuration may be in addition to, or related to, a phenomenon known as "hyper-ramification," due to ramified cells subtly responding to noxious stimuli such as chronic stress (Hinwood et al., 2012).

Potentially relevant to quantitating hyper-ramification is that many methods depend on changes in markers constitutively expressed at low or undetected levels then upregulated during responses to compromise such as chronic stress. This opens up the possibility of morphological information being underrepresented in ramified cells being used as comparisons to identify activated cells. Further research about the extent to which a cell has become hyper-ramified, vice having possibly had more existing detail made visible (e.g., as revealed by upregulated MHC-II in the absence of actual morphological changes) is required (Kanaan et al., 2010; Kettenmann et al., 2011; Hinwood et al., 2012).

Differences according to experimental paradigm

Over and above the finding of a fundamental relative pattern hangs the issue of the actual differences in fractal dimensions found with different investigations. There are important practical considerations in fractal analysis to consider that may underlie such differences. Accordingly, below we discuss several factors affecting microglial morphology that may be relevant to fractal analysis.

Downward shift in D_B for microglia grown in the laboratory. The D_B for cultured microglia showed the fundamental relative pattern for microglia described above, but actual values tended to be lower than for other protocols. This may be related to certain differences that distinguish these cells from other preparations due to the culture environment and have the potential to affect the results of fractal analysis. Such cells, depicted in the drawings in **Figure 6**, typically look different from other microglia. On ramified cells, secondary branches are often less apparent or appear as extremely fine extensions, and cellular contours generally appear smoother. Lamellopodia are frequently more evident, and hairy-looking fringes, not normally detected in light microscope examinations of microglia from tissue, may be seen. The drawings show morphologies from unstimulated (left) to becoming activated (right) but lack an initially rounded morphology that immature cells in culture often have before ramifying. In addition, depending on many conditions, cultured microglia adopt other morphologies not shown in these drawings (de Groot et al., 1992; Bohatschek et al., 2001; Kettenmann et al., 2011; Olah et al., 2011).

Some of the differences such as seen with lamellopodia translate into less detail than is typical of microglia in tissue and lead to a lower D_F for ramified cells in particular. Our lab did find in one investigation that the D_B of cultured cells increased as subjective ratings of activation increased. This result was attributed to a technical issue that rendered secondary branches invisible on many of the ramified cells (Karperien, 2004). The cytoskeletal reorganization seen in microglia is known to distinguish microglial ramification from ramification of other cells, so it might be informative to separate cytoskeletal rearrangements from strictly membrane features (Faff et al., 1996; Tanaka et al., 1999; Faff and Nolte, 2000; Ohsawa et al., 2000). Perhaps comparing cytoskeletal components in cultured and *in vivo* microglia would eliminate the difference, or perhaps the difference is indeed inherent to the spatial context of microglia in the culture environment. In this respect, there are a host of factors that influence microglial morphology in culture (e.g., the presence of astroglia). In general, work is needed to confirm if the D_F is lower in other types of fractal analysis and to further clarify quantifiable differences between *in vitro* and *in vivo* morphologies with respect to different microglia classes and interpretation of microglia cycling.

FIGURE 6 | Drawings of representative microglia grown in the laboratory. Cells grown in culture tend to appear different than microglia found in live animals. Art by Thomas R. Roy.

The unknown influence of species on the D_F. Another element that may have been a factor in different studies reporting different values from fractal analysis is species. Microglia are presumed to reside in all mammals and have been found in several other vertebrates and invertebrates (e.g., birds, frogs, fish, snails, and leeches) (Dowding et al., 1991; Dowding and Scholes, 1993; Sonetti et al., 1994; Magazine et al., 1996; Dobrenis, 1998; Bernhardi and Nicholls, 1999; McMenamin, 1999; Navascues et al., 2000), which has led many researchers to generally classify microglial morphology assuming the fundamentals apply across species. But this assumption is questionable because microglia from one species are not necessarily comparable to microglia from another. In some cases, significant differences in various features have been noted even between strains of one species (Humphrey and Moore, 1995; Klyushnenkova and Vanguri, 1997). Microglia differ in how they are distributed in any particular species (Hutchins et al., 1990; Andjelkovic et al., 1998; Maslinska et al., 1998; Rezaie and Male, 1999; Rezaie et al., 1999; Navascues et al., 2000; Male and Rezaie, 2001); the evidence suggests that some amphibians and rodents have smaller, and some fish considerably larger, proportions than humans (Lawson et al., 1990; Dowding et al., 1991; Sonetti et al., 1994). Other differences have been found in function and staining, as well as fundamental morphology (Chen et al., 2002; Hayakawa et al., 2005; Jinno and Yamada, 2011). A difference affecting ramified microglia in particular is that processes branch subtly differently according to species (Finch et al., 2002). Broadly speaking, any of a host of species-related differences in microglia may have accounted for some of the difference across paradigms, but the significance of species differences for fractal analysis is unknown. A future challenge for fractal analysts, then, is to explore differences amongst microglia from different species.

Branching angle. The difference noted above in microglial branching angle with species deserves further consideration. It is known that microglia tend to sprout essentially orthogonally near their middle and end, filling the outer portion of the spread of processes with nearly perpendicular branch points. They are more orthogonal than astroglia, for instance, which tend to branch more acutely (Karperien, 2001c, 2004; Jelinek and Karperien, 2008). Branching angle in general may have special relevance to fractal analysis of microglia, because not just the degree of

branching but in particular branching angle influences both flow within branched structures and the D_B (Hahn et al., 2005).

Potential influences of texture and space. Another factor that may have contributed to differences in the D_F is texture. Microglia respond to textures such as glass and various biomaterials (Dobrenis, 1998; Tanaka et al., 1999; de Groot et al., 2000; Wollmer et al., 2001). Research into mechanisms behind the rejection of biomedical implants has shown that microglia respond to coatings used on electrodes and implants, for instance (Leung et al., 2008). Perhaps future applications of fractal analysis will move our understanding of biomedical implant rejection forward, but whether or not fractal analysis is sensitive to the effects of various textures is currently unknown.

Space may also have accounted for some differences. Some morphological variability of microglia grown in the laboratory and identified in tissue is known to be accounted for by 3-dimensional space and how microglia orient themselves in it. Given room, for instance, the processes of microglia grown in culture tend to avoid each other, and under astrocyte layers, whole microglia appear flatter than cells above, and rounder in more open than confined spaces (Dobrenis, 1998; Tanaka et al., 1999; de Groot et al., 2000; Wollmer et al., 2001). A topic currently being investigated is how microglia in the human CNS are oriented with respect to each other and with respect to neurons, and how this influences brain function (e.g., in autism in humans). Microglia are more dynamic in space and time than neurons and may therefore play important roles in real-time signal processing (Morgan et al., 2012). One study has used both 2D and 3D data to analyze the D_F of microglia in pathological scenarios, and as this technology continues to evolve (Sheets et al., 2010; Xiao et al., 2011), fractal analysis may prove valuable for modeling and understanding the significance of orientation in space and interdigitation amongst these mutually-existing branched structures (Pow et al., 1989; Hahn et al., 2005). Currently, however, this is another topic that needs to be explored.

Location and subtype. Other factors that affect microglial morphology are location and subtype. In CNS tissue samples, microglia adopt different conformations depending on where they are from and local characteristics within that area, to the extent that investigators formally classify subcategories based on location and different staining characteristics (Lawson et al., 1990; Mittelbronn et al., 2001). For instance, whereas microglia in gray matter are usually more stellate, hugging neuron cell bodies, sprawling alongside of oligodendroglia, or intertwining with astroglial processes, when in intra- and inter-cortical tracts, they are more often found in a bipolar, lengthwise orientation with a leading process, suggesting they may be in motion and giving them the appearance of a less ramified cell even though they are not "activated" (Kreutzberg, 1995; Rezaie et al., 1997; Rezaie and Male, 1997, 1999; Bayer et al., 1999; Radewicz et al., 2000; Male and Rezaie, 2001). Perivascular microglia, too, adopt conformations distinguishing them from other microglia. Characteristically elongated and conforming to the surfaces of the vessels they lie along like lichens clinging along branches, they are less stellate than ramified microglia networked throughout the CNS. A relatively sprawling and untortuous morphology of ramified microglia can be observed in the retina, where they may be found in a horizontal laminar distribution from the nerve fiber layer to the outer plexiform layer (Liu et al., 2012). One pilot investigation of differences reported in the literature suggests the D_B can be affected by the location at which microglia are found in the brain (Karperien, 2004). More and more phenoytpes of microglia are being proposed (Olah et al., 2011) and the details of such differences still need to be charted.

THE LANGUAGE OF MORPHOLOGICAL CLASSIFICATION AND HOW IT RELATES TO THE D_B

In addition to characterizing how the D_F changes with morphology, research has looked at how the D_F corresponds to subjective morphological categories. Underpinning virtually all recent microglial research is the system of morphological/functional categories we discussed in the introduction to this review. In the scientific literature, people describing microglial morphology use primarily words grounded in this model of *reactive microgliosis*, a term for the response of microglia to compromise in the CNS (Streit, 2000). Typically, this system includes ramified and activated categories and forms between, which have variously been referred to as "intermediately activated," "bipolar," "rod-like," "hypertrophied," and "bushy." (Note that "rod" cells are presumed to be fused microglia) (Ziaja and Janeczko, 1999; Streit, 2000). Along these same lines, **Figure 7** lists an operational definition used in research carried out in our lab (Karperien, 2004). The list synthesizes descriptions from various authors of what microglia look like at places along the cycle. Investigators commonly use systems of two, three, or four categories according to their needs, and usually specify features of each category in their operational definitions but do not always publish their precise definitions.

With no classification standard, from one published study to the next, definitions of microglial morphology differ, categories overlap, and comparisons are awkward. Moreover for people applying objective criteria in order to classify microglia, shifts and overlaps in categories are often evident but difficult to quantify. At worst, microglia may be assessed using techniques and categories insensitive to certain subtle variations and disruptions that may have grave consequences for people harboring them. Thus, quantitating that model is of great interest in neuroscience.

D_B discriminates a 4-category classification

Studies of how D_Fs correspond to visually assigned morphological categories have so far yielded three main results. (1) Fractal dimensions discriminated between all categories in four-category systems. (2) D_Fs did not consistently discriminate three-category systems, failing to differentiate intermediate from ramified cells in some studies. And (3), despite failing to discriminate three-category systems, they nevertheless discriminated other subtle and visually undetectable differences within a category, ramified normal and ramified compromised (Soltys et al., 2001; Karperien, 2004; Karperien et al., 2005).

These results suggest that classifying microglia using a three-category system ignores differences in complexity that a

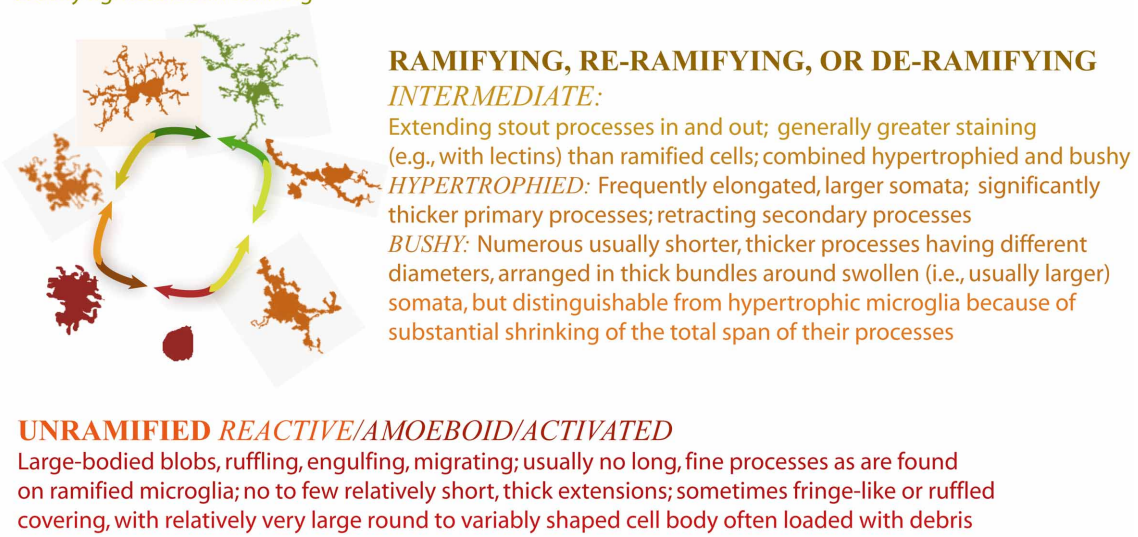


FIGURE 7 | Criteria for morphological categories of microglia.

four-category system detects. That is, where people subjectively group some "bushy" and some "hypertrophied" cells with ramified, the D_F objectively draws lines quantitating these categories (as well as activated). Moreover, that fractal dimensions discriminate between subtle differences within ramified cells from compromised vs. normal tissue suggests a fifth category, presumably the peak cells noted above, may be quantifiable.

These results may be particularly relevant to counting cells classified into a category. Because the changes in microglial morphology occur in so many domains and reflect so many influences and events, finding the same number of cells in a morphological category might obscure subtle but important differences between samples in what the cells are doing. By clarifying the functional and morphological correlates of differences in complexity within categories, fractal analysis may be a major contributor to making models of microglial function more quantitatively sensitive to subtle but meaningful changes in morphology. Further study of the quantifiable differences may also lead to identification of rules that will assist human observers in categorizing microglia.

QUANTITATING RESPONSES

Another potential application of fractal analysis is objectively quantitating effects of treatments without classifying cells. Researchers have successfully used D_Fs to quantitate responses of cultured microglia to different treatments including the addition and removal of as well as graded levels of activating stimuli (Jelinek et al., 2008), subtle effects of LPS and naloxone when those effects were not visually detectable (Karperien et al., 2008a), and nonsteroidal anti-inflammatories in models of age-related disease (Varvel et al., 2009).

Other investigations have demonstrated the potential to use fractal analysis for quantitating pathological status based on the average D_F and on the overall distribution of complexity in samples containing microglia (Soltys et al., 2005). One pilot study (Karperien, 2004) indicated that the D_B clustered within distinct ranges respectively for control, acute, and chronic responses to spinal cord injury. This was based on a very small convenience sample, so the results should be considered with caution. Another study showed that for microglia in postmortem tissue, the average D_B distinguished control from both overtly and subtly pathological human tissue (Karperien et al., 2008c). Additional work suggests that the distribution itself of complexity can provide important clues about pathological status, where rather than an average, the proportions of cells with certain constellations of quantitative features may provide information about what is happening in a particular location at a particular time (Karperien, 2004).

Injury, disease, and drugs

Further work is necessary to verify and expand this small body of work, but the results so far suggest that the discriminating capacities of the D_B may be powerful for gauging microglial activation in incipient or ongoing responses associated with injury, disease, and drug use (Soltys et al., 2005). This could affect clinical decision-making as well as laboratory work. Based on the work described above, one application that could be explored is measuring the fractal dimension of microglia in spinal fluid to monitor and quantify pathological status, stages of an ongoing response after injury, or effects of drugs in patients (e.g., for schizophrenia) (Nikkila et al., 1999; Stoll et al., 2006; Blackbeard

et al., 2007). Another area we can speculate that fractal analysis of microglia may be helpful in is in making mathematical models to predict drug responses. Whether or not average D_Bs or profiles of the distribution of complexity will be useful for differentiating different diseases or states by quantifiably characterizing the numbers of various types of morphology without the need for subjective classification is a question for future research.

Tracking microglial morphology

Another area with promise for the future is building on recent work in tracking microglia with *in vivo* imaging.

Diabetes. In diabetes and glycemic status in human and animal models microglia have been implicated as playing important roles in pathological sequelae (Chahed et al., 2010; Polito et al., 2011). Liu et al. (2012), for example, have tracked the association of retinal microgliosis with retinal ganglion cell degeneration in rodents. Several studies of rodent models of diabetes have shown that the function and morphology of microglia in different areas of the CNS can be affected (e.g., marked shortening of processes) by compromised glycemic status without cells becoming activated (Gaucher et al., 2007; Arden and Sivaprasad, 2011). Based on work cited earlier in this paper describing how similar changes affect the D_B, it is likely that the types of changes that have been noted in diabetic sequelae are likely to influence the D_B, suggesting another potential use of fractal analysis that could be developed. This may be especially relevant with respect to diabetes research for live monitoring, but will depend on *in vivo* visualization techniques being further developed.

Alcohol and thiamine deficiency. The link to diabetes research brings up the point that diet and nutritional status in general are factors that can have multiple, compound effects on microglia. It is well documented that a host of factors in the milieu affect microglia (e.g., acidity and temperature) (Faff and Nolte, 2000; Rezaie et al., 2002). Researchers have shown that microglia in mice respond rapidly to a high fat diet by infiltrating the hypothalamic arcuate nucleus (Yi et al., 2012). Effects on microglia of thiamine deficiency, which may occur in alcoholics or nutritionally deprived people, have also been studied. Like all cells, microglia depend on energy, thus can become dysregulated by metabolic compromise (e.g., ATP may be unavailable or lactate may accumulate with reduced thiamine dependent enzymes) (Park et al., 1999). Researchers have shown that microglia in rats are exquisitely sensitive to such compromise, changing both their profile of proteins expressed and their morphology (Zhao et al., 1996), but not necessarily being classifiable as responding. It has been reported, for instance, that with thiamine deficiency, especially perivascular microglia become "plump," although still have processes, prior to and probably mediating eventual overt neuronal damage (Sonetti et al., 1994; Dickson, 1999; Todd and Butterworth, 1999a,b; Calingasan and Gibson, 2000a,b). Again, because the changes noted are subtle and difficult to objectively classify but also the kinds typically quantifiable by the D_B, this may be another area that could be explored by fractal analysis.

AGE-RELATED CHANGES

Microglial morphology in the very young: from rounded to ramified

Another subject in which fractal analysis has been used to quantitate typical changes in microglia is early development. Microglia play many roles in neurodevelopment, at different times, places, and forms. Proposed roles include "fine-tuning" CNS structure, promoting axonal growth, directing neuronal migration, determining neuronal phenotypes, interacting with oligodendrocytes in myelination, influencing vascularization, and disposing of debris and normally dying cells. Through epigenetic, structural, and other mechanisms, they may also contribute to developmental disruptions and subsequent neuropathology such as in fetal alcohol spectrum disorder, cerebral palsy, Down's syndrome, autism, and schizophrenia (Hao et al., 2001a,b; Aarum et al., 2003; McAllister and Miller, 2010; Maezawa et al., 2011; Paolicelli et al., 2011). There are many opportunities to test the ability of fractal analysis to contribute to our knowledge of early development.

In humans, rather than there being one general early microglial morphology, there are characteristic temporal and spatial patterns. Many of these appear to be very much the purview of fractal analysis. For instance, microglia appear very early and then colonize the CNS mainly during the second and to some extent the third trimester of fetal development (in rodents, microglia start to populate the CNS around or after birth). In tissue from humans gestated a few weeks, investigators first see "rounded" cells, then "intermediately ramified" cells migrating along white matter tracts and blood vessels; then, later in development, mainly fully ramified cells. This basic temporal pattern is superimposed on several spatial patterns—different brain areas are populated at different times but according to usually the same basic morphologic sequence. Orlowski et al. (2003) have demonstrated an elegant quantification of this progression using fractal analysis as cells becoming increasingly complex over time. There is evidence that distinct morphological and functional subtypes of microglia appear even in the earliest stages of development, which requires further investigation (Hutchins et al., 1990; Andjelkovic et al., 1998; Cuadros and Navascues, 1998; Maslinska et al., 1998; Alliot et al., 1999; Navascues et al., 2000; Rezaie et al., 2002).

Another topic that could be explored with fractal analysis is variation in different areas. Billiards et al. (2006) have found that human fetus and infant cerebral white matter is densely populated with "intermediate" and "amoeboid" microglia and that there is a transiently increased density of activated microglia (i.e., cells in the typical morphology marked by CD68) in the cerebral white matter prior to 37 weeks gestation. This peak has been proposed to translate into a window of increased vulnerability to injury mediated by microglia (e.g., hypoxic injury underlying cerebral palsy). Fractal analysis may add to our knowledge by quantitating such differences and providing a finer level of knowledge about what cells are doing in different places and times in development.

Rounded cells seen early on in development are generally considered to be "immature" rather than activated. Many authors call a cell "amoeboid," regardless of its origins or maturity, as long as its cell body is amorphous, long, fine processes are absent, and pseudopodia are (usually) present, but other authors object,

saying that immature and unbranched as opposed to reactive microglia differ. It is not clear if the microglia that are first seen as rounded cells are rounded because they are immature or because that is the morphology in which they are best able to migrate through, prune, and clean up the developing CNS. Further, the extent to which microglia deramify and reramify in developing CNS is not known. These are questions for which fractal analysis may be able to provide additional insight.

Microglial morphology in the very old

Not only do microglia change over early development, microglia in the very young are not the same as microglia in the very old. Microglia generally increase from sparse cells with few branches in infancy to a ramified network in adulthood to an increasingly dense network of increasingly "reactive looking" microglia in old age. Primary microglial processes are usually shorter, thicker, and more numerous in adults as compared to infants, and again in nonelderly adults compared to elderly adults. Researchers have also characterized a tendency toward more aberrant features, including twisting and fragmenting of processes, with age (Sheng et al., 1997a,b, 1998a,b; Rozovsky et al., 1998; Sheffield and Berman, 1998; Nichols, 1999; Sheffield et al., 2000; Finch et al., 2002; Yu et al., 2002; Olah et al., 2011).

Within the overall spectrum of age-related changes, other factors such as gender and brain region can further influence microglia and may be reflected in the morphology itself or at least how that morphology is revealed under the microscope. To illustrate, some researchers have found that HLA-DR expression is higher in normal elderly human males than females (but higher in females with Alzheimer's disease), and significantly so only in white matter. In a study of rodents, Morgan and colleagues (1999) found that food restriction lessened age-related increases in complement receptor expression but not MHC-II expression in microglia in the basal ganglia, but food restriction and age did not affect these two measures in the outer molecular layer of the dentate gyrus (Kreutzberg, 1995; Carson et al., 1998; Hurley et al., 1999; Morgan et al., 1999; Overmyer et al., 1999; Ren et al., 1999; Wierzba-Bobrowicz et al., 2000a,b; Cross and Woodroofe, 2001; Kanaan et al., 2010).

The changes of age are accompanied by functional changes in elderly adults, whereby from development to old age, microglia lose their ability to protect and become more likely to react or more likely to react abnormally. Why microglia become "senescent" or "dystrophic" is unknown, but it has been suggested that the changes may be at least partly mediated by and contribute to the changing hormonal environment of aging as well as a reflection of accumulating pathology.

Modeling studies suggest that the changes described in the literature would affect the D_B. For instance, in space-emulating models simulating process swelling in isolation from other changes—i.e., changing only the diameter of primary branches—as process diameter increased relative to soma diameter, the measured D_B decreased despite the fact that the complexity input to the models did not. Another factor relevant to age-related changes that affected results was the number of primary branches. The D_B tended to increase with the number of primary branches even when the fundamental complexity of the branches was not changed. Tortuousness was another factor having an effect: in keeping with results for known fractal patterns, the average D_B for more tortuous models generally deviated more from theoretical than for straighter patterns (e.g., ∼5 vs. 3%) and models with curled rather than sprawled processes had higher D_Bs (Jelinek et al., 2002; Karperien, 2004; Karperien et al., 2005; Jelinek and Karperien, 2008).

Little research has been published describing actual measurements of these changes with fractal analysis. One investigation measuring the D_B for microglia from elderly human brain illustrated that the distribution of complexity in healthy elderly human brain differed from that for pathological elderly human brain (e.g., for the D_B range from 1.30 to 1.43, pathological brain had more cells, and for the range from 1.40 to 1.48 healthy elderly had more), and that microglia in the healthy brain had a different complexity profile than more typically noted for younger adult brain (Karperien, 2004). Future research exploring age-related changes in microglia and correlates in the D_B may shed light on the topic of age-related disease and dysfunction.

RESULTS OF SUPPLEMENTARY MEASURES

Lacunarity and other morphometrics

Virtually all of the fractal analysis studies in the literature compared D_Fs against other metrics. The utility of the D_F varied with different methods. In many cases where multiple measures were compared, the D_F was the most consistently useful measure of, and in some cases, the only measure refined enough to detect, the most subtle and difficult to identify changes. One consistent result was that D_Fs were generally complemented by various measures of lacunarity, where using them together gave the most sensitive correlation to morphological categories (Soltys et al., 2001, 2005; Orlowski et al., 2003; Karperien, 2004).

In some work, measures of the size and shape of the 2-dimensional space occupied by a cell were less sensitive to differences in microglial morphology, where the D_B was superior to Λ in detecting the subtlest changes, but in some studies Λ was shown to be especially good at detecting changes in soma shape and size that D_Fs did not detect (Soltys et al., 2001, 2005; Orlowski et al., 2003; Karperien, 2004).

Some investigations found some D_Fs to be correlated with certain features, but the D_B in particular was generally deemed not superfluous with other measures. In one study, the D_B was correlated with Λ, but only for cells at certain levels of activation. The DB was correlated with the number of pixels in some cases, but generally not strongly correlated with the density of pixels, and the number of pixels was not as useful an index of function-related change as was the DB. Modeling studies showed that Λ was sensitive to many of the changes affecting the D_B but as an independent measure; the two were not consistently correlated, except for tortuousness, for which they were strongly negatively correlated (Karperien, 2004; Karperien et al., 2008c; Jelinek et al., 2011).

Multifractality and local dimensions

Modeling and other studies using multifractal measures suggest mutlifractality and local dimension analysis may be useful for identifying particularly intermediate forms of microglia.

Modeling studies have shown that microglia modeled with the same branching parameters on all branches did not scale as multifractals, but models with disparate parts were more likely to scale as multifractals and showed variation in the local dimension. These results are consistent with other results (Karperien, 2004; Karperien et al., 2011; Jelinek et al., 2013) indicating that multifractal scaling is rare overall but that ramified microglia are more likely than others to show multifractal scaling, and when they do, to have disparate parts. One challenge for the future, then, is to clarify the nature of multifractal scaling in microglia, and pursue the potential for multifractal analysis to reveal cells in a transitional state having elements of more than one typical level of activation.

SUMMARY AND CONCLUSION

In summary, microglia have emerged as tiny wielders of formidable power and are now serious targets for study and intervention in essentially anything that goes on in the nervous system. We know well that their form and function are tightly coupled, but to take our investigations and therapies to the next level, we need to know this more quantitatively. An obstacle to moving forward is the very nature of microglial morphology. Microglia undergo changes in a host of domains along multiple time scales, changes that are indeed challenging to distil into practical metrics. Fractal analysis, a well-established technique in neuroscience, is coming into its own as a tool to help quantitate our models of microglial form and function. Able to quantify not only gross morphological differences but also subtleties of microglial morphology that may hold clues to understanding normal and pathological CNS, it has provided a fundamental quantitative model relating complexity to morphology and has provided insight into how to quantitate and improve our classification systems.

It has begun to provide insight into how to answer the questions put to the reader at the beginning of this review, and in the future, combined with measures of other features from complementary morphological analysis techniques may provide full answers. Questions posed about the point at which to consider a cell to have crossed over from ramified to activated, or about whether it is de- or re-ramifying, for instance, might be investigated by combining live video techniques with the power of fractal analysis to discriminate and quantitate subtle morphological differences. Questions posed about subtle differences in branching angle and other features not accounted for in traditional classification systems might be investigated through fractal and multifractal methods combined with traditional metrics such as soma size. Questions about interpreting the distribution of different morphologies or other features of microglia in space might also be investigated using fractal analysis of sections and grayscale analysis rather than individual cells in binary images.

As noted throughout this paper, the future holds many challenges on several levels. Theoretically, 3-dimensional and 2-dimensional methods should be comparable, but this remains to be tested. Key issues that need to be explored in the laboratory are the application of 2-dimensional fractal analysis methods to live video monitoring and comparisons to 3-dimensional fractal analysis methods in order to quantitate and understand microglial form and function in real time and space. Nonetheless, complexity measured by box-counting fractal analysis of 2-dimensional images has been shown to be a robust and powerful measure of the subtlest changes in one feature of microglial morphology that is associated with function. Basic groundwork has been laid to move forward in some areas and explore the utility of fractal analysis in clinical applications where these subtlest of changes may matter such as tracking disease progression, healing after trauma, drug responses, implant rejection, etc. Indeed it may be the case that clinical application will precede full knowledge of the implications of fractal analysis.

REFERENCES

Aarum, J., Sandberg, K., Haeberlein, S. L., and Persson, M. A. (2003). Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15983–15988.

Ahammer, H. (2011). Higuchi dimension of digital images. *PLoS ONE* 6:e24796. doi: 10.1371/journal.pone.0024796

Alliot, F., Godin, I., and Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* 117, 145–152.

Andjelkovic, A. V., Nikolic, B., Pachter, J. S., and Zecevic, N. (1998). Macrophages/microglial cells in human central nervous system during development: an immunohistochemical study. *Brain Res.* 814, 13–25.

Arden, G. B., and Sivaprasad, S. (2011). Hypoxia and oxidative stress in the causation of diabetic retinopathy. *Curr. Diabetes Rev.* 7, 291–304.

Baatz, M., Schäpe, A., Schmidt, G., Athelogou, M., and Binnig, G. (2005). "Cognition network technology: object orientation and fractal topology in biomedical image analysis. Method and applications," in *Fractals in Biology and Medicine*, eds G. A., Losa, D., Merlini, T. F. Nonnenmacher, and E. R. Weibel (Basel: Birkhäuser Verlag Basel), 67–74.

Baksi, B. G., and Fidler, A. (2012). Image resolution and exposure time of digital radiographs affects fractal dimension of periapical bone. *Clin. Oral Investig.* 16, 1507–1510.

Banati, R. B., Goerres, G. W., Myers, R., Gunn, R. N., Turkheimer, F. E., Kreutzberg, G. W., et al. (1999). [11C](R)-PK11195 positron emission tomography imaging of activated microglia *in vivo* in Rasmussen's encephalitis. *Neurology* 53, 2199–2203.

Bayer, T. A., Buslei, R., Havas, L., and Falkai, P. (1999). Evidence for activation of microglia in patients with psychiatric illnesses. *Neurosci. Lett.* 271, 126–128.

Bernhardi, R. V., and Nicholls, J. G. (1999). Transformation of leech microglial cell morphology and properties following co-culture with injured central nervous system tissue. *J. Exp. Biol.* 202(Pt 6), 723–728.

Billiards, S. S., Haynes, R. L., Folkerth, R. D., Trachtenberg, F. L., Liu, L. G., Volpe, J. J., et al. (2006). Development of microglia in the cerebral white matter of the human fetus and infant. *J. Comp. Neurol.* 497, 199–208.

Blackbeard, J., O'Dea, K. P., Wallace, V. C., Segerdahl, A., Pheby, T., Takata, M., et al. (2007). 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.

Bohatschek, M., Kloss, C. U., Kalla, R., and Raivich, G. (2001). *In vitro* model of microglial deramification: ramified microglia transform into amoeboid phagocytes following addition of brain cell membranes to microglia-astrocyte cocultures. *J. Neurosci. Res.* 64, 508–522.

Busse, S., Busse, M., Schiltz, K., Bielau, H., Gos, T., Brisch, R., et al. (2012). Different distribution patterns of lymphocytes and microglia in the hippocampus of patients with residual versus paranoid schizophrenia: Further evidence for disease course-related immune alterations? *Brain Behav. Immun.* 26, 1273–1279.

Calingasan, N. Y., and Gibson, G. E. (2000a). Dietary restriction attenuates the neuronal loss, induction of heme oxygenase-1 and blood-brain barrier breakdown induced by impaired oxidative metabolism. *Brain Res.* 885, 62–69.

Calingasan, N. Y., and Gibson, G. E. (2000b). Vascular endothelium is a site of free radical production and inflammation in areas of neuronal loss in thiamine-deficient brain. *Ann. N.Y. Acad. Sci.* 903, 353–356.

Carson, M. J., Reilly, C. R., Sutcliffe, J. G., and Lo, D. (1998). Mature microglia resemble immature antigen-presenting cells. *Glia* 22, 72–85.

Chahed, S., Leroyer, A. S., Benzerroug, M., Gaucher, D., Georgescu, A., Picaud, S., et al. (2010). Increased vitreous shedding of microparticles in proliferative diabetic retinopathy stimulates endothelial proliferation. *Diabetes* 59, 694–701.

Chen, L., Yang, P., and Kijlstra, A. (2002). Distribution, markers, and functions of retinal microglia. *Ocul. Immunol. Inflamm.* 10, 27–39.

Chhabra, A., and Jensen, R. V. (1989). Direct determination of the f(α) singularity spectrum. *Phys. Rev. Lett.* 62, 1327–1330.

Colton, C. A., Chernyshev, O. N., Gilbert, D. L., and Vitek, M. P. (2000). Microglial contribution to oxidative stress in Alzheimer's disease. *Ann. N.Y. Acad. Sci.* 899, 292–307.

Crews, F., Nixon, K., Kim, D., Joseph, J., Shukitt-Hale, B., Qin, L., et al. (2006). BHT blocks NF-kappaB activation and ethanol-induced brain damage. *Alcohol. Clin. Exp. Res.* 30, 1938–1949.

Cross, A. K., and Woodroofe, M. N. (2001). Immunoregulation of microglial functional properties. *Microsc. Res. Tech.* 54, 10–17.

Cuadros, M. A., and Navascues, J. (1998). The origin and differentiation of microglial cells during development. *Prog. Neurobiol.* 56, 173–189.

Dailey, M. E., and Waite, M. (1999). Confocal imaging of microglial cell dynamics in hippocampal slice cultures. *Methods* 18, 222–230, 177.

de Groot, C. J., Huppes, W., Sminia, T., Kraal, G., and Dijkstra, C. D. (1992). Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive in situ hybridization and immunoperoxidase techniques. *Glia* 6, 301–309.

de Groot, C. J., Montagne, L., Janssen, I., Ravid, R., Van Der Valk, P., and Veerhuis, R. (2000). Isolation and characterization of adult microglial cells and oligodendrocytes derived from postmortem human brain tissue. *Brain Res. Brain Res. Protoc.* 5, 85–94.

Dickson, D. W. (1999). Microglia in Alzheimer's disease and transgenic models. How close the fit? *Am. J. Pathol.* 154, 1627–1631.

Dobrenis, K. (1998). Microglia in cell culture and in transplantation therapy for central nervous system disease. *Methods* 16, 320–344.

Donnelly, D. J., Gensel, J. C., Ankeny, D. P., Van Rooijen, N., and Popovich, P. G. (2009). An efficient and reproducible method for quantifying macrophages in different experimental models of central nervous system pathology. *J. Neurosci. Methods* 181, 36–44.

Dowding, A. J., Maggs, A., and Scholes, J. (1991). Diversity amongst the microglia in growing and regenerating fish CNS: immunohistochemical characterization using FL.1, an anti-macrophage monoclonal antibody. *Glia* 4, 345–364.

Dowding, A. J., and Scholes, J. (1993). Lymphocytes and macrophages outnumber oligodendroglia in normal fish spinal cord. *Proc. Natl. Acad. Sci. U.S.A.* 90, 10183–10187.

Eder, C., Klee, R., and Heinemann, U. (1998). Involvement of stretch-activated Cl- channels in ramification of murine microglia. *J. Neurosci.* 18, 7127–7137.

Faff, L., and Nolte, C. (2000). Extracellular acidification decreases the basal motility of cultured mouse microglia via the rearrangement of the actin cytoskeleton. *Brain Res.* 853, 22–31.

Faff, L., Ohlemeyer, C., and Kettenmann, H. (1996). Intracellular pH regulation in cultured microglial cells from mouse brain. *J. Neurosci. Res.* 46, 294–304.

Fernandez, E., and Jelinek, H. F. (2001). Use of fractal theory in neuroscience: methods, advantages, and potential problems. *Methods* 24, 309–321.

Finch, C. E., Todd, E. M., Rozovsky, I., Xie, Z., Weindruch, R., and Prolla, T. (2002). "Microglia and aging in the brain," in *Microglia in the Regenerating and Degenerating Central Nervous System*, ed W. J. Streit (New York, NY: Springer), 275–305.

Garcia-Segura, L. M., Chowen, J. A., and Naftolin, F. (1996). Endocrine glia: roles of glial cells in the brain actions of steroid and thyroid hormones and in the regulation of hormone secretion. *Front. Neuroendocrinol.* 17:5. doi: 10.1006/frne.1996.0005

Gaucher, D., Chiappore, J. A., Paques, M., Simonutti, M., Boitard, C., Sahel, J. A., et al. (2007). Microglial changes occur without neural cell death in diabetic retinopathy. *Vision Res.* 47, 612–623.

Hahn, H. K., Manfred, G., and Peitgen, H.-O. (2005). "Fractal aspects of three-dimensional vascular constructive optimization," in *Fractals in Biology and Medicine*, eds G. A. Losa, D. Merlini, T. F. Nonnenmacher, and E. R. Weibel (Basel: Birkhäuser Verlag Basel), 55–66.

Hao, A. J., Dheen, S. T., and Ling, E. A. (2001a). Induction of cytokine expression in the brain macrophages/amoeboid microglia of the fetal rat exposed to a teratogen. *Neuroreport* 12, 1391–1397.

Hao, A. J., Dheen, S. T., and Ling, E. A. (2001b). Response of amoeboid microglia/brain macrophages in fetal rat brain exposed to a teratogen. *J. Neurosci. Res.* 64, 79–93.

Hayakawa, T., Angata, T., Lewis, A. L., Mikkelsen, T. S., Varki, N. M., and Varki, A. (2005). A human-specific gene in microglia. *Science* 309, 1693.

Hinwood, M., Tynan, R. J., Charnley, J. L., Beynon, S. B., Day, T. A., and Walker, F. R. (2012). Chronic sress induced remodeling of the prefrontal cortex: structural re-organization of microglia and the inhibitory effect of minocycline. *Cereb. Cortex* 22, 1442–1454.

Humphrey, M. F., and Moore, S. R. (1995). Strain differences in the distribution of NDP-ase labelled microglia in the normal rabbit retina. *Glia* 15, 367–376.

Hurley, S. D., Walter, S. A., Semple-Rowland, S. L., and Streit, W. J. (1999). Cytokine transcripts expressed by microglia *in vitro* are not expressed by ameboid microglia of the developing rat central nervous system. *Glia* 25, 304–309.

Hutchins, K. D., Dickson, D. W., Rashbaum, W. K., and Lyman, W. D. (1990). Localization of morphologically distinct microglial populations in the developing human fetal brain: implications for ontogeny. *Brain Res. Dev. Brain Res.* 55, 95–102.

Inoue, K. (2006). ATP receptors of microglia involved in pain. *Novartis Found. Symp.* 276, 263–272, discussion 273–281.

Jelinek, H. F., De Mendonça, M. B. D. M., Fernando, O., Garcia, C. A. D. A., Nogueira, R. A., Soares, J. V. B., et al. (2010). Fractal analysis of the normal human retinal vasculature. *Internet J. Ophthalmol. Vis. Sci.* 8:2. doi: 10.5580/1a6a

Jelinek, H., Karperien, A., Buchan, A., and Bossomaier, T. (2008). Differentiating grades of microglia activation with fractal analysis. *Complexity International* 12, Paper ID: msid14.

Jelinek, H. F., Elston, N., and Zietsch, B. (2005). "Fractal analysis: pitfalls and revelations in neuroscience," in *Fractals in Biology and Medicine*, eds G. A. Losa, D. Merlini, T. F. Nonnenmacher, and E. R. Weibel (Basel: Birkhäuser Verlag Basel), 85–94.

Jelinek, H. F., and Fernandez, E. (1998). Neurons and fractals: how reliable and useful are calculations of fractal dimensions? *J. Neurosci. Methods* 81, 9–18.

Jelinek, H. F., and Karperien, A. (2008). Microglia modelling and analysis using L-systems grammar. *Biosignals* 2, 289–294.

Jelinek, H. F., Karperien, A., Cornforth, D., Cesar, R. M. J., and Leandro, J. (2002). "MicroMod—an L-systems approach to neuron modelling," in *6th Australasia-Japan Joint Workshop, Australia's National University* (Canberra, ACT), 156–163.

Jelinek, H. F., Karperien, A., and Milosevic, N. T. (2011). "Lacunarity analysis and classification of microglia in neuroscience," in *Proceedings of the 8th European Conference on Mathematical and Theoretical Biology, European Society for Mathematical and Theoretical Biology (ESMTB)* (Cracow).

Jelinek, H. F., Milošević, N. T., Karperien, A., and Krstonošić, B. (2013). "Box-counting and multifractal analysis in neuronal and glial classification," in *Advances in Intelligent Control Systems and Computer Science*, ed I. Dumitrache (Berlin, Heidelberg: Springer), 177–189.

Jinno, S., and Yamada, J. (2011). Using comparative anatomy in the axotomy model to identify distinct roles for microglia and astrocytes in synaptic stripping. *Neuron Glia Biol.* 7, 55–66.

Kam, Y., Karperien, A., Weidow, B., Estrada, L., Anderson, A. R., and Quaranta, V. (2009). Nest expansion assay: a cancer systems biology approach to *in vitro* invasion measurements. *BMC Res. Notes* 2:130. doi: 10.1186/1756-0500-2-130

Kanaan, N. M., Kordower, J. H., and Collier, T. J. (2010). Age-related changes in glial cells of dopamine midbrain subregions in rhesus monkeys. *Neurobiol. Aging* 31, 937–952.

Kane, C. J., Phelan, K. D., Han, L., Smith, R. R., Xie, J., Douglas, J. C., et al. (2011). Protection of

neurons and microglia against ethanol in a mouse model of fetal alcohol spectrum disorders by peroxisome proliferator-activated receptor-gamma agonists. *Brain Behav. Immun.* 25(Suppl. 1), S137–S145.

Karperien, A. (2001a). *FracLac for ImageJ; JavaDoc, source code, and jar, (Version 2.5) [Software]*. Albury, NSW: Charles Sturt University. Retrieved October 1, 2011. Available from: US National Institutes of Health. http://rsb.info.nih.gov/ij/plugins/fraclac/Frac_Lac.jar

Karperien, A. (2001b). *FracLac User's Guide,* Available Online At: http://rsbweb.nih.gov/ij/plugins/fraclac/FLHelp/Introduction.htm [Accessed 2012].

Karperien, A. (2001c). *MicroMod Biological Modelling Tool (Version 6.0b.) [Software]*. Albury, NSW: Charles Sturt University.

Karperien, A. (2004). *Defining Microglial Morphology; Form, Function, and Fractal dimension.* Albury, NSW: MHlthSci(Hon) bound, Charles Sturt University.

Karperien, A., Jelinek, H. F., and Bossomaier, T. (2008a). Fractal analysis quantitates overt and subtle effects of naloxone and lipopolysaccharide on cultured rat microglia. *Complexity International* 12, Paper ID: msid12.

Karperien, A., Jelinek, H. F., Leandro, J. J., Soares, J. V., Cesar, R. M., and Luckie, A. (2008b). Automated detection of proliferative retinopathy in clinical practice. *Clin. Ophthalmol.* 2, 109–122.

Karperien, A. L., Jelinek, H. F., and Buchan, A. M. (2008c). Box-counting analysis of microglia form in schizophrenia, Alzheimer's disease and affective disorder. *Fractals* 16, 103.

Karperien, A., Jelinek, H. F., and Milošević, N. T. (2011). "Multifractals: a review with an application in neuroscience," in *CSCS18-18th International Conference on Control Systems and Computer Science: Fifth Symposium on Interdisciplinary Approaches in Fractal Analysis IAFA 1.5.* (Bucharest).

Karperien, A., Lucas, C., Depardieu, C., Aurel, G., and Jelinek, H. F. (2005). "Fractal analysis of microglial morphology," in *European Conference on Complex Systems*, 226–227.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Kim, J., Kwon, N., Chang, S., Kim, K. T., Lee, D., Kim, S., et al. (2011). Altered branching patterns of Purkinje cells in mouse model for cortical development disorder. *Sci. Rep.* 1:122. doi: 10.1038/srep00122

Klyushnenkova, E. N., and Vanguri, P. (1997). Ia expression and antigen presentation by glia: strain and cell type-specific differences among rat astrocytes and microglia. *J. Neuroimmunol.* 79, 190–201.

Kozlowski, C., and Weimer, R. M. (2012). An automated method to quantify microglia morphology and application to monitor activation state longitudinally *in vivo*. *PLoS ONE* 7:e31814. doi: 10.1371/journal.pone.0031814

Kreutzberg, G. W. (1995). Microglia, the first line of defence in brain pathologies. *Arzneimittelforschung* 45, 357–360.

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170.

Lee, J. E., Liang, K. J., Fariss, R. N., and Wong, W. T. (2008). *Ex vivo* dynamic imaging of retinal microglia using time-lapse confocal microscopy. *Inves. Ophthalmol. Vis. Sci.* 49, 4169–4176.

Leung, B. K., Biran, R., Underwood, C. J., and Tresco, P. A. (2008). Characterization of microglial attachment and cytokine release on biomaterials of differing surface chemistry. *Biomaterials* 29, 3289–3297.

Liu, S., Li, Z. W., Weinreb, R. N., Xu, G., Lindsey, J. D., Ye, C., et al. (2012). Tracking retinal microgliosis in models of retinal ganglion cell damage. *Invest. Ophthalmol. Vis. Sci.* 53, 6254–6262.

Losa, G. A., Merlini, D., Nonnenmacher, T. F., and Weibel, E. R. (eds.). (2005). *Fractals in Biology and Medicine.* Basel: Birkhäuser Verlag Basel.

Losa, G. N., Theo, F., Merlini, D., Weibel, E. R. (ed.). (1997). *Fractals in Biology and Medicine.* Basel: Birkhauser.

Lyons, S. A., and Kettenmann, H. (1998). Oligodendrocytes and microglia are selectively vulnerable to combined hypoxia and hypoglycemia injury *in vitro*. *J. Cereb. Blood Flow Metab.* 18, 521–530.

Maezawa, I., Calafiore, M., Wulff, H., and Jin, L. W. (2011). Does microglial dysfunction play a role in autism and Rett syndrome? *Neuron Glia Biol.* 7, 85–97.

Magazine, H. I., Liu, Y., Bilfinger, T. V., Fricchione, G. L., and Stefano, G. B. (1996). Morphine-induced conformational changes in human monocytes, granulocytes, and endothelial cells and in invertebrate immunocytes and microglia are mediated by nitric oxide. *J. Immunol.* 156, 4845–4850.

Male, D., and Rezaie, P. (2001). Colonisation of the human central nervous system by microglia: the roles of chemokines and vascular adhesion molecules. *Prog. Brain Res.* 132, 81–93.

Mancardi, D., Varetto, G., Bucci, E., Maniero, F., and Guiot, C. (2008). Fractal parameters and vascular networks: facts and artifacts. *Theor. Biol. Med. Model.* 5:12. doi: 10.1186/1742-4682-5-12

Mandelbrot, B. B. (1983). *The Fractal Geometry of Nature.* New York, NY: W.H. Freeman.

Marker, D. F., Tremblay, M. E., Lu, S. M., Majewska, A. K., and Gelbard, H. A. (2010). A thin-skull window technique for chronic two-photon *in vivo* imaging of murine microglia in models of neuroinflammation. *J. Vis. Exp.* 2059. doi: 10.3791/2059

Maslinska, D., Laure-Kamionowska, M., and Kaliszek, A. (1998). Morphological forms and localization of microglial cells in the developing human cerebellum. *Folia Neuropathol.* 36, 145–151.

McAllister, J., and Miller, J. (2010). Minocycline inhibits glial proliferation in the H-Tx rat model of congenital hydrocephalus. *Cerebrospinal Fluid Res.* 7:7. doi: 10.1186/1743-8454-7-7

McMenamin, P. G. (1999). Subretinal macrophages in the developing eye of eutherian mammals and marsupials. *Anat. Embryol.* 200, 551–558.

Mittelbronn, M., Dietz, K., Schluesener, H. J., and Meyermann, R. (2001). Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol.* 101, 249–255.

Morgan, J. T., Chana, G., Abramson, I., Semendeferi, K., Courchesne, E., and Everall, I. P. (2012). Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. *Brain Res.* 1456, 72–81.

Morgan, T. E., Xie, Z., Goldsmith, S., Yoshida, T., Lanzrein, A. S., Stone, D., et al. (1999). The mosaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. *Neuroscience* 89, 687–699.

Nakajima, K., and Shinichi, K. (2002). "Neuroprotective roles of microglia in the central nervous system," in *Microglia in the Regenerating and Degenerating Central Nervous System*, ed W. J. Streit (New York, NY: Springer), 188–208.

Navascues, J., Calvente, R., Marin-Teva, J. L., and Cuadros, M. A. (2000). Entry, dispersion and differentiation of microglia in the developing central nervous system. *An. Acad. Bras. Cienc.* 72, 91–102.

Nichols, N. R. (1999). Glial responses to steroids as markers of brain aging. *J. Neurobiol.* 40, 585–601.

Nikkila, H. V., Muller, K., Ahokas, A., Miettinen, K., Rimon, R., and Andersson, L. C. (1999). Accumulation of macrophages in the CSF of schizophrenic patients during acute psychotic episodes. *Am. J. Psychiatry* 156, 1725–1729.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318.

Ohsawa, K., Imai, Y., Kanazawa, H., Sasaki, Y., and Kohsaka, S. (2000). Involvement of Iba1 in membrane ruffling and phagocytosis of macrophages/microglia. *J. Cell. Sci.* 113(Pt 17), 3073–3084.

Olah, M., Biber, K., Vinet, J., and Boddeke, H. W. (2011). Microglia phenotype diversity. *CNS Neurol Disord. Drug. Targets* 10, 108–118.

Orlowski, D., Soltys, Z., and Janeczko, K. (2003). Morphological development of microglia in the postnatal rat brain. A quantitative study. *Int. J. Dev. Neurosci.* 21, 445–450.

Overmyer, M., Helisalmi, S., Soininen, H., Laakso, M., Riekkinen, P. Sr., and Alafuzoff, I. (1999). Reactive microglia in aging and dementia: an immunohistochemical study of postmortem human brain tissue. *Acta Neuropathol.* 97, 383–392.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Park, L. C., Zhang, H., Sheu, K. F., Calingasan, N. Y., Kristal, B. S., Lindsay, J. G., et al. (1999). Metabolic impairment induces oxidative stress, compromises inflammatory responses, and inactivates a key mitochondrial enzyme in microglia. *J. Neurochem.* 72, 1948–1958.

Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2012). Microglia activation triggers astrocyte-mediated modulation

of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U.S.A.* 109, E197–E205.

Perego, C., Fumagalli, S., and De Simoni, M. G. (2011). Temporal pattern of expression and colocalization of microglia/macrophage phenotype markers following brain ischemic injury in mice. *J. Neuroinflammation* 8:174. doi: 10.1186/1742-2094-8-174

Perry, V. H. (1998). A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J. Neuroimmunol.* 90, 113–121.

Polito, A., Brouland, J. P., Porcher, R., Sonneville, R., Siami, S., Stevens, R. D., et al. (2011). Hyperglycaemia and apoptosis of microglial cells in human septic shock. *Crit. Care* 15, R131.

Pow, D. V., Perry, V. H., Morris, J. F., and Gordon, S. (1989). Microglia in the neurohypophysis associate with and endocytose terminal portions of neurosecretory neurons. *Neuroscience* 33, 567–578.

Radewicz, K., Garey, L. J., Gentleman, S. M., and Reynolds, R. (2000). Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. *J. Neuropathol. Exp. Neurol.* 59, 137–150.

Ren, L., Lubrich, B., Biber, K., and Gebicke-Haerter, P. J. (1999). Differential expression of inflammatory mediators in rat microglia cultured from different brain regions. *Brain Res. Mol. Brain Res.* 65, 198–205.

Rezaie, P., Cairns, N. J., and Male, D. K. (1997). Expression of adhesion molecules on human fetal cerebral vessels: relationship to microglial colonisation during development. *Brain Res. Dev. Brain Res.* 104, 175–189.

Rezaie, P., and Male, D. (1997). Expression of adhesion molecules on human foetal cerebral vessels: relationship to colonisation by microglial precursors. *Biochem. Soc. Trans.* 25, 170S.

Rezaie, P., and Male, D. (1999). Colonisation of the developing human brain and spinal cord by microglia: a review. *Microsc. Res. Tech.* 45, 359–382.

Rezaie, P., Patel, K., and Male, D. K. (1999). Microglia in the human fetal spinal cord–patterns of distribution, morphology and phenotype. *Brain Res. Dev. Brain Res.* 115, 71–81.

Rezaie, P., Trillo-Pazos, G., Greenwood, J., Everall, I. P., and Male, D. K. (2002). Motility and ramification of human fetal microglia in culture: an investigation using time-lapse video microscopy and image analysis. *Exp. Cell Res.* 274, 68–82.

Ristanović, D., Milošević, N. T., Jelinek, H. F., and Stefanovic, I. B. (2009). The mathematical modelling of neuronal dendritic branching patterns in two dimensions: application to retinal ganglion cells in the cat and rat. *Biol. Cybern.* 100, 97–108.

Rozovsky, I., Finch, C. E., and Morgan, T. E. (1998). Age-related activation of microglia and astrocytes: *in vitro* studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation. *Neurobiol. Aging* 19, 97–103.

Sant, S. P., and Fairgrieve, S. I. (2012). Exsanguinated blood volume estimation using fractal analysis of digital images. *J. Forensic Sci.* 57, 610–617.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Meth.* 9, 671–675.

Sheets, K. G., Gordon, B. W. C., and Bazan, N. G. (2010). "Topical NPD1 promotes microglia ramification in experimental CNV," in *ARVO (2012)*, eds L. Wu and D. Vavvas (Rockville, MD: The Association for Research in Vision and Ophthalmology), 5829.

Sheffield, L. G., and Berman, N. E. (1998). Microglial expression of MHC class II increases in normal aging of nonhuman primates. *Neurobiol. Aging* 19, 47–55.

Sheffield, L. G., Marquis, J. G., and Berman, N. E. (2000). Regional distribution of cortical microglia parallels that of neurofibrillary tangles in Alzheimer's disease. *Neurosci. Lett.* 285, 165–168.

Sheng, J. G., Griffin, W. S., Royston, M. C., and Mrak, R. E. (1998a). Distribution of interleukin-1-immunoreactive microglia in cerebral cortical layers: implications for neuritic plaque formation in Alzheimer's disease. *Neuropathol. Appl. Neurobiol.* 24, 278–283.

Sheng, J. G., Mrak, R. E., and Griffin, W. S. (1998b). Enlarged and phagocytic, but not primed, interleukin-1 alpha-immunoreactive microglia increase with age in normal human brain. *Acta Neuropathol.* 95, 229–234.

Sheng, J. G., Mrak, R. E., and Griffin, W. S. (1997a). Glial-neuronal interactions in Alzheimer disease: progressive association of IL-1alpha+ microglia and S100beta+ astrocytes with neurofibrillary tangle stages. *J. Neuropathol. Exp. Neurol.* 56, 285–290.

Sheng, J. G., Mrak, R. E., and Griffin, W. S. (1997b). Neuritic plaque evolution in Alzheimer's disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms. *Acta Neuropathol.* 94, 1–5.

Smith, T. G. Jr., Lange, G. D., and Marks, W. B. (1996). Fractal methods and results in cellular morphology–dimensions, lacunarity and multifractals. *J. Neurosci. Methods* 69, 123–136.

Soltys, Z., Orzylowska-Sliwinska, O., Zaremba, M., Orlowski, D., Piechota, M., Fiedorowicz, A., et al. (2005). Quantitative morphological study of microglial cells in the ischemic rat brain using principal component analysis. *J. Neurosci. Methods* 146, 50–60.

Soltys, Z., Ziaja, M., Pawlinski, R., Setkowicz, Z., and Janeczko, K. (2001). Morphology of reactive microglia in the injured cerebral cortex. Fractal analysis and complementary quantitative methods. *J. Neurosci. Res.* 63, 90–97.

Sonetti, D., Ottaviani, E., Bianchi, F., Rodriguez, M., Stefano, M. L., Scharrer, B., et al. (1994). Microglia in invertebrate ganglia. *Proc. Natl. Acad. Sci. U.S.A.* 91, 9180–9184.

Stence, N., Waite, M., and Dailey, M. E. (2001). Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. *Glia* 33, 256–266.

Stoll, G., and Jander, S. (1999). The role of microglia and macrophages in the pathophysiology of the CNS. *Prog. Neurobiol.* 58, 233–247.

Stoll, M., Capper, D., Dietz, K., Warth, A., Schleich, A., Schlaszus, H., et al. (2006). Differential microglial regulation in the human spinal cord under normal and pathological conditions. *Neuropathol. Appl. Neurobiol.* 32, 650–661.

Streit, W. J. (2000). Microglial response to brain injury: a brief synopsis. *Toxicol. Pathol.* 28, 28–30.

Streit, W. J., and Kreutzberg, G. W. (1987). Lectin binding by resting and reactive microglia. *J. Neurocytol.* 16, 249–260.

Streit, W. J., Walter, S. A., and Pennell, N. A. (1999). Reactive microgliosis. *Prog. Neurobiol.* 57, 563–581.

Takayasu, H. (1990). *Fractals in the Physical Sciences.* Manchester, New York: St. Martin's Press.

Tanaka, J., Toku, K., Sakanaka, M., and Maeda, N. (1999). Morphological differentiation of microglial cells in culture: involvement of insoluble factors derived from astrocytes. *Neurosci. Res.* 34, 207–215.

Todd, K., and Butterworth, R. F. (1999a). Mechanisms of selective neuronal cell death due to thiamine deficiency. *Ann. N.Y. Acad. Sci.* 893, 404–411.

Todd, K. G., and Butterworth, R. F. (1999b). Early microglial response in experimental thiamine deficiency: an immunohistochemical analysis. *Glia* 25, 190–198.

Toth, C. C., Jedrzejewski, N. M., Ellis, C. L., and Frey, W. H. 2nd. (2010). Cannabinoid-mediated modulation of neuropathic pain and microglial accumulation in a model of murine type I diabetic peripheral neuropathic pain. *Mol. Pain* 6:16. doi: 10.1186/1744-8069-6-16

Tremblay, M. E., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069.

Varvel, N. H., Bhaskar, K., Kounnas, M. Z., Wagner, S. L., Yang, Y., Lamb, B. T., et al. (2009). NSAIDs prevent, but do not reverse, neuronal cell cycle reentry in a mouse model of Alzheimer disease. *J. Clin. Invest.* 119, 3692–3702.

Vicsek, T. (1992). *Fractal Growth Phenomena*. Singapore, New Jersey: World Scientific.

Vinet, J., Weering, H. R., Heinrich, A., Kalin, R. E., Wegner, A., Brouwer, N., et al. (2012). Neuroprotective function for ramified microglia in hippocampal excitotoxicity. *J. Neuroinflammation* 9:27. doi: 10.1186/1742-2094-9-27

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Watkins, L. R., Milligan, E. D., and Maier, S. F. (2001). Glial activation: a driving force for pathological pain. *Trends Neurosci.* 24, 450–455.

Wen, Y. R., Tan, P. H., Cheng, J. K., Liu, Y. C., and Ji, R. R. (2011). Microglia: a promising target for treating neuropathic and postoperative pain, and morphine tolerance. *J. Formos. Med. Assoc.* 110, 487–494.

Wierzba-Bobrowicz, T., Kosno-Kruszewska, E., Gwiazda, E., and Lechowicz, W. (2000a). Major histocompatibility complex class II (MHC II) expression during the development of human fetal cerebral occipital lobe, cerebellum, and hematopoietic organs. *Folia Neuropathol.* 38, 111–118.

Wierzba-Bobrowicz, T., Schmidt-Sidor, B., Gwiazda, E., Lechowicz, W., and Kosno-Kruszewska, E. (2000b).

Major histocompatibility complex class II expression in the frontal and temporal lobes in the human fetus during development. *Folia Neuropathol.* 38, 73–77.

Wollmer, M. A., Lucius, R., Wilms, H., Held-Feindt, J., Sievers, J., and Mentlein, R. (2001). ATP and adenosine induce ramification of microglia *in vitro*. *J. Neuroimmunol.* 115, 19–27.

Won, S. J., Yoo, B. H., Kauppinen, T. M., Choi, B. Y., Kim, J. H., Jang, B. G., et al. (2012). Recurrent/moderate hypoglycemia induces hippocampal dendritic injury, microglial activation, and cognitive impairment in diabetic rats. *J. Neuroinflammation* 9:182. doi: 10.1186/1742-2094-9-182

Xiao, H., Li, Y., Du, J., and Mosig, A. (2011). Ct3d: tracking microglia motility in 3D using a novel cosegmentation approach. *Bioinformatics* 27, 564–571.

Yi, C. X., Tschop, M. H., Woods, S. C., and Hofmann, S. M. (2012). High-fat-diet exposure induces IgG accumulation in hypothalamic microglia. *Dis. Model. Mech.* 5, 686–690.

Yrjanheikki, J., Keinanen, R., Pellikka, M., Hokfelt, T., and Koistinaho, J. (1998). Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15769–15774.

Yu, W. H., Go, L., Guinn, B. A., Fraser, P. E., Westaway, D., and McLaurin, J. (2002). Phenotypic and functional changes in glial cells as a function of age. *Neurobiol. Aging* 23, 105–115.

Zhao, Y. N., Wang, F., Fan, Y. X., Ping, G. F., Yang, J. Y., and Wu, C. F. (2012). Activated microglia are implicated in cognitive deficits, neuronal death, and successful recovery following intermittent ethanol exposure. *Behav. Brain Res.* 236, 270–282.

Zhao, Z., Hertz, L., and Code, W. E. (1996). Effects of benzodiazepines on potassium-induced increase in free cytosolic calcium concentration in astrocytes: interactions with nifedipine and the peripheral-type benzodiazepine antagonist PK 11195. *Can. J. Physiol. Pharmacol.* 74, 273–277.

Ziaja, M., and Janeczko, K. (1999). Spatiotemporal patterns of microglial proliferation in rat brain injured at the postmitotic stage of postnatal development. *J. Neurosci. Res.* 58, 379–386.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 November 2012; accepted: 08 January 2013; published online: 30 January 2013.

Citation: Karperien A, Ahammer H and Jelinek HF (2013) Quantitating the subtleties of microglial morphology with fractal analysis. Front. Cell. Neurosci. 7:3. doi: 10.3389/fncel.2013.00003

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 14 May 2013
doi: 10.3389/fncel.2013.00065

Functional diversity of microglia – how heterogeneous are they to begin with?

Uwe-Karsten Hanisch*

Institute of Neuropathology, University of Göttingen, Göttingen, Germany

Edited by:
Amanda Sierra, University of the Basque Country Euskal Herriko Unibertsitatea/Universidad del País Vasco, Spain

Reviewed by:
Astrid E Cardona, The University of Texas at San Antonio, USA
Marco Prinz, University of Freiburg, Germany

***Correspondence:**
Uwe-Karsten Hanisch, Institute of Neuropathology, University of Göttingen, Robert-Koch-Straße 40, D-37075 Göttingen, Germany.
e-mail: ukhanisch@med.uni-goettingen.de

Microglia serve in the surveillance and maintenance, protection and restoration of the central nervous system (CNS) homeostasis. By their parenchymal location they differ from other CNS-associated myeloid cells, and by origin as well as functional characteristics they are also–at least in part–distinct from extraneural tissue macrophages. Nevertheless, microglia themselves may not comprise a uniform cell type. CNS regions vary by cellular and chemical composition, including white matter (myelin) content, blood–brain barrier properties or prevailing neurotransmitters. Such a micromilieu could instruct as well as require local adaptions of microglial features. Yet even cells within circumscribed populations may reveal some specialization by subtypes, regarding house-keeping duties and functional capacities upon challenges. While diversity of reactive phenotypes has been established still little is known as to whether all activated cells would respond with the same program of induced genes and functions or whether responder subsets have individual contributions. Preferential synthesis of a key cytokine could asign a master control to certain cells among a pool of activated microglia. Critical functions could be sequestered to discrete microglial subtypes in order to avoid interference, such as clearance of endogenous material and presentation of antigens. Indeed, several and especially a number of recent studies provide evidence for the constitutive and reactive heterogeneity of microglia by and within CNS regions. While such a principle of "division of labor" would influence the basic notion of "the" microglia, it could come with the practival value of addressing separate microglia types in experimental and therapeutic manipulations.

Keywords: diversity, cytokines, immunity, innate, microglia, subtypes, TLR, experience

INTRODUCTION

Microglia are myeloid cells of the central nervous system (CNS). They are found with parenchymal distribution throughout the various regions of the brain, the spinal cord and also in the retina where they engage with numerous tasks in the surveillance and maintenance of the tissue homeostasis as well as the protection of the structural and functional integrity of the CNS (Hanisch and Kettenmann, 2007; Ransohoff and Cardona, 2010; Kettenmann et al., 2011; Prinz et al., 2011; Saijo and Glass, 2011). We are currently vitnessing an enormous development in microglial research as literally all aspects of their physiology undergo in-depth clarification and revision. Was it for a long time a common believe that microglia are under healthy conditions "resting" and functionally dormant it is now clear that they actively monitor their local environment with motile processes, nurse synapses in periodical interactions and assist in the myelin turnover by the clearance of oligodenroglia-derived exosomes (Davalos et al., 2005; Nimmerjahn et al., 2005; Haynes et al., 2006; Wake et al., 2009; Graeber, 2010; Fitzner et al., 2011; Paolicelli et al., 2011; Tremblay et al., 2011; Schafer et al., 2012; Kettenmann et al., 2013). Microglia participate in the development and plasticity of the CNS (Saijo and Glass, 2011; Tremblay et al., 2011; Kettenmann et al., 2013). Fundamental questions concerning their developmental origin and fate from embryonic states to aging, their turnover under normal (healthy) and replenishment under disease conditions have been addressed as well, employing sophisticated approaches for unraveling lineage relations and principles of microglial in-tissue maintenance (Mildner et al., 2007; Ginhoux et al., 2010; Ajami et al., 2011; Schulz et al., 2012; Gomez Perdiguero et al., 2013; Greter and Merad, 2013; Kierdorf et al., 2013; Yona et al., 2013). Most notably, features and functions as sentinels and innate immune cell are of foremost interest as they regard crucial roles played by microglia in emergency situations and chronic diseases.

Their striking ability to rapidly react to infection, trauma, ischemia or other real or potential threats has been known as microglial "activation". However, this term does not adequately reflect the diversity of response options, nor does it define the net impact on the CNS (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011; Hanisch, 2012). Against earlier notions that dominated the text book knowledge for a long time, microglia are not notorious miscreants lurking in the CNS to harm neurons on any occasion. They are not placed there simply as a risk factor. Their association with a lesion does not automatically identify them as a harming component, and even proven involvement in neuropathologic events and processes might be outnumbered by the cases in which their activity limits the damage or eliminates a localized minor defect. However, most of their protective actions probably remain unnoticed, while our view on pathological implications got biased for situations in which such

activities failed (Schwartz et al., 2006; Hanisch and Kettenmann, 2007). On the other hand, harmful contributions of microglia have been convincingly demonstrated, indicating that the actual impact can differ dramatically. Indeed, eligibility for diverse responses and complexity of the functional repertoire are key elements in microglial physiology.

The concept of reactive phenotypes, originally and largely founded by investigations on extraneural macrophages, has meanwhile been expanded to microglia (Mantovani et al., 2004; Town et al., 2005; Butovsky et al., 2006c; Schwartz et al., 2006; Hanisch and Kettenmann, 2007; Mosser and Edwards, 2008; Ransohoff and Perry, 2009; David and Kroner, 2011; Olah et al., 2011). It encompasses the recognition of a plethora of "activating" signals, their integration in the context of a given situation and their translation into adapted profiles of induced (or suppressed) genes and functions (Gordon and Taylor, 2005; Mosser and Edwards, 2008; Murray and Wynn, 2011; Hanisch, 2012, 2013). It also allows to better understand at the molecular, cellular and systemic level why and how microglial activation can result in rather protective outcomes or in a worsening of the damage. Proper selection and initiation, maturation and termination of reactive phenotypes are essential not only for the immediate responses to acute insults. They are immanent to the development and progression of autoimmune, neurodegenerative and age-related diseases, primary tumors and metastases, certain neuropsychiatric disorders as well as the compensatory and restorative attempts of the CNS (Miller and Streit, 2007; Perry et al., 2007; Monji et al., 2009; Pollard, 2009; Neumann et al., 2009; Chen et al., 2010; Prinz et al., 2011; Derecki et al., 2012; Cunningham, 2013; Aguzzi et al., 2013).

A question not yet addressed properly concerns the simple reflection as to whether the large number of microglia in a CNS represents a homogeneous population with identical duties and functional capacities. Microglia can account for 5–12% of the cell numbers, depending on the anatomical region (Lawson et al., 1990). These regions differ by biochemical and cellular composition, circuitry and functions – and thus probably differ by their needs of support and assistance. Diversity in morphology, physiology and even aspects of immunity is established or getting more and more recognized for subtypes of neurons and astrocytes (Landgraf and Evers, 2005; Matyash and Kettenmann, 2010; Cho et al., 2013). Immune cells, such as lymphocytes, dendritic and also monocytic cells are classified by subsets that diversify by expression patterns and functions (Yona and Jung, 2010; Satpathy et al., 2012). Microglia, however, the principal immunocompetent element of the CNS, would still be considered as a uniform community with standardized properties, just varying by regional density, obscure cell shape parameters or some protein expression levels? This is rather unlikely. It appears especially doubtful in the light of evidence for constitutive heterogeneity and responder diversity.

While this idea still awaits more experimental validation as well as conceptual maturation, first findings and interpretations, implications and consequences have been presented and discussed previously already (Hanisch and Kettenmann, 2007; Hanisch, 2012, 2013), and essentials as well as new elements will be stressed below. They concern a principle by which some microglia can act as a master subset due to a privileged release of a cytokine, such as tumor necrosis factor α (TNFα). Such a cell may take the role of a *primus inter pares* within a local community to govern activities of neighboring cells (**Figure 1**). They also address the preferential expression of major histocompatibility complex (MHC) structures for antigen presentation and of a molecular machinery that would allow a discrete routing of phagocytotic cargo to subsets of microglia. Organized in distinct subpopulations potentially interfering functions could thereby segregate. Notably, the reactive phenotypes with their profiles of transcribed genes and executed functions could build up on the individual contributions of responding sets of microglia, rather than representing the mere sum of equal performances. Nevertheless, whether and how such specialized capacities are pre-determined or randomly assigned to subsets among a population of reactive microglia is not known yet.

HETEROGENEITY OF TISSUE MACROPHAGES

Macrophages are found in many tissues and organs. They share a core of duties for which they are equipped with an array of constitutively expressed or inducible molecules, such as receptors, enzymes, or cytokines. While macrophage-associated tasks in homeostatic control or responses to damage and infection obviously rely on similar sets of genes and functions the various tissue environments must also determine adaptations of features. Kupffer cells in the liver, alveolar macrophages in the lung, osteoclasts in the bones or the macrophages in the spleen are embedded within characteristic cellular communities and are exposed to particular bio/chemical milieus. Accordingly, gene expression patterns and the organization of functions under normal conditions and in emergency situations likely require adjustment and control to suite the particular needs. Indeed, sophisticated analyses reveal a substantial diversity in the transcriptomes of tissue macrophages (Gautier et al., 2012) as well as in the factors that guide their development (Greter and Merad, 2013).

MACROPHAGES AS A FAMILY OF MYELOID CELLS

An assessment of the gene expression profiles in resting peritoneal, pulp splenic and lung macrophages as well as CNS microglia revealed a considerable degree of diversity between these populations, more than among populations of dendritic cells (DCs) studied in parallel (Gautier et al., 2012). Several genes distinguished macrophages from DCs. They comprised a signature that was more or less definite depending on the criteria of strict exclusiveness, levels of expression or presence in all or only some of the macrophage types. It included the immunoglobulin Fc receptors FcγRI (CD64) and FcγRIII (CD16), Toll-like receptor (TLR) 4 and its coreceptor CD14, the receptor of granulocyte colony stimulating factor (G-CSF), the MHC class I-related molecule MR1 and some enzymes with metabolic implications or (other) roles in signaling. Molecules commonly serving detection of (tissue) macrophages – besides F4/80 as the prototypical marker-, such as CD11b (αM integrin, complement receptor 3 CR3), CD68 (macrosialin), or CD206 (mannose receptor), did not fulfill the demanding properties of exclusive and/or panpopulational expression by macrophages (Gautier et al., 2012).

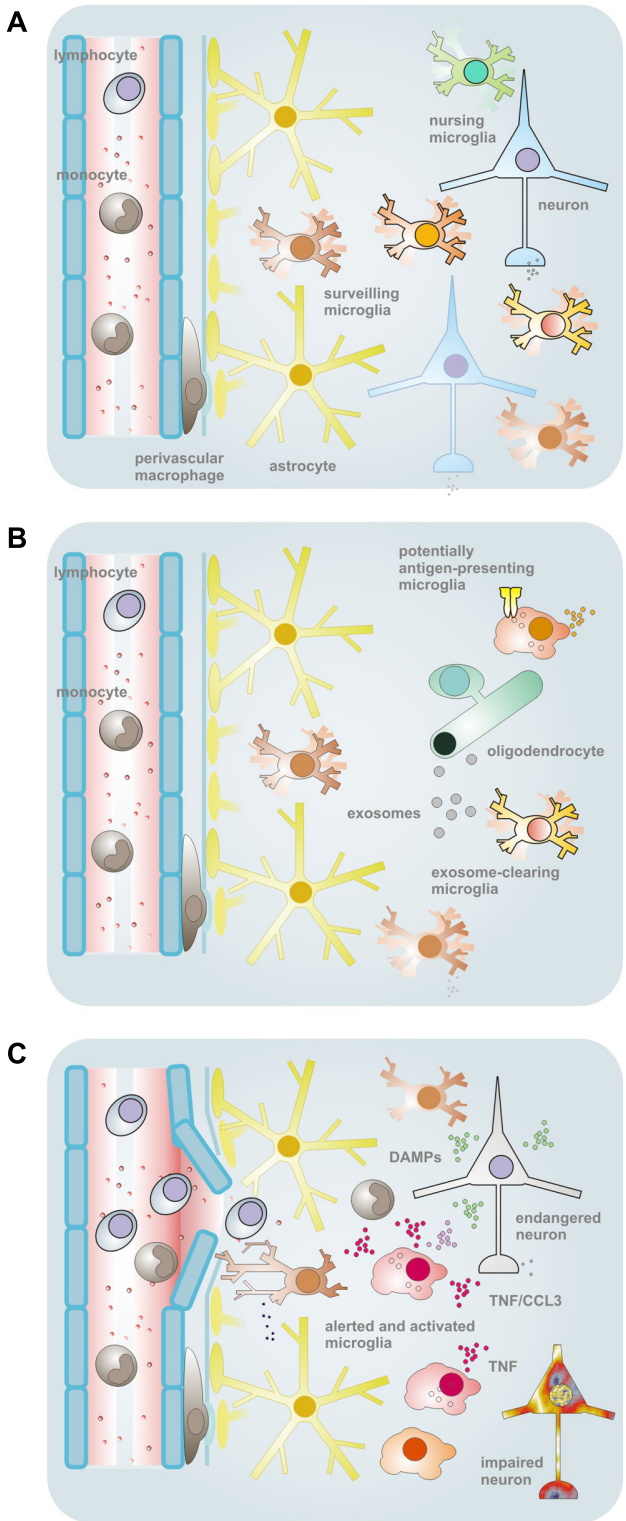


FIGURE 1 | Schematic overview of the conceptual framework and some supporting findings pointing to a heterogeneity of microglial properties. (A) In the normal CNS tissues, microglia comprise a population of myeloid cells with parenchymal distribution. The ramified morphology and a low expression of immune function-related molecules

(Continued)

FIGURE 1 | Continued
were formerly taken as signs of a "resting" status. This view got corrected upon demonstration of active tissue surveillance and periodic inspection of synapses with their motile processes. Nevertheless, by morphology and immunophenotype, microglia may still largely appear as a homogeneous cell type, although regional differences exist regarding density, morphology, capacity for proliferation and expression of certain proteins (or antigenic structures). On the other hand, subtle or yet unidentified variations in house-keeping duties (such as the nursing of synapses) and latent capacities may exist that define subtypes among and within anatomical subdivisions. **(B)** One of the duties unequally performed by microglia under physiological conditions relates to the macropinocytotic uptake of myelin-laden exosomes as they are emitted by oligodendrocytes in a principle of "outsourced" myelin turnover. Upon a challenge, such as by IFNγ, this subset of microglia reveals a lack of MHC II expression, in opposition to a largely complementary portion of cells that readily upregulate the surface structure for (potential) professional antigen presentation – but, in turn, do not engage with the exosomal clearance. As a purpose of this division of labor, immunologically silent disposal of endogenous material can take place in a sequestered compartment. **(C)** Challenges by bacterial agents, like LPS, or probably also appearance of endogenous factors with a connotation of damage and a similar TLR4-agonistic activity, can induce the synthesis of TNFα, which can then affect the vitality and functionality of resident as well as infiltrating (immune) cells. Since the production appears to be a privilege of some microglia only (even within local cell communities), they could claim the role of an instructor role or *maître de plaisir* during a response. TNFα-producing cells further subdivide by the ability or inability to also release CCL3. Yet the organization of such responder subtypes could be based on entirely different principles. Cellular subsets could be predetermined as to their functional capacities, acquire such distinction by environmental cues or regulate activities in a stochastic process. The scheme was adapted from previous own work (Hanisch and Kettenmann, 2007; Scheffel et al., 2012).

DIVERSITY AMONG THE MACROPHAGES FROM DIFFERENT TISSUES

Macrophage populations also differ themselves by numerous molecules that they can express. TLRs, C-type lectins as well as chemokine and efferocytic receptors were identified as gene families with heterogeneous expression patterns, thus pointing to tissue-specific adjustments in typical innate immune and macrophage effector activities (Gautier et al., 2012). Other studies on the relatedness of microglia to myeloid cells in particular as well as to immune and non-immune cells in general concluded on a strong similarity to the bone marrow-derived macrophages (BMDM), more than to peritoneal macrophages (Saijo and Glass, 2011). In our own work, when comparing the organization and properties of TLR-stimulated responses, we also see more similarities of microglia to BMDM than to peritoneal macrophages, suggesting that relationships are reflected by the reactive behavior as well. Tissue macrophages may thus vary by molecular equipment due to functional requirements as well as the daily or situational exposure to different signals (Davoust et al., 2008; Murray and Wynn, 2011; Hanisch, 2012). While alveolar macrophages are continuously confronted by airborne microbes, their peritoneal counterparts may occasionally sense traces of agents that derive from commensal microbiota. Microglia, on the contrary, would normally never face confrontations with such factors. Their appearance must then be interpreted as a true threat.

Cluster analyses of gene profiles, protein expression or functional activities in myeloid cells might be less affected by the mouse strain or even the species of the laboratory animals, as long as cell

types are compared within each category and assuming that such fundamental relationships are conserved. Nevertheless, systematic comparisons of the mouse and human cell repertoire are still lacking to ensure that classifications relying on animals studies are representative. Comparisons of the molecular and functional features are certainly prone to pitfalls when routine preparations of cells involve animals of different ages. Cultures of microglia served in a countless number of studies and delivered invaluable information on their basic properties (Kettenmann et al., 2011). Yet the popular standard *in vitro* setting uses newborn mice and rats, whereas peritoneal macrophages, for example, are more easily isolated from (young) adults. Differences deliberately assigned to the tissue may thus rather be determined by the ontogenetic stage.

Indeed, microglial cells reveal a remarkable maturation of basic features and response options during postnatal development (Scheffel et al., 2012). Moreover, if a property is well established for these cells, then it is the ability to rapidly react to even subtle changes in their environment as brought about by cellular impairments, blood–brain barrier (BBB) disruption and tissue injury (Hanisch and Kettenmann, 2007). Any preparational procedure that would result in a liberation of microglia-active substances and would allow them to exert influences on gene regulation at the time point of sampling may suffer from confounding responses to damage. Even mild cell isolations unavoidably associate with tissue disintegration. This has been stimulating efforts to develop appropriate techniques by wich undesired stimulation of microglia is largely circumvented or excluded from affecting the actual status of the cells at the time point of their isolation (Mertsch et al., 2001; de Haas et al., 2008). As an alternative, isolation and characterization can be separated in time, to allow accommodation of the cells. Such a strategy bears the risk that features acquired by cells – and in particular microglia – in the intact tissue at a given time, location and state are lost upon sampling and passing through cultivation. Surprisingly, however, critical properties can still be preserved. Analyses of molecular and physiological properties of microglia *ex vivo* under basal and stimulated conditions revealed a developmental profile, with gradual and transient adjustments according to the animal age at cell isolation, rather than as a function of time in culture (Scheffel et al., 2012). Apparently, cells can maintain a status (or critical aspects of) relating to the situation *in vivo*.

MICROGLIA AS A CNS-SPECIFIC POPULATION OF MYELOID CELLS

Microglia comprise myeloid cells with parenchymal distribution. They thereby differ by location from other cells of the mononuclear phagocyte system that are contained within the bony compartment harboring the CNS, such as the perivascular cells or the macrophages of the choroid plexus and the meninges (Kettenmann et al., 2011). The fine processes that can be visualized by antibody and lectin staining for marker molecules or that are revealed in transgenic animals expressing fluorescent proteins under the control of (more or less) microglia-selective promotors (Kettenmann et al., 2011) were commonly taken as an indication of a "resting" state. As meanwhile known better, this ramified morphology is a cytoarchitectural reflection of their surveillance function in the healthy adult tissue (Nimmerjahn et al., 2005). By territorial organization and with a cell body staying faithful to the location, the processes are in constant motion to sample information about the homeostatic situation, to receive inputs about the activity of nearby neurons (and other glia) and to scan the synaptic interconnections. It is probably a fine-anatomical adaptation to the complex tissue organization that enables microglia to reach with their tiny arms into narrow spaces between axons and dendritic trees without disturbing their structure and functionality. Besides a surveillance, it guarantees precision in shaping neuronal connections, as a suggested role of microglia in CNS development and plasticity as well as in response to altered neuronal activity. The phenomenon, termed synaptic stripping or pruning, has been elegantly shown by a number of studies (Wake et al., 2009; Paolicelli et al., 2011; Schafer et al., 2012; Kettenmann et al., 2013).

The delicate CNS architecture also determines that innate immune reactions of microglia are tightly controlled. The proinflammatory response repertoire is essential for fighting infections, but it also contributes – with many facets – to the events following tissue damage, non-infectious injuries as well as subsequent attempts of repair, while carrying enormous potential for aggravating destructive cascades (Bitsch et al., 2000; Butovsky et al., 2006b; Schwartz et al., 2006; Block et al., 2007; Chen and Nunez, 2010). Uncontrolled inflammation and tissue swelling are not tolerated by the CNS. Upon such disturbances and insults, microglia depart from the surveillance mode and activate programs for executive actions that must be kept in magnitude and organized by quality to serve as much in the defense as simultaneously avoiding unnecessary impairment (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011). Along with a functional transition, microglial processes are rapidly reorientated, the ramified morphology resolves, cells turn into a more ameboid shape and even migrate to the site of a lesion. This remarkable change has been taken as a prototypical sign of a microgial response, and it was noted already at the dawn of microglia research (Kettenmann et al., 2011). Nevertheless, whereas morphology changes with and in support of function, and often also with complex spatiotemporal pattern (Morrison and Filosa, 2013), it is not automatically a reliable surrogate of the actual activities (Ilschner and Brandt, 1996). On the other hand, variations in the cellular shape, as seen in the various anatomical divisions, may still point to adjustments to the environment. When considering heterogeneity of microglia, these adjustments should not be ignored.

While the BBB guarantees a special milieu in terms of the ionic, molecular and cellular CNS composition it is not an impenetrable wall, but rather an interface and check point where a controlled exchange of nutrients and metabolites is managed. Also for the so-called "immune privilege" of the CNS, its roles and mechanisms in immune surveillance and the organization of immune reactions are now better understood as to their anatomical fundaments as well as physiological processes (Bechmann et al., 2007; Ransohoff and Engelhardt, 2012). The BBB yet also differs in organizational features throughout the CNS and can change by integrity and functional properties which, in turn, can affect local populations of microglia. In other words, the CNS and its vascular (and also ependymal) lining create a

special compartment in which microglia are embedded. Suited for the neuronal functions, this milieu instructs and requires also the functions of microglia. They may vary as vascular and other anatomical properties differ throughout space. Nevertheless, local variations are likely also imposed by intrinsic, namely cellular, elements of the tissue regions, as stressed below. Most essentially, microglia may after all identify themselves as being (i) distinct from extraneural tissue macrophages, (ii) and distinct from other CNS-associated myeloid cell populations as well as (iii) representing a blend with closely related phenotypes but still discrete functional capacities (Carson et al., 2007; Hanisch and Kettenmann, 2007).

HETEROGENEITY OF MICROGLIAL RESPONSES AND RESPONDERS

While the concept of monocyte/macrophage diversity due to origin and location has a longer history (Treves, 1984; Naito et al., 1996; Sunderkotter et al., 2004), a conscious transfer to microglia could now take ground as well. More and more studies also address microglia in the aging brain, variations by species and gender or the surprising differences among individuals (Mouton et al., 2002; Meeuwsen et al., 2005; Sierra et al., 2007; Streit et al., 2008). Analyses of this kind may need to deal with a sophisticated characterization of microglial properties at close inspection and even at the single-cell level.

CONSTITUTIVE HETEROGENEITY BY PROTEIN EXPRESSION

In contrast to the morphology, expression of proteins suited for the identification of microglia among neural cells (i.e., neurons, astrocytes, oligodendrocytes) is not equally selective when distinguishing microglia from other myeloid cells. Certain molecules (namely proteins and carbohydrates) allow the visualization of microglia in animal and human tissues as well as cell cultures although their physiological functions and sometimes even the exact nature are largely unknown. This concerns, for example, moieties bound by certain lectins. Other molecules are characterized as receptors, adhesion molecules or as an intracellular calcium-binding factor, including CD11b/CD18 (αMβ2 integrin, CR3, MAC1) or Iba1 (Kettenmann et al., 2011; Prinz et al., 2011; Greter and Merad, 2013). CD16/32/64 (FcγRIII/II/I), CD45 (leukocyte common antigen, LCA), CD68 (macrosialin), CD115 (CSF1R), CD163 (scavenger receptor, ED2), CD169 (sialoadhesin, siglec 1), CD204 (MSR), CD206 (mannose receptor), dectin-1 (β-glucan receptor), and F4/80 have been serving in the detection of microglia, although levels of expression vary (mostly increase) with or even depend on activating challenges, thus potentially biasing detection for activity states (Kettenmann et al., 2011).

Due to shared expression, most of them would not allow an unequivocal identification of microglial cells when monocytic infiltrates intermingle with them in a lesion, especially when also the microglial morphology is altered. Factors, like CD45, still discriminate microglia from perivascular and peripheral cells since expression is low(er) and can thus serve discrimination especially in flow cytometry. Other proteins are suggested to identify circulating and recently invaded monocytes/macrophages, like macrophage-related proteins (MRPs) 8 and 14 (S100A8 and S100A9), whereas again others fail to be unique to microglia or have limited value for certain species (Kettenmann et al., 2011). Accordingly, combinations of two or more markers are more practicable or even required, depending on the study and the biological material. The discriminating potential of newly identified genes with selective expression in microglia still needs confirmation, but could offer additional options (Donnou et al., 2005). Inside the CNS, expression of CX_3CR1, the receptor of the chemokine CX_3CL1 (fractalkine), is restricted to microglia (and other brain macrophages). In turn, CCR2 as the receptor for the monocyte-attracting chemokine CCL2 (MCP-1) is expressed by cells in the blood stream, but under non-inflammatory conditions not found within the CNS – a patho/physiological relevant pattern that has been technically extremely useful (Mildner et al., 2007; Saederup et al., 2010; Prinz et al., 2011; Tremblay et al., 2011; Mizutani et al., 2012). The availability of transgenic animals with fluorescent reporter proteins has thus tremendously fostered studies on the rate and impact of monocytic infiltration of the CNS.

Several of the above listed as well as additional molecules representing surface receptors, cytokines and trophic factors or enzymes reveal inhomogeneous expression by microglia, at least in terms of detectable presence at mRNA and protein level or signaling organization. A short list has been compiled in recent reviews and includes CD11c, CD34, CD40, CD45, CD86, CXCL14, FcγRII, IGF-I, IL-6, inducible nitric oxide synthase (iNOS), certains integrins, MHC II, neurotrophins, Tim-3, TNFα, Trem-2 as well as ion channels (Kettenmann et al., 2011; Hanisch, 2012, 2013).

One has to acknowledge that consciously presented and interpreted evidence for heterogeneity among microglia in terms of selective or preferential expressions of a molecular structure can be found in the literature records of more than a decade ago (Rezaie and Male, 1999). Even though respective glyo/protein(s) had occasionally unidentified functions such work pointed to the uniqueness of microglia in the myelomonocytic lineage and documented variations among microglia. In rat brain and spinal cord, two cell populations were identified by the expression of the 5D4 keratan sulfate (KS) epitope. Occurring as of postnatal week 21, KS associated exclusively with ramified, but not with (activated) ameboid microglia (Wilms et al., 1999). It was found to be absent from other CNS macrophages (namely in the choroid plexus, vessel walls or meninges). Also peripheral monocytes/macrophages lacked its expression. KS^+ microglia distributed ubiquitously, but not uniformly throughout the CNS. Expressing cells were enriched in some regions and almost absent in others. These cells prefered gray over white matter as location and the global pattern suggested a favor of tissues with neurogenesis potential. The partially intimate contact to neurons pointed to a nursing function. Like for the *in situ* investigation, KS^+ microglia comprised a subset *in vitro*, among cells expressing the CR3 or ED1 epitope without being affected by lipopolysaccharide (LPS) or interferon-γ (IFNγ) stimulations. Thus, while certain molecules (or at least epitopes) reveal a more or less pan-microglial or even general macrophage expression under basal and/or activated conditions (e.g., CR3, or in one of our studies MHC I as shown below), others can apparently discriminate microglial

subpopulations by discrete anatomical distribution, white *versus* gray matter association and timed or transient appearance during ontogeny. Notably, subtypes can occur within a circumscribed region.

One of the most systematic studies thus far could determine regional expression profiles based on *ex vivo* flow cytometric analysis of CD11b, CD40, CD45, CD80, CD86, F4/80, TREM-2b, MHC II, CXCR3, CCR7, and CCR9 in isolated microglia (de Haas et al., 2008). Another study from this group related the CXCL10/CXCR3 system to the differential involvement of the local microglia in excitotoxic actions on neurons in in the mouse hippocampal regions CA1 and CA3, as triggered by *N*-methyl-D-aspartic acid (NMDA) administration (van Weering et al., 2010). This demonstration goes beyond mere detection of expression levels by identifying functional implications of a ligand-receptor signaling in microglia, by presenting a consequence for the neuronal environment as well as by showing the distinguished microglial impact for tissue regions in close vicinity. Expression and activity features would therefore not only vary between gross-anatomical structures but even within short ranges.

Going even further by the niveau of local resolution, microglial cells in side-by-side position could be shown to dissociate as to an altered protein expression – indicating a split in their individual duties. The triggering receptor expressed on myeloid cells-2 (Trem-2) is expressed by microglia under normal conditions and down-regulated when the cells are challenged. Such challenges can be achieved with LPS, a major cell wall constituent of Gram-negative bacteria signaling through TLR4, or with IFNγ, the immune IFN produced by natural killer (NK) cells and T cells of the Th1 type (Schmid et al., 2002). Both factors can drive a microglial phenotype that is well-known from studies on macrophages and has been termed as classical (also M1) or innate activation, as discussed below (Gordon and Taylor, 2005; Hanisch and Kettenmann, 2007; Mosser and Edwards, 2008). As a receptor with relevance for microglia and phagocytosis, Trem-2 is in the focus of research on autoimmune, neurodegenerative and (neuro)inflammatory diseases, including multiple sclerosis and Morbus Alzheimer (Piccio et al., 2008; Neumann and Daly, 2013). Besides other proteins, Trem-2 expression may also give hints to a heterogenous organization of microglia (Schmid et al., 2009). Not all microglia do express Trem-2 and this distinction is seen between as well as within CNS regions (Schmid et al., 2002). Interestingly, microglia in areas with incomplete BBB exhibited lowest expression. It is also interesting to speculate that plasma factors probably sensed by such a microglia are responsible for the reduced expression. Indeed, the plasma contains factors that can signal via TLR4 and that install in microglia a response that is in part similar to the reactive phenotype induced by LPS (Regen et al., 2011; Hanisch, 2013). Intriguingly, microglial cells in immediate vicinity to each other were reported to clearly differ by Trem-2 expression (Schmid et al., 2002). This is a conscious notion of intralocational distinction by protein expression – published a decade ago. In other studies, such information can likely be contained in the data without an explicit interpretation. Along this line and with conscious conclusion, microglia subtypes were recently described with regard to myelin removal under both physiological and pathophysiological conditions (van Rossum et al., 2008; Fitzner et al., 2011; Regen et al., 2011; Scheffel et al., 2012). Moreover, also TLR4- or IFNγ receptor-driven responses by cytokine release and expression of other surface molecules unraveled the existence of responder subtypes (Scheffel et al., 2012).

HETEROGENEITY IN HOUSE-KEEPING FUNCTIONS

The never-resting microglia seem to cover also a particular function in the normal turnover of myelin (Fitzner et al., 2011). Oligodendrocytes create the extremely well-organized and also tremendously elaborate myelin structures that require constant upkeep. Apparently, they thereby rely on the assistance of microglia. Oligodendrocytes can wrap myelin material from their turnover process in exosomes and deliver them to the microglia in their surrounding. The principle of outsourcing has been recently described, both *in vitro* and *in vivo*, and involves a subpopulation of microglia for clearance (Fitzner et al., 2011). Microglia taking up these myelin-laden exosomes would not upregulate MHC II when, for example, being stimulated by IFNγ. The assumption that this group of microglia would then also not function as antigen-presenting cells got confirmed in respective T cell activation assays. The exosome uptake, that is carried out by macropinocytosis, does otherwise not trigger concomitant responses, such as release of proinflammatory mediators, nor does it lead to overt signs of "activation" within the tissue. On the other hand, challenges by inflammatory stimuli, such as IFNγ and LPS, led to a decrease in exosome internalization. In turn, those microglial cells increasing MHC II levels upon IFNγ treatment were obviously devoid of exosomal clearing activity. As a conclusion, we formulated the hypothesis of an immunologically silent disposal of endogenous material (Fitzner et al., 2011). As a service for oligodendrocytes, myelin would be constantly routed to a specialized cellular compartment, i.e., a pool of microglia, from which it could not be "accidentally" presented to the adaptive immunity. One may speculate that a disturbance in this segregation could be detrimental, and there might be actual links to the triggering of myelin-afflicting autoimmune diseases, such as multiple sclerosis. Such a daily function in the turnover of self-derived material would be distinct from and exceed phagocytotic removal of aberrant cells and structures during development (Schlegelmilch et al., 2011).

The necessity of such microglial functions during development as well as later on is further illustrated by the impact of dysfunctional microglia on higher CNS functions. In an animal model of the human obsessive-compulsive disorder spectrum, microglia with a mutation in Hoxb8 associate with behavioral symptoms of compulsive grooming and hair removal (Chen et al., 2010). Microglia with a MECP2 gene mutation and showing insufficient phagocytotic performance were characterized as a cellular substrate of pathology in a mouse model of Rett syndrome, which is known in humans as an X-linked autism spectrum disorder (Derecki et al., 2012). Paving the way for therapeutic strategies, both studies presented improvement of the respective symptoms by substitution of the defective microglia via a transfer of wildtype bone marrow and monocytic engraftment. In part, the data may also indicate that a genetic defect can deteriorate the performance of especially some yet not all microglia (Chen et al., 2010).

Life-long consequences for the CNS and the functional behavior of its microglia can also result from complications of non-genetic nature. In this regard, intrauterine and postnatal infections may not only impede proper development but leave traces in form of an increased susceptibility of the CNS to subsequent challenges later in life, involving altered responses of microglia as a causative element (Bilbo et al., 2005; Stolp et al., 2005, 2009; Bilbo and Schwarz, 2009; Monji et al., 2009), as also discussed recently (Hanisch, 2013). Yet little is known how exactly such early challenges of the immune defense actions of microglia are preserved in altered properties to be conveyed to later ontogenetic periods and whether such a priming would occur in any or preferentially in certain cells. Specialized microglia may, in addition, be required for the support of neurogenesis and oligodendrogenesis, roles that have emerged more recently (Butovsky et al., 2006b).

The principle of specialization by functions of homeostatic maintenance seems to include the in-tissue renewal of microglia itself. Tissue macrophages in general can maintain themselves (Yona et al., 2013). In the absence of a pathology or experimental stimuli the replenishment of microglia from extraneural sources, such as the bone marrow and circulating monocytes, is very low to negligible (Ajami et al., 2007, 2011; Mildner et al., 2007). Or better to say, there is no clear evidence for any replenishment during adulthood. The stability of the CNS population must thus be guaranteed by – at least some – self-renewal, which may depend on specialized cells with some "stem cell" attributes (Gomez Perdiguero et al., 2013). Microglia with such a capacity may (or even should) scatter throughout the CNS tissues, even though some regions seem to harbor populations of a higher proliferative potential than others (Marshall et al., 2008). In this regard, the subventricular zone can claim a prominent status and microglia in this niche apparently also support neurogenesis (Walton et al., 2006; Thored et al., 2009). Proliferation under hoemostatic as well as pathological conditions may thus rely on subsets rather than the entire population. Such a notion could be supported by the assumption that a cell passing through the cell cycle for division may not simultaneously mount a production of cytokines, chemokines or other proteins for executive function. If challenged by activating factors and situations that require rapid functional responses as well as an expansion of the local cell numbers the affected population of microglia may split these duties by subsets.

Increase in microglial numbers will support the capacity to fight an infection and to cope with the demands of an injured tissue. Yet inflammatory infiltrates will also contain monocytic cells, that present with functional heterogeneity and distinct roles throughout the course of a disease (Butovsky et al., 2012; London et al., 2013). They are probably needed, in addition to merely provide more cells *per se*, for some functional expertise not sufficiently covered by resident microglia. Would dividing microglia, on the other hand, create also offsprings with different properties or cells that are omnipotent at a more immature level? In some aspects, microglia may also base their functional plasticity on a kind of immaturity (Carson et al., 1998; Ladeby et al., 2005). Furthermore, microglia can migrate to a lesion site, experimentally shown by an injection of a neurotoxin or by the local puncture of a blood vessel using a laser. Areas from which they emigrated would need some subsequent repopulation to avoid a drop in microglial support activity. This could be achieved either by remigration or limited proliferation – or via monocytic engraftment (Varvel et al., 2012). Would such areas regain diversity of microglia subtypes or just be helped by a "stopgap solution"? If constant (phagocytotic) clearance of endogenous material is one of the important roles of microglia in their territorial organization, understaffed tissues could run into trouble. Phagocytic deficiency likely causes severe complications (Fitzner et al., 2011; Derecki et al., 2012). On the other hand, microglial clearance functions may have distinct and different impacts under the various disease conditions, depending on the particular pathology as well as the phagocytic cargo material, and with other cell populations proving sometimes more relevant (Mildner et al., 2011). Yet homeostatic phagocytosis is still not properly understood and would conceivably represent just one of several house-keeping functions. The importance of synaptic inspections may just be raised again as another example (Wake et al., 2009).

The use of the term "heterogeneity" in direct combination with "microglia" has thus far almost exclusively been reserved for the different responses that are triggered when exposing cells to a range of experimental stimuli and activating conditions, or when dealing with the respective diversity of reactive phenotypes in *in vivo* settings. Indeed, macrophages – and as more and more appreciated also microglia – display an elaborate spectrum of programs for adapted gene inductions and supragenetic organization of functions in order to react to diverse disturbances of the tissue homeostasis. For further reading, a number of original contributions and reviews could be suggested (Gordon and Taylor, 2005; Martinez et al., 2006, 2013; Kono and Rock, 2008; Mosser and Edwards, 2008; Michelucci et al., 2009; Ransohoff and Perry, 2009; Rivest, 2009; Pollard, 2009; Biswas and Mantovani, 2010; Piccinini and Midwood, 2010; Pukrop et al., 2010; Kettenmann et al., 2011; Olah et al., 2011; Saijo and Glass, 2011; Hanisch, 2012, 2013).

DIVERSITY OF REACTIVE PHENOTYPES

Not so long ago, macrophages were underrated for the flexibility in their responses, regarding both the versatility of reactive programs as well as the options to further adjust or shift their individual profiles. This has changed drastically. Seminal discoveries and ground-preparing achievements being done by immunological research on (initially extraneural) macrophages resulted in refined concepts of reactive phenotype diversity (Treves, 1984; Mantovani et al., 2004; Gordon and Taylor, 2005; Martinez et al., 2006, 2009; Weber et al., 2007; Mosser and Edwards, 2008; van Rossum et al., 2008; Pollard, 2009; Biswas and Mantovani, 2010; Daley et al., 2010; Wong et al., 2010; Mikita et al., 2011; Murray and Wynn, 2011). Over the last years, this concept was rapidly implemented in the research on microglia as well, based on diverse approaches as well as CNS-adapted models and revealing both similarities in principles and profiles – but also indicating tissue- and cell type-specific response properties. The following references, covering original contributions and surveys, are selected for further reading (Popovich et al., 1999; Schmid et al., 2002; Town et al., 2005; Butovsky et al., 2006c; Colton et al., 2006; Schwartz et al., 2006;

Hanisch and Kettenmann, 2007; Ponomarev et al., 2007; Davoust et al., 2008; van Rossum et al., 2008; Colton, 2009; Kigerl et al., 2009; Michelucci et al., 2009; Tierney et al., 2009; Pukrop et al., 2010; David and Kroner, 2011; Kettenmann et al., 2011; Saijo and Glass, 2011; Olah et al., 2011, 2012; Hanisch, 2012; Starossom et al., 2012; Zhou et al., 2012).

In its fundaments, the notion relies on the observation that exposure to certain cytokines, such as the T helper (Th) cell type 1 cytokine IFNγ or the pluripotent TNFα, and challenges by microbial agents, such as LPS, other TLR-agonistic compounds or pathogen-associated molecular patterns (PAMPs), would install a "classical" activation of macrophages, that has been more or less synonymously termed as a M1 phenotype. Such cells are defense-oriented, release proinflammatory and cytotoxic factors, support Th1 type of immune response – yet potentially and occasionally also at the costs of damage. Th2 cytokines, like IL-4 or IL-13, instead organize for an "alternative" activation of macrophages, also known as M2 phenotype. The respective profile is more anti-inflammatory, inflammation-resolving. It is involved in parasite killing and supports tissue repair. Differing from the uncommitted M0 condition, the M1 and M2 phenotypes demonstrate distinct sets of active genes. Their profiles are largely discrete and partially reciprocal. By certain genes, however, they can also overlap, rendering an isolated factor often rather inappropriate to reliably classify the orientation. For simplicity, the reciprocal expression of IL-10 (M2) and IL-12 (M1) served as a phenotype indication.

Classically activated macrophages were then also reported for an expression and/or release of TNFα, IL-1, IL-6, IL-23, CCL2, CCL3, CCL5, reactive oxygen and nitrogen species, matrix metalloproteinases (including MMP-1, -2, -7, -9, and -12), cyclooxygenase 2 (COX2), iNOS, the FcR types CD16/32/64, MHC II and the accessory molecules B7.1/7.2 (CD80/86), that serve in antigen presentation. Actual sets, combinations and intensities vary with the type of macrophage and stimulus. Alternatively activated macrophages show, besides the typical IL-10, expression or release of IL-1 receptor antagonist (IL-1ra), CCL17, CCL22, IL-1RII, CD163, scavenger receptors, factor XIII, fibronectin, COX1, arginase 1, and enzymes for extracellular matrix (ECM) repair (Hanisch, 2012).

The various genes and their products are not necessarily induced under every phenotype-conform condition, as mentioned above. Furthermore, the M1-*versus*-M2 classification does not reflect the versatility – and even the kind of continuum – of the observed spectra. IFNγ, for example, induces MHC II in both macrophages and microglia. LPS, driving an innate or M1-like activation, however, fails to induce it in microglia (van Rossum et al., 2008; Scheffel et al., 2012). So, not all M1 forms come with an congruent gene set, whereas certain gene products may be found in otherwise distinct phenotypes (Mantovani et al., 2004; Edwards et al., 2006). While these variations enrich the phenotype diversity even within the M1 domain (Starossom et al., 2012), different types of M2 orientations were also noted already early in these studies (Mantovani et al., 2004). Exposure to either IL-10, antibody-antigen (immune) complexes or their combinations with TLR and other agonists, contact to cellular breakdown material along with its phagocytotic removal, contact to apoptotic cells or association with a tumor install phenotypes that share M2 likeness, while simultaneously displaying sufficient differences to allow for subclassification as to M2a, M2b, and M2c. Consequently, not isolated markers but profiles define the actual state of the cells, while stages in a response process, lack of typically indicative or concomitant expression of "opposite" markers may hamper simple definitions and blur phenotype borders (Gerber and Mosser, 2001; Anderson and Mosser, 2002; Anderson et al., 2002; Town et al., 2005; Edwards et al., 2006; Mosser and Edwards, 2008; van Rossum et al., 2008; Kettenmann et al., 2011). The dynamic nature of responses cannot be emphasized enough (London et al., 2013). Monocytes/macrophages likely display distinct molecular signatures as they correlate with distinct activities and functions during the initiation, progression and resolution of a disease and the reaction to it. In addition, phenotype inductions in microglia and extraneural macrophages by standard stimuli, such as LPS, IFNγ, or IL-4, can lead to similar yet discrete profiles of induced genes and functional consequences (Butovsky et al., 2006c; van Rossum et al., 2008; Girard et al., 2013), as previously discussed (Kettenmann et al., 2011; Hanisch, 2012, 2013).

New candidate proteins and carbohydrate moieties allow now a fairly reliable association with reactive phenotypes as to their bifurcated polarity or their distinguishable varieties. To name a few, claudin (Cldn) 1, 2, and 11, found in inflammatory zone 1 (FIZZ1), mouse macrophage galactose-type C-type lectins (mMGL) 1 and 2, sphingosine kinase-1, TNF superfamily member 14 (LIGHT), transglutaminase 2, the secretory lectin chitinase 3-like protein 3 Ym1 or O- and N-glycans sort with M1 and M2 orientations, although functions in general and in regard to the phenotype are often unclear (Chang et al., 2001; Raes et al., 2002, 2005; Colton et al., 2006; Edwards et al., 2006; Maresz et al., 2008; Starossom et al., 2012; Van den Bossche et al., 2012; Zhou et al., 2012; Martinez et al., 2013).

It is important to note – also for our discussion on heterogeneity – that phenotypes are chosen at the beginning of the activation process, based on initial sets of information on threatening events. Gene expression may subsequently shift, organized by cell-autonomous mechanisms (taking late induction of IL-10 as an example), or due to altered conditions (e.g., when primary stimuli fade upon successful clearance, as for microbes) or with modulatory factors gaining influence (like cytokines, resolvins, and other mediators that are emitted by infiltrating neutrophils, monocytes, or T cell populations). Reactions may start with a defense orientation that later on deescalates and fades when profiles are required that facilitate structural repair and functional restoration. In the living tissue, phenotypes ideally induced in experimental settings as of M1- or M2-like blends could probably develop as transient stages of a complete response. We have discussed these issues in recent reviews (Kettenmann et al., 2011; Hanisch, 2012).

Notebaly, macrophages do not only commit to diverse phenotypes upon defined challenges. More or less pronounced overlaps between functionally related profiles could (and actually do) exist – especially in the more complex *in vivo* situations – as many genes, including those with an "inflammatory connotation," are important in several responses to (as well as following to) infection and

injury. Phagocytotic activity and proinflammatory components are needed for wound healing and repair. On the contrary, inflammation is a double-edged sword in cancer development and progression. It can even be supportive, and macrophages can display a bewildering variety of phenotypic markers and their combination in tumor association, as they do in many other disease situations, such as in atherosclerosis, or repair (Mantovani et al., 2004; Mantovani et al., 2009; Pukrop et al., 2010; Mantovani et al., 2013). They can exhibit phenotypic profiles that do not easily sort by a strict M1/M2 dichotomy. Also microglial populations can present with more complex locotemporal patterns of their morphological, molecular and functional features (Kigerl et al., 2009; Morrison and Filosa, 2013). Evidence for the parallel existence of M1- and M2-oriented cells in a mouse spinal cord injury model either points to hybrid states expressing genes of either activation type or suggests discrete cellular subsets following own time courses and having independently organized programs (Kigerl et al., 2009). Orientations could be split between the resident microglia on one and infiltrating monocytes on the other side – or the lines of demarcation are not that simply drawn by CNS-intrinsic *versus* extraneural origin. While it is obviously not a trivial task to assign a certain reactive phenotype as a dominat as well as persisting response pattern of macrophages and microglia to a given pathology, in some cases a major orientation and the respective molecular and cellular mechanisms can be successfully linked to a disease (Heneka et al., 2013).

RESPONSE HETEROGENEITY UPON CHALLENGES

An increasing number of already characterized (and probably still to be identified) factors of exogenous (microbial), endogenous and especially CNS-intrinsic (homeostatic and damage- or degeneration-related) factors takes influences on the activity state and activity recruitment of microglia, as listed previously (Hanisch, 2002; Hanisch and Kettenmann, 2007; Kettenmann et al., 2011; Hanisch, 2012, 2013). They differ by origin and the situations in which they appear in microglia activation-relevant concentration and format, they belong to most disparate classes as to their chemical structure, they employ diverse modes of action and they definitely vary by their phenotype instructions.

As a principle to set an alert and to trigger a rapid transition from the surveillance ("resting") mode to executive states, signals appear in the form of receptor agonists that are normally not found in the microglial environment – not at all, not with relevant modification or arrangement (e.g., oxidized or aggregated forms) or at effective concentration. Many classes of compounds that play roles in microglial instructions for phenotypic commitments follow this principe of "on signaling," in which already expressed surface or intracellular receptors are stimulated by the arrival of their cognate ligands (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011; Hanisch, 2012). Major examples relate to cytokines. Typically, "on signaling" occurs with the assortment of evolutionary conserved structural motifs in glyproteins, lipopeptides, glycolipids, RNA, and DNA of virus, bacteria, fungi, and protozoans, commonly described as PAMPs, which are sensed by germ line-encoded pattern recognition receptors (PRRs), notably NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), C-type lectin receptors (CLRs), absence in melanoma 2-like receptors and TLRs (Kawai and Akira, 2010; Takeuchi and Akira, 2010).

In other situations, when damage or impairment has to be sensed by microglia in the absence of infection, endogenous factors can act as "activators" to drive an innate immune reaction as a sterile inflammation (Chen and Nunez, 2010; Stewart et al., 2010). Classified as damage- or danger-associated molecular patterns (DAMPs), these disparate molecules (mainly but not only proteins) usually serve most diverse functions and gain the role as danger signs only upon unphysiological release (from a cell), translocation (from the plasma to the parenchyma) and modification (e.g., oxidation; Rubartelli and Lotze, 2007; Lotze et al., 2007; Kono and Rock, 2008; Zhang and Mosser, 2008; Piccinini and Midwood, 2010). DAMP activities have been established for (to name a few) chromatin-associated and other intracellular proteins, such as high mobilioty group box protein (HMGB) 1, S100A8, and S100A9, a range of heat shock proteins, ECM and plasma components, such as tenascin, versican, fibronectin, lactoferrin, lipoprotein A, and oxidized low density lipoprotein, but also serum amyloid and amyloid β (Aβ; Hanisch, 2012). New entries to the list are reported on a constant basis, recently regarding fetuin (Pal et al., 2012). Interestingly, many of these DAMPs are recognized by the binding to PAMP receptors, most notably TLRs. By the recognition of alarming structural patterns a rather small number of such receptors can cover a wide range of pathogens as well as indicators of dangerous situations – and the reactions to these threats would be immediately in the hands of the sentinel cells, like microglia in the CNS (Hanisch, 2012, 2013).

In contrast to this "on signaling," microglia can, in turn, also be alerted and "activated" when certain calming influences fade or disappear. Accordingly, such an organization would be a kind of "off signaling," the term being introduced as a microglial principle already in 2004 (van Rossum and Hanisch, 2004; Biber et al., 2007; Hanisch and Kettenmann, 2007; Hanisch, 2012). For first pairs of ligands and their receptors, reciprocal expression by neurons and microglia has been shown to have a controlling influence, such as in the cases of CD200/CD200R, CD47-SIRPα, CX_3CL1/CX_3CR1 and CD22/CD45. Yet microglia may sense the decline of neighboring neurons also via altered neurotransmitter levels. Both sources of information could be important for microglia to rapidly respond to any homeostatic disturbance affecting neurons simply on the basis of lost inputs, and without the need of expressing a sweeping array of particular receptors (Hanisch, 2013). In other words, danger is recognized, also by microglia, via a whole tool box of receptors and signaling systems (Lotze et al., 2007; Matzinger, 2007; Rubartelli and Lotze, 2007; Lehnardt et al., 2008; Kettenmann et al., 2011). In harsh contrast to the growing knowledge on signals and signaling principles that keep the control over microglia as a major cell type in the CNS, much less information is yet delivered as to the individuality of the cellular responses.

Injections of LPS into rat neocortex, hippocampus or the substantia nigra revealed a markedly distinct susceptibility in terms of neurodegeneration, with a profound impact on mesencephalic and especially dopaminergic neurons (Kim et al., 2000). The critical

role of microglia was addressed in respective neuron-glia culture preparations. Sensitivity of mesencephalic and resistance of cortical and hippocampal neurons both *in vivo* as well as *in vitro* apparently matched the region-selective microglial responsiveness and responses. The substantia nigra is the CNS region in rodents that accommodates the highest density of microglia (Lawson et al., 1990). Respective cell transfer (supplementation) experiments could associate cell number and outcome. Yet quantity might not explain all regional differences. Neonatal rat microglia isolated form brainstem, cortex, hippocampus, striatum, and thalmus differently responded to glutamate, ATP and LPS (Lai et al., 2011). This report and other work with functional demonstrations (van Weering et al., 2010) correlate to the inventories of distinct expression profiles (de Haas et al., 2008) and precipitate the conclusion as to region-specific microglial populations. Of course, the ontogenetic stage of the microglial sample cannot be neglected since several features, especially the organization of receptor signaling and stimulation consequences, obviously mature not only prior but also after birth (Draheim et al., 1999; Scheffel et al., 2012). Nevertheless, regional distinction with varying resolution is on the way to depict responder heterogeneity in rather circumscribed areas.

In a recent study, we addressed the postnatal reorganization of microglial responses to TLR activation (Scheffel et al., 2012). Along the characterization of inducible genes and profiles of synthesized factors we also observed responder subsets, i.e., cells being responsible for the release of certain cytokines and chemokines. The respective distinction of producers from the non-producing subpopulations developed as part of a more general maturation of microglial features. This concerned profiles of genes and activities expressed under basal conditions or subsequent to a defined stimulation. Microglia were harvested from mice at birth or at the postnatal days P21 and P49 and kept *ex vivo* for various periods of time. Interestingly, and against the common expectation, several features that were studied in repeated sessions did not reveal obvious changes with the time in culture but differed, partially in a drastic fashion, with the time point during postnatal development, namely the day at which the microglia were isolated. Even though such an *ex vivo* approach would never claim to properly reflect tissue-encoded features of the cells in their entire complexity, at least some of the critical aspects seemed to be carried over to the culture where they were maintained with surprising stability.

When microglia was challenged with defined structural varianst of LPS they produced, among other cytokines and chemokines, TNFα (Regen et al., 2011; Scheffel et al., 2012). Microglia could even discriminate among the LPS chemotypes as to the actual release profiles, and this ability developed postnatally. Notably, synthesis of TNFα related to a subpopulation which increased from about 30% in neonatal to 75% in the young adult cells. At the same time, the boundaries defining the responder *versus* non-responder subpopulations – as revealed in flow cytometry – gained precision (i.e., by increased peak distance). Moreover, the two subtypes were then also seen in isolates of microglia from different regions of the adult CNS (Scheffel et al., 2012). Their existence was confirmed by immunocytochemistry on both cells and organotypic brain slice preparations *in vitro* (using also confocal microscopy) and on tissue sections from mice that underwent targeted focal experimental autoimmune encephalomyelitis (EAE).

Selective induction of TNFα was accompanied by a subset-restricted synthesis of CCL3, an important chemokine also known as macrophage inflammatory protein 1α (MIP-1α), that serves in the attraction of monocytes, T and B cells, eosinophils as well as DCs (Scheffel et al., 2012). The rather small subpopulation would apparently suffice an impressive release, since CCL3 can be induced in high amounts. Most of the CCL3-synthesizing cells were contained in the (larger) population of TNFα producers, as revealed by combined detection. This $TNF\alpha^{+}CCL3^{+}$ subpopulation increased in size as the postnatal development of microglia features proceeded – ascertained by expression and cell cycle analyses as well as electrophysiological recordings (Scheffel et al., 2012; Hanisch, 2013).

In support of these observations, a similar subset-selective TNFα induction by LPS was also reported for rat microglia (Kawahara et al., 2009; Shen et al., 2009), ruling out a species phenomenon. Employing FluoroSpot technology for single cell analyses among populations, a comprehensive characterization of TLR-activated cytokine secretion recently revealed distinct responder subtypes among human monocytes (Smedman et al., 2011). Production of TNFα was determined in subpopulations of TLR2- and TLR4-stimulated cells. Moreover, also other cytokines and chemokines depended for their release on subsets. Interestingly, combinatory detections identified cells with co-secretion capacity for pairs of factors, such as TNFα and MIP-1β (CCL4), whereas fractions of cells in the same population revealed only singular synthesis. Moreover, the release of certain cytokines relied on surprisingly small numbers of cells. IL-12p40 production, for example, was confined to less than 10% of the monocytes. Findings of this study – focusing on human monocytes – are thus very similar to those obtained with rodent microglia.

While a developmental reorganization of the microglial TLR systems is also in line with the data by others (Kaul et al., 2012), our findings point to a rearrangement in the associated signaling cascades, rather than a change in receptor expression. Evidence indicates an altered organization of the pathway requiring the signaling adaptor myeloid differentiation primary response gene 88 (MyD88), which participates in intracellular consequences of almost all TLRs (except for TLR3). On the contrary, the adaptor protein TIR-domain containing adaptor protein inducing IFNβ (TRIF), that conveys information under TLR3 and also for TLR4, seemd to be spared. Noteworthy, we found critical TLR4 functions to be distinctly governed by MyD88 and TRIF (Regen et al., 2011) and to undergo transient reorganization during postnatal development (Scheffel et al., 2012). Exemplified for the reactions to TLR1/2, TLR4, and TLR6/2, microglia rebuild their mechanisms during a critical window within the first few weeks after birth (marked by a peak around p21) – while simultaneously passing through a gradual change of other properties. Microglia will acquire the typical morphology of the "resting" cell (which is not dormant, as we know), accompanying the maturation of the CNS in terms of its neuronal circuitry and myelination (Scheffel et al., 2012). As this period coincides with a suggested phase of increased susceptibility to CNS infection, we also tested mice for the outcome

of an intracerebral bacteria deposition and noticed a higher mortality for those at P21, in comparison to animals at an age of P49.

The formation of TNFα- and CCL3-producing subsets of microglia under LPS was not related to a lack of TLR(4) in the complementary cell fraction. When studying microglial inductions upon TLR4 challenge, we found a strong expression of MHC I in almost all cells. While failing to induce MHC II under these conditions, LPS caused a panpopulational response in microglia at any age and in all regions examined (Scheffel et al., 2012). MHC $I^+TNF\alpha^-$ and MHC $I^+TNF\alpha^+$ microglia thus do not split by their TLR4 expression and responsiveness as such. Among the TLRs, TLR4 appears to be homogeneously distributed over macrophage populations (Gautier et al., 2012). In the case of microglia, cells must rather differ by TLR4 signaling. Under the influence of TLR4-agonistic PAMPs or DAMPs, subsets could provide individual elements to compose the reactive phenotype of a responding microglia population. Some deliver, for example, TNFα to which other cells have then to obey, while all of them identify themselves with MHC I expression. Since microglia differ by their TNFα receptor expression (Kraft et al., 2009), this could create a hierarchy of cells, with some in control of the synthesis and others with a varying level of perception. Cells with preferential expression of enzymes, such as COX2 or iNOS, could similarly serve as (privileged) sources of prostaglandines and NO (Kawahara et al., 2009; Scheffel et al., 2012). Reactive phenotypes may thus reflect an ensemble of individual cellular engagements. Inhomogeneous responses of microglia in circumscribed populations (i.e., of a defined tissue region) to a homogeneously presented stimulus could be taken as a hint to their differential organization. Distinct response capacities could be established even before the challenge. Heterogeneity could be, at least in some part, a constitutive one.

In contrast to MHC I, microglial expression of MHC II for professional antigen presentation was not triggered by TLR4 activation, but readily induced by IFNγ, both *in vitro* as well as *in vivo,* and then in a subset (Fitzner et al., 2011; Scheffel et al., 2012). Also upon injection of IFNγ *in vivo*, microglia reveal MHC II immunoreactivity (Fitzner et al., 2011). B7.1 and B7.2 (CD80 and CD86), costimulatory molecules for MHC II function and interaction with $CD4^+$ T cells, also revealed induction by subsets. IFNγ injection in animals caused a scattered MHC II^+ induction, a pattern ruling out that it just followed a gradient (Fitzner et al., 2011; Scheffel et al., 2012). Moreover, evidence for restricted expression can also be derived from other reports (Ponomarev et al., 2005, 2006; Starossom et al., 2012). We presented data from a histopathological examination of human tissue that expression of respective HLA structures in hypoxic lesions is confined to individual microglia (Scheffel et al., 2012). TLR4 signaling organization by subsets could probably relate to the organization of subsets by MHC II expression. A recent study identified a previously unknown importance of the intracellular MHC II pool in acting as an adaptor in TLR signaling (Liu et al., 2011). Conceivably, MHC II, as it is also inducible by IFNγ, would then participate in the TLR reactions of some cells, whereas TLR4 itself is functionally expressed by the majority of microglia. The well-known phenomenon of IFNγ priming as affecting microglial challenges by LPS (Häusler et al., 2002) may consequently involve unequal mechanisms in cellular subsets (Scheffel et al., 2012).

While phagocytotic and other clearance mechanisms are essential elements of the microglial activity spectrum to support CNS development as well as maintenance, removal of foreign and endogenous material is most critical in infections, injuries, autoimmune and degenerative processes (Hanisch and Kettenmann, 2007; Kettenmann et al., 2011). Successful clearance of bacteria decides already in the initial phase of an infection on the extent of damage and sequelae, while efficient removal of myelin debris is a known prerequisite for attempts of remyelination in multiple sclerosis. In other disease, deficiency in the clearance of protein deposits, such as Aβ plaques in Alzheimer's disease, may have harmful outcomes, with the role of microglia being different in comparison to extraneural monocytes. Mechanisms of a functional – and dysfunctional – clearance are of an imaginable huge clinical importance (see also above). On the other hand, these functions have been addresses mainly for *the* microglia, with thus far little conscious distinction of potential subtypes.

Myelin uptake activity in pathophysiological conditions apparently associates with a fraction of microglia (van Rossum et al., 2008; Regen et al., 2011; Scheffel et al., 2012), similarly as it was described for the physiological disposal of exosomally encapsulated myelin (Fitzner et al., 2011). Even though the underlying mechanisms and the responsible molecular machinery of incorporation will differ between clearances of the exosomes *versus* free myelin, both are suppressed by inflammatory conditions (van Rossum et al., 2008; Fitzner et al., 2011; Regen et al., 2011; Scheffel et al., 2012). Interestingly, TLR activity regulates the myelin clearance with MyD88 signaling dependence and with opposite outcome in comparison to the phagocytosis and killing of Gram-negative as well as -positive bacterial strains (Ribes et al., 2009, 2010a,b, Ribes et al., 2011). The latter functions might also be reserved for subtypes, as work in progress suggests. Probably, this qualifies only partially overlapping sets of microglia as specialists that are either called up for duty when the CNS is under an infectious attack or take the part of clearing the terrain from debris.

The collecton of evidence for microglial responder heterogeneity could be continued as the literature can serve as a source for data and images that contain such information, although explicit mentioning is not always found in the text. Of course, gene expression studies reveal signatures that apply to microglia as a CNS-resident entity. Microglia show low expression of many transcripts which are readily found in extraneural macrophages, whereas they present with a particular profile of other genes, such as those encoding for molecules of the oxidative metabolism (Gautier et al., 2012). While such classes will be used to understand the gross-anatomical organization of myeloid cell distribution and function, it will be the identification of also minute differences that could provide an image of microglial response diversity and its probably underlying constitutive heterogeneity at high resolution. There is a list of molecules and activities, responses as well as susceptibilities with particular expression and distribution patterns, which would deserve consideration for subset

analyses (Butovsky et al., 2006a; He et al., 2006; Sriram et al., 2006; Ensinger et al., 2010; Iwama et al., 2011). Some molecules, such as galectin-3/Mac-2, could thereby prove as suitable candidates for delineating sets of cells by expression and associated function (Venkatesan et al., 2010).

INSTRUCTION AND ORGANIZATION OF HETEROGENEITY

Since the concept of microglial heterogeneity is just emerging, potential principles of its organization are still enigmatic. Several sources of instruction can, however, be envisaged. One may define orientations of cells during development. Alternatively, cells may acquire their individuality upon arrival at their tissue destination and within the respective local cell community. Still another model could be based on probability.

HETEROGENEITY BY LINEAGE ORIGIN AND MICROENVIRONMENT

Substantial efforts based on genetic strategies and fate mapping have led to the identification of progenitors and lineages that give rise to the various myeloid cell types and thereby covering macrophages of various tissues, including microglia. Against the common believe of a mandatory monocytic replenishment, this work can strengthen concepts claiming primitive progenitors from the yolk sac as a source. It shows that major tissue macrophage populations are established before birth and kept independent of monocytes from the blood (Naito et al., 1996; Yona et al., 2013). Transcription factors have been nominated to be distinctly involved in the development of DCs, monocytes, tissue macrophages and microglia, such as Batf3, Flt3, PU.1, or Myb (Ginhoux et al., 2010; Gautier et al., 2012; Schulz et al., 2012; Satpathy et al., 2012; Gomez Perdiguero et al., 2013). Work published now adds factors and steps for microgliogenesis, confirming PU.1 and introducing Irf8 (Kierdorf et al., 2013). Accordingly, microglia derive from primitive c-Kit-positive erythromyeloid precursors in the yolk sac of the mouse that develope through stages with distinct expression patterns of CD31, CD45, c-Kit, CX_3CR1, F4/80 as well as MCSFR and that depend for proper development and settlement also on MMP-8 and MMP-9 (Kierdorf et al., 2013). This fascinating research will continue to fill gaps in precursor sequences and help to draw road maps of tissue population (colonization). Conceivably, some late differentiation steps include further splitting of the microglial lineage to create diversity by subsets. Earlier work suggested, based on a gene defect and respective lineage labeling, that not the entire microglia population was affected, leaving room for speculation as to its heterogeneity (Chen et al., 2010). If microglial lineage branching during development would give rise to subtypes their distribution throughout the CNS would need to be outlined as well – especially to explain their settlement in overlapping territories.

Alternatively to a predisposition of cells as to their origin, tissue adaptation of molecular and functional properties could derive from local instructions and the requirements of particular micromilieus. The anatomical divisions of the CNS come with variable composition as it regards their cellular and molecular constituents. Simply sorted by white and gray matter, the regions differ by the content of myelin, a complex and vulnerable structure that by itself may determine microglial properties or set directions for the flow of messengers. Expression of receptors does vary by region, and inspection of data published earlier may indicate trends by white matter content (de Haas et al., 2008). These differences could impact on development, normal functionality or the vulnerability of CNS structures to inflammation (McKay et al., 2007; Hristova et al., 2010). Microglia from white and gray matter differ by expression of Tim-3, an immunoregulatory receptor (Anderson et al., 2007). The authors linked its differential and timed expression by microglia, DC-like as well as Th1 cells to a regulation of inflammation and immune responses in defined phases. Interestingly, heterogeneity of microglia might be complemented by heterogeneity of oligodendroglial subpopulations in subregions of gray and white matter (Kitada and Rowitch, 2006). The biochemical milieu is also determined by the vascular and ECM features. Exposure to some plasma factors at certain sites – and not only upon a BBB disruption – may affect neighboring microglia, as reflected by the distinct sialoadhesion expression (Perry et al., 1992). Microglia are not just embedded in an ECM environment for structural support. Interactions are not limited to the manipulation and rebuilding of ECM by microglial proteases, such as upon an injury or cell migration, but include signals to microglia, for example via integrins. Certain factors are – in varied format – present in both the plasma and the ECM, like fibronectin, that can potentially trigger microglial responses through TLR4 (Regen et al., 2011).

Structures of the CNS vary by prevalent neurotransmitters, for example in the cerebral cortex by the layers. Microglia express a range of classical neurotransmitter and purinergic receptors and also respond to their activation (Pocock and Kettenmann, 2007). Accordingly, they may sense acetylcholine, dopamine, glutamate, noradrenaline, GABA, and purines, namely ATP and metabolites (Kettenmann et al., 2011). In addition, the repertoire of receptors covers those for neurohormones, neuromodulators, steroids, prostaglandins, leukotrienes, complement, and immunoglobulins in order to respond by various outcomes to opiodis, angiotensin, bradykinin, endothelin, neurokinins, neurotrophins, somatostatin, vasoactive intestinal polypeptide, cannabinoids, histamine, platelet activating factor, glucocorticoids, and mineralocorticoids (Kettenmann et al., 2011). Microglia have been shown to express receptors for proteases, such as thrombin, which may come into play when an injury causes inundation of the parenchyma by plasma content but also when such factors are operating within the intact CNS (Balcaitis et al., 2003; Hanisch et al., 2004). Their importance may differ and vary with the ontogenic stage or homoeostatic situation. Some may serve as modulators to synergize with or to contain the consequences of cytokines, chemokines or microbial agents (Kettenmann et al., 2011). Undoubted is, however, that they would exert control on different microglial cell populations differently depending on the local levels of their agonists. Distinguishable expression profiles of such receptors would determine distinct functional adjustments. Yet even more classical neurotransmitter receptors may have some not yet fully appreciated implications in a general, phasic or disease-related control of microglia and with specificity by receptor properties and outcomes by region (Zerrate et al., 2007; Heneka et al., 2010). For CNS tissue structures and their microglia, neurochemical cues could be

critical determinants. However, would tiny differences or flat gradients of instructing factors be sufficient to govern differential subtype orientation for cells in close vicinity?

RESPONDER SUBTYPES BY STOCHASTIC VARIATION

The above mechanisms would define individual capacities of microglia by some constitutive principle. Cells would be predetermined by lineage commitment or by their environment. Yet variety in responses, such as profiles of induced genes, could also be explained by probability (Hume, 2000). This exciting concept describes transcriptional regulation in individual cells by probabilistic events and could explain heterogeneous expression among a cell population as occurring by chance. The concept explicitly applies to leukocyte differentiation and activities. It thereby even offers tempting options for explaining the observation of subpopulations. Key to the model is the variable stability of the respective mRNAs and their translation products, i.e., proteins. A physiological "purpose" of such a simply stochastic organization could derive from the generation of an entire spectrum of individual responses in individual responders. Such a repertoire could then cope with any challenge. Indeed, heterogeneity in the responses of a macrophage cell line to LPS exposure was subsequently shown to arise from autonomous probability in the transcription of individual genes, including TNFα (Ravasi et al., 2002).

A "hatched" expression of genes could be an argument against the existence of "real," namely pre-determined subtypes. In subpopulation analyses of any kind, some quantitative variation could pretend qualitative distinctions simply due to insufficient detection sensitivity or a variation by chance. The former situation would be a technical pitfall, the latter would stand for a basic phenomenon. A probabilistic organization of response diversity would thus not require any pre-instruction and leave the decision on subtypes to the moment of a challenge. It remains to be shown whether this is enough to explain all facets of functional diversity of microglia (and other myeloid cell populations), including housekeeping activities and activation in emergency settings. Indeed, transcriptional regulation in macrophages presents with some previously unforeseen complexity (Lawrence and Natoli, 2011). Multiplicity of approaches will avoid that conclusions are rashly drawn from single lines of evidence. In this regard, populational context may determine single-cell heterogeneity in a combination of intrinsic and extrinsic factors. Modeling approaches aim at explaining the phenomenon and to predict differences among a cell population with regard to gene transcription, phosphorylation events, cell morphology as well as drug perturbations (Snijder et al., 2009). Stochastic fluctuations in transcription factors at critical decision-making phases could participate in creating and determining (also local) diversity of microglia. Most convincing support for an existence of distinct microglia could be taken from a demonstration of the vital importance of a particular subtype and by dissecting the mechanisms by which it is instructed.

ADJUSTMENT OF RESPONSE CAPACITIES BY EXPERIENCE

All this has to consider that microglia are a rather long-lived population that is probably maintained with some in-tissue renewal but – most likely – without replenishment from bone marrow sources, at least under healthy adult conditions (Mildner et al., 2007; Ajami et al., 2011; Gomez Perdiguero et al., 2013). If instructed for a set of (potential) functions by local cues, the setting might be either constantly maintained, similarly driven by receptor signaling as it was discussed for control of the activity level by "on" and "off" signals, or it might be induced once and preserved. Could a microglia subpopulation, for example, be experimentally translocated from its natural location to another tissue region and thereby change prominent features? It would also be important to clarify whether commitments stay stable throughout the life span of the individual cell and/or whether they may change – especially also following to a challenge. Would, for example, a transient activation episod simply lead to a return of microglia to their naïve pre-activation status or leave an engram affecting functional behavior in the post-activation phase? Would this lead to a (locally) distinct microglia?

This question has been raised but awaits further answers from experimental demonstrations of either preserved or altered microglial properties subsequent to activation (Hanisch and Kettenmann, 2007; Saijo and Glass, 2011; Hanisch, 2012). Phenomena of "priming" and "tolerance," however, are already described. Also microglia change responses to a stimulus after being exposed to the same or another stimulus before (Häusler et al., 2002). If lasting for a longer period of time, such an altered phenotype setting could be termed "experience" or even "memory." Traces of former gene activation remain in form of epigenetic modifications and thereby affect transcriptional activity for a long time and even through a cell cycle (Probst et al., 2009; Suderman et al., 2012). Covalent modifications of chromatin proteins or the DNA itself are major epigenetic principles. In contrast to cells of the adaptive immunity (such as T lymphocytes), which reveal distinct epigenetic changes during their differentiation (Wei et al., 2009), epigenetic regulation in innate immune cells is still largely unknown. As macrophages maintain a high degree of plasticity to commit to diverse reactive phenotypes, epigenetic modifications could be expected to take a part therein. Indeed, regulations of this kind seem to play fundamental roles in inflammatory processes and the functional control of innate immunity (Foster et al., 2007; Foster and Medzhitov, 2009).

CONCLUSION

After all, the plural term "microglia" may include a true meaning as to the existence of subsets with preferential or exclusive duties. Subsets may specialize for day-to-day functions as well as harbor selective capacities that become effective on demand. Whether such an individuality is predestined before or organized upon arrival of microglia at their locations in the tissue – or whether it occurs upon challenge as an entirely stochastic phenomenon – still remains to be shown. Whether and how a specialization can be found similarly throughout the CNS is not known either. The concept of functional diversity is worth more consideration not for the sake of an inventory of modifications or a sorting of cells and cell activities

of every description. Practical value derives from a refined classification of activities and activity states in lesions or during the course of a neuropathology. Most importantly, individual microglial subsets and (or) their functions could be addressed by targeted manipulation in experimental settings and therapeutic interventions.

ACKNOWLEDGMENTS

This work was supported by the German Research Council (DFG) and respective grants within the SFB/TRR43 and FOR1336. The inspiring discussions with Hugh Perry deserve special mentioning as several critical comments helped to consider alternative explanations and concepts.

REFERENCES

Aguzzi, A., Barres, B. A., and Bennett, M. L. (2013). Microglia: scapegoat, saboteur, or something else? *Science* 339, 156–161.

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M., and Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* 14, 1142–1149.

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

Anderson, A. C., Anderson, D. E., Bregoli, L., Hastings, W. D., Kassam, N., Lei, C., et al. (2007). Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. *Science* 318, 1141–1143.

Anderson, C. F., Gerber, J. S., and Mosser, D. M. (2002). Modulating macrophage function with IgG immune complexes. *J. Endotoxin Res.* 8, 477–481.

Anderson, C. F., and Mosser, D. M. (2002). A novel phenotype for an activated macrophage: the type 2 activated macrophage. *J. Leukoc. Biol.* 72, 101–106.

Balcaitis, S., Xie, Y., Weinstein, J. R., Andersen, H., Hanisch, U. K., Ransom, B. R., et al. (2003). Expression of proteinase-activated receptors in mouse microglial cells. *Neuroreport* 14, 2373–2377.

Bechmann, I., Galea, I., and Perry, V. H. (2007). What is the blood–brain barrier (not)? *Trends Immunol.* 28, 5–11.

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci.* 30, 596–602.

Bilbo, S. D., Biedenkapp, J. C., Der-Avakian, A., Watkins, L. R., Rudy, J. W., and Maier, S. F. (2005). Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition. *J. Neurosci.* 25, 8000–8009.

Bilbo, S. D., and Schwarz, J. M. (2009). Early-life programming of later-life brain and behavior: a critical role for the immune system. *Front. Behav. Neurosci.* 3:14. doi: 10.3389/neuro.08.014.2009

Biswas, S. K., and Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* 11, 889–896.

Bitsch, A., Schuchardt, J., Bunkowski, S., Kuhlmann, T., and Bruck, W. (2000). Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 123(Pt 6), 1174–1183.

Block, M. L., Zecca, L., and Hong, J. S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69.

Butovsky, O., Koronyo-Hamaoui, M., Kunis, G., Ophir, E., Landa, G., Cohen, H., et al. (2006a). Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. *Proc. Natl. Acad. Sci U.S.A.* 103, 11784–11789.

Butovsky, O., Landa, G., Kunis, G., Ziv, Y., Avidan, H., Greenberg, N., et al. (2006b). Induction and blockage of oligodendrogenesis by differently activated microglia in an animal model of multiple sclerosis. *J. Clin. Invest.* 116, 905–915.

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A. E., Pluchino, S., et al. (2006c). Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31, 149–160.

Butovsky, O., Siddiqui, S., Gabriely, G., Lanser, A. J., Dake, B., Murugaiyan, G., et al. (2012). Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. *J. Clin. Invest.* 122, 3063–3087.

Carson, M. J., Bilousova, T. V., Puntambekar, S. S., Melchior, B., Doose, J. M., and Ethell, I. M. (2007). A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease. *Neurotherapeutics* 4, 571–579.

Carson, M. J., Reilly, C. R., Sutcliffe, J. G., and Lo, D. (1998). Mature microglia resemble immature antigen-presenting cells. *Glia* 22, 72–85.

Chang, N. C., Hung, S. I., Hwa, K. Y., Kato, I., Chen, J. E., Liu, C. H., et al. (2001). A macrophage protein, Ym1, transiently expressed during inflammation is a novel mammalian lectin. *J. Biol. Chem.* 276, 17497–17506.

Chen, G. Y., and Nunez, G. (2010). Sterile inflammation: sensing and reacting to damage. *Nat. Rev. Immunol.* 10, 826–837.

Chen, S. K., Tvrdik, P., Peden, E., Cho, S., Wu, S., Spangrude, G., et al. (2010). Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* 141, 775–785.

Cho, H., Proll, S. C., Szretter, K. J., Katze, M. G., Gale, M. Jr., and Diamond, M. S. (2013). Differential innate immune response programs in neuronal subtypes determine susceptibility to infection in the brain by positive-stranded RNA viruses. *Nat. Med.* 19, 458–464.

Colton, C. A. (2009). Heterogeneity of microglial activation in the innate immune response in the brain. *J. Neuroimmune Pharmacol.* 4, 399–418.

Colton, C. A., Mott, R. T., Sharpe, H., Xu, Q., Van Nostrand, W. E., and Vitek, M. P. (2006). Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD. *J. Neuroinflammation* 3, 27.

Cunningham, C. (2013). Microglia and neurodegeneration: the role of systemic inflammation. *Glia* 61, 71–90.

Daley, J. M., Brancato, S. K., Thomay, A. A., Reichner, J. S., and Albina, J. E. (2010). The phenotype of murine wound macrophages. *J. Leukoc. Biol.* 87, 59–67.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* 8, 752–758.

David, S., and Kroner, A. (2011). Repertoire of microglial and macrophage responses after spinal cord injury. *Nat. Rev. Neurosci.* 12, 388–399.

Davoust, N., Vuaillat, C., Androdias, G., and Nataf, S. (2008). From bone marrow to microglia: barriers and avenues. *Trends Immunol.* 29, 227–234.

de Haas, A. H., Boddeke, H. W., and Biber, K. (2008). Region-specific expression of immunoregulatory proteins on microglia in the healthy CNS. *Glia* 56, 888–894.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

Donnou, S., Fisson, S., Mahe, D., Montoni, A., and Couez, D. (2005). Identification of new CNS-resident macrophage subpopulation molecular markers for the discrimination with murine systemic macrophages. *J. Neuroimmunol.* 169, 39–49.

Draheim, H. J., Prinz, M., Weber, J. R., Weiser, T., Kettenmann, H., and Hanisch, U. K. (1999). Induction of potassium channels in mouse brain microglia: cells acquire responsiveness to pneumococcal cell wall components during late development. *Neuroscience* 89, 1379–1390.

Edwards, J. P., Zhang, X., Frauwirth, K. A., and Mosser, D. M. (2006). Biochemical and functional characterization of three activated macrophage populations. *J. Leukoc. Biol.* 80, 1298–1307.

Ensinger, E. M., Boekhoff, T. M., Carlson, R., Beineke, A., Rohn, K., Tipold, A., et al. (2010). Regional topographical differences of canine microglial immunophenotype and function in the healthy spinal cord. *J. Neuroimmunol.* 227, 144–152.

Fitzner, D., Schnaars, M., van Rossum, D., Krishnamoorthy, G., Dibaj, P., Bakhti, M., et al. (2011). Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J. Cell Sci.* 124, 447–458.

Foster, S. L., Hargreaves, D. C., and Medzhitov, R. (2007). Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 447, 972–978.

Foster, S. L., and Medzhitov, R. (2009). Gene-specific control of the TLR-induced inflammatory response. *Clin. Immunol.* 130, 7–15.

Gautier, E. L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., et al. (2012). Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 13, 1118–1128.

Gerber, J. S., and Mosser, D. M. (2001). Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. *J. Immunol.* 166, 6861–6868.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Girard, S., Brough, D., Lopez-Castejon, G., Giles, J., Rothwell, N. J., and Allan, S. M. (2013). Microglia and macrophages differentially modulate cell death after brain injury caused by oxygen-glucose deprivation in organotypic brain slices. *Glia* 61, 813–824.

Gomez Perdiguero, E., Schulz, C., and Geissmann, F. (2013). Development and homeostasis of "resident" myeloid cells: the case of the microglia. *Glia* 61, 12–20.

Gordon, S., and Taylor, P. R. (2005). Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* 5, 953–964.

Graeber, M. B. (2010). Changing face of microglia. *Science* 330, 783–788.

Greter, M., and Merad, M. (2013). Regulation of microglia development and homeostasis. *Glia* 61, 121–127.

Hanisch, U. K. (2002). Microglia as a source and target of cytokines. *Glia* 40, 140–155.

Hanisch, U. K. (2012). "Factors controlling microglial activation", in *Neuroglia*, eds H. Kettenmann and B. Ransom (New York: Oxford University Press), 614–625.

Hanisch, U. K. (2013). Proteins in microglial activation – inputs and outputs by subsets. *Curr. Protein Pept. Sci.* 14, 3–15.

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394.

Hanisch, U. K., van, R. D., Xie, Y., Gast, K., Misselwitz, R., Auriola, S., et al. (2004). The microglia-activating potential of thrombin: the protease is not involved in the induction of proinflammatory cytokines and chemokines. *J. Biol. Chem.* 279, 51880–51887.

Häusler, K. G., Prinz, M., Nolte, C., Weber, J. R., Schumann, R. R., Kettenmann, H., et al. (2002). Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages. *Eur. J. Neurosci.* 16, 2113–2122.

Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W. B., et al. (2006). The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* 9, 1512–1519.

He, B. P., Wang, J. J., Zhang, X., Wu, Y., Wang, M., Bay, B. H., et al. (2006). Differential reactions of microglia to brain metastasis of lung cancer. *Mol. Med.* 12, 161–170.

Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., et al. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* 493, 674–678.

Heneka, M. T., Nadrigny, F., Regen, T., Martinez-Hernandez, A., Dumitrescu-Ozimek, L., Jardanhazi-Kurutz, D., et al. (2010). Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6058–6063.

Hristova, M., Cuthill, D., Zbarsky, V., costa-Saltos, A., Wallace, A., Blight, K., et al. (2010). Activation and deactivation of periventricular white matter phagocytes during postnatal mouse development. *Glia* 58, 11–28.

Hume, D. A. (2000). Probability in transcriptional regulation and its implications for leukocyte differentiation and inducible gene expression. *Blood* 96, 2323–2328.

Ilschner, S., and Brandt, R. (1996). The transition of microglia to a ramified phenotype is associated with the formation of stable acetylated and detyrosinated microtubules. *Glia* 18, 129–140.

Iwama, S., Sugimura, Y., Suzuki, H., Suzuki, H., Murase, T., Ozaki, N., et al. (2011). Time-dependent changes in proinflammatory and neurotrophic responses of microglia and astrocytes in a rat model of osmotic demyelination syndrome. *Glia* 59, 452–462.

Kaul, D., Habbel, P., Derkow, K., Kruger, C., Franzoni, E., Wulczyn, F. G., et al. (2012). Expression of Toll-like receptors in the developing brain. *PLoS ONE* 7:e37767. doi: 10.1371/journal.pone.0037767

Kawahara, K., Yoshida, A., Koga, K., Yokoo, S., Kuniyasu, A., Gotoh, T., et al. (2009). Marked induction of inducible nitric oxide synthase and tumor necrosis factor-alpha in rat CD40+ microglia by comparison to CD40- microglia. *J. Neuroimmunol.* 208, 70–79.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Kettenmann, H., Kirchhoff, F., and Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. *Neuron* 77, 10–18.

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* 16, 273–280.

Kigerl, K. A., Gensel, J. C., Ankeny, D. P., Alexander, J. K., Donnelly, D. J., and Popovich, P. G. (2009). Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J. Neurosci.* 29, 13435–13444.

Kim, W. G., Mohney, R. P., Wilson, B., Jeohn, G. H., Liu, B., and Hong, J. S. (2000). Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J. Neurosci.* 20, 6309–6316.

Kitada, M., and Rowitch, D. H. (2006). Transcription factor co-expression patterns indicate heterogeneity of oligodendroglial subpopulations in adult spinal cord. *Glia* 54, 35–46.

Kono, H., and Rock, K. L. (2008). How dying cells alert the immune system to danger. *Nat. Rev. Immunol.* 8, 279–289.

Kraft, A. D., McPherson, C. A., and Harry, G. J. (2009). Heterogeneity of microglia and TNF signaling as determinants for neuronal death or survival. *Neurotoxicology* 30, 785–793.

Ladeby, R., Wirenfeldt, M., Dalmau, I., Gregersen, R., Garcia-Ovejero, D., Babcock, A., et al. (2005). Proliferating resident microglia express the stem cell antigen CD34 in response to acute neural injury. *Glia* 50, 121–131.

Lai, A. Y., Dhami, K. S., Dibal, C. D., and Todd, K. G. (2011). Neonatal rat microglia derived from different brain regions have distinct activation responses. *Neuron Glia Biol.* 7, 5–16.

Landgraf, M., and Evers, J. F. (2005). Control of dendritic diversity. *Curr. Opin. Cell Biol.* 17, 690–696.

Lawrence, T., and Natoli, G. (2011). Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat. Rev. Immunol.* 11, 750–761.

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170.

Lehnardt, S., Schott, E., Trimbuch, T., Laubisch, D., Krueger, C., Wulczyn, G., et al. (2008). A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *J. Neurosci.* 28, 2320–2331.

Liu, X., Zhan, Z., Li, D., Xu, L., Ma, F., Zhang, P., et al. (2011). Intracellular MHC class II molecules promote TLR-triggered innate immune responses by maintaining activation of the kinase Btk. *Nat. Immunol.* 12, 416–424.

London, A., Benhar, I., Mattapallil, M. J., Mack, M., Caspi, R. R., and Schwartz, M. (2013). Functional macrophage heterogeneity in a mouse model of autoimmune central nervous system pathology. *J. Immunol.* 190, 3570–3578.

Lotze, M. T., Zeh, H. J., Rubartelli, A., Sparvero, L. J., Amoscato, A. A., Washburn, N. R., et al. (2007). The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol. Rev.* 220, 60–81.

Mantovani, A., Biswas, S. K., Galdiero, M. R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. *J. Pathol.* 229, 176–185.

Mantovani, A., Sica, A., Allavena, P., Garlanda, C., and Locati, M. (2009). Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. *Hum. Immunol.* 70, 325–330.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., and Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 25, 677–686.

Maresz, K., Ponomarev, E. D., Barteneva, N., Tan, Y., Mann, M. K., and Dittel, B. N. (2008). IL-13 induces the expression of the alternative activation marker Ym1 in a subset of testicular macrophages. *J. Reprod. Immunol.* 78, 140–148.

Marshall, G. P., Demir, M., Steindler, D. A., and Laywell, E. D. (2008). Subventricular zone microglia possess a unique capacity for massive in vitro expansion. *Glia* 56, 1799–1808.

Martinez, F. O., Gordon, S., Locati, M., and Mantovani, A. (2006). Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J. Immunol.* 177, 7303–7311.

Martinez, F. O., Helming, L., and Gordon, S. (2009). Alternative activation of macrophages: an immunologic functional perspective. *Annu. Rev. Immunol.* 27, 451–483.

Martinez, F. O., Helming, L., Milde, R., Varin, A., Melgert, B. N., Draijer, C., et al. (2013). Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. *Blood* 121, e57–e69.

Matyash, V., and Kettenmann, H. (2010). Heterogeneity in astrocyte morphology and physiology. *Brain Res. Rev.* 63, 2–10.

Matzinger, P. (2007). Friendly and dangerous signals: is the tissue in control? *Nat. Immunol.* 8, 11–13.

McKay, S. M., Brooks, D. J., Hu, P., and McLachlan, E. M. (2007). 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.

Meeuwsen, S., Bsibsi, M., Persoon-Deen, C., Ravid, R., and van Noort, J. M. (2005). Cultured human adult microglia from different donors display stable cytokine, chemokine and growth factor gene profiles but respond differently to a pro-inflammatory stimulus. *Neuroimmunomodulation* 12, 235–245.

Mertsch, K., Hanisch, U. K., Kettenmann, H., and Schnitzer, J. (2001). Characterization of microglial cells and their response to stimulation in an organotypic retinal culture system. *J. Comp. Neurol.* 431, 217–227.

Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E., and Heuschling, P. (2009). 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.

Mikita, J., Dubourdieu-Cassagno, N., Deloire, M. S., Vekris, A., Biran, M., Raffard, G., et al. (2011). Altered M1/M2 activation patterns of monocytes in severe relapsing experimental rat model of multiple sclerosis. Amelioration of clinical status by M2 activated monocyte administration. *Mult. Scler.* 17, 2–15.

Mildner, A., Schlevogt, B., Kierdorf, K., Bottcher, C., Erny, D., Kummer, M. P., et al. (2011). Distinct and non-redundant roles of microglia and myeloid subsets in mouse models of Alzheimer's disease. *J. Neurosci.* 31, 11159–11171.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., et al. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* 10, 1544–1553.

Miller, K. R., and Streit, W. J. (2007). The effects of aging, injury and disease on microglial function: a case for cellular senescence. *Neuron Glia Biol.* 3, 245–253.

Mizutani, M., Pino, P. A., Saederup, N., Charo, I. F., Ransohoff, R. M., and Cardona, A. E. (2012). The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. *J. Immunol.* 188, 29–36.

Monji, A., Kato, T., and Kanba, S. (2009). Cytokines and schizophrenia: microglia hypothesis of schizophrenia. *Psychiatry Clin. Neurosci.* 63, 257–265.

Morrison, H. W., and Filosa, J. A. (2013). A quantitative spatiotemporal analysis of microglia morphology during ischemic stroke and reperfusion. *J. Neuroinflammation.* 10:4. doi: 10.1186/1742-2094-10-4 [Epub ahead of print].

Mosser, D. M., and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8, 958–969.

Mouton, P. R., Long, J. M., Lei, D. L., Howard, V., Jucker, M., Calhoun, M. E., et al. (2002). Age and gender effects on microglia and astrocyte numbers in brains of mice. *Brain Res.* 956, 30–35.

Murray, P. J., and Wynn, T. A. (2011). Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* 11, 723–737.

Naito, M., Umeda, S., Yamamoto, T., Moriyama, H., Umezu, H., Hasegawa, G., et al. (1996). Development, differentiation, and phenotypic heterogeneity of murine tissue macrophages. *J. Leukoc. Biol.* 59, 133–138.

Neumann, H., and Daly, M. J. (2013). Variant TREM2 as risk factor for Alzheimer's disease. *N. Engl. J. Med.* 368, 182–184.

Neumann, H., Kotter, M. R., and Franklin, R. J. (2009). Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain* 132, 288–295.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314–1318.

Olah, M., Amor, S., Brouwer, N., Vinet, J., Eggen, B., Biber, K., et al. (2012). Identification of a microglia phenotype supportive of remyelination. *Glia* 60, 306–321.

Olah, M., Biber, K., Vinet, J., and Boddeke, H. W. (2011). Microglia phenotype diversity. *CNS Neurol. Disord. Drug Targets* 10, 108–118.

Pal, D., Dasgupta, S., Kundu, R., Maitra, S., Das, G., Mukhopadhyay, S., et al. (2012). Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat. Med.* doi: 10.1038/nm.2851[Epub ahead of print].

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Perry, V. H., Crocker, P. R., and Gordon, S. (1992). The blood–brain barrier regulates the expression of a macrophage sialic acid-binding receptor on microglia. *J. Cell Sci.* 101(Pt 1), 201–207.

Perry, V. H., Cunningham, C., and Holmes, C. (2007). Systemic infections and inflammation affect chronic neurodegeneration. *Nat. Rev. Immunol.* 7, 161–167.

Piccinini, A. M., and Midwood, K. S. (2010). DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm.* 2010, 672395.

Piccio, L., Buonsanti, C., Cella, M., Tassi, I., Schmidt, R. E., Fenoglio, C., et al. (2008). Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. *Brain* 131, 3081–3091.

Pocock, J. M., and Kettenmann, H. (2007). Neurotransmitter receptors on microglia. *Trends Neurosci.* 30, 527–535.

Pollard, J. W. (2009). Trophic macrophages in development and disease. *Nat. Rev. Immunol.* 9, 259–270.

Ponomarev, E. D., Maresz, K., Tan, Y., and Dittel, B. N. (2007). CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. *J. Neurosci.* 27, 10714–10721.

Ponomarev, E. D., Shriver, L. P., and Dittel, B. N. (2006). CD40 expression by microglial cells is required for their completion of a two-step activation process during central nervous system autoimmune inflammation. *J. Immunol.* 176, 1402–1410.

Ponomarev, E. D., Shriver, L. P., Maresz, K., and Dittel, B. N. (2005). Microglial cell activation and proliferation precedes the onset of CNS autoimmunity. *J. Neurosci. Res.* 81, 374–389.

Popovich, P. G., Guan, Z., Wei, P., Huitinga, I., van, R. N., and Stokes, B. T. (1999). Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. *Exp. Neurol.* 158, 351–365.

Prinz, M., Priller, J., Sisodia, S. S., and Ransohoff, R. M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* 13, 1227–1235.

Probst, A. V., Dunleavy, E., and Almouzni, G. (2009). Epigenetic inheritance during the cell cycle. *Nat. Rev. Mol. Cell Biol.* 10, 192–206.

Pukrop, T., Dehgani, F., Han-Ning, C., Lohaus, R., Byanga, K., Heermann, S., et al. (2010). Microglia promote colonization of brain tissue by breast cancer cells in a Wnt-dependent way. *Glia* 58, 1477–1489.

Raes, G., Brys, L., Dahal, B. K., Brandt, J., Grooten, J., Brombacher, F., et al. (2005). Macrophage galactose-type C-type lectins as novel markers for alternatively activated macrophages elicited by parasitic infections and allergic airway inflammation. *J. Leukoc. Biol.* 77, 321–327.

Raes, G., De, B. P., Noel, W., Beschin, A., Brombacher, F., and Hassanzadeh, G. G. (2002). Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. *J. Leukoc. Biol.* 71, 597–602.

Ransohoff, R. M., and Cardona, A. E. (2010). The myeloid cells of the central nervous system parenchyma. *Nature* 468, 253–262.

Ransohoff, R. M., and Engelhardt, B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat. Rev. Immunol.* 12, 623–635.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Ravasi, T., Wells, C., Forest, A., Underhill, D. M., Wainwright, B. J.,

Aderem, A., et al. (2002). Generation of diversity in the innate immune system: macrophage heterogeneity arises from gene-autonomous transcriptional probability of individual inducible genes. *J. Immunol.* 168, 44–50.

Regen, T., van Rossum, D., Scheffel, J., Kastriti, M. E., Revelo, N. H., Prinz, M., et al. (2011). CD14 and TRIF govern distinct responsiveness and responses in mouse microglial TLR4 challenges by structural variants of LPS. *Brain Behav. Immun.* 25, 957–970.

Rezaie, P., and Male, D. (1999). Colonisation of the developing human brain and spinal cord by microglia: a review. *Microsc. Res. Tech.* 45, 359–382.

Ribes, S., Adam, N., Ebert, S., Regen, T., Bunkowski, S., Hanisch, U. K., et al. (2011). The viral TLR3 agonist poly(I:C) stimulates phagocytosis and intracellular killing of *Escherichia coli* by microglial cells. *Neurosci. Lett.* 482, 17–20.

Ribes, S., Ebert, S., Czesnik, D., Regen, T., Zeug, A., Bukowski, S., et al. (2009). Toll-like receptor prestimulation increases phagocytosis of *Escherichia coli* DH5alpha and *Escherichia coli* K1 strains by murine microglial cells. *Infect. Immun.* 77, 557–564.

Ribes, S., Ebert, S., Regen, T., Agarwal, A., Tauber, S. C., Czesnik, D., et al. (2010a). Toll-like receptor stimulation enhances phagocytosis and intracellular killing of nonencapsulated and encapsulated *Streptococcus pneumoniae* by murine microglia. *Infect. Immun.* 78, 865–871.

Ribes, S., Ebert, S., Regen, T., Czesnik, D., Scheffel, J., Zeug, A., et al. (2010b). Fibronectin stimulates *Escherichia coli* phagocytosis by microglial cells. *Glia* 58, 367–376.

Rivest, S. (2009). Regulation of innate immune responses in the brain. *Nat. Rev. Immunol.* 9, 429–439.

Rubartelli, A., and Lotze, M. T. (2007). Inside,outside, upside down: damage-associated molecular pattern molecules (DAMPs) and redox. *Trends Immunol.* 28, 429–436.

Saederup, N., Cardona, A. E., Croft, K., Mizutani, M., Cotleur, A. C., Tsou, C. L., et al. (2010). Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS ONE* 5:e13693. doi: 10.1371/journal.pone.0013693

Saijo, K., and Glass, C. K. (2011). Microglial cell origin and phenotypes in health and disease. *Nat. Rev. Immunol.* 11, 775–787.

Satpathy, A. T., Wu, X., Albring, J. C., and Murphy, K. M. (2012). Re(de)fining the dendritic cell lineage. *Nat. Immunol.* 13, 1145–1154.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Scheffel, J., Regen, T., van Rossum, D., Seifert, S., Ribes, S., Nau, R., et al. (2012). Toll-like receptor activation reveals developmental reorganization and unmasks responder subsets of microglia. *Glia* 60, 1930 –1943.

Schlegelmilch, T., Henke, K., and Peri, F. (2011). Microglia in the developing brain: from immunity to behaviour. *Curr. Opin. Neurobiol.* 21, 5–10.

Schmid, C. D., Melchior, B., Masek, K., Puntambekar, S. S., Danielson, P. E., Lo, D. D., et al. (2009). Differential gene expression in LPS/IFNgamma activated microglia and macrophages: in vitro versus in vivo. *J. Neurochem.* 109(Suppl. 1), 117–125.

Schmid, C. D., Sautkulis, L. N., Danielson, P. E., Cooper, J., Hasel, K. W., Hilbush, B. S., et al. (2002). Heterogeneous expression of the triggering receptor expressed on myeloid cells-2 on adult murine microglia. *J. Neurochem.* 83, 1309–1320.

Schulz, C., Gomez, P. E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336, 86–90.

Schwartz, M., Butovsky, O., Brück, W., and Hanisch, U. K. (2006). Microglial phenotype: is the commitment reversible? *Trends Neurosci.* 29, 68–74.

Shen, A., Zhou, D., Shen, Q., Liu, H. O., Sun, L., Liu, Y., et al. (2009). The expression of tumor necrosis factor-alpha (TNF-alpha) by the intrathecal injection of lipopolysaccharide in the rat spinal cord. *Neurochem. Res.* 34, 333–341.

Sierra, A., Gottfried-Blackmore, A. C., McEwen, B. S., and Bulloch, K. (2007). Microglia derived from aging mice exhibit an altered inflammatory profile. *Glia* 55, 412–424.

Smedman, C., Ernemar, T., Gudmundsdotter, L., Gille-Johnson, P., Somell, A., Nihlmark, K., et al. (2011). FluoroSpot analysis of TLR-activated monocytes reveals several distinct cytokine secreting subpopulations. *Scand. J. Immunol.* doi: 10.1111/j.1365-3083.2011.02641 [Epub ahead of print].

Snijder, B., Sacher, R., Ramo, P., Damm, E. M., Liberali, P., and Pelkmans, L. (2009). Population context determines cell-to-cell variability in endocytosis and virus infection. *Nature* 461, 520–523.

Sriram, K., Matheson, J. M., Benkovic, S. A., Miller, D. B., Luster, M. I., and O'Callaghan, J. P. (2006). Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-alpha. *FASEB J.* 20, 670–682.

Starossom, S. C., Mascanfroni, I. D., Imitola, J., Cao, L., Raddassi, K., Hernandez, S. F., et al. (2012). Galectin-1 deactivates classically activated microglia and protects from inflammation-induced neurodegeneration. *Immunity* 37, 249–263.

Stewart, C. R., Stuart, L. M., Wilkinson, K., van Gils, J. M., Deng, J., Halle, A., et al. (2010). CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat. Immunol.* 11, 155–161.

Stolp, H. B., Dziegielewska, K. M., Ek, C. J., Potter, A. M., and Saunders, N. R. (2005). Long-term changes in blood–brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat. *Eur. J. Neurosci.* 22, 2805–2816.

Stolp, H. B., Ek, C. J., Johansson, P. A., Dziegielewska, K. M., Bethge, N., Wheaton, B. J., et al. (2009). Factors involved in inflammation-induced developmental white matter damage. *Neurosci. Lett.* 451, 232–236.

Streit, W. J., Miller, K. R., Lopes, K. O., and Njie, E. (2008). Microglial degeneration in the aging brain-bad news for neurons? *Front. Biosci.* 13, 3423–3438.

Suderman, M., McGowan, P. O., Sasaki, A., Huang, T. C., Hallett, M. T., Meaney, M. J., et al. (2012). Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. *Proc. Natl. Acad. Sci U.S.A.* 109(Suppl. 2), 17266–17272.

Sunderkotter, C., Nikolic, T., Dillon, M. J., van, R. N., Stehling, M., Drevets, D. A., et al. (2004). Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J. Immunol.* 172, 4410–4417.

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* 140, 805–820.

Thored, P., Heldmann, U., Gomes-Leal, W., Gisler, R., Darsalia, V., Taneera, J., et al. (2009). Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* 57, 835–849.

Tierney, J. B., Kharkrang, M., and La Flamme, A. C. (2009). Type II-activated macrophages suppress the development of experimental autoimmune encephalomyelitis. *Immunol. Cell Biol.* 87, 235–240.

Town, T., Nikolic, V., and Tan, J. (2005). The microglial "activation" continuum: from innate to adaptive responses. *J. Neuroinflammation.* 2, 24.

Tremblay, M. E., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069.

Treves, A. J. (1984). The origin of monocyte-macrophage heterogeneity: possible alternatives. *Med. Hypotheses* 14, 335–346.

Van den Bossche, J., Laoui, D., Morias, Y., Movahedi, K., Raes, G., De, B. P., et al. (2012). Claudin-1, claudin-2 and claudin-11 genes differentially associate with distinct types of anti-inflammatory macrophages in vitro and with parasite- and tumour-elicited macrophages in vivo. *Scand. J. Immunol.* 75, 588–598.

van Rossum, D., and Hanisch, U. K. (2004). Microglia. *Metab. Brain Dis.* 19, 393–411.

van Rossum, D., Hilbert, S., Strassenburg, S., Hanisch, U. K., and Bruck, W. (2008). Myelin-phagocytosing macrophages in isolated sciatic and optic nerves reveal a unique reactive phenotype. *Glia* 56, 271–283.

van Weering, H. R., Boddeke, H. W., Vinet, J., Brouwer, N., de Haas, A. H., van, R. N., et al. (2010). CXCL10/CXCR3 signaling in glia cells differentially affects NMDA-induced cell death in CA and DG neurons of the mouse hippocampus. *Hippocampus* 21, 220–232.

Varvel, N. H., Grathwohl, S. A., Baumann, F., Liebig, C., Bosch, A., Brawek, B., et al. (2012). Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc. Natl. Acad. Sci U.S.A.* 109, 18150–18155.

Venkatesan, C., Chrzaszcz, M., Choi, N., and Wainwright, M. S. (2010).

Chronic upregulation of activated microglia immunoreactive for galectin-3/Mac-2 and nerve growth factor following diffuse axonal injury. *J. Neuroinflammation* 7, 32.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Walton, N. M., Sutter, B. M., Laywell, E. D., Levkoff, L. H., Kearns, S. M., Marshall, G. P., et al. (2006). Microglia instruct subventricular zone neurogenesis. *Glia* 54, 815–825.

Weber, M. S., Prod'Homme, T., Youssef, S., Dunn, S. E., Rundle, C. D., Lee, L., et al. (2007). Type II monocytes modulate T cell-mediated central nervous system autoimmune disease. *Nat. Med.* 13, 935–943.

Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., et al. (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity* 30, 155–167.

Wilms, H., Wollmer, M. A., and Sievers, J. (1999). In vitro-staining specificity of the antibody 5-D-4 for microglia but not for monocytes and macrophages indicates that microglia are a unique subgroup of the myelomonocytic lineage. *J. Neuroimmunol.* 98, 89–95.

Wong, S. C., Puaux, A. L., Chittezhath, M., Shalova, I., Kajiji, T. S., Wang, X., et al. (2010). Macrophage polarization to a unique phenotype driven by B cells. *Eur. J. Immunol.* 40, 2296–2307.

Yona, S., and Jung, S. (2010). Monocytes: subsets, origins, fates and functions. *Curr. Opin. Hematol.* 17, 53–59.

Yona, S., Kim, K. W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 38, 79–91.

Zerrate, M. C., Pletnikov, M., Connors, S. L., Vargas, D. L., Seidler, F. J., Zimmerman, A. W., et al. (2007). Neuroinflammation and behavioral abnormalities after neonatal terbutaline treatment in rats: implications for autism. *J. Pharmacol. Exp. Ther.* 322, 16–22.

Zhang, X., and Mosser, D. M. (2008). Macrophage activation by endogenous danger signals. *J. Pathol.* 214, 161–178.

Zhou, X., Spittau, B., and Krieglstein, K. (2012). TGFbeta signalling plays an important role in IL-4-induced alternative activation of microglia. *J. Neuroinflammation* 9:210. doi: 10.1186/1742-2094-9-210 [Epub ahead of print].

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 January 2013; paper pending published: 04 March 2013; accepted: 19 April 2013; published online: 14 May 2013.

*Citation: Hanisch U-K (2013) Functional diversity of microglia – how heterogeneous are they to begin with? Front. Cell. Neurosci. **7**:65. doi: 10.3389/fncel.2013.00065*

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 23 April 2013
doi: 10.3389/fncel.2013.00044

Factors regulating microglia activation

Katrin Kierdorf [1,2] ***and Marco Prinz*** [1,3] *******

[1] *Institute of Neuropathology, University of Freiburg, Freiburg, Germany*
[2] *Faculty of Biology, University of Freiburg, Freiburg, Germany*
[3] *BIOSS Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany*

Edited by:
Amanda Sierra, Ikerbasque Foundation, University of the Basque Country, Spain

Reviewed by:
Colm Cunningham, Trinity College of Dublin, Ireland
William E. Seaman, University of California at San Francisco, USA

****Correspondence:***
Marco Prinz, Institute of Neuropathology, University of Freiburg, Breisacher Str. 64, D-79106 Freiburg, Germany.
e-mail: marco.prinz@uniklinik-freiburg.de

Microglia are resident macrophages of the central nervous system (CNS) that display high functional similarities to other tissue macrophages. However, it is especially important to create and maintain an intact tissue homeostasis to support the neuronal cells, which are very sensitive even to minor changes in their environment. The transition from the "resting" but surveying microglial phenotype to an activated stage is tightly regulated by several intrinsic (e.g., Runx-1, Irf8, and Pu.1) and extrinsic factors (e.g., CD200, CX_3CR1, and TREM2). Under physiological conditions, minor changes of those factors are sufficient to cause fatal dysregulation of microglial cell homeostasis and result in severe CNS pathologies. In this review, we discuss recent achievements that gave new insights into mechanisms that ensure microglia quiescence.

Keywords: microglia, activation, development, transcription factors, silencing

MICROGLIA – GATEKEEPER OF TISSUE HOMEOSTASIS IN THE CNS

Tissue macrophages are found in virtually all organs of the body. In the central nervous system (CNS), specialized tissue macrophages were first identified within the "third element," or "mesoglia," by Pio del Rio-Hortega (1882–1945). He first characterized a small cell in the neuroectodermal tissue, which apparently was of mesodermal origin and seemed to be related to other tissue macrophages in the body (del Rio-Hortega, 1919, 1932). The specialized tissue macrophage of the CNS is known today as microglia.

The postulated mesodermal origin of microglia by Pio del Rio-Hortega was under investigation for several decades. However, the issue about the exact origin of microglia was not solved. An increasing number of studies pointed to a very early colonization of the CNS by mesodermal progenitors (Kaur et al., 2001) and indicated that microglial progenitors arise from the yolk sac (Alliot et al., 1999). More recently, we and others showed that microglia are derived from the primitive hematopoiesis in the yolk sac and excluded a contribution of definitive hematopoietic stem cells (HSCs) to the generation of microglia (Ginhoux et al., 2010; Schulz et al., 2012). It was further shown that microglia are derived from an uncommitted F4/80-negative erythromyeloid precursor in the yolk sac that develops via immature $F4/80^{-}CX_3CR1^{-}$ myeloid progenitor subsets into $F4/80^{+}CX_3CR1^{+}$ mature macrophages, which finally colonize the CNS to give rise to microglia (Kierdorf et al., 2013).

Upon infection or insults within the adult CNS parenchyma, microglia are rapidly activated and efficiently phagocytose pathogens and dying cells (Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009). Furthermore, microglia release many effector molecules for the recruitment of other immune cells from the blood to limit infections in the CNS, or which serve as antigen presenting cells of phagocytosed material (Saijo and Glass, 2011). In addition, they help in the regeneration of damaged tissue by secretion of growth factors and anti-inflammatory molecules (Saijo and Glass, 2011). Therefore, microglia are indispensable in the adult CNS as stabilizers and modulators of tissue homeostasis under physiological conditions.

Microglia are well integrated in the neuronal glial network of the healthy CNS. They are distributed in all brain regions with varying density between 5% in the *corpus callosum* and 12% in the *substantia nigra* (Lawson et al., 1990). However, the reason for this difference in cell frequency has not been resolved. The morphology of a "resting" microglial cell is characterized by a very small cell soma with elongated ramified processes (Cuadros and Navascués, 1998). Under healthy conditions, microglial cell processes do not overlap with processes of neighboring cells and each cell seems to have a scavenger function for its own immediate area. The position of the cell soma remains stable, whereas the processes of the resting microglia are continuously elongating and retracting to explore the tissue environment. *In vivo* imaging of microglia in intact brain tissue demonstrated highly dynamic processes which continuously scan the surrounding microenvironment (Nimmerjahn et al., 2005). Upon recognition of a pathogen or other inflammatory stimuli, microglia can rapidly retract their processes and become efficient mobile effector cells (Davalos et al., 2005). These observations highlighted the important immune surveillant function of microglia in the healthy CNS parenchyma. In general, microglia activation is triggered by a plethora of well described subsets of immune receptors such as Toll-like receptors (TLRs), scavenger receptors, and numerous cytokine and chemokine receptors.

In this review, however, we focus on several distinct exogenous as well as endogenous signals and factors that are important for the maintenance of the "resting" state of microglia. First, we

describe the interaction between microglia and their surrounding cells, such as neurons, which have a major role in transmitting survival and inhibitory signals to resting microglia. Next, we discuss endogenous microglial signals, mainly transcription factors, which modulate the transcriptional program in these cells that either lead to changes in microglial features or maintain their resting state. Small perturbations in one of these signaling cascades can lead to a spontaneous activation of microglia without any infection or injury to the CNS. However, this spontaneous activated phenotype may be harmful to the neuronal network via induced major changes in the tissue environment, and result in severe damage of the neuronal integrity and function.

EXOGENOUS SIGNALS FOR MICROGLIA ACTIVATION

In the non-diseased adult CNS, microglia communicate with the surrounding glial cells as well as with neighboring neurons. This communication is enabled by a versatile subset of different cell surface molecules on the microglial cell membrane. Indeed, most of the surface molecules of microglia belong to the families of cytokine receptors, scavenger receptors, and pattern recognition receptors (PRRs), as well as to the chemokine receptors, which recognize pro-inflammatory mediators upon inflammation or infection. Most of these receptors are binding ligands that are secreted by, or expressed on the membrane of healthy neurons. Activation of these receptor subsets by inflammatory molecules or pathogens result in a rapid activation of the "resting" microglia to a motile effector cell which contributes to the ongoing inflammation. However, for a small heterogeneous subset of molecules known as "inhibitory molecules" on the surface of microglia, their ligand recognition and binding does not result in a pro-inflammatory activation (Ransohoff and Perry, 2009). Otherwise, the binding of these ligands is necessary for keeping up the resting ramified phenotype of microglia in the healthy CNS (Linnartz and Neumann, 2012).

The surface molecule CD200 is widely expressed not only on neurons, but also on astrocytes and oligodendrocytes (Barclay et al., 2002). Its receptor CD200R is exclusively expressed on macrophages in the CNS, including microglia. The interaction of neuronal CD200 with CD200R leads to inactivation of microglia and keeps them in a resting state (Hoek et al., 2000; Biber et al., 2007) (**Figure 1**). Analysis of microglia of CD200-deficient mice revealed a less ramified morphology with shorter processes and upregulation of CD45 (leukocyte common antigen) and CD11b (complement receptor 3/integrin $\alpha_m\beta_2$), which are also markers of activation (Hoek et al., 2000). Additionally, microglia in non-immunized CD200-deficient animals seemed to form aggregate-like structures, which are typically only found in neurodegenerative disease with strong microglial activation. Following facial nerve axotomy, a model for local neuronal degeneration, CD200-deficient neurons elicited an accelerated microglial response in the lesioned nucleus. In the animal model for multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE), deficiency of CD200 resulted in a more rapid onset of disease (Hoek et al., 2000). These findings indicate that without the CD200–CD200R signaling, microglia develop an activated phenotype in the CNS.

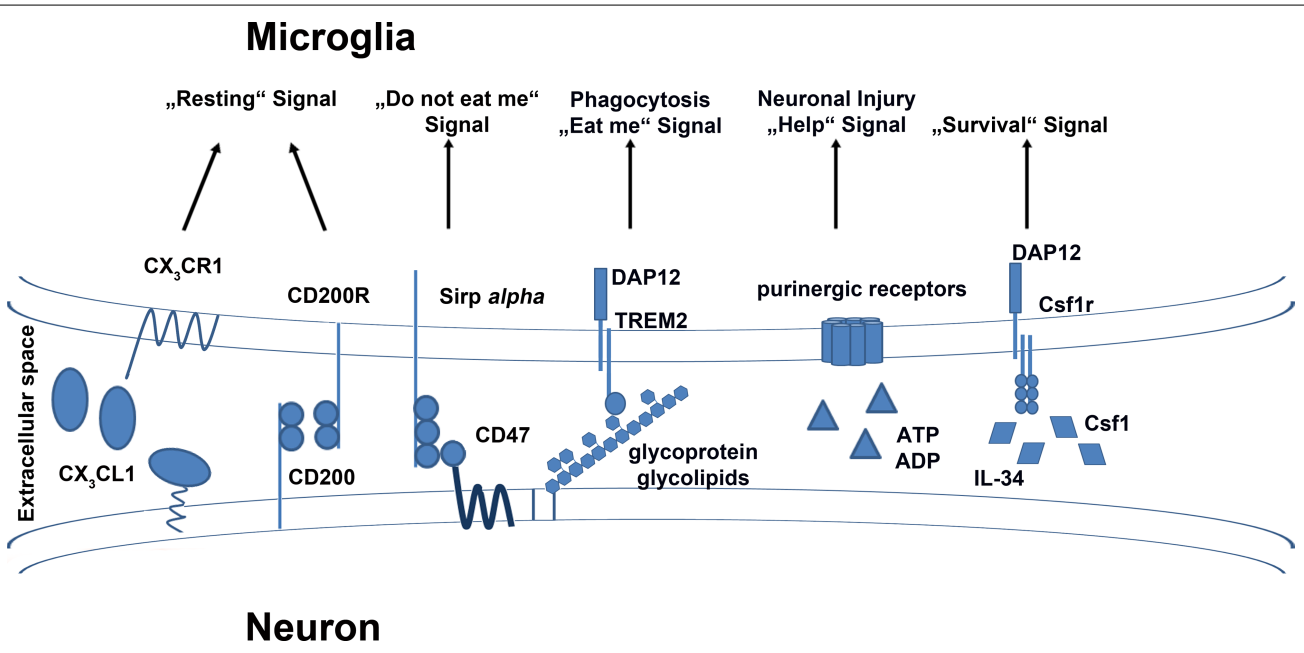


FIGURE 1 | Exogenous signals and their receptors on microglia. Microglia are equipped with a group of surface receptors which trigger signals in microglia under "resting" conditions. Many of these signals are ligands which are released or expressed on the surface of neurons. Inhibitory receptors like CX_3CR1, CD200R, and CD172a/Sirp *alpha* have their ligands on the surface of healthy neurons. A loss of the signal also indicates a loss of neuronal integrity. Receptors such as TREM2 and purinergic receptors are essential to mimic neuronal injury and induce phagocytotic and anti-inflammatory functions in microglia. The crosstalk between microglia and neurons is also important for the survival of microglia. Neurons release factors like IL34 and Csf1 which bind to Csf1r on the surface of microglia and induce cell survival or proliferation.

The G-protein-coupled seven-transmembrane chemokine receptor, CX_3CR1, is expressed on monocytes, macrophages, dendritic cells (DCs), as well as on natural killer (NK) cells. In the CNS parenchyma, CX_3CR1 expression is restricted to the macrophage population, e.g., microglia (Jung et al., 2000). Its only known ligand, CX_3CL1, is expressed on different neuronal subsets in the adult CNS (Kim et al., 2011). Also called fractalkine or neurotactin, CX_3CL1 was first described as a member of a new chemokine class with a specific cysteine motif containing three amino acids separating both cysteines in the chemokine domain (Bazan et al., 1997; Pan et al., 1997). The ligand can be found in a secreted form as well as in a membrane-bound variant (Hughes et al., 2002). Harrison et al. (1998) showed that the binding between of neuronal CX_3CL1 to the microglial CX_3CR1 seems to play a fundamental role in the interaction of neurons and microglia in the healthy and diseased brain. Furthermore, they postulated in this study that CX_3CL1 binding to CX_3CR1 is a fundamental signaling pair in neurophysiology.

Cardona et al. (2006) also found high levels of secreted CX_3CL1 in the CNS parenchyma under healthy conditions. They further demonstrated that CX_3CR1-deficient microglia show an over-activated phenotype in three different diseases models. The loss of fractalkine signaling led to an enhanced neuronal cell death in animal models for Parkinson's disease and other motor neuron disorders. The accelerated neurotoxicity of the CX_3CR1-deficient microglia seemed to worsen neurodegenerative diseases. Nevertheless, several studies on the role of CX_3CR1-deficiency in animal models for Alzheimer's disease (AD) revealed quite divergent results (Fuhrmann et al., 2010; Lee et al., 2010; Liu et al., 2010; Prinz et al., 2011). In AD, CX_3CR1 deficiency appeared to be beneficial by resulting in reduced neuronal loss and improved behavioral deficits. Altogether, these results indicated that CX_3CR1-deficiency has several effects on the course and pathology of inflammatory or neurodegenerative CNS diseases. Therefore, neuron–microglia communication via CX_3CL1 and CX_3CR1 could play different roles under inflammatory and neurodegenerative CNS conditions (Prinz and Priller, 2010).

Notably, recent investigations showed a pivotal role for the CX_3CR1–CX_3CL1 signaling under physiological conditions (**Figure 1**). In the postnatal brain, the formation of mature neural circuits depends on the elimination of redundant synapses. Little was known about the mechanisms of this process called "synaptic pruning" (Hua and Smith, 2004). Recent investigations showed that the ligand CX_3CL1 is highly expressed during this time of synapse maturation (Paolicelli et al., 2011). The authors could elegantly demonstrate that microglia are in direct contact with these synapses and remove unwanted synapses by phagocytosis. This study highlighted the non-redundant role of CX_3CR1 on microglia for this process. CX_3CR1-deficient animals showed a reduced number of microglia during the first weeks after birth (Paolicelli et al., 2011). However, during the embryonic phase and in adult animals there was no alteration in microglial cell numbers detected in these animals (Kierdorf et al., 2013). The authors pointed out that CX_3CR1 is an important regulator of microglial surveillance (Paolicelli et al., 2011). Reduced microglial cell number in CX_3CR1-deficient animals resulted in the development of immature neuronal circuits. In contrast, CX_3CR1-deficient animals revealed a high density of spines and functional excitatory synapses, which are more known to be related to a mature phenotype than delayed brain development.

In addition, another study reported that CX_3CR1-deficient animals exhibited an inhibition in microglial recruitment to forming synapses in the barrel field of the somatosensory cortex, leading to an abnormal synapse formation in this area (Hoshiko et al., 2012). Therefore, interaction of neurons and microglia appears to be essential for proper synapse formation in the postnatal cortex.

Earlier publications did not indicate an essential role of CX_3CR1 in the healthy brain. Most studies were dealing with the role of this receptor under inflammatory conditions (Jung et al., 2000; Cardona et al., 2006). Recent investigations in healthy CX_3CR1-deficient animals showed a harmful effect of this mutation for adult neurogenesis and hippocampal circuit integrity. The number of neuronal precursors in the hippocampus was massively decreased and led to diminished adult neurogenesis (Bachstetter et al., 2011). Reintroducing CX_3CL1 in the hippocampus of aged animals could rescue of the decreasing neurogenesis generally observed in aging animals. Furthermore, a reduction of CX_3CR1 in adult animals resulted in a decrease in neurogenesis. The authors showed that a lower level of neurogenesis is mediated by highly elevated levels of hippocampal pro-inflammatory cytokines such as interleukin (IL)-1β, which is secreted by microglia, and is toxic to the developing neuronal progenitors. This supports the role of CX_3CR1 signaling in keeping microglia in a quiescent state.

Another study further evaluated this theory and found that CX_3CR1-deficient animals display cognitive impairment (Rogers et al., 2011). They established performance deficits in fear conditioning as well as in the Morris water maze, which is associated with learning, in CX_3CR1-deficient mice. Mice lacking this receptor had significant impairment in long-term potentiation, which is widely considered to underlie learning and memory. The authors demonstrated an essential role of IL-1β in these CX_3CR1-mediated impairments by reversing the deficits of CX_3CR1-deficiency via application of IL-1R antagonists.

There are many other inhibitory surface receptors or molecules on the surface of microglia which are mostly interacting with ligands secreted or expressed on the surface of neurons, such as CD47. CD47 is expressed ubiquitously, including on neurons, transmits a "do not eat me" signal to the microglia via CD172a/Sirp *alpha* (van Beek et al., 2005; Biber et al., 2007) (**Figure 1**). Another glycoprotein identified on microglia is known as the triggering receptor expressed on myeloid cells 2 (TREM2), which was linked to an anti-inflammatory phenotype (Colonna, 2003). TREM2 is associated with the adaptor protein DNAX-activating protein of 12-kDa (DAP12), which transmits the signal from the receptor to the intracellular signaling cascade. TREM2 is essential for phagocytosis of, for example, apoptotic cell membranes by microglia (Neumann and Takahashi, 2007). Furthermore, TREM2 was shown to have beneficial effects in autoimmune CNS demyelination (Takahashi et al., 2007). Here, myeloid precursors from bone marrow cultures, which were lentivirally transduced to overexpress TREM2, were transplanted into EAE animals.

The resultant disease amelioration in this animal model for MS indicated that TREM2 may be the key receptor that triggers tissue repair and limits CNS damage. Mutations in TREM2 or DAP12 are associated with a severe neurodegenerative disease known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), which is further characterized by a form of early onset dementia and formation of bone cysts (Paloneva et al., 2001; Klünemann et al., 2005).

Microglia also express receptors that trigger essential cellular survival and developmental signals. One of the receptors which plays a major role in microglial development and survival is the receptor for colony stimulating factor 1 (Csfr1), which is also known to regulate the differentiation and survival of peripheral macrophages (Chitu and Stanley, 2006) (**Figure 1**). Csf1r binds Csf1, which is important for the maintenance of many macrophage subsets (Cecchini et al., 1994). Csf1r-deficient animals show deficiency in several subsets of mononuclear phagocytes and microglia are completely absent in these mice (Dai et al., 2002; Ginhoux et al., 2010). Ginhoux et al. (2010) further showed that the development of microglia and primitive yolk sac macrophages is completely dependent on Csf1r signaling. However, the $Csf1^{op/op}$ mouse strain with a natural occurring null mutation in Csf1 did not reveal the same severe phenotype observed in the Csf1r-deficient animals (Yoshida et al., 1990). In fact, detailed examination of $Csf1^{op/op}$ mice revealed the presence of microglia but at reduced numbers (Blevins and Fedoroff, 1995). These findings clearly indicated that survival and maintenance of microglia are majorly influenced by Csf1r function. However, Csf1r may have Csf1-independent functions in microglial homeostasis, suggesting the existence of another ligand for this receptor. Indeed, (Lin et al., 2008) discovered another ligand of the Csf1r known as IL-34. It was shown that IL-34 and Csf1 share some similar signaling functions via Csf1r and that both ligands can compensate for each other (Wei et al., 2010). However, both ligands showed a differential expression pattern *in vivo*. Wang et al. (2012) generated IL-34-deficient animals with an insertion of the LacZ reporter gene in the IL-34 gene locus, allowing its expression to be traced to neurons. They showed that mostly microglia and Langerhans cells of the epidermis are affected by the loss of IL-34. IL-34-deficient animals had reduced numbers of microglia and were consequently more susceptible to viral infections in the CNS. Similar results were recently obtained by Greter et al. (2012). They demonstrated that IL-34 is expressed by specific neuronal subsets restricted to defined brain regions such as the cortex and hippocampus (Greter et al., 2012). The authors found that microglial development was not influenced by IL-34 deficiency. In contrast, microglial cell number was decreased in specific regions of the adult brain. These results indicated that IL-34 plays a role in the adult brain for microglial survival and homeostasis. In another recent study, Erblich et al (2011) analyzed microglia in $Csf1^{op/op}$ mice and Csf1r-deficient animals. They observed a reduced number of microglial cell in the absence of Csf1, and a complete loss of microglia in Csf1r-deficient animals. These severe defects in microglial developments resulted in disturbed brain development with a prominent phenotype of a thinned cortex. Additionally, a recent study showed that the signaling of both IL-34 and Csf1 are important for microglial proliferation upon neurodegeneration during prion infection and AD (Gómez-Nicola et al., 2013). Injection of a blocking antibody against Csf1r on activated microglia induced a strong reduction of proliferating cells in both diseases. In turn, microglial proliferation could be induced by administration of Csf1 and IL-34, whereas IL-34 showed a stronger proliferative capacity then Csf1. In addition to the studies from Wang et al. (2012) and Greter et al. (2012), it was shown here that not only are neurons a source of IL-34, astrocytes could also express this ligand under neurodegenerative conditions. Therefore, the sources of IL-34 might differ depending on the cellular conditions and the time points of investigation. Interestingly, heterozygous mutations in the Csf1r locus can be found in patients with hereditary diffuse leukoencephalopathy with spheroids, characterized by demyelination of the cerebral white matter and formation of spheroids which lead to progressive cognitive and motor dysfunction (Rademakers et al., 2012). However, it is still unclear, what roles IL-34 and Csf1 play in microglial development or homeostasis in these patients. Future studies will elucidate the detailed function of these important physiological signaling molecules in microglia.

Release of nucleotides such as adenosine triphosphate (ATP) in the CNS mimics an inflammatory insult in the parenchyma, especially after nerve injury, and could efficiently activate microglia (Davalos et al., 2005). This activation is mediated by several purinergic receptors on the microglial surface. Microglia are equipped with a wide range of ionotropic ($P2X_4$ and $P2X_7$) and metabotropic ($P2Y_1$, $P2Y_2$, and $P2Y_{12}$) purinergic receptors (Inoue, 2002; Haynes et al., 2006). Many studies focused on the role of these nucleotides for microglial activation and showed an important function in their early response to injury (Haynes et al., 2006; Ohsawa et al., 2007; Ulmann et al., 2008). Nucleotides are versatile effector molecules which initiate the fast recruitment of microglia to injury sites for the release of neurotrophic factors (Haynes et al., 2006; Ohsawa et al., 2007; Ulmann et al., 2008). The purinergic receptors seem to be another subset of microglial surface receptors which are important for the maintenance of injured neurons and facilitating tissue homeostasis by microglia (Inoue, 2002).

Therefore, it can be assumed that microglia are very important for the development and maintenance of the neuronal network. As soon as there is a microglial activation without any insult or infection that is solved, this activation can be disruptive and harmful for developing and already existing neuronal networks.

ENDOGENOUS FACTORS REGULATING MICROGLIA ACTIVATION

The state of the microglial cell is not only regulated by exogenous signal via surface receptors. Their activation and maturation states are tightly controlled by a subset of endogenous transcription factors. Factors like Runt-related transcription factor 1 (Runx1), ETS (E-twenty six) family transcription factor Pu.1, and interferon regulatory factor 8 (Irf8) are indispensable regulators of the differentiation process during embryonic development (Ginhoux et al., 2010; Kierdorf et al., 2013).

Runx1 was already known for its crucial function during definitive hematopoiesis. Loss of Runx1 leads to a complete lack of HSC development from the aortic endothelium, accompanied by an abnormal fetal liver hematopoiesis (Okuda et al., 1996; North et al.,

1999). Okuda et al. (1996) generated Runx1-deficient animals and showed that they die very early during embryonic development at around 12.5 days post conception (dpc). Another study indicated that Runx1-deficient embryos also suffer from necrosis and hemorrhages in the CNS (Wang et al., 1996). Cell tracing studies in the early mouse embryo revealed that Runx1 is a transcription factor which is already expressed in hematopoietic progenitors in the extraembryonic blood islands (Samokhvalov et al., 2007). Ginhoux et al. (2010) used an inducible Cre-recombinase under the control of the Runx1 promoter for their pulse labeling experiments during early embryonic stages. Here, they targeted microglia by pulse labeling Runx1$^+$ progenitors in the yolk sac around 7.5 dpc, since Runx1 is expressed in early microglial progenitors. The decisive role of Runx1 in myelopoiesis was recently described in zebrafish (Jin et al., 2012). Both transcription factors Pu.1 and Runx1 were shown to act in a negative feedback loop for the regulation of Pu.1 expression levels and thereafter regulating myeloid cell fate (Jin et al., 2012). These findings indicated a key role of Runx1 for myeloid and microglia development. However, it was unclear at this point whether Runx1 is also involved in the cell homeostasis of microglia. In a transcriptome analysis of laser-microdissected microglia from the corpus callosum of rats, Runx1 was found to be down regulated in ramified microglia of 4 week old rats, compared to amoeboid microglia of 5 days old pubs (Parakalan et al., 2012). Zusso et al. (2012) performed a detailed analysis for the function of Runx1 in postnatal microglia. They showed that Runx1 is not only a regulator of differentiation, but further regulates proliferation and homeostasis of postnatal microglia. They further suggested that Runx1 might play an important function in microglia by modulating the transition of amoeboid microglia to ramified ones. Therefore, Runx1 is a non-redundant transcription factor that is important for the activation and resting states of microglia(**Table 1**).

The transcription factor Pu.1 is a master regulator of myeloid development which is already required during the first stages of myeloid differentiation programs. Pu.1-deficient animals indicated a fundamental role of Pu.1 for myeloid and microglial development (Scott et al., 1994; McKercher et al., 1996). These animals died during the first days after birth due to severe septicemia. Their lifespan could be extended by antibiotic treatment or bone marrow transplantation (Beers et al., 2006). Mature B cells and myeloid cells are completely missing in these mice. Examination of several tissues revealed a complete loss of tissue macrophages such as Kupffer cells, microglia, or other tissue macrophages (Schulz et al., 2012). Another study demonstrated that CNS cultures from Pu.1-deficient animals showed reduced proliferation of cortical precursors and reduced astrogliogenesis (Antony et al., 2011). Until now, there is no direct evidence whether Pu.1 is also involved in the normal homeostasis of adult microglia. However, several studies suggest a regulatory function in the microglia/macrophage activation state. Ponomarev et al. (2011) down regulated Pu.1 in macrophages by overexpression of miRNA-124. The reduced levels of Pu.1 led to deactivated phenotype of macrophages. Further studies are needed to verify the function of Pu.1 in activation and homeostasis of microglia (**Table 1**).

Recent investigations on the development of microglia showed that microglial development is tightly regulated by distinct transcriptional programs. Schulz and colleagues defined microglia as myeloid cells that develop from a HSC-independent progenitor, independent of the transcription factor c-myb, which, however, is indispensable for the development of definitive HSCs (Mucenski et al., 1991; Schulz et al., 2012). Microglia developed normally in c-myb-deficient animals, indicating that c-myb is not regulating microglial development (**Table 1**). Furthermore, they showed that an induced ablation of c-myb in adult mice did not affect

Table 1 | Transcription factors regulating microglia development and homeostasis.

Transcription factor	Microglia development	Microglia homeostasis
c-myb	→ Independent of c-myb (Schulz et al., 2012; Kierdorf et al., 2013)	→ After induced ablation of c-myb, no change in microglia cell number (Schulz et al., 2012)
Runx-1	→ Expressed on microglia progenitors in the yolk sac (Ginhoux et al., 2010)	→ Regulating proliferation and tissue homeostasis of postnatal microglia (Zusso et al., 2012) → Transition of amoeboid to ramified morphology (Zusso et al., 2012)
Pu.1	→ Absence of microglia progenitors in the yolk sac; no development of microglia (Scott et al., 1994; McKercher et al., 1996; Beers et al., 2006; Schulz et al., 2012; Kierdorf et al., 2013)	→ No direct evidence yet for a functional role during microglia cell homeostasis
Irf8	→ Reduced number of microglia and microglial progenitors in the yolk sac (Kierdorf et al., 2013)	→ Dysregulation of microglial cell morphology and function (Horiuchi et al., 2012; Minten et al., 2012) → Defects in microglial activation (Masuda et al., 2012)
Hoxb8	→ No direct evidence yet for a functional role during microglia development	→ Mutant Hoxb8 microglia induce pathological grooming in mice (Chen et al., 2010)

adult microglia. C-myb could therefore be considered a transcription factor that is redundant for microglia development and homeostasis.

In a very recent study on microglial development, a detailed characterization of microglial progenitors and factors which are important for their development was performed (Kierdorf et al., 2013). The transcriptional programing of microglial development is tightly regulated by Pu.1 and the myeloid transcription factor Irf8. Irf8 is a transcription factor which is known to be important for the development of B cells and myeloid cells in the bone marrow (Holtschke et al., 1996). It was shown that Irf8-deficient animals have a severe defect in the generation of mature myeloid cells, finally resulting in chronic myelogenous leukemia (CML)-like symptoms (Holtschke et al., 1996). Furthermore, Irf8-deficient animals were more susceptible to infections (Holtschke et al., 1996). Mutations in the Irf8 locus have been found to result in severe immunodeficiency in humans (Hambleton et al., 2011). These patients had decreased mature DCs and monocytes in the blood and suffered from recurrent bacterial infections after Bacillus Calmette–Guérin (BCG) vaccination. The authors described two mutations in the DNA-binding domain of Irf8 which were shown to induce a reduced binding to target gene promoter regions like IL-12 and inducible nitric oxide synthetase (iNOS; Hambleton et al., 2011). Additionally, Irf8 was found to be a susceptibility gene for autoimmune diseases such as Lupus erythematodes and MS (De Jager et al., 2009; The International Multiple Sclerosis Genetics Consortium, 2011; Lessard et al., 2012).

Besides the detrimental role of Irf8 for microglial and myeloid development, recent studies indicated a role of Irf8 in adult microglia homeostasis and activation (**Table 1**). One report demonstrated the involvement of Irf8 for the activation of microglia during nerve injury (Masuda et al., 2012). A massive upregulation of this transcription factor upon lesion induction was detected. Irf8-deficient animals showed less hypersensitivity after nerve injury, decreased activation marker levels in microglia, and no changes in microglial proliferation. In this study, no morphological abnormalities of Irf8-deficient microglia were described. In contrast, two recent studies showed that loss of Irf8 has severe effects on microglia already under physiological conditions. Horiuchi et al. (2012) reported no changes in cell number but found an abnormal cell morphology with reduced ramification and altered expression of ionized calcium-binding adapter molecule (Iba)-1. The authors further described reduced microglia proliferation in culture and diminished phagocytotic capacity in the absence of Irf8. In addition, the authors investigated microglial activation and detected an altered cytokine expression level similar to Masuda et al. (2012). Another study found increased microglial cell numbers and showed gross alterations in morphology and surface area in the absence of Irf8 (Minten et al., 2012).

Another important transcription factor essential for microglial homeostasis is Hoxb8. Hoxb8-deficient animals showed excessive grooming behavior (Greer and Capecchi, 2002). Chen et al. (2010) were able to define Hoxb8 expression in the adult CNS in regions associated with the grooming behavior in mice. In humans, these brain areas correspond to the obsessive-compulsive disorder (OCD) circuitry. OCD is a condition that is often characterized by excessive behaviors dealing with cleanliness, including grooming. Whereas the exact microglia-mediated mechanisms of this pathological grooming were not identified in this study, the authors could elucidate a role of Hoxb8 mutant microglia in a behavioral disorder. Hoxb8 was found to be expressed only in microglia in the CNS, but just in approximately 40% of the cell population which should be derived from bone marrow cells as the authors stated in this study. Loss of Hoxb8 led to a slightly reduced microglial cell number, but no obvious change in cell morphology. The authors showed that wild type bone marrow transplantation in Hoxb8 mutants after total body irradiation rescued the pathological grooming behavior. The authors obtained surprisingly high engraftment rates (up to 30%) of infiltrating bone marrow-derived phagocytes (BMDPs) in the mutant CNS. Furthermore, when they restricted the Hoxb8 deletion to the hematopoietic system, they found the same pathological grooming behavior and hair loss symptoms. This study is one of the first that shows that mutations and defects in microglia can result in prominent behavioral syndromes in mice. Deciphering novel functions of microglia for psychiatric disorders and behavioral anomalies will open new avenues in neuroimmunology in the future.

We conclude that microglia have different surface receptors that are essential for microglial cell homeostasis by regulating either survival and or activation properties. Dysregulation of these receptors induce severe changes in the microglia, which could potentially be harmful for neuronal networks, and result in developmental defects and/or neuropathological changes in the adult CNS.

ACKNOWLEDGMENTS

We want to thank Tuan L. Tay for critical reading and editing. Marco Prinz is supported by the DFG funded research unit (FOR) 1336, the BMBF-funded Competence Network of Multiple Sclerosis (KKNMS), the Competence Network of Neurodegenerative Disorders (KNDD), and the DFG (PR 577/8-2).

REFERENCES

Alliot, F., Godin, I., and Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* 117, 145–152.

Antony, J. M., Paquin, A., Nutt, S. L., Kaplan, D. R., and Miller, F. D. (2011). Endogenous microglia regulate development of embryonic cortical precursor cells. *J. Neurosci. Res.* 89, 286–298.

Bachstetter, A. D., Morganti, J. M., Jernberg, J., Schlunk, A., Mitchell, S. H., Brewster, K. W., et al. (2011). Fractalkine and CX3CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol. Aging* 32, 2030–2044.

Barclay, A. N., Wright, G. J., Brooke, G., and Brown, M. H. (2002). CD200 and membrane protein interactions in the control of myeloid cells. *Trends Immunol.* 23, 285–290.

Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., et al. (1997). A new class of membrane-bound chemokine with a CX3C motif. *Nature* 385, 640–644.

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., et al. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16021–16026.

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. G. M. (2007). Neuronal "On" and "Off" signals control microglia. *Trends Neurosci.* 30, 596–602.

Blevins, G., and Fedoroff, S. (1995). Microglia in colony-stimulating factor 1-deficient op/op mice. *J. Neurosci. Res.* 40, 535–544.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., et al. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* 9, 917–924.

Cecchini, M. G., Dominguez, M. G., Mocci, S., Wetterwald, A., Felix, R., Fleisch, H., et al. (1994). Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development* 120, 1357–1372.

Chen, S. -K., Tvrdik, P., Peden, E., Cho, S., Wu, S., Spangrude, G., et al. (2010). Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* 141, 775–785.

Chitu, V., and Stanley, E. R. (2006). Colony-stimulating factor-1 in immunity and inflammation. *Curr. Opin. Immunol.* 18, 39–48.

Colonna, M. (2003). TREMs in the immune system and beyond. *Nat. Rev. Immunol.* 3, 445–453.

Cuadros, M. A., and Navascués, J. (1998). The origin and differentiation of microglial cells during development. *Prog. Neurobiol.* 56, 173–189.

Dai, X.-M., Ryan, G. R., Hapel, A. J., Dominguez, M. G., Russell, R. G., Kapp, S., et al. (2002). Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. *Blood* 99, 111–120.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* 8, 752–758.

De Jager, P. L., Jia, X., Wang, J., de Bakker, P. I. W., Ottoboni, L., Aggarwal, N. T., et al. (2009). Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat. Genet.* 41, 776–782.

del Rio-Hortega, P. (1919). El "tercer elemento" de los centros nerviosus. I. La microglia en estado normal. II. Intervencion de la microglia en los procesos patologicos (Celulas en bastoncito y cuerpos granuloadiposos). III. Naturaleza probable de la microglia. *Bol. R. Soc. Esp. Hist. Nat. Secc. Biol.* 9, 68–120.

del Rio-Hortega, P. (1932). "Microglia," in *Cytology and Cellular Pathology of the Nervous System*, ed. W. Penfield (New York: P. B. Hoeber), 483–534.

Erblich, B., Zhu, L., Etgen, A. M., Dobrenis, K., and Pollard, J. W. (2011). Absence of colony stimulation factor-1 receptor results in loss of microglia, disrupted brain development and olfactory deficits. *PLoS ONE* 6:e26317. doi: 10.1371/journal.pone.0026317

Fuhrmann, M., Bittner, T., Jung, C. K. E., Burgold, S., Page, R. M., Mitteregger, G., et al. (2010). Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nat. Neurosci.* 13, 411–413.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Gómez-Nicola, D., Fransen, N. L., Suzzi, S., and Perry, V. H. (2013). Regulation of microglial proliferation during chronic neurodegeneration. *J. Neurosci.* 33, 2481–2493.

Greer, J. M., and Capecchi, M. R. (2002). Hoxb8 is required for normal grooming behavior in mice. *Neuron* 33, 23–34.

Greter, M., Lelios, I., Pelczar, P., Hoeffel, G., Price, J., Leboeuf, M., et al. (2012). Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. *Immunity* 37, 1050–1060.

Hambleton, S., Salem, S., Bustamante, J., Bigley, V., Boisson-Dupuis, S., Azevedo, J., et al. (2011). IRF8 mutations and human dendritic-cell immunodeficiency. *N. Engl. J. Med.* 365, 127–138.

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

Harrison, J. K., Jiang, Y., Chen, S., Xia, Y., Maciejewski, D., McNamara, R. K., et al. (1998). Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 10896–10901.

Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W.-B., et al. (2006). The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* 9, 1512–1519.

Hoek, R. M., Ruuls, S. R., Murphy, C. A., Wright, G. J., Goddard, R., Zurawski, S. M., et al. (2000). Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 290, 1768–1771.

Holtschke, T., Löhler, J., Kanno, Y., Fehr, T., Giese, N., Rosenbauer, F., et al. (1996). Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. *Cell* 87, 307–317.

Horiuchi, M., Wakayama, K., Itoh, A., Kawai, K., Pleasure, D., Ozato, K., et al. (2012). Interferon regulatory factor 8/interferon consensus sequence binding protein is a critical transcription factor for the physiological phenotype of microglia. *J. Neuroinflammation* 9, 227.

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012). Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J. Neurosci.* 32, 15106–15111.

Hua, J. Y., and Smith, S. J. (2004). Neural activity and the dynamics of central nervous system development. *Nat. Neurosci.* 7, 327–332.

Hughes, P. M., Botham, M. S., Frentzel, S., Mir, A., and Perry, V. H. (2002). Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. *Glia* 37, 314–327.

Inoue, K. (2002). Microglial activation by purines and pyrimidines. *Glia* 40, 156–163.

Jin, H., Li, L., Xu, J., Zhen, F., Zhu, L., Liu, P. P., et al. (2012). Runx1 regulates embryonic myeloid fate choice in zebrafish through a negative feedback loop inhibiting Pu.1 expression. *Blood* 119, 5239–5249.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M. J., Kreutzberg, G. W., Sher, A., et al. (2000). Analysis of fractalkine receptor CX3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol. Cell. Biol.* 20, 4106–4114.

Kaur, C., Hao, A. J., Wu, C. H., and Ling, E. A. (2001). Origin of microglia. *Microsc. Res. Tech.* 54, 2–9.

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* 16, 273–280.

Kim, K.-W., Vallon-Eberhard, A., Zigmond, E., Farache, J., Shezen, E., Shakhar, G., et al. (2011). In vivo structure/function and expression analysis of the CX3C chemokine fractalkine. *Blood* 118, e156–e167.

Klünemann, H. H., Ridha, B. H., Magy, L., Wherrett, J. R., Hemelsoet, D. M., Keen, R. W., et al. (2005). The genetic causes of basal ganglia calcification, dementia, and bone cysts: DAP12 and TREM2. *Neurology* 64, 1502–1507.

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170.

Lee, S., Varvel, N. H., Konerth, M. E., Xu, G., Cardona, A. E., Ransohoff, R. M., et al. (2010). CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. *Am. J. Pathol.* 177, 2549–2562.

Lessard, C. J., Adrianto, I., Ice, J. A., Wiley, G. B., Kelly, J. A., Glenn, S. B., et al. (2012). Identification of IRF8, TMEM39A, and IKZF3-ZPBP2 as susceptibility loci for systemic lupus erythematosus in a large-scale multiracial replication study. *Am. J. Hum. Genet.* 90, 648–660.

Lin, H., Lee, E., Hestir, K., Leo, C., Huang, M., Bosch, E., et al. (2008). Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* 320, 807–811.

Linnartz, B., and Neumann, H. (2012). Microglial activatory (immunoreceptor tyrosine-based activation motif)- and inhibitory (immunoreceptor tyrosine-based inhibition motif)-signaling receptors for recognition of the neuronal glycocalyx. *Glia* 61, 37–46.

Liu, Z., Condello, C., Schain, A., Harb, R., and Grutzendler, J. (2010). CX3CR1 in microglia regulates brain amyloid deposition through selective protofibrillar amyloid-β phagocytosis. *J. Neurosci.* 30, 17091–17101.

Masuda, T., Tsuda, M., Yoshinaga, R., Tozaki-Saitoh, H., Ozato, K., Tamura, T., et al. (2012). IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep.* 1, 334–340.

McKercher, S. R., Torbett, B. E., Anderson, K. L., Henkel, G. W., Vestal, D. J., Baribault, H., et al. (1996). Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J.* 15, 5647–5658.

Minten, C., Terry, R., Deffrasnes, C., King, N. J. C., and Campbell, I. L. (2012). IFN regulatory factor 8 is a key constitutive determinant of the morphological and molecular properties of microglia in the CNS. *PLoS ONE* 7:e49851. doi: 10.1371/journal.pone.0049851

Mucenski, M. L., McLain, K., Kier, A. B., Swerdlow, S. H., Schreiner, C. M., Miller, T. A., et al. (1991).

A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. *Cell* 65, 677–689.

Neumann, H., and Takahashi, K. (2007). Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. *J. Neuroimmunol.* 184, 92–99.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314–1318.

North, T., Gu, T. L., Stacy, T., Wang, Q., Howard, L., Binder, M., et al. (1999). Cbfa2 is required for the formation of intra-aortic hematopoietic clusters. *Development* 126, 2563–2575.

Ohsawa, K., Irino, Y., Nakamura, Y., Akazawa, C., Inoue, K., and Kohsaka, S. (2007). Involvement of P2X4 and P2Y12 receptors in ATP-induced microglial chemotaxis. *Glia* 55, 604–616.

Okuda, T., van Deursen, J., Hiebert, S. W., Grosveld, G., and Downing, J. R. (1996). AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell* 84, 321–330.

Paloneva, J., Autti, T., Raininko, R., Partanen, J., Salonen, O., Puranen, M., et al. (2001). CNS manifestations of Nasu–Hakola disease: a frontal dementia with bone cysts. *Neurology* 56, 1552–1558.

Pan, Y., Lloyd, C., Zhou, H., Dolich, S., Deeds, J., Gonzalo, J.-A., et al. (1997). Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 387, 611–617.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Parakalan, R., Jiang, B., Nimmi, B., Janani, M., Jayapal, M., Lu, J., et al. (2012). Transcriptome analysis of amoeboid and ramified microglia isolated from the corpus callosum of rat brain. *BMC Neurosci.* 13:64. doi: 10.1186/1471-2202-13-64

Ponomarev, E. D., Veremeyko, T., Barteneva, N., Krichevsky, A. M., and Weiner, H. L. (2011). MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. *Nat. Med.* 17, 64–70.

Prinz, M., and Priller, J. (2010). Tickets to the brain: role of CCR2 and CX3CR1 in myeloid cell entry in the CNS. *J. Neuroimmunol.* 224, 80–84.

Prinz, M., Priller, J., Sisodia, S. S., and Ransohoff, R. M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* 14, 1227–1235.

Rademakers, R., Baker, M., Nicholson, A. M., Rutherford, N. J., Finch, N., Soto-Ortolaza, A., et al. (2012). Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nat. Genet.* 44, 200–205.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., et al. (2011). CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J. Neurosci.* 31, 16241–16250.

Saijo, K., and Glass, C. K. (2011). Microglial cell origin and phenotypes in health and disease. *Nat. Rev. Immunol.* 11, 775–787.

Samokhvalov, I. M., Samokhvalova, N. I., and Nishikawa, S. (2007). Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. *Nature* 446, 1056–1061.

Schulz, C., Perdiguero, E. G., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336, 86–90.

Scott, E. W., Simon, M. C., Anastasi, J., and Singh, H. (1994). Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. *Science* 265, 1573–1577.

Takahashi, K., Prinz, M., Stagi, M., Chechneva, O., and Neumann, H. (2007). TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. *PLoS Med.* 4:e124. doi: 10.1371/journal.pmed.0040124

The International Multiple Sclerosis Genetics Consortium. (2011). The genetic association of variants in CD6, TNFRSF1A and IRF8 to multiple sclerosis: a multicenter case–control study. *PLoS ONE* 6:e18813. doi: 10.1371/journal.pone.0018813

Ulmann, L., Hatcher, J. P., Hughes, J. P., Chaumont, S., Green, P. J., Conquet, F., et al. (2008). Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J. Neurosci.* 28, 11263–11268.

van Beek, E. M., Cochrane, F., Barclay, A. N., and van den Berg, T. K. (2005). Signal regulatory proteins in the immune system. *J. Immunol.* 175, 7781–7787.

Wang, Q., Stacy, T., Binder, M., Marin-Padilla, M., Sharpe, A. H., and Speck, N. A. (1996). Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. *Proc. Natl. Acad. Sci. U.S.A.* 93, 3444–3449.

Wang, Y., Szretter, K. J., Vermi, W., Gilfillan, S., Rossini, C., Cella, M., et al. (2012). IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nat. Immunol.* 13, 753–760.

Wei, S., Nandi, S., Chitu, V., Yeung, Y.-G., Yu, W., Huang, M., et al. (2010). Functional overlap but differential expression of CSF-1 and IL-34 in their CSF-1 receptor-mediated regulation of myeloid cells. *J. Leukoc. Biol.* 88, 495–505.

Yoshida, H., Hayashi, S., Kunisada, T., Ogawa, M., Nishikawa, S., Okamura, H., et al. (1990). The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature* 345, 442–444.

Zusso, M., Methot, L., Lo, R., Greenhalgh, A. D., David, S., and Stifani, S. (2012). Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor runx1. *J. Neurosci.* 32, 11285–11298.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 February 2013; paper pending published: 15 February 2013; accepted: 30 March 2013; published online: 23 April 2013.

Citation: Kierdorf K and Prinz M (2013) Factors regulating microglia activation. Front. Cell. Neurosci. 7:44. doi: 10.3389/fncel.2013.00044

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 16 May 2013
doi: 10.3389/fncel.2013.00071

The brain's best friend: microglial neurotoxicity revisited

Sabine Hellwig*[1], *Annette Heinrich*[1] *and Knut Biber*[1,2]

[1] *Department of Psychiatry and Psychotherapy, University Hospital Freiburg, Freiburg, Germany*
[2] *Department of Neuroscience, University Medical Center Groningen, Groningen, Netherlands*

Edited by:
Marie-Eve Tremblay, Université Laval, Canada

Reviewed by:
Alain Bessis, Institut de Biologie of the Ecole Nomale Superieure, France
Erik Boddeke, University Medical Centre Groningen, Netherlands

***Correspondence:**
Knut Biber, Department of Psychiatry and Psychotherapy, University Hospital Freiburg, Hauptstrasse 5, 79104 Freiburg, Germany.
e-mail: knut.biber@uniklinik-freiburg.de

One long standing aspect of microglia biology was never questioned; their involvement in brain disease. Based on morphological changes (retracted processes and amoeboid shape) that inevitably occur in these cells in case of damage in the central nervous system, microglia in the diseased brain were called "activated." Because "activated" microglia were always found in direct neighborhood to dead or dying neuron, and since it is known now for more than 20 years that cultured microglia release numerous factors that are able to kill neurons, microglia "activation" was often seen as a neurotoxic process. From an evolutionary point of view, however, it is difficult to understand why an important, mostly post-mitotic and highly vulnerable organ like the brain would host numerous potential killers. This review is aimed to critically reconsider the term microglia neurotoxicity and to discuss experimental problems around microglia biology, that often have led to the conclusion that microglia are neurotoxic cells.

Keywords: microglia, neuroprotection, mouse models, innate immunity, CX3CR1, microglia depletion

INTRODUCTION

Microglia research has intensified enormously in the last decade, and many surprising findings have been published. However, microglia still rank among the most mysterious cells of the brain and only recent results have begun to provide answers to the most basic questions in microglial biology, for example the origin of these cells, or the fact that microglia are not replaced by peripheral monocytes/macrophages in the healthy situation (Ajami et al., 2007; Ginhoux et al., 2010; Kierdorf et al., 2013). Moreover, it has become clear that "resting" microglia are by no means just idle cells (Nimmerjahn et al., 2005; Sierra et al., 2010; Tremblay et al., 2010; Paolicelli et al., 2011; Vinet et al., 2012) and there is good evidence to suggest that microglia are not only important in brain pathology but also play important roles in the healthy brain (Wake et al., 2009; Sierra et al., 2010; Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2012). Thus, the general and simple concept of microglia "activation" is now questionable (see for recent reviews: Hanisch and Kettenmann, 2007; Colton, 2009; Ransohoff and Perry, 2009; Yong and Rivest, 2009; Graeber, 2010; Parkhurst and Gan, 2010; Ransohoff and Cardona, 2010; Kettenmann et al., 2011; Prinz et al., 2011; Tremblay et al., 2011; Aguzzi et al., 2013; Kettenmann et al., 2013). One long standing aspect of microglia biology, however, has never been challenged; namely, their involvement in brain disease, which was proposed many decades ago. This assumption was first based on morphological data, whereby ramified microglia in the healthy brain were described as "resting," the rounded, macrophage-like microglia in the diseased brain were designated as "activated" microglia. Later it was shown that "activated" microglia sometimes express potential harmful substances, which led to the suggestion that these cells are detrimental during brain disease. On top of this, numerous cell culture experiments, most of which involved lipopolysaccharide (LPS)-treated microglia that have the potential to kill neurons, have further corroborated the assumption that "activated" microglia are neurotoxic cells (see for recent reviews: Block et al., 2007). However, from an evolutionary point of view it is difficult to understand why a highly sensitive, but otherwise long-lived, post-mitotic organ like the brain would serve as a host to such a large number of potentially toxic cells.

This review therefore aims to critically reconsider the common view that "activated" microglia are neurotoxic cells, and to highlight studies in which the role of microglia *in vivo* was specifically targeted, often revealing a protective function of these cells.

MICROGLIA *IN VITRO* STUDIES

The first direct evidence concerning microglia as neurotoxic cells was published some 20 years ago (see for example: Boje and Arora, 1992; Chao et al., 1992). These experiments utilized standard microglia cultures (shake-off microglia from cultured neonatal brain homogenate) that were stimulated with rather high concentrations of single or combined pro-inflammatory stimuli such as LPS, interferon-gamma (IFN-γ), or tumor necrosis factor-α (TNF-α). These cells (or the resulting supernatant) were transferred to plates containing cultured neurons, and incubated for some time before neuronal survival was assessed (Boje and Arora, 1992; Chao et al., 1992). Ever since these pioneering experiments were performed, numerous variations of this experimental paradigm have identified a plethora of toxic microglial secretory products and/or detrimental microglia functions that obviously add weight to the notion that microglia are neurotoxic cells (see for recent examples: Lehnardt et al., 2008; Pais et al., 2008; Levesque et al., 2010; Burguillos et al., 2011; Gao et al., 2011). Thus, from the numerous papers that have investigated the influence of *in vitro* microglia on the survival of neurons, the majority has described a detrimental microglia

role. Fewer studies have also found a neuroprotective function of cultured microglia showing that not all functions of cultured microglia are detrimental for neurons (see for recent review: Polazzi and Monti, 2010).

Cell culture experiments, however, should be approached with caution, especially when highly sensitive and reactive cells such as microglia are used. Standard cultured microglia have at least three major disadvantages: First, since standard cultured microglia are derived from the neonatal brain, these cells have missed the potential maturation process that occurs *in vivo*. Second, cultured microglia are grown in serum-containing (usually 10%) medium, whereas *in vivo* microglia normally never come in contact with serum components. Third, nowadays it is also very well known that *in vivo* microglia are kept under constant restraint by a variety inhibitory inputs such as CX3CL1, CD200, CD22, or CD172 (see for review: Biber et al., 2007; Ransohoff and Cardona, 2010; Prinz et al., 2011), which, of course, is not the case in culture. Indeed, the genetic removal of even just one of these inhibitory factors in animal models dramatically changes the reaction profile of microglia, often causing overshooting microglia reactions and sometimes even toxic microglia responses (Hoek et al., 2000; Cardona et al., 2006); therefore, it is very likely that the complete lack of normal inhibition has a dramatic influence on the reactivity of cultured microglia.

Despite the caveats associated with studying microglial function *in vitro*, there is surprisingly little research regarding the question of whether cultured microglia can be reliably compared to their *in vivo* counterparts. One such report by Boucsein et al. (2000) investigated the electrophysiological properties of microglia by comparing cultured (with or without LPS treatment) and ramified microglia in acute brain slice preparations. It was found that ramified microglia barely display membrane currents, in stark contrast to primary cultured microglia, which elicited inward and outward rectifying currents (depending on LPS treatment) that were similar to those found in cultured macrophages (Boucsein et al., 2000). More recently, Schmid et al., 2009 compared mRNA expression profiles between cultured microglia and alveolar macrophages stimulated with LPS/IFN-γ and microglia rapidly isolated from the brain of LPS/IFN-γ treated animals. This study also reported that cultured microglia and macrophages are much more alike than the microglia that have been acutely derived from brain tissue (Schmid et al., 2009). Recently, a similar comparative analysis was performed for post-mortem human microglia and macrophages derived from the choroid plexus (Melief et al., 2012). These authors not only provided convincing evidence for major differences in surface marker and mRNA expression pattern between brain-derived microglia and macrophages, they further showed that acutely isolated microglia are not able to respond to LPS stimulation, most likely because these cells lack CD14 (Melief et al., 2012). It is yet not known whether this lack of CD14 and LPS response is due to the isolation technique used in the study. However, overnight incubation in culture increased CD14 levels and rendered the cells sensitive to LPS treatment, again suggesting that growing microglia in culture can have a tremendous influence on the reactivity of these cells (Melief et al., 2012). These results strongly implicate that cultured microglia share few similarities with their *in vivo* counterparts, which leads to the conclusion that *in vitro* evidence concerning microglia should be interpreted with the utmost caution when extrapolating data into the context of the brain.

NEURONAL LOSS AND THE PRESENCE OF AMOEBOID MICROGLIA: CHICKEN OR EGG?

Histological studies by Del Rio-Hortega identified microglia almost a century ago (see Kettenmann et al., 2011 for an excellent overview on microglia history) and even back in the early days of microglia research, the potential importance of these cells in brain disease was already been recognized. Indeed, these seminal histological studies on microglia morphology also gave rise to the concept of microglia "activation," which states that ramified microglia in the healthy brain are in a resting state, and that upon any potential danger signal these cells morph into an amoeboid or macrophage-like shape. Because the complexity of this morphological transition is limited, microglia responses were generally seen as graded and stereotypic (see for review: Kettenmann et al., 2011). In other words it was more or less believed that microglia always had the same role once they become amoeboid. Numerous *in vivo* reports have concluded that amoeboid microglia can potentially confer neurotoxicity by expressing substances (that are toxic *in vitro*) such as pro-inflammatory cytokines (Blais and Rivest, 2004; Xie et al., 2004; Allan et al., 2005; Kim and de Vellis, 2005; Walker and Lue, 2005). Other reports used minocycline (a potential microglia inhibitor) or other anti-inflammatory drugs to ameliorate damage in different models of brain diseases and since at the same time a decreased morphological transition of microglia was observed these results were often discussed in favor of a neurotoxic role of amoeboid microglia (see for example: Yrjänheikki et al., 1998; He et al., 2001; Tikka et al., 2001; Kriz et al., 2002; Hunter et al., 2004; Fan et al., 2005).

However, it could very well be that the morphological transition of microglia is the result rather than the cause of neuronal damage, as was shown in a model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine (METH)-induced neurotoxicity (O'Callaghan et al., 2008). In addition, the fact that microglia may undergo morphological transition in the absence neuronal loss is often disregarded. For example, although the injection of LPS *in vivo* causes rapid morphological transition of microglia, yet it does not induce massive neuronal death, except when injected into the substantia nigra (Kim et al., 2000; Nadeau and Rivest, 2002, 2003). Moreover, it has been known for more than 10 years that microglia in the spinal cord become amoeboid in response to peripheral nerve injury, which is important for the development of neuropathic pain. However, despite the presence of activated microglia, neuronal loss is not a hallmark of neuropathic pain.

Taken together, we think that there is a bias in the field regarding the function of amoeboid microglia. The temporal and spatial relationship between amoeboid microglia-like cells (see next chapters) and dead or dying neurons -which inevitably occurs in case of neuronal damage- is often seen as indication for a neurotoxic role of microglia. Such correlation, of course, does not allow conclusions about causality.

TARGETING MICROGLIA IN MOUSE MODELS OF DISEASE

Using an experimental approach to understand microglial function is a challenging task and as a result most studies, although carefully performed, did not specifically target microglia. In numerous studies is a microglia reaction induced by exogenous application of pro-inflammatory molecules (injection of IL-1, TNFα, LPS, and others) that unleash an uncontrollable immune response, not only in the brain (which also potentially involves astrocytes, oligodendrocytes, endothelial cells, perivascular macrophages, and neurons), but also most likely causes an immune response in the periphery. The second-generation tetracycline minocycline is often referred to as a specific inhibitor of microglia (see for example: Raghavendra et al., 2003; Ledeboer et al., 2005; Mika et al., 2007; Osikowicz et al., 2009). However, it should be kept in mind that minocycline is much less specific than often stated, as it clearly affects peripheral macrophages (Dunston et al., 2011), and T cells (Szeto et al., 2011), and can have a direct influence on the survival of neuronal cell lines and cultured neurons (Hashimoto and Ishima, 2010; Huang et al., 2010; Schildknecht et al., 2011; Ossola et al., 2012), thus, numerous microglia-independent effects of minocycline have been published (Hughes et al., 2004). Moreover, it was reported that the microglia reaction in response to facial nerve injury is unchanged in the presence of even high concentrations of minocycline (Fendrick et al., 2005). Similarly, other anti-inflammatory drugs are not specific for microglia either; for example, the neuroprotective effect of the anti-inflammatory compound triflusal was found not to depend on the presence of microglia (Montero Domínguez et al., 2009). It can thus be concluded that more specific methods are needed to address microglia function.

MICROGLIA VS. OTHER PERIPHERAL MONOCYTES/MACROPHAGES

THE PROBLEM OF IDENTIFICATION

Microglia are derived from early myeloid precursor cells that appear in the yolk sac before major vascularization or hematopoiesis occurs in the developing embryo (Sorokin et al., 1992; Alliot et al., 1999; Herbomel et al., 2001; Ginhoux et al., 2010; Mizutani et al., 2012). Strikingly, it was found that microglia stem from primitive erythromyeloid progenitor cells that develop via a special program into mature microglia, and most importantly that these cells form a stable self-contained population that is not replaced by peripheral monocytes in the unchallenged brain (Kierdorf et al., 2013; Neumann and Wekerle, 2013).

While microglia can be easily identified in the healthy brain, this changes under pathological conditions in which peripheral monocytes/macrophages enter the brain. Despite the fact that microglia and peripheral monocytes/macrophages have different developmental origins, both cell populations share many properties. The expression of general innate pattern recognition receptors such as Toll-like receptors (TLR), nucleotide-binding oligomerization domain (NOD)-lime receptors (NLR), or complement receptors are common to both microglia and peripheral monocytes/macrophages, as is the ability to secrete a whole variety of different cytokines (pro- and anti-inflammatory), growth factors, chemokines, reactive oxygen, and nitrogen species (Kettenmann et al., 2011; Jung and Schwartz, 2012). The lack of reliable microglia-specific markers makes it very difficult both to discriminate between microglia and peripheral monocytes/macrophages, to allocate functions to either cell type (Jung and Schwartz, 2012). This difficulty may have added confusion to the question whether or not microglia in the diseased brain are neurotoxic cells: an example here is the chemokine receptor CCR2. On one hand there are various reports in which CCR2 expressing cells are suggested to be microglia (Abbadie et al., 2003; Zhang et al., 2007; Fernández-López et al., 2012) or described as microglia/macrophages (Yao and Tsirka, 2012) or referred to as amoeboid microglia cells (Deng et al., 2009). Often CCR2 is discussed to be an important receptor for the recruitment of microglia to injured brain areas (El Khoury et al., 2007; Zhang et al., 2007; Deng et al., 2009; Raber et al., 2013) and the inhibition or lack of CCR2 signaling is related to improved disease outcome (Abbadie et al., 2003; Dimitrijevic et al., 2007; Zhang et al., 2007; Fernández-López et al., 2012; Yao and Tsirka, 2012) implicating that CCR2-expressing microglia at least contribute to disease progression.

On the other hand there is convincing evidence from different transgenic mouse models and bone-marrow transplantation experiments that microglia do not express CCR2 in the healthy or diseased brain (Mildner et al., 2007; Jung et al., 2009; Saederup et al., 2010; Mizutani et al., 2012), moreover mouse microglia lack the mRNA for CCR2 (Zuurman et al., 2003; Olah et al., 2012). In bone-marrow transplantation experiments it was shown that the response of endogenous microglia to stroke was not affected in CCR2 deficient animals, showing that CCR2 is not regulating microglia responses here (Schilling et al., 2009a,b). In these studies it was shown that the infiltration of peripheral monocytes into the brain was greatly reduced, which is in agreement with other reports clearly showing that peripheral monocytes require CCR2 in order to invade the diseased central nervous system (CNS; Mildner et al., 2007; Schilling et al., 2009a,b; Prinz and Priller, 2010; Prinz and Mildner, 2011; Mizutani et al., 2012).

Thus CCR2 in the brain should be regarded as marker of peripheral monocytes/macrophages making it questionable whether the published (mostly detrimental) effects of CCR2 expressing cells can be allocated to microglia. CCR2 most likely is not the only example in this respect, showing that without a proper identification it is not possible to draw conclusions about microglia function.

HOW TO TELL THEM APART?

One way to discriminate between monocytes/macrophages and microglia is offered by flow cytometry analysis [(fluorescence assisted cell sorting (FACS)] of acutely isolated cell preparations from the diseased brain using CD11b and CD45 antibodies. Although monocytes/macrophages and microglia are both positive for CD11b and CD45, microglia can be identified by their relatively low expression levels of CD45 ("CD45dim") compared to those of peripheral monocytes/macrophages ("CD45high"). Thus microglia and monocytes/macrophages appear as separate cell populations in FACS analysis (Sedgwick et al., 1998; de Haas et al., 2007, 2008; Remington et al., 2007).

We used such a FACS-based identification approach in a mouse model of cuprizone-induced loss of oligodendrocytes, whereby

microglia were specifically isolated from the corpus callosum of mice before cuprizone treatment, during demyelination and after remyelination (Olah et al., 2012). Thus, pure preparations of ramified microglia (healthy controls), amoeboid microglia (peak of demyelination) and microglia returning to the quiescent state (2 weeks post-remyelination) were subjected to genome-wide gene expression analysis. One aim of this study was to describe the two-sides of microglia activity, based on our expectation of finding a pro-inflammatory profile in the amoeboid microglia isolated during the demyelination phase, and a more anti-inflammatory expression profile in microglia isolated from the corpus callosum during the remyelination period. To our surprise, we did not find any evidence for a double-edged function of microglia during the disease course in this model. Instead we observed a microglial phenotype that supported remyelination at the onset of demyelination, a function which persisted throughout the remyelination process (Olah et al., 2012). Our data showed that microglia are involved in the phagocytosis of myelin debris and apoptotic cells during demyelination (Olah et al., 2012). Furthermore, microglia displayed cytokine and chemokine expression profiles that were associated with the activation and recruitment of endogenous oligodendrocyte precursor cells to the lesion site, as well as the delivery of trophic support during remyelination (Olah et al., 2012). In other words, although corpus callosal microglia displayed an amoeboid morphology under demyelinating conditions, these cells expressed proteins that, rather than potentially contributing to oligodendrocyte death, actually initiated a repair response, even during the early onset phase of the disease.

Another way to discriminate peripheral monocytes/macrophages vs. endogenous microglia are bone-marrow transplantation or parabiosis experiments. Both have been utilized in experimental allergic encephalomyelitis (EAE) and it was observed that peripheral myeloid cells utilize CCR2 in order to invade the diseased brain and that an inhibition of this invasion resulted in a significantly diminished EAE disease course, leading the authors to conclude that peripheral monocytes/macrophages but not resident microglia cells are responsible for disease progression in EAE (Mildner et al., 2009; Ajami et al., 2011). Using bone-marrow transplantation and different genetic models it was further demonstrated that in acute spinal cord injury, endogenous microglia also differ functionally from invading peripheral monocytes/macrophages (see for review: Jung and Schwartz, 2012).

Thus there are various studies that convincingly demonstrate that peripheral monocytes/macrophages may have significantly different functions compared to endogenous microglia in the diseased brain. Interestingly, a more specific analysis of microglia (as discussed above) often (but not always, see Jung and Schwartz, 2012) revealed beneficial, or at least non-toxic microglia responses.

LACK OF CX3CR1 OR CD200r IN MICROGLIA

As mentioned above, nowadays it is clear that microglia in the brain are under constant restraint, particularly because they specifically express receptors for a variety of inhibitory factors that are constitutively expressed in the brain, mostly by neurons (Biber et al., 2007; Ransohoff and Perry, 2009). The most prominent ligand-receptor pairs in this respect are CX3CL1-CX3CR1 and CD200-CD200r. Regarding the CX3CR1-CX3CL1 axis, one of the most used mouse model in microglia research is the CX3CR1-EGFP mouse line in which all microglia are green fluorescent protein (GFP)-positive (Jung et al., 2000). This mouse line, has since contributed enormously to our current understanding of microglia biology, and CX3CR1-deficient homozygotes have been used extensively to study the role of CX3CR1 in various models of brain disease. Indeed, the consequences of CX3CR1 deletion in microglia largely depends on the mouse model used (see for extensive review: Prinz et al., 2011; Ransohoff and Prinz, 2013; Wolf et al., 2013); however, the overall idea at the moment is that a lack of CX3CR1 leads to the "hyperactivity" of microglia in the diseased brain, thereby unleashing potential neurotoxic properties (Wolf et al., 2013). Accordingly, administration of CX3CL1 into the brain causes neuroprotection in experimental stroke and two models of Parkinsons disease (Cipriani et al., 2011; Pabon et al., 2011; Morganti et al., 2012) Similarly, removing the inhibitory input that is normally modulated by CD200 [i.e., as in CD200r knockout (KO) mice] reportedly promotes microglial morphological transition even in the healthy brain (Hoek et al., 2000) and leads to an exaggerated disease course both in EAE (Broderick et al., 2002) and retinal inflammation (Hoek et al., 2000).

What remains to be established is the question whether the CX3CL1-CX3CR1 or CD200-CD200r axes are affected in the diseased brain. To this end, there are only a few reports about CXC3L1 levels in the brain and CX3CR1 expression levels in microglia during the course of disease. For example, Cardona et al. (2006) detected rather high levels of free CX3CL1 in the brain (around 300pg/mg), which is suggestive of constitutive CX3CL1 release under normal physiological conditions. In the diseased (rodent or human) brain, the levels of CX3CL1 and/or CX3CR1 were found either to be unchanged or increased (Hughes et al., 2002; Tarozzo et al., 2002; Hulshof et al., 2003; Lindia et al., 2005; Xu et al., 2012), indicating that the inhibitory function of the CX3CL1-CX3CR1 axis is not generally weakened under diseased conditions. This might be different in the aged brain or brains of Alzheimer patients where a downregulation of CX3CL1 was recently observed (Wynne et al., 2010; Cho et al., 2011).

Even less is known about the regulation of CD200 or CD200r expression in disease. It was reported that in human multiple sclerosis (MS) patients, CD200 expression in neurons diminishes around the periphery and in the center of MS lesions (Koning et al., 2007); however, astrocytes in these lesions acquire CD200 expression (Koning et al., 2009). In a mouse model of hippocampal excitotoxicity, an increase in neuronal CD200 expression was observed (Yi et al., 2012), while a decrease in CD200 and CD200r expression was reported in a mouse model of Alzheimer's disease (Walker et al., 2009)

Thus, it is at the moment unclear whether CX3CR1 or CD200r signaling is diminished in the diseased brain. To gain more knowledge about the regulation of the microglia inhibitory environment during brain disease is, however, of importance for our interpretation of the results gained in mice with mutated CX3CL1-CX3CR1 or CD200-CD200r signaling. In other words it remains to be established whether or not in a given brain disease the inhibitory input for microglia is decreased and as a result these cells become "hyperactivated" and potentially neurotoxic.

OTHER MOUSE MODELS WITH MUTATED MICROGLIA (FUNCTION)

In amyotrophic lateral sclerosis (ALS), mutations within the ubiquitously expressed enzyme superoxide dismutase 1 (SOD1) gene are responsible for about a quarter of the inherited disease cases. Accordingly, mice that express mutant human SOD1 exhibit motoneuron degeneration and a decreased life span (see for review: Lobsiger and Cleveland, 2007). The role of microglia in this disease has been investigated in various elegant experiments in which mutated SOD1 was expressed in specific cell types (Pramatarova et al., 2001; Lino et al., 2002; Clement et al., 2003; Beers et al., 2006; Boillée et al., 2006). The conclusion that arose from these experiments was that microglia with mutated SOD1 do not initiate motor neuron degeneration but rather accelerate disease progression (see for review: Lobsiger and Cleveland, 2007), since the replacement of SOD1 mutated microglia with wild-type cells slowed down disease progression and prolonged the life span of the animals (Beers et al., 2006); this effect required functional MyD88 signaling in microglia, indicating that proper immune function of these cells is necessary in order to inhibit disease progression and prolong the lifespan of animals (Kang and Rivest, 2007). Similarly, it was recently reported that transplantation of wild-type microglia into the brains of mice deficient for methyl-CpG binding protein (MECP2−/−; a model for Rett syndrome) ameliorated disease progression and significantly increased the life span of the animals (Derecki et al., 2012).

MUTATED HUMAN MICROGLIA

Triggering receptor expressed on myeloid cells-2 (TREM2) is another receptor that is in brain exclusively expressed in microglia (for review see: Linnartz et al., 2010). TREM2 belongs to the family of immunoreceptor tyrosine-based activation motif (ITAM) receptors for which the ligand has yet not been identified. Activation of TREM2 stimulates phagocytic activity in microglia and downregulates TNFα and inducible nitric oxide synthase (iNOS) expression (Takahashi et al., 2005). TREM2 is thus an anti-inflammatory receptor that at the same time promotes phagocytic activity. TREM2 is intracellularly coupled to the adapter protein DAP12 (see for review: Linnartz et al., 2010), and interestingly, loss of function mutations of either TREM2 or DAP12 lead to a rare chronic neurodegenerative disease known as Nasu–Hakola or polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), an inherited autosomal recessive human disease characterized by early onset presenile dementia (Colonna, 2003).

It can thus be appreciated from the studies discussed in the last three chapters that milder disease or less neuronal loss in the presence of mutated microglia is seldom occurring. In contrary, the perturbation of proper microglia function by various mutations regularly leads to neuronal dysfunction and/or neurodegeneration, findings that would not corroborate the idea that microglia can become neurotoxic cells. It should be noted that the problem of microglia vs. peripheral monocytes/macrophages, however, also is of importance here since none of the above described mutated genes is exclusively found in microglia.

MODELS OF MICROGLIAL INHIBITION OR DEPLETION

MICROGLIA DEPLETION WITH CLODRONATE

The bisphosphonate drug clodronate is toxic to cells of the myeloid lineage and can be used to selectively deplete them *in vivo* and *in vitro* (Buiting and Van Rooijen, 1994). Since microglia are of myeloid origin, clodronate can also be used to deplete microglia in cell culture, organotypic hippocampal slice cultures (OHSC) and *in vivo* (Kohl et al., 2003; Lauro et al., 2010; Drabek et al., 2012). OHSC are *in vitro* explant cultures that reflect many aspects of the hippocampus *in vivo* situation by maintaining a certain degree of intrinsic connectivity and lamination (see for example: Frotscher et al., 1995). With respect to microglia, it is known that after 10 days in culture these cells acquire a ramified morphology that is comparable to their *in vivo* counterparts (Hailer et al., 1996). OHSC neurons of the CA1, CA3, and DG regions display distinct and selective neuronal vulnerability toward *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity, with CA1 neurons being the most susceptible, followed by CA3 and DG neurons, respectively (Vornov et al., 1991; Cronberg et al., 2005; Boscia et al., 2006; Gee et al., 2006). Importantly, this effect has also been observed *in vivo* (Kirino and Sano, 1984; Horn and Schlote, 1992; Acarin et al., 1996; Won et al., 1999; Schauwecker, 2002). In addition, there is a strict correlation between the amount of neuronal loss occurring and the morphological profile of microglia in OHSC subjected to excitotoxicity (Heppner et al., 1998; van Weering et al., 2011; Vinet et al., 2012). We have used OHSC to address the function of microglia in NMDA-induced neuronal loss by depleting microglia and then replenishing them with microglia (Vinet et al., 2012). It was found that neuronal cell loss was prominently increased in the absence of microglia and that even neurons of the DG were affected by the NMDA treatment when microglia cells were not present (Vinet et al., 2012). These findings are in agreement with earlier reports from our group, as well as from others (Montero et al., 2009; van Weering et al., 2011). In addition to earlier findings, we also showed that when microglia-free OHSCs were replenished with microglia, these cells invaded the tissue, distributed themselves evenly across the slice and acquired an *in vivo*-like, ramified morphology (Vinet et al., 2012). Most importantly, neurons in the presence of these ectopic microglia were protected from NMDA-induced toxicity to the same extent as in non-depleted control slices (Vinet et al., 2012). These findings convincingly show not only that microglia have a neuroprotective capacity, but also that this property applies to ramified microglia (Vinet et al., 2012). Thus, neurons are protected in the vicinity of ramified microglia, while removing microglia from the local environment renders neurons more vulnerable to excitotoxicity.

Although it is yet not clear whether similar processes also occur *in vivo*, it is tempting to speculate that numerous protective properties of microglia have simply gone unnoticed because ramified microglia were generally long considered to be inactive cells. This speculation is corroborated by recent findings in the neonatal brain subjected to middle cerebral artery occlusion (MCAO), a widely used stroke model. Here it was reported by Faustino et al. (2011) that the depletion (or reduction) of ramified microglia *in vivo* (by intracerebral injection of clodronate-filled liposomes)

exacerbated injury after stoke. Interestingly, the initial effect of the MCAO did not change in the absence of microglia, however, the volume of the lesion gradually increased over time (Faustino et al., 2011), again suggesting that the absence of ramified microglia is detrimental to the injured brain.

CD11b HSVTK MOUSE LINES

Another way to specifically target microglia is through the use of transgenic mouse strains in which the herpes simplex virus thymidine kinase (HSVTK) is placed under the control of the CD11b promoter (Heppner et al., 2005; Gowing et al., 2006). Treating OHSCs from these animals with ganciclovir efficiently depletes microglia from the tissue slice culture (Falsig et al., 2008; Vinet et al., 2012). As a result the lack of microglia increases prion titers by 15 times and thereby also the susceptibility of the slices to prion infection was enhanced (Falsig et al., 2008). From these results the authors concluded that microglia are important elements in containment of prion infections (Falsig et al., 2008).

Application of ganciclovir to CD11b-HSVTK animals leads to the death of proliferating CD11b+ cells (Heppner et al., 2005; Gowing et al., 2006). The effects of ganciclovir on microglia *in vivo* are dependent on the application route of the drug in these animals. If peripheral ganciclovir application (intraperitoneal injection or oral application) is used, transplantation of wild-type bone-marrow is required to spare the peripheral myeloid compartment from ganciclovir treatment. In these resulting chimeric animals, ganciclovir application leads to the inhibition of morphological microglia transition to amoeboid cells in the case of EAE (referred to as microglia paralysis Heppner et al., 2005), or to the death of microglia undergoing proliferation after experimental stroke (Lalancette-Hébert et al., 2007). No effects of peripheral ganciclovir application on ramified microglia were found in these studies (Heppner et al., 2005; Lalancette-Hébert et al., 2007). Whereas the inhibition of morphological microglia transition (microglia paralysis) was protective in EAE (delayed disease onset and reduced clinical scores; Heppner et al., 2005), the ablation of microglia proliferation in the stroke model led to a larger stroke lesion area and increased neuronal death (Lalancette-Hébert et al., 2007).

More recent studies using these mouse lines have changed the application route of ganciclovir from peripheral to central, which has a twofold benefit. First, the need for bone-marrow transplantation is circumvented, and second ramified microglia are also sensitive to centrally delivered ganciclovir, which depletes the treated brain tissue of ramified microglia (Gowing et al., 2008; Grathwohl et al., 2009; Mirrione et al., 2010; Varvel et al., 2012). In the corresponding studies it was shown that the depletion of microglia by ganciclovir did not affect the development of beta amyloid plaques in two different mouse models of Alzheimer's disease (Grathwohl et al., 2009), nor did the absence of microglia change disease progression and motor neuron degeneration in the SOD mouse model of ALS (Gowing et al., 2008). However, in the case of pilocarpine-induced seizures, the depletion of microglia prevented the protective effect of LPS pre-conditioning, indicating that the inflammatory capacity of microglia is beneficial in this mouse model (Mirrione et al., 2010). Taken together, it can be concluded that ganciclovir-dependent inhibition of microglia function in CD11b-HSVTK animals was only advantageous in a disease model in one reported case (Heppner et al., 2005). All other reports either provided evidence for a beneficial role of microglial function *in vivo* (Lalancette-Hébert et al., 2007; Mirrione et al., 2010) or showed no effect of blunting the microglial response (Gowing et al., 2008; Grathwohl et al., 2009). It should be noted here that the latter studies inhibited or depleted microglia for a limited time at rather late stages of chronic disease models (Gowing et al., 2008; Grathwohl et al., 2009), which may explain the surprising lack of effect. The inhibition or depletion of microglial function may have been too late or too short to unravel the role of these cells in mouse models of AD and ALS (Gowing et al., 2008; Grathwohl et al., 2009). Thus, inhibition or depletion of microglia for longer time periods may be required for chronic disease models.

CD11b-DTR MICE

Another mouse model that allows the depletion of myeloid cells is the CD11b-DTR mouse line that expresses diphtheria toxin receptor under the control of the CD11b promoter (Duffield et al., 2005). This mouse was very recently used to study the role of microglia in the development of the cortex (Ueno et al., 2013). This study shows that impaired microglia function or depletion of these cells by diphtheria toxin injection leads to enhanced neuronal loss of layer V neurons. The authors furthermore provide evidence that microglia provide trophic support for layer V neurons through the synthesis and release of IGF1. Interestingly, this vital role of microglia for layer V neurons was attributed to amoeboid microglia (Ueno et al., 2013).

Taken together, depleting microglia is rarely correlated to improved outcome in various brain disease models. These findings are thus not in favor of a major neurotoxic function of microglia in the brain but would argue more for a protective role of the innate immune cells of the brain.

CONCLUSION

Innate immunity was originally seen as a stereotypic response to exogenous pathogens. However, the "danger model" formulated by Polly Matzinger more than a decade ago (Matzinger, 2002) couple with more recent findings that pattern recognition receptors are also activated by endogenous ligands [so-called danger-associated molecular patterns (DAMP)] have dramatically challenged this original view (Gordon, 2002). Even the classical immune defense components, such as the complement system, which are activated to ward off pathogens have now been recognized as danger signals that are not necessarily linked to pathogen infection (Köhl, 2006). Moreover, innate immune cells are no longer considered to elicit a stereotypic response. In striking contrast, it now is apparent that in the face of different kinds of threats, innate immune cells are able to interpret signals and launch an appropriate response (see for review: Gordon, 2003; Martinez et al., 2008; Mosser and Edwards, 2008; Gordon and Mantovani, 2011). Hence, it is now clear that tissue damage or cellular stress is also a potent inducer of innate immunity, where the ultimate goal is to protect and restore cellular function, thereby guaranteeing the functional integrity of the body (Medzhitov, 2010). Microglia, the innate immune cells of the brain, should be viewed along the same lines, because

infections with exogenous pathogens are (luckily) scarce events in the brain making cellular stress or tissue damage more likely signals for microglia.

In this review we argue that amoeboid microglia have a bad reputation. Based on correlative histological data and corresponding *in vitro* experiments these cells are often discussed to be neurotoxic. Moreover, their striking similarity with peripheral monocytes/macrophages has blurred our picture concerning the function of these cells. Microglia colonize the CNS very early and are sequestered there throughout a lifetime, making it very likely that these cells have an elaborate repertoire of brain specific functions that may not be appropriately taken over by peripheral monocytes/macrophages (Neumann and Wekerle, 2013). Therefore to discriminate microglia from peripheral monocytes/macrophages and to elucidate the functional spectrum of microglia in the (healthy and diseased) brain will be a major challenge.

New mouse models may be helpful here. It was recently shown that following chemical depletion of microglia (CD11b-HSVTK mouse) there is a rapid and efficient repopulation of the brain with CD45 high and CCR2+ blood monocytes, which gradually engrafted into the microglia-free tissue with an overall distribution and morphology reminiscent (yet different) to that of endogenous microglia (Varvel et al., 2012). This mouse model thus offers an opportunity to investigate the question whether or not invaded monocytes/macrophages can become true brain microglia (Varvel et al., 2012; Neumann and Wekerle, 2013). Other new mouse models may also be helpful to distinguish microglia (function) from peripheral monocytes macrophages. The double knock-in mouse expressing red fluorescent protein (RFP) and GFP in the CCR2 and CX3CR1 locus, respectively, allows a reliable discrimination of both cell types even in tissue sections (Mizutani et al., 2012), and the newly described CX3CR1^{creER}-line can be utilized to construct KO or overexpression models that will enable us to investigate the *in vivo* functions of receptors, signaltransduction pathways or inflammatory mediators in a microglia specific context (Yona et al., 2013; Wolf et al., 2013).

Using such models we may now be able to investigate the overall picture of microglia responses, for example in the development of neuropathic pain. There is compelling evidence that amoeboid microglia in the spinal cord release brain-derived neurotrophic factor (BDNF) in response to peripheral nerve lesion, which is crucial for the development of neuropathic pain (Tsuda et al., 2003; Coull et al., 2005; Ulmann et al., 2008). Accordingly, microglia are seen as inducers of a pathological pain reaction, again indicating that their action in response to nervous damage is detrimental (see for recent review: Tsuda et al., 2013). The whole functional spectrum of microglia derived BDNF under these circumstances, however, is not known at the moment. Since BDNF is a neuronal survival factor it is tempting to speculate that one reason for its release from microglia is to protect neurons from nerve injury. It may therefore be of interest to analyze the neuronal fate after peripheral nerve injury in microglia-specific BDNF KO animals.

Taken together, mutating or deleting microglia very often leads to the development or worsening of a brain disease, results that are favoring a beneficial role of these cells in the CNS. It is discussed here that several experimental problems around these cells have led to potential misinterpretations concerning the role of microglia. It is thus clear that without (i) a detailed analysis of the causal relationship between microglial function and neuronal fate, and (ii) a thorough understanding of all aspects of the microglia response in a given disease (model), it is difficult to fully appreciate what microglia are doing in the brain. As new techniques and mouse models are now emerging to do such analysis, it is anticipated that more surprising findings about "the brain's best friend" will be published.

ACKNOWLEDGMENTS

Knut Biber is supported by the DFG (FOR 1336 "From monocytes to brain macrophages-conditions influencing the fate of myeloid cells in the brain"; DFG BI 668/5-1), DFG grant BI 668/2-2 and BMBF-funded Competence Network Degenerative Diseases (KNDD). The authors are grateful to Dr. Sandra Dieni for critical reading and editing.

REFERENCES

Abbadie, C., Lindia, J. A., Cumiskey, A. M., Peterson, L. B., Mudgett, J. S., Bayne, E. K., et al. (2003). Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. *Proc. Natl. Acad. Sci. U.S.A.* 100, 7947–7952.

Acarin, L., Gonzalez, B., Castellano, B., and Castro, A. J. (1996). Microglial response to *N*-methyl-D-aspartate-mediated excitotoxicity in the immature rat brain. *J. Comp. Neurol.* 367, 361–374.

Aguzzi, A., Barres, B. A., and Bennett, M. L. (2013). Microglia: scapegoat, saboteur, or something else? *Science* 339, 156–161.

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M., and Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* 14, 1142–1149.

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

Allan, S. M., Tyrrell, P. J., and Rothwell, N. J. (2005). Interleukin-1 and neuronal injury. *Nat. Rev. Immunol.* 5, 629–640.

Alliot, F., Godin, I., and Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* 117, 145–152.

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., et al. (2006). Wild-type microglia extend survival in PU. 1 knockout mice with familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16021–16026.

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci.* 30, 596–602.

Blais, V., and Rivest, S. (2004). Effects of TNF-alpha and IFN-gamma on nitric oxide-induced neurotoxicity in the mouse brain. *J. Immunol.* 172, 7043–7052.

Block, M. L., Zecca, L., and Hong, J. S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69.

Boillée, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., et al. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312, 1389–1392.

Boje, K. M., and Arora, P. K. (1992). Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Res.* 587, 250–256.

Boscia, F., Annunziato, L., and Taglialatela, M. (2006). Retigabine and flupirtine exert neuroprotective actions in organotypic hippocampal cultures. *Neuropharmacology* 51, 283–294.

Boucsein, C., Kettenmann, H., and Nolte, C. (2000). Electrophysiological properties of microglial cells in normal and pathologic rat brain slices. *Eur. J. Neurosci.* 12, 2049–2058.

Broderick, C., Hoek, R. M., Forrester, J. V., Liversidge, J., Sedgwick, J. D., and Dick, A. D. (2002). Constitutive retinal CD200 expression regulates

resident microglia and activation state of inflammatory cells during experimental autoimmune uveoretinitis. *Am. J. Pathol.* 161, 1669–1677.

Buiting, A. M., and Van Rooijen, N. (1994). Liposome mediated depletion of macrophages: an approach for fundamental studies. *J. Drug Target.* 2, 357–362.

Burguillos, M. A., Deierborg, T., Kavanagh, E., Persson, A., Hajji, N., Garcia-Quintanilla, A., et al. (2011). Caspase signalling controls microglia activation and neurotoxicity. *Nature* 472, 319–324.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., et al. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* 9, 917–924.

Chao, C. C., Hu, S., Molitor, T. W., Shaskan, E. G., and Peterson, P. K. (1992). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. *J. Immunol.* 149, 2736–2741.

Cho, S. H., Sun, B., Zhou, Y., Kauppinen, T. M., Halabisky, B., Wes, P., et al. (2011). CX3CR1 protein signaling modulates microglial activation and protects against plaque-independent cognitive deficits in a mouse model of Alzheimer disease. *J. Biol. Chem.* 286, 32713–32722.

Cipriani, R., Villa, P., Chece, G., Lauro, C., Paladini, A., Micotti, E., et al. (2011). CX3CL1 is neuroprotective in permanent focal cerebral ischemia in rodents. *J. Neurosci.* 31, 16327–16335.

Clement, A. M., Nguyen, M. D., Roberts, E. A., Garcia, M. L., Boillée, S., Rule, M., et al. (2003). Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302, 113–117.

Colonna, M. (2003). DAP12 signaling: from immune cells to bone modeling and brain myelination. *J. Clin. Invest.* 111, 313–314.

Colton, C. A. (2009). Heterogeneity of microglial activation in the innate immune response in the brain. *J. Neuroimmune. Pharmacol.* 4, 399–418.

Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., et al. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021.

Cronberg, T., Jensen, K., Rytter, A., and Wieloch, T. (2005). Selective sparing of hippocampal CA3 cells following in vitro ischemia is due to selective inhibition by acidosis. *Eur. J. Neurosci.* 22, 310–316.

de Haas, A. H., Boddeke, H. W., and Biber, K. (2008). Region-specific expression of immunoregulatory proteins on microglia in the healthy CNS. *Glia* 56, 888–894.

de Haas, A. H., Boddeke, H. W., Brouwer, N., and Biber, K. (2007). Optimized isolation enables ex vivo analysis of microglia from various central nervous system regions. *Glia* 55, 1374–1384.

Deng, Y. Y., Lu, J., Ling, E. A., and Kaur, C. (2009). Monocyte chemoattractant protein-1 (MCP-1) produced via NF-kappaB signaling pathway mediates migration of amoeboid microglia in the periventricular white matter in hypoxic neonatal rats. *Glia* 57, 604–621.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

Dimitrijevic, O. B., Stamatovic, S. M., Keep, R. F., and Andjelkovic, A. V. (2007). Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. *Stroke* 38, 1345–1353.

Drabek, T., Janata, A., Jackson, E. K., End, B., Stezoski, J., Vagni, V. A., et al. (2012). Microglial depletion using intrahippocampal injection of liposome-encapsulated clodronate in prolonged hypothermic cardiac arrest in rats. *Resuscitation* 83, 517–526.

Duffield, J. S., Forbes, S. J., Constandinou, C. M., Clay, S., Partolina, M., Vuthoori, S., et al. (2005). Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J. Clin. Invest.* 115, 56–65.

Dunston, C. R., Griffiths, H. R., Lambert, P. A., Staddon, S., and Vernallis, A. B. (2011). Proteomic analysis of the anti-inflammatory action of minocycline. *Proteomics* 11, 42–51.

El Khoury, J., Toft, M., Hickman, S. E., Means, T. K., Terada, K., Geula, C., et al. (2007). Ccr2 deficiency impairs microglial accumulation and accelerates progression of Alzheimer-like disease. *Nat. Med.* 13, 432–438.

Falsig, J., Julius, C., Margalith, I., Schwarz, P., Heppner, F. L., and Aguzzi, A. (2008). A versatile prion replication assay in organotypic brain slices. *Nat. Neurosci.* 11, 109–117.

Fan, L. W., Pang, Y., Lin, S., Rhodes, P. G., and Cai, Z. (2005). Minocycline attenuates lipopolysaccharide-induced white matter injury in the neonatal rat brain. *Neuroscience* 133, 159–168.

Faustino, J. V., Wang, X., Johnson, C. E., Klibanov, A., Derugin, N., Wendland, M. F., et al. (2011). Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. *J. Neurosci.* 31, 12992–13001.

Fendrick, S. E., Miller, K. R., and Streit, W. J. (2005). Minocycline does not inhibit microglia proliferation or neuronal regeneration in the facial nucleus following crush injury. *Neurosci. Lett.* 385, 220–223.

Fernández-López, D., Faustino, J., Derugin, N., Wendland, M., Lizasoain, I., Moro, M. A., et al. (2012). Reduced infarct size and accumulation of microglia in rats treated with WIN 55,212-2 after neonatal stroke. *Neuroscience* 207, 307–315.

Frotscher, M., Zafirov, S., and Heimrich, B. (1995). Development of identified neuronal types and of specific synaptic connections in slice cultures of rat hippocampus. *Prog. Neurobiol.* 45, vii–xxviii.

Gao, H. M., Zhou, H., Zhang, F., Wilson, B. C., Kam, W., and Hong, J. S. (2011). HMGB1 acts on microglia Mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration. *J. Neurosci.* 31, 1081–1092.

Gee, C. E., Benquet, P., Raineteau, O., Rietschin, L., Kirbach, S. W., and Gerber, U. (2006). NMDA receptors and the differential ischemic vulnerability of hippocampal neurons. *Eur. J. Neurosci.* 23, 2595–2603.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Gordon, S. (2002). Pattern recognition receptors: doubling up for the innate immune response. *Cell* 111, 927–930.

Gordon, S. (2003). Alternative activation of macrophages. *Nat. Rev. Immunol.* 3, 23–35.

Gordon, S., and Mantovani, A. (2011). Diversity and plasticity of mononuclear phagocytes. *Eur. J. Immunol.* 41, 2470–2472.

Gowing, G., Philips, T., Van Wijmeersch, B., Audet, J. N., Dewil, M., Van Den Bosch, L., et al. (2008). Ablation of proliferating microglia does not affect motor neuron degeneration in amyotrophic lateral sclerosis caused by mutant superoxide dismutase. *J. Neurosci.* 28, 10234–10244.

Gowing, G., Vallières, L., and Julien, J. P. (2006). Mouse model for ablation of proliferating microglia in acute CNS injuries. *Glia* 53, 331–337.

Graeber, M. B. (2010). Changing face of microglia. *Science* 330, 783–788.

Grathwohl, S. A., Kälin, R. E., Bolmont, T., Prokop, S., Winkelmann, G., Kaeser, S. A., et al. (2009). Formation and "maintenance" of Alzheimer's disease beta-amyloid plaques in the absence of microglia. *Nat. Neurosci.* 12, 1361–1363.

Hailer, N. P., Jarhult, J. D., and Nitsch, R. (1996). Resting microglial cells in vitro: analysis of morphology and adhesion molecule expression in organotypic hippocampal slice cultures. *Glia* 18, 319–331.

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394.

Hashimoto, K., and Ishima, T. (2010). A novel target of action of minocycline in NGF-induced neurite outgrowth in PC12 cells: translation initiation [corrected] factor eIF4AI. *PLoS ONE* 5:e15430. doi: 10.1371/journal.pone.0015430

He, Y., Appel, S., and Le, W. (2001). Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. *Brain Res.* 909, 187–193.

Heppner, F. L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hövelmeyer, N., et al. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat. Med.* 11, 146–152.

Heppner, F. L., Skutella, T., Hailer, N. P., Haas, D., and Nitsch, R. (1998). Activated microglial cells migrate towards sites of excitotoxic neuronal injury inside organotypic hippocampal slice cultures. *Eur. J. Neurosci.* 10, 3284–3290.

Herbomel, P., Thisse, B., and Thisse, C. (2001). Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. *Dev. Biol.* 238, 274–288.

Hoek, R. M., Ruuls, S. R., Murphy, C. A., Wright, G. J., Goddard, R., Zurawski, S. M., et al. (2000). Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 290, 1768–1771.

Horn, M., and Schlote, W. (1992). Delayed neuronal death, and delayed neuronal recovery in the human brain following global ischemia. *Acta Neuropathol.* 85, 79–87.

Huang, W. C., Qiao, Y., Xu, L., Kacimi, R., Sun, X., Giffard, R. G., et al. (2010). Direct protection of cultured neurons from ischemia-like injury by minocycline. *Anat. Cell Biol.* 43, 325–331.

Hughes, E. H., Schlichtenbrede, F. C., Murphy, C. C., Broderick, C., van Rooijen, N., Ali, R. R., et al. (2004). Minocycline delays photoreceptor death in the rds mouse through a microglia-independent mechanism. *Exp. Eye Res.* 78, 1077–1084.

Hughes, P. M., Botham, M. S., Frentzel, S., Mir, A., and Perry, V. H. (2002). Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. *Glia* 37, 314–327.

Hulshof, S., van Haastert, E. S., Kuipers, H. F., van den Elsen, P. J., De Groot, C. J., van der Valk, P., et al. (2003). CX3CL1 and CX3CR1 expression in human brain tissue: noninflammatory control versus multiple sclerosis. *J. Neuropathol. Exp. Neurol.* 62, 899–907.

Hunter, C. L., Bachman, D., and Granholm, A. C. (2004). Minocycline prevents cholinergic loss in a mouse model of Down's syndrome. *Ann. Neurol.* 56, 675–688.

Jung, H., Bhangoo, S., Banisadr, G., Freitag, C., Ren, D., White, F. A., et al. (2009). Visualization of chemokine receptor activation in transgenic mice reveals peripheral activation of CCR2 receptors in states of neuropathic pain. *J. Neurosci.* 29, 8051–8062.

Jung, S., and Schwartz, M. (2012). Non-identical twins – microglia and monocyte-derived macrophages in acute injury and autoimmune inflammation. *Front. Immunol.* 3:89. doi: 10.3389/fimmu.2012.00089

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M. J., Kreutzberg, G. W., Sher, A., et al. (2000). Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol. Cell. Biol.* 20, 4106–4114.

Kang, J., and Rivest, S. (2007). MyD88-deficient bone marrow cells accelerate onset and reduce survival in a mouse model of amyotrophic lateral sclerosis. *J. Cell Biol.* 179, 1219–1230.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Kettenmann, H., Kirchhoff, F., and Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. *Neuron* 77, 10–18.

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat. Neurosci.* 16, 273–280.

Kim, S. U., and de Vellis, J. (2005). Microglia in health and disease. *J. Neurosci. Res.* 81, 302–313.

Kim, W. G., Mohney, R. P., Wilson, B., Jeohn, G. H., Liu, B., and Hong, J. S. (2000). Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J. Neurosci.* 20, 6309–6316.

Kirino, T., and Sano, K. (1984). Selective vulnerability in the gerbil hippocampus following transient ischemia. *Acta Neuropathol.* 62, 201–208.

Kohl, A., Dehghani, F., Korf, H. W., and Hailer, N. P. (2003). The bisphosphonate clodronate depletes microglial cells in excitotoxically injured organotypic hippocampal slice cultures. *Exp. Neurol.* 181, 1–11.

Köhl, J. (2006). Self, non-self, and danger: a complementary view. *Adv. Exp. Med. Biol.* 586, 71–94.

Koning, N., Bö, L., Hoek, R. M., and Huitinga, I. (2007). Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. *Ann. Neurol.* 62, 504–514.

Koning, N., Swaab, D. F., Hoek, R. M., and Huitinga, I. (2009). Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron-glia and glia-glia interactions. *J. Neuropathol. Exp. Neurol.* 68, 159–167.

Kriz, J., Nguyen, M. D., and Julien, J. P. (2002). Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol. Dis.* 10, 268–278.

Lalancette-Hébert, M., Gowing, G., Simard, A., Weng, Y. C., and Kriz, J. (2007). Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. *J. Neurosci.* 27, 2596–2605.

Lauro, C., Cipriani, R., Catalano, M., Trettel, F., Chece, G., Brusadin, V., et al. (2010). Adenosine A1 receptors and microglial cells mediate CX3CL1-induced protection of hippocampal neurons against Glu-induced death. *Neuropsychopharmacology* 35, 1550–1559.

Ledeboer, A., Sloane, E. M., Milligan, E. D., Frank, M. G., Mahony, J. H., Maier, S. F., et al. (2005). Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. *Pain* 115, 71–83.

Lehnardt, S., Schott, E., Trimbuch, T., Laubisch, D., Krueger, C., Wulczyn, G., et al. (2008). A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *J. Neurosci.* 28, 2320–2331.

Levesque, S., Wilson, B., Gregoria, V., Thorpe, L. B., Dallas, S., Polikov, V. S., et al. (2010). Reactive microgliosis: extracellular micro-calpain and microglia-mediated dopaminergic neurotoxicity. *Brain* 133(Pt 3), 808–821.

Lindia, J. A., McGowan, E., Jochnowitz, N., and Abbadie, C. (2005). Induction of CX3CL1 expression in astrocytes and CX3CR1 in microglia in the spinal cord of a rat model of neuropathic pain. *J. Pain* 6, 434–438.

Linnartz, B., Wang, Y., and Neumann, H. (2010). Microglial immunoreceptor tyrosine-based activation and inhibition motif signaling in neuroinflammation. *Int. J. Alzheimers Dis.* 2010, pii: 587463.

Lino, M. M., Schneider, C., and Caroni, P. (2002). Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J. Neurosci.* 22, 4825–4832.

Lobsiger, C. S., and Cleveland, D. W. (2007). Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nat. Neurosci.* 10, 1355–1360.

Martinez, F. O., Sica, A., Mantovani, A., and Locati, M. (2008). Macrophage activation and polarization. *Front. Biosci.* 13, 453–461.

Matzinger, P. (2002). An innate sense of danger. *Ann. N. Y. Acad. Sci.* 961, 341–342.

Medzhitov, R. (2010). Innate immunity: quo vadis? *Nat. Immunol.* 11, 551–553.

Melief, J., Koning, N., Schuurman, K. G., Van De Garde, M. D., Smolders, J., Hoek, R. M., et al. (2012). Phenotyping primary human microglia: tight regulation of LPS responsiveness. *Glia* 60, 1506–1517.

Mika, J., Osikowicz, M., Makuch, W., and Przewlocka, B. (2007). Minocycline and pentoxifylline attenuate allodynia and hyperalgesia and potentiate the effects of morphine in rat and mouse models of neuropathic pain. *Eur. J. Pharmacol.* 560, 142–149.

Mildner, A., Mack, M., Schmidt, H., Brück, W., Djukic, M., Zabel, M. D., et al. (2009). CCR2+Ly-6Chi monocytes are crucial for the effector phase of autoimmunity in the central nervous system. *Brain* 132(Pt 9), 2487–2500.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., et al. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* 10, 1544–1553.

Mirrione, M. M., Konomos, D. K., Gravanis, I., Dewey, S. L., Aguzzi, A., Heppner, F. L., et al. (2010). Microglial ablation and lipopolysaccharide preconditioning affects pilocarpine-induced seizures in mice. *Neurobiol. Dis.* 39, 85–97.

Mizutani, M., Pino, P. A., Saederup, N., Charo, I. F., Ransohoff, R. M., and Cardona, A. E. (2012). The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. *J. Immunol.* 188, 29–36.

Montero Domínguez, M., González, B., and Zimmer, J. (2009). Neuroprotective effects of the anti-inflammatory compound triflusal on ischemia-like neurodegeneration in mouse hippocampal slice cultures occur independent of microglia. *Exp. Neurol.* 218, 11–23.

Montero, M., Gonzalez, B., and Zimmer, J. (2009). Immunotoxic depletion of microglia in mouse hippocampal slice cultures enhances ischemia-like neurodegeneration. *Brain Res.* 1291, 140–152.

Morganti, J. M., Nash, K. R., Grimmig, B. A., Ranjit, S., Small, B., Bickford, P. C., et al. (2012). The soluble isoform of CX3CL1 is necessary for neuroprotection in a mouse model of Parkinson's disease. *J. Neurosci.* 32, 14592–14601.

Mosser, D. M., and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8, 958–969.

Nadeau, S., and Rivest, S. (2002). Endotoxemia prevents the cerebral inflammatory wave induced by intraparenchymal lipopolysaccharide injection: role of glucocorticoids and CD14. *J. Immunol.* 169, 3370–3381.

Nadeau, S., and Rivest, S. (2003). Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *J. Neurosci.* 23, 5536–5544.

Neumann, H., and Wekerle, H. (2013). Brain microglia: watchdogs with pedigree. *Nat. Neurosci.* 16, 253–255.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314–1318.

O'Callaghan, J. P., Sriram, K., and Miller, D. B. (2008). Defining "neuroinflammation". *Ann. N. Y. Acad. Sci.* 1139, 318–330.

Olah, M., Amor, S., Brouwer, N., Vinet, J., Eggen, B., Biber, K., et al. (2012). Identification of a microglia phenotype supportive of remyelination. *Glia* 60, 306–321.

Osikowicz, M., Skup, M., Mika, J., Makuch, W., Czarkowska-Bauch, J., and Przewlocka, B. (2009). Glial inhibitors influence the mRNA and protein levels of mGlu2/3, 5, and 7 receptors and potentiate the analgesic effects of their ligands in a mouse model of neuropathic pain. *Pain* 147, 175–186.

Ossola, B., Lantto, T. A., Puttonen, K. A., Tuominen, R. K., Raasmaja, A., and Männistö, P. T. (2012). Minocycline protects SH-SY5Y cells from 6-hydroxydopamine by inhibiting both caspase-dependent and -independent programmed cell death. *J. Neurosci. Res.* 90, 682–690.

Pabon, M. M., Bachstetter, A. D., Hudson, C. E., Gemma, C., and Bickford, P. C. (2011). CX3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease. *J. Neuroinflammation* 8, 9.

Pais, T. F., Figueiredo, C., Peixoto, R., Braz, M. H., and Chatterjee, S. (2008). Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway. *J. Neuroinflammation* 5, 43.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Parkhurst, C. N., and Gan, W. B. (2010). Microglia dynamics and function in the CNS. *Curr. Opin. Neurobiol.* 20, 595–600.

Polazzi, E., and Monti, B. (2010). Microglia and neuroprotection: from in vitro studies to therapeutic applications. *Prog. Neurobiol.* 92, 293–315.

Pramatarova, A., Laganière, J., Roussel, J., Brisebois, K., and Rouleau, G. A. (2001). Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J. Neurosci.* 21, 3369–3374.

Prinz, M., and Mildner, A. (2011). Microglia in the CNS: immigrants from another world. *Glia* 59, 177–187.

Prinz, M., and Priller, J. (2010). Tickets to the brain: role of CCR2 and CX3CR1 in myeloid cell entry in the CNS. *J. Neuroimmunol* 224, 80–84.

Prinz, M., Priller, J., Sisodia, S. S., and Ransohoff, R. M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* 14, 1227–1235.

Raber, J., Allen, A. R., Rosi, S., Sharma, S., Dayger, C., Davis, M. J., et al. (2013). Effects of 56Fe radiation on hippocampal function in mice deficient in chemokine receptor 2 (CCR2). *Behav. Brain Res.* 246, 69–75.

Raghavendra, V., Tanga, F., and DeLeo, J. A. (2003). Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *J. Pharmacol. Exp. Ther.* 306, 624–630.

Ransohoff, R. M., and Cardona, A. E. (2010). The myeloid cells of the central nervous system parenchyma. *Nature* 468, 253–262.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Ransohoff, R. M., and Prinz, M. (2013). Editors' preface: microglia – a new era dawns. *Glia* 61, 1–2.

Remington, L. T., Babcock, A. A., Zehntner, S. P., and Owens, T. (2007). Microglial recruitment, activation, and proliferation in response to primary demyelination. *Am. J. Pathol.* 170, 1713–1724.

Saederup, N., Cardona, A. E., Croft, K., Mizutani, M., Cotleur, A. C., Tsou, C. L., et al. (2010). Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS ONE* 5:e13693. doi: 10.1371/journal.pone.0013693

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Schauwecker, P. E. (2002). Modulation of cell death by mouse genotype: differential vulnerability to excitatory amino acid-induced lesions. *Exp. Neurol.* 178, 219–235.

Schildknecht, S., Pape, R., Müller, N., Robotta, M., Marquardt, A., Bürkle, A., et al. (2011). Neuroprotection by minocycline caused by direct and specific scavenging of peroxynitrite. *J. Biol. Chem.* 286, 4991–5002.

Schilling, M., Strecker, J. K., Ringelstein, E. B., Schäbitz, W. R., and Kiefer, R. (2009a). The role of CC chemokine receptor 2 on microglia activation and blood-borne cell recruitment after transient focal cerebral ischemia in mice. *Brain Res.* 1289, 79–84.

Schilling, M., Strecker, J. K., Schäbitz, W. R., Ringelstein, E. B., and Kiefer, R. (2009b). Effects of monocyte chemoattractant protein 1 on blood-borne cell recruitment after transient focal cerebral ischemia in mice. *Neuroscience* 161, 806–812.

Schmid, C. D., Melchior, B., Masek, K., Puntambekar, S. S., Danielson, P. E., Lo, D. D., et al. (2009). Differential gene expression in LPS/IFNgamma activated microglia, and macrophages: in vitro versus in vivo. *J. Neurochem.* 109(Suppl. 1), 117–125.

Sedgwick, J. D., Ford, A. L., Foulcher, E., and Airriess, R. (1998). Central nervous system microglial cell activation and proliferation follows direct interaction with tissue-infiltrating T cell blasts. *J. Immunol.* 160, 5320–5330.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Sorokin, S. P., Hoyt, R. F. Jr., Blunt, D. G., and McNelly, N. A. (1992). Macrophage development: II. Early ontogeny of macrophage populations in brain, liver, and lungs of rat embryos as revealed by a lectin marker. *Anat. Rec.* 232, 527–550.

Szeto, G. L., Pomerantz, J. L., Graham, D. R., and Clements, J. E. (2011). Minocycline suppresses activation of nuclear factor of activated T cells 1 (NFAT1) in human CD4+ T cells. *J. Biol. Chem.* 286, 11275–11282.

Takahashi, K., Rochford, C. D., and Neumann, H. (2005). Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J. Exp. Med.* 201, 647–657.

Tarozzo, G., Campanella, M., Ghiani, M., Bulfone, A., and Beltramo, M. (2002). Expression of fractalkine, and its receptor, CX3CR1, in response to ischaemia-reperfusion brain injury in the rat. *Eur. J. Neurosci.* 15, 1663–1668.

Tikka, T., Fiebich, B. L., Goldsteins, G., Keinanen, R., and Koistinaho, J. (2001). Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J. Neurosci.* 21, 2580–2588.

Tremblay, M. È., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Tremblay, M. È., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069.

Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M. W., et al. (2003). P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424, 778–783.

Tsuda, M., Beggs, S., Salter, M. W., and Inoue, K. (2013). Microglia and intractable chronic pain. *Glia* 61, 55–61.

Ueno, M., Fujita, Y., Tanaka, T., Nakamura, Y., Kikuta, J., Ishii, M., et al. (2013). Layer V cortical neurons require microglial support for survival during postnatal development. *Nat. Neurosci.* 16, 543–551.

Ulmann, L., Hatcher, J. P., Hughes, J. P., Chaumont, S., Green, P. J., Conquet, F., et al. (2008). Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J. Neurosci.* 28, 11263–11268.

van Weering, H. R., Boddeke, H. W., Vinet, J., Brouwer, N., de Haas, A. H., van Rooijen, N., et al. (2011). CXCL10/CXCR3 signaling in glia cells differentially affects NMDA-induced cell death in CA and DG neurons of the mouse hippocampus. *Hippocampus* 21, 220–232.

Varvel, N. H., Grathwohl, S. A., Baumann, F., Liebig, C., Bosch, A., Brawek, B., et al. (2012). Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 18150–18155.

Vinet, J., Weering, H. R., Heinrich, A., Kälin, R. E., Wegner, A., Brouwer, N., et al. (2012). Neuroprotective function for ramified microglia in hippocampal excitotoxicity. *J. Neuroinflammation* 9, 27.

Vornov, J. J., Tasker, R. C., and Coyle, J. T. (1991). Direct observation of the agonist-specific regional vulnerability to glutamate, NMDA, and kainate neurotoxicity in organotypic hippocampal cultures. *Exp. Neurol.* 114, 11–22.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Walker, D. G., Dalsing-Hernandez, J. E., Campbell, N. A., and Lue, L. F. (2009). Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. *Exp. Neurol.* 215, 5–19.

Walker, D. G., and Lue, L. F. (2005). Investigations with cultured human microglia on pathogenic mechanisms of Alzheimer's disease and other neurodegenerative diseases. *J. Neurosci. Res.* 81, 412–425.

Wolf, Y., Yona, S., Kim, K. W., and Jung, S. (2013). Microglia, seen from the CX3CR1 angle. *Front. Cell. Neurosci.* 7:26. doi: 10.3389/fncel.2013.00026

Won, S. J., Ko, H. W., Kim, E. Y., Park, E. C., Huh, K., Jung, N. P., et al. (1999). Nuclear factor kappa B-mediated kainate neurotoxicity in the rat and hamster hippocampus. *Neuroscience* 94, 83–91.

Wynne, A. M., Henry, C. J., Huang, Y., Cleland, A., and Godbout, J. P. (2010). Protracted downregulation of CX3CR1 on microglia of aged mice after lipopolysaccharide challenge. *Brain Behav. Immun.* 24, 1190–1201.

Xie, Z., Smith, C. J., and Van Eldik, L. J. (2004). Activated glia induce neuron death via MAP kinase signaling pathways involving JNK and p38. *Glia* 45, 170–179.

Xu, Y., Zeng, K., Han, Y., Wang, L., Chen, D., Xi, Z., et al. (2012). Altered expression of CX3CL1 in patients with epilepsy and in a rat model. *Am. J. Pathol.* 180, 1950–1962.

Yao, Y., and Tsirka, S. E. (2012). The CCL2-CCR2 system affects the progression and clearance of intracerebral hemorrhage. *Glia* 60, 908–918.

Yi, M. H., Zhang, E., Kang, J. W., Shin, Y. N., Byun, J. Y., Oh, S. H., et al. (2012). Expression of CD200 in alternative activation of microglia following an excitotoxic lesion in the mouse hippocampus. *Brain Res.* 1481, 90–96.

Yona, S., Kim, K. W., Wolf, Y., Mildner, A., Varol, D., Breker, M., et al. (2013). Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 38, 79–91.

Yong, V. W., and Rivest, S. (2009). Taking advantage of the systemic immune system to cure brain diseases. *Neuron* 64, 55–60.

Yrjänheikki, J., Keinänen, R., Pellikka, M., Hökfelt, T., and Koistinaho, J. (1998). Tetracyclines inhibit microglial activation, and are neuroprotective in global brain ischemia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15769–15774.

Zhang, J., Shi, X. Q., Echeverry, S., Mogil, J. S., De Koninck, Y., and Rivest, S. (2007). Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. *J. Neurosci.* 27, 12396–12406.

Zuurman, M. W., Heeroma, J., Brouwer, N., Boddeke, H. W., and Biber, K. (2003). LPS-induced expression of a novel chemokine receptor (L-CCR) in mouse glial cells in vitro and in vivo. *Glia* 41, 327–336.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 January 2013; accepted: 26 April 2013; published online: 16 May 2013.

Citation: Hellwig S, Heinrich A and Biber K (2013) The brain's best friend: microglial neurotoxicity revisited. Front. Cell. Neurosci. ***7****:71. doi: 10.3389/fncel.2013.00071*

frontiers in
CELLULAR NEUROSCIENCE

HYPOTHESIS AND THEORY ARTICLE
published: 08 April 2013
doi: 10.3389/fncel.2013.00034

Microglia and monocyte-derived macrophages: functionally distinct populations that act in concert in CNS plasticity and repair

Anat London, Merav Cohen and Michal Schwartz*

Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Edited by:
Amanda Sierra, Ikerbasque Foundation, University of the Basque Country EHU/UPV, Spain

Reviewed by:
Shaoyu Ge, State University of New York at Stony Brook, USA
Monica J. Carson, University of California, Riverside, USA

***Correspondence:**
Michal Schwartz, Department of Neurobiology, Weizmann Institute of Science, P O. Box 26, Rehovot 76100, Israel.
e-mail: michal.schwartz@weizmann.ac.il

Functional macrophage heterogeneity is recognized outside the central nervous system (CNS), where alternatively activated macrophages can perform immune-resolving functions. Such functional heterogeneity was largely ignored in the CNS, with respect to the resident microglia and the myeloid-derived cells recruited from the blood following injury or disease, previously defined as blood-derived microglia; both were indistinguishably perceived detrimental. Our studies have led us to view the myeloid-derived infiltrating cells as functionally distinct from the resident microglia, and accordingly, to name them monocyte-derived macrophages (mo-MΦ). Although microglia perform various maintenance and protective roles, under certain conditions when they can no longer provide protection, mo-MΦ are recruited to the damaged CNS; there, they act not as microglial replacements but rather assistant cells, providing activities that cannot be timely performed by the resident cells. Here, we focus on the functional heterogeneity of microglia/mo-MΦ, emphasizing that, as opposed to the mo-MΦ, microglia often fail to timely acquire the phenotype essential for CNS repair.

Keywords: microglia, monocytes, CNS, innate, resolution of inflammation, macrophages, neuroprotection, monocyte-derived macrophages

INTRODUCTION

Outside the central nervous system (CNS), macrophages are known to acquire distinct phenotypes, and accordingly, perform various different and even opposing functions. Macrophages are generally polarized into two major phenotypes: Th1-related cytokines, such as interferon-gamma (IFN-γ), and microbial challenge by products such as lipopolysaccharides (LPS) induce the classically activated M1 phenotype, driving macrophages toward a pro-inflammatory microbicidal function, whereas Th2 cytokines, such as interleukin-4 (IL-4) and IL-13 polarize macrophages to an alternatively activated M2 phenotype associated with wound healing and immune resolution (Gordon, 2003; Gordon and Taylor, 2005; Mantovani et al., 2005; Mosser and Edwards, 2008; Auffray et al., 2009; Martinez et al., 2009; Gordon and Martinez, 2010; Sica and Mantovani, 2012). These different macrophage populations act following various insults outside the CNS where the $CCR2^{+}CX_3CR1^{low}Ly6C^{high}$ subset, corresponding to the M1 phenotype, is the first recruited to the damage site and is typically pro-inflammatory, while the $CCR2^{-}CX_3CR1^{high}Ly6C^{low}$ cells, matching the M2 phenotype, subsequently terminate the local inflammation as well as promoting regeneration and healing (Arnold et al., 2007; Nahrendorf et al., 2007). These two polarized phenotypes are further classified, based on their diverse surface markers, phenotypes and functions, into a more continuum spectrum of macrophage repertoire (Mosser and Edwards, 2008).

In the CNS, however, such diversity of macrophage functions was largely overlooked, as microglia, the native immune cells of the CNS, were considered its exclusive innate components. Initially discovered by Cajal (1913) and his student Del Rio-Hortega (1919), microglia are currently accepted as self-renewing cells with a unique embryonic origin (Ginhoux et al., 2010; Schulz et al., 2012; Gomez Perdiguero et al., 2013), distributed along CNS parenchymal tissues. Their primary roles are the maintenance of normal CNS functions (Elkabes et al., 1996; Nakajima et al., 2001; Aarum et al., 2003; Nimmerjahn et al., 2005; Walton et al., 2006; Ziv et al., 2006; Ransohoff and Perry, 2009; Wake et al., 2009; Sierra et al., 2010; Tremblay, 2011) and the continuous search for alterations in homeostasis through their constantly scanning dynamic ramifications (Nimmerjahn et al., 2005; Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009; Kettenmann et al., 2011). Under pathological conditions, microglial functions are largely dependent on their activation stimuli; whereas moderate CNS damage evokes protection by microglia (Prewitt et al., 1997; Rabchevsky and Streit, 1997; Batchelor et al., 1999; Chung et al., 1999; Butovsky et al., 2001; Kotter et al., 2001; Stadelmann et al., 2002; Streit, 2002; Shaked et al., 2004, 2005; Neumann et al., 2006, 2008; Schwartz et al., 2006; Yin et al., 2006; Majumdar et al., 2007; Muzio et al., 2007; Thored et al., 2009; Kettenmann et al., 2011), intensive acute activation (for example in spinal cord injury, optic nerve crush, or stroke) and chronic activation, which characterizes neurodegenerative diseases, render these cells neurotoxic, potentially impairing neuronal activity (Munn, 2000; Stalder et al., 2001; Wegiel et al., 2001; Ekdahl et al., 2003; Monje et al., 2003; Stirling et al., 2004; Block and Hong, 2005; Heppner et al., 2005; Block et al., 2007; Hanisch and Kettenmann, 2007; Muzio et al., 2007; Ovanesov et al., 2008; Centonze et al., 2009; Maezawa and Jin, 2010; Perry et al., 2010; Derecki et al., 2012, 2013; Scheffel et al., 2012). Such a phenotype not only prohibits

microglia from resolving inflammatory damage but rather contributes to the vicious cycle of toxicity and calls for additional assistance to terminate the local inflammation.

As an immune privileged site, the CNS was, for decades, considered sealed for leukocyte entry, protected from the circulation behind the walls of the blood–brain barrier (Wilson et al., 2010; Ransohoff and Engelhardt, 2012). Any recruitment of immune cells to the CNS was perceived as either a technical artifact (Ajami et al., 2007; Mildner et al., 2007) or as part of the ongoing inflammatory damage (McGeer et al., 1990; Popovich et al., 1999; Gris et al., 2004; Stirling et al., 2004; Boster et al., 2008). Further confusion resulted from the fact that blood-derived myeloid cells, recruited following CNS damage, were considered microglial reinforcements of comparable functions, and were accordingly termed "blood-derived microglia" (Eglitis and Mezey, 1997; Priller et al., 2001; Bechmann et al., 2005; Simard et al., 2006). A series of recent innovative studies demonstrated that such infiltrating cells, which we defined as monocyte-derived macrophages (mo-MΦ), perform indispensable roles that cannot be provided by their resident counterparts (Shechter et al., 2009, 2011; London et al., 2011). These studies challenged the traditional perception of macrophages in the CNS as a functionally homogeneous population. Moreover, they set the ground for a new era of research employing sophisticated techniques including parabiosis, head-protected bone marrow chimeras, transgenic mice, and fate mapping analysis (Carson et al., 2007; Ginhoux et al., 2010; Saederup et al., 2010; Ajami et al., 2011; Mildner et al., 2011; Prinz et al., 2011; Butovsky et al., 2012; Derecki et al., 2012; Schulz et al., 2012; Colton, 2013; Gomez Perdiguero et al., 2013), aimed at revealing the differential origin, phenotype, and function of distinct myeloid populations within the CNS. In this perspective, we will focus on the functional heterogeneity of microglia/mo-MΦ, addressing microglial functions as the first immunological support, the failure of these cells to provide significant protection under intensive acute or chronic activation, and the subsequent unique contribution of the mo-MΦ.

MICROGLIA IN MAINTENANCE AND DEFENSE

Similar to other tissue-resident macrophages outside the CNS, the primary role of microglia is to support normal tissue function, in this case neuronal integrity (Nimmerjahn et al., 2005; Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009; Kettenmann et al., 2011; Scheffel et al., 2012). The development of *in vivo* two-photon microscopy revolutionized our understanding of microglial functions under steady state. It allowed the study of non-activated microglia in intact brains of living animals (Davalos et al., 2005; Hanisch and Kettenmann, 2007). In these studies, using *Cx3cr1*$^{GFP/+}$ or Iba1-EGFP transgenic mice, in which microglia are fluorescently labeled (Jung et al., 2000; Hirasawa et al., 2005), these cells present highly motile processes, which directly contact astrocytes, neurons, and blood vessels, allowing the microglia to perform surveillance functions, constantly sensing subtle changes in their microenvironment (Nimmerjahn et al., 2005). Microglia provide several housekeeping functions. For instance, these cells are involved in the maintenance of synapses; microglial ramifications directly interact with termini, spines, astrocytic processes, and synaptic clefts (Murabe and Sano, 1982; Wake et al., 2009; Tremblay, 2011). These interactions enable the recognition of neuronal activity or structural alterations, according to which microglia facilitate synapse elimination, pruning or maturation, thereby preserve and organize neuronal networks (Murabe and Sano, 1982; Wake et al., 2009; Paolicelli et al., 2011; Tremblay, 2011). Microglia have been reported to support neurogenesis; they rapidly and efficiently clear out, by phagocytosis, the numerous apoptotic neural progenitor cells (NPCs) that do not incorporate into the circuitry (Sierra et al., 2010), direct the migration and differentiation of NPCs, as well as secreting soluble factors promoting neurogenesis (Aarum et al., 2003; Butovsky et al., 2006c; Walton et al., 2006; Choi et al., 2008). Moreover, microglial activation following exercise and by local interaction with adaptive immune cells strongly supports neurogenesis and enhances cognitive functions (Ziv et al., 2006; Ziv and Schwartz, 2008; Wolf et al., 2009; Vukovic et al., 2012). Additionally, several *in vitro* and *in vivo* studies demonstrated the capacity of microglia to secrete neurotrophic factors, e.g., nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4 (Elkabes et al., 1996; Nakajima et al., 2001). Under certain conditions microglia upregulate their brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) expression; both factors have protective and growth-promoting effects and are essential for learning and memory skills (Mizuno et al., 2000; Hsieh et al., 2004; Lee et al., 2004; Butovsky et al., 2006c; Wang et al., 2012).

Being the native immune cells of the CNS, microglia act as the first line of defense, protecting the CNS from invading agents as well as internal enemies; microglia are involved in infection, inflammation, autoimmune disease, trauma, ischemia, and neurodegeneration. After initial exposure to a danger signal, microglia become activated; they upregulate expression levels of certain molecules such as CD11b and Iba1, and gain expression of molecules associated with antigen presentation, such as major histocompatibility complex (MHC)-II, B7.1, and B7.2 (CD80/86), which are absent in naïve microglia. Microglia then lose their ramified morphology and surveillance mode, and convert to amoeboid-like, functional cells (Kettenmann et al., 2011).

Microglial functions under pathological conditions may reflect their diverse phenotypes acquired contingent to their activation signals. For example, activation of microglia by T cells that recognize CNS antigens or T cell-derived cytokines such as IFN-γ (at low concentrations) and IL-4 supports differentiation of NPCs and provides neuroprotection by regulating IGF-1 and tumor necrosis factor-alpha (TNF-α) levels. However, stimulation with LPS, amyloid-β or high concentrations of IFN-γ diminishes these effects. Moreover, activation of microglia by IL-4 prior to the LPS stimulation prevents the LPS-mediated-inhibition of the microglial neuroprotective effects (Avidan et al., 2004; Shaked et al., 2004; Butovsky et al., 2005, 2006a,b,c; Scheffel et al., 2012). Thus, microglia are highly versatile cells; their regulated activation and proper termination might help in tissue preservation, repair, and renewal, while intensive acute or chronic activation may result in irreversible tissue loss.

Microglia exert several protective roles. These include removal by phagocytosis of pathogens and microbes, as well as clearance of toxic molecules, cell debris, remains of extracellular matrix, myelin derivatives, and protein deposits (e.g., amyloid-β or p-tau), all of which further contribute to the local inflammation

and are inhibitory to regeneration and repair (Chung et al., 1999; Magnus et al., 2001; Ravichandran, 2003; Shaked et al., 2005; Liu et al., 2006; Majumdar et al., 2007; Kettenmann et al., 2011). Microglia can promote regeneration; other than removal of growth-inhibitory compounds (Kettenmann et al., 2011), these cells produce classical growth factors required for remyelination and regeneration (Kotter et al., 2001; Stadelmann et al., 2002). Microglia were reported to support regeneration of the optic nerve as well as sensory axons in the injured spinal cord (Prewitt et al., 1997; Rabchevsky and Streit, 1997; Yin et al., 2006) and to induce dopaminergic sprouting in the injured striatum (Batchelor et al., 1999). Additionally, microglia were shown to support neurogenesis and reduce neuronal death following stroke (Neumann et al., 2006; Thored et al., 2009).

MICROGLIAL MALFUNCTION FOLLOWING INTENSIVE ACUTE OR CHRONIC ACTIVATION – A CALL FOR PERIPHERAL HELP

Substantial evident suggest that microglial activity is not always optimal and is often not sufficient to support significant CNS repair; on the contrary in many cases their insufficient support turns detrimental. Under intensive acute or chronic activation, microglia not only fail to provide the needed functions, but there are ample evidence implying that they can be actively deleterious; these cells secrete reactive oxygen species, nitric oxide (NO), and pro-inflammatory cytokines that can endanger neurons, oligodendrocytes, or essential structures of the extracellular matrix (Monje et al., 2003; Stirling et al., 2004; Block and Hong, 2005; Block et al., 2007; Hanisch and Kettenmann, 2007; Perry et al., 2010). Microglial malfunction was suggested as a possible etiology in schizophrenia, resulting in impaired pruning during neurodevelopment, disturbance of normal neurotransmitter function, and uncontrolled production of pro-inflammatory cytokines such as TNF and IL-6, as well as failure in clearance of neuronal corpses (Munn, 2000). Microglial abnormal response is also evident in Rett syndrome, a neurodevelopmental disease resulting from mutation of the gene encoding methyl-CpG binding protein (*Mecp2*). *Mecp2*-null microglia release glutamate at neurotoxic levels (Maezawa and Jin, 2010) and have impaired phagocytic activity (Derecki et al., 2012, 2013), possibly contributing to disease development. Impaired microglial function is also reported in amyotrophic lateral sclerosis (ALS), where microglia derived from mutant superoxide dismutase 1 (SOD1) mouse, an established disease model of ALS, induce more oxidative stress and cause higher neuronal loss compared with wt microglia (Beers et al., 2006). Interestingly, down regulation of the mutant levels in microglia results in reduced disease progression (Boillée et al., 2006). In experimental autoimmune encephalomyelitis (EAE), a neurodegenerative disease model, microglial activation was inhibited by administration of ganciclovir to chimeric mice in which only the microglia express thymidine kinase that converts this drug into its cytotoxic form. Such specific microglial paralysis inhibits disease development and attenuates inflammatory CNS lesions (Heppner et al., 2005). Moreover, in Alzheimer's disease, characterization of fibrillar plaque development in brains of transgenic APP(SW) mice revealed that microglia are not only unable to clear amyloid-β deposits, but rather, contribute to plaque formation (Stalder et al., 2001; Wegiel et al., 2001). Additionally, microglia-derived chronic inflammation was shown to precede neuronal loss in neonatal borna disease virus (BDV) infection (Ovanesov et al., 2008).

Collectively, these evident suggest that under intensive acute or chronic activation microglia fail to acquire the desired phenotype, lose their essential functions and turn actively deleterious, and thus cannot provide immune resolution and subsequent CNS protection. In such scenarios, the recruitment of additional myeloid cells from the blood, comparable to microglia, is not likely. Rather, there is a need for peripheral intervention, in the form of unique cells, capable of providing the functions that cannot be delivered by the resident microglia.

FUNCTIONAL MACROPHAGE HETEROGENEITY

Indeed, intensive acute or chronic microglial activation drives these cells to produce a chemoattractive profile favoring the recruitment of monocytes and lymphocytes (Hausler et al., 2002; Sargsyan et al., 2009; Okamura et al., 2012). However, as the CNS is an immune privileged site, it was assumed to exclude leukocyte trafficking (Wilson et al., 2010; Ransohoff and Engelhardt, 2012). Consequently, several studies suggested that the recruitment of myeloid cells to the CNS reflected non-physiological entry, imposed by the unnatural experimental model (Ajami et al., 2007; Mildner et al., 2007). Other studies, although recognizing leukocyte entry to the CNS, interpreted their presence as a sign of pathology or malfunction that is detrimental and should be avoided (McGeer et al., 1990; Popovich et al., 1999; Gris et al., 2004; Stirling et al., 2004; Boster et al., 2008). Moreover, the previous technical limited ability to distinguish between the infiltrating blood-derived cells and the resident microglia resulted in the view of the newly recruited cells as part of the microglial reservoir, leading to their inaccurate tagging as "blood-derived microglia" (Eglitis and Mezey, 1997; Priller et al., 2001; Bechmann et al., 2005; Simard et al., 2006). This misleading nomenclature resulted in the erroneous perception of these cells as phenotypically and functionally comparable to microglia. Since then, advanced techniques have been developed to allow the blood-derived cells to be distinctly tracked and manipulated (Popovich and Hickey, 2001; Wright et al., 2001; Carson et al., 2007; Rolls et al., 2008; Shechter et al., 2009; Ajami et al., 2011; Colton, 2013). A series of recent studies used head shielded [$Cx3cr1^{GFP/+}$→WT] bone marrow chimeric mice, whose wt bone marrow was replaced with that of $Cx3cr1^{GFP/+}$ mice (Jung et al., 2000). This approach allows the infiltrating myeloid cells, derived from donor bone marrow and labeled with GFP, to be distinguished from their resident counterparts, while avoiding any artifacts related to brain irradiation (Shechter et al., 2009). These studies revealed the unique and non-redundant functions of the newly recruited cells and suggested the term "monocyte-derived macrophages (mo-MΦ)" to identify these cells as an entity separate from the resident microglia (Shechter et al., 2009, 2011; London et al., 2011). mo-MΦ restrict amyloid-β plaques in a mouse model of Alzheimer's disease (Simard et al., 2006; Butovsky et al., 2007), contribute to motor function recovery following spinal cord injury (Shechter et al., 2009), promote survival of neurons and cell renewal in the injured retina (London et al., 2011), and were recently shown to arrest disease progression in Rett syndrome (Derecki et al., 2012). These cells display immune-resolving characteristics and

express anti-inflammatory cytokines, which are crucial for their neuroprotective function. Moreover, they restrict accumulation of other inflammatory leukocytes including neutrophils and resident microglia (Shechter et al., 2009; London et al., 2011), mediate debris clearance by phagocytosis (Derecki et al., 2012), and regulate the extracellular matrix and glial scar surrounding the damaged area (Shechter et al., 2011). Importantly, inhibition or attenuation of the infiltration of mo-MΦ results in exacerbated damage, indicating that the resident microglia, which were spared in these experiments, cannot fulfill the protective functions provided by the mo-MΦ (Butovsky et al., 2007; Shechter et al., 2009, 2011; London et al., 2011).

Additional reinforcement for the disparity of these two myeloid populations is the fact that resident microglia and mo-MΦ development is dependent on different transcription factors. While development of both microglia and mo-MΦ requires the transcription factor, *Pu.1* (McKercher et al., 1996), the latter necessitates *Myb* and FLT3, whereas microglial development is *csf1*-receptor-dependent and FLT3- and *Myb*-independent (Ginhoux et al., 2010; Schulz et al., 2012; Gomez Perdiguero et al., 2013). Each of these two myeloid populations has a unique set of transcription factors and regulators leading to a diverse pattern of gene expression. Advanced analysis methods compared the profile of gene expression, microRNAs (miRNAs) and transcription factors, of splenic Ly6C^{hi} monocytes and CD39^{+} resident microglia in the SOD1 mouse, and of CD14^{+}CD16^{-} peripheral monocytes in ALS patients (Butovsky et al., 2012). In this study, microglial apoptosis was demonstrated along disease progression, while the macrophages derived from Ly6C^{hi} monocytes proliferated in the spinal cord parenchyma and
were associated with motor neuron loss (Butovsky et al., 2012). Notably, in this study, resident microglia were compared only to the inflammatory Ly6C^{hi} mo-MΦ population, whereas a recent study reported the recruitment of two blood-derived macrophage populations following spinal cord injury: the Ly6C^{hi}CX$_3$CR1^{low} pro-inflammatory and Ly6C^{low}CX$_3$CR1^{hi} anti-inflammatory cells, which acquire their phenotype via their trafficking route (Shechter et al., 2013). Thus, it will be interesting to characterize the gene signature of both Ly6C^{hi} and Ly6C^{low} mo-MΦ compared to resident microglia, and also to address the issue of where, how and when microglia acquire their phenotype.

All together, the evidence collected above indicates that under extensive inflammatory conditions, microglia lose their beneficial functions and instead display a deleterious role, further contributing to the spread of damage. Withstanding this vicious inflammatory cycle requires the recruitment of mo-MΦ, which induce resolution of the local immune response, rather than simply acting as microglial reinforcements. This perception leads, however, to the question of what drives the functional disparity between resident microglia and mo-MΦ. Specifically, what prevents microglia from acquiring the essential immune-resolving "alternatively activated" phenotype that is provided by the mo-MΦ? The answers to these questions may lie in the distinct origin of the two myeloid populations; microglial progenitors are yolk sac-derived macrophages that infiltrate the CNS during early embryogenesis, when bone marrow-derived hematopoiesis, from which mo-MΦ originate, is not yet established (Ginhoux et al., 2010; Schulz et al., 2012; Gomez Perdiguero et al., 2013). Educated in the CNS from early ontogeny, microglia were never exposed to any other environment; they have a relatively long life-span and undergo moderate and limited turn-over by self-renewal of primitive myeloid precursors (Ajami et al., 2007; Ginhoux et al., 2010; Schulz et al., 2012; Gomez Perdiguero et al., 2013). In contrast, mo-MΦ are freshly recruited from the circulation. Their differences may also be related to the fact that microglia are the first to encounter the damaged tissue, which might dictate their phenotype, while the mo-MΦ, recruited at a slightly later stage, acquire their nature via their trafficking route to the CNS (Shechter et al., 2013). Interestingly, such timely recruitment of myeloid subsets with differential functions is also evident in insults of non-CNS organs (Arnold et al., 2007; Nahrendorf et al., 2007). Clearly, further research is needed in order to address these issues.

FUNCTIONAL RELATIONSHIPS BETWEEN THE MICROGLIA AND mo-Mϕ – A CASCADE OF EVENTS

Based on the data reviewed above, we suggest here a cascade of events representing microglial functions within the CNS and the distinct contribution of mo-MΦ. Microglia enter the CNS during early developmental stages. By continuous scanning and sampling their environment via their dynamic processes, microglia are able to maintain CNS homeostasis; they preserve and modify (upon need) the synapse complex, support neurogenesis, secrete essential growth factors, and sustain normal CNS performance. Once encountering an unbalanced milieu, microglia become fully activated; retract their long ramifications, proliferate and shift toward a "ready to act" mode. Their subsequent function is very much dependent on their activation signal. A short and moderate stimulus will direct microglia to rapidly eliminate the source of damage without evoking a further immune response. Such stimuli are part of routine CNS maintenance and are generally resolved without activating or affecting other systems in the body. Even when the stimulus is stronger but short-lived, microglia can potentially cope with the danger signal, performing clearance of neurotoxic factors, supporting regeneration, and secreting neurotrophic factors supportive of remyelination. However, when the stimulus is intense or chronic, microglia can no longer handle the ongoing damage; these cells become neurotoxic and release reactive oxygen species, NO, proteases and pro-inflammatory cytokines, all of which endanger neuronal activity. Such microglial malfunction results in signals for recruitment of mo-MΦ to the damage site, which provide functions that cannot be delivered by the resident cells; mo-MΦ restrict the local inflammation, attenuate accumulation of misfolded proteins or any other intruders, restore homeostasis, and support healing and renewal. Unfortunately, the spontaneous response of mo-MΦ is often insufficient to achieve complete recovery. Thus, several therapeutic attempts to boost such a protective response by either direct administration of monocytes or indirectly augmenting their recruitment are currently underway (**Figure 1**).

LESSONS FROM OTHER TISSUE-SPECIFIC RESIDENT MACROPHAGES

Although unique, microglia are not the sole tissue-specific resident myeloid-derived cells. Many organs in the body contain

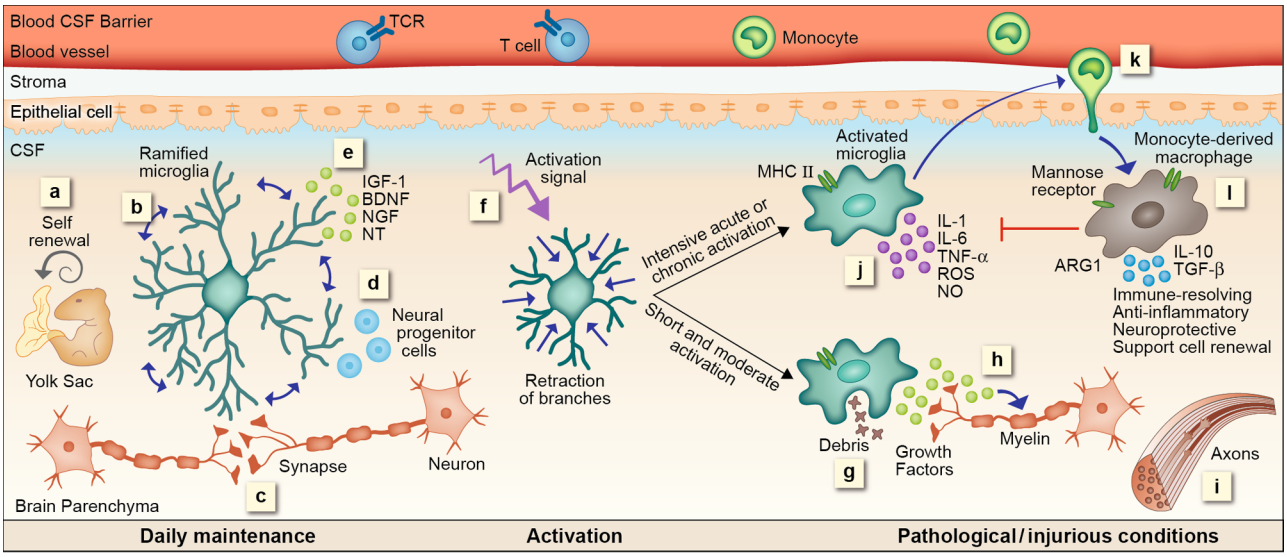


FIGURE 1 | microglial and mo-MΦ functions – cascade of events. (a) Resident microglia originate from yolk sac macrophages that repopulate CNS parenchyma during early development and are self-renewed locally, independent from bone marrow-derived monocytes, by proliferation of primitive progenitors. (b) In the steady state microglia are constantly scanning their environment through their highly motile processes. These cells facilitate the maintenance of synapses (c) and neurogenesis (d), as well as secrete growth factors essential for normal CNS performance (e). Upon recognition of a danger signal, microglia retract their branches, become round and ameboid, and convert into an activated mode (f). A short or moderate signal directs microglia toward a neuroprotective phenotype; these cells clear debris by phagocytosis (g), secrete growth factors associated with remyelination (h) and support regeneration (i). Intensive acute or chronic activation renders microglia neurotoxic; under such conditions microglia fail to acquire a neuroprotective phenotype. Instead, these cells produce reactive oxygen species (ROS), nitric oxide (NO), proteases, and pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, all of which endanger neuronal activity (j). Microglial malfunction results in the recruitment of mo-MΦ to the damage site (k). mo-MΦ secrete anti-inflammatory cytokines such as IL-10 and TGF-β, express factors associated with immune resolution such as manose receptor and arginase 1 (ARG1), and promote neuroprotection and cell renewal (l), all of which are functions that cannot be provided, under these conditions, by the resident microglia.

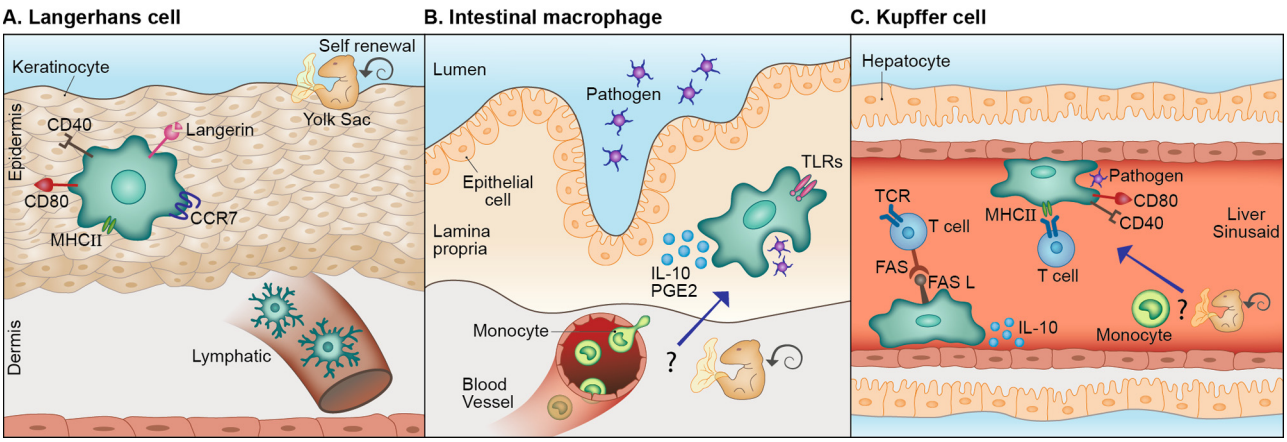


FIGURE 2 | Lessons from other tissue-specific resident macrophages. (A) Langerhans cells (LCs), the resident myeloid cells of the epidermis share with microglia their scanning capacity, their activation mode and possibly, their embryonic origin. Different from microglia these cells migrate to the lymph node where they act as antigen presenting cells. **(B)** Analogous to microglia, intestinal macrophages act as the first line of defense, protecting the mucosa from harmful pathogens and removing dead cells and debris. Unlike most other tissue macrophages, upon activation by certain stimuli, these cells produce immune-resolving factors. Distinct from microglia, circulating monocytes are largely accepted as the source of intestinal macrophages, however, the possibility of local self-renewal by embryonic precursors, under steady state, was also raised. **(C)** Kupffer cells are the macrophages of the liver. Similar to microglia, these cells perform clearance of host-related debris and pathogens. Kupffer cells are classical antigen presenting cells; however can also display immune-resolving functions. Moreover, they are largely assumed to be self-renewed independently from circulating monocytes, but a certain Kupffer cell subset was reported to originate from hematogenous precursors.

distinctive resident macrophages whose properties are tailored to the host tissue (**Figure 2**). Langerhans cells (LCs), for example, are the resident myeloid cells of the epidermis. Similar to microglia, they have extended dendritic processes that embrace neighboring keratinocytes (Langerhans, 1868; Bilzer et al., 2006) and scan the epidermis for pathogens and toxic molecules (de Jong and Geijtenbeek, 2010). These cells are endowed with the C-type lectin, Langerin, used for interaction with bacteria, fungi, and viruses (Turville et al., 2002; de Witte et al., 2007; Merad et al., 2008; de Jong and Geijtenbeek, 2010). Like microglia, LCs descend from embryonic precursors, possibly yolk sac macrophages or fetal liver monocytes, and are renewed independently of the bone marrow, by *in situ* proliferation upon need (Merad et al., 2002, 2008; Chorro et al., 2009; Chorro and Geissmann, 2010; Hoeffel et al., 2012). Moreover, as in microglial activation, upon capture of pathogens, LCs undergo phenotypic changes including increased expression of MHC-I and -II, and of the co-stimulatory molecules CD80, CD86, and CD40 (Merad et al., 2008; de Jong and Geijtenbeek, 2010). However, unlike microglia, which are restricted to the CNS parenchyma, LCs upregulate the lymph node-homing receptor, CCR7, which eventually leads to their migration to peripheral lymph nodes where they induce a specific adaptive immune response against skin invading pathogens (Merad et al., 2008). Intestinal macrophages are the largest population of mononuclear phagocytes in the body (Smith et al., 2005; Varol et al., 2010; Mowat and Bain, 2011). Similar to microglia, they have essential functions under both normal and pathological conditions; intestinal macrophages preserve a delicate equilibrium between commensal bacteria and the host, maintaining epithelial integrity and mucosal homeostasis. These cells act as the first line of defense protecting the highly exposed mucosa from harmful pathogens, removing dead cells and debris, and modulating the local inflammatory response (Smith et al., 2005; Varol et al., 2010; Mowat and Bain, 2011). Unlike other tissue macrophages, upon activation, for instance by certain Toll-like receptor (TLR) ligands, intestinal macrophages do not express high levels of co-stimulatory molecules nor do they secrete pro-inflammatory cytokines (Rogler et al., 1998; Hirotani et al., 2005; Uematsu et al., 2006; Mowat and Bain, 2011; Smith et al., 2011). Rather, they produce anti-inflammatory mediators such as IL-10 and prostaglandin E2 that restrict the local immune response (Mowat and Bain, 2011). Unlike microglia, the replenishment of intestinal macrophages is mostly associated with the recruitment of circulating monocytes. However, the possibility of self-renewal under steady state has also been raised (Mowat and Bain, 2011). Kupffer cells are the macrophages of the liver. These cells are mainly involved in clearance of pathogens and host-derived waste; they are constantly exposed to bacterial endotoxin (LPS) and microbial debris delivered from the gastrointestinal tract (Naito et al., 2004) and are involved in removal of senescent or malformed red blood cells and phagocytosis of soluble immunoglobulin G (IgG) complexes, microorganisms and eukaryotic cells (Naito et al., 2004; Parker and Picut, 2012). In addition to their role as phagocytes, Kupffer cells act as effective antigen presenting cells; upon Hepatitis C virus infection, human Kupffer cells elevate MHC-I and -II expression, upregulate co-stimulatory molecules, and interact with T cells (Burgio et al., 1998). However, several studies also demonstrated the immune-resolving nature of Kupffer cells, which were shown to suppress lymphocytes in culture (Callery et al., 1991), secrete IL-10 in response to LPS challenge (Knolle et al., 1995) and facilitate Fas ligand (FasL)-mediated apoptosis of T cells in a liver transplant model (Miyagawa-Hayashino et al., 2007). Thus, similarly to CNS heterogeneous macrophages, Kupffer cells seem to perform highly versatile functions. These cells, like microglia and LCs appear to self-renew independently of bone marrow-derived precursors (Schulz et al., 2012; Gomez Perdiguero et al., 2013). However, a study addressing Kupffer cell heterogeneity identified two subsets of Kupffer cells; one of them is radiosensitive and rapidly replaced from hematogenous precursors (Klein et al., 2007), indicating that the issue of Kupffer cell renewal is still unresolved.

CONCLUSIONS AND FUTURE DIRECTIONS

The evidence collected in this perspective supports the concept of functional macrophage heterogeneity within the CNS. Due to their similar morphology, previously assumed shared origin and subsequent misleading nomenclature, as well as the lack of available techniques to distinguish between the two populations, microglia and mo-MΦ were erroneously assumed to comprise a single population. Alternatively, and based on the ample findings addressed above, our model suggests that when it comes to CNS macrophages initial impressions can be deceiving; although they appear similar, mo-MΦ and microglia present different gene expression patterns and phenotypes, and are functionally distinct. Additional research is needed in order to further reveal the different function of these two distinct populations and the conditions that determine their unique phenotype. Such research will help resolving the misunderstanding that resulted from the previously held blanket view of these cells as homogeneously destructive, and might assist in employing specific manipulations of the two subsets as a potential therapeutic approach.

REFERENCES

Aarum, J., Sandberg, K., Haeberlein, S. L., and Persson, M. A. (2003). Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15983–15988.

Ajami, B., Bennett, J. L., Krieger, C., Mcnagny, K. M., and Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat. Neurosci.* 14, 1142–1149.

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

Arnold, L., Henry, A., Poron, F., Baba-Amer, Y., Van Rooijen, N., Plonquet, A., et al. (2007). Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J. Exp. Med.* 204, 1057–1069.

Auffray, C., Sieweke, M. H., and Geissmann, F. (2009). Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu. Rev. Immunol.* 27, 669–692.

Avidan, H., Kipnis, J., Butovsky, O., Caspi, R. R., and Schwartz, M. (2004). Vaccination with autoantigen protects against aggregated beta-amyloid and glutamate toxicity by controlling microglia: effect of CD4+CD25+ T cells. *Eur. J. Immunol.* 34, 3434–3445.

Batchelor, P. E., Liberatore, G. T., Wong, J. Y., Porritt, M. J., Frerichs, F., Donnan, G. A., et al. (1999). Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor

and glial cell line-derived neurotrophic factor. *J. Neurosci.* 19, 1708–1716.

Bechmann, I., Goldmann, J., Kovac, A. D., Kwidzinski, E., Simburger, E., Naftolin, F., et al. (2005). Circulating monocytic cells infiltrate layers of anterograde axonal degeneration where they transform into microglia. *FASEB J.* 19, 647–649.

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., et al. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16021–16026.

Bilzer, M., Roggel, F., and Gerbes, A. L. (2006). Role of Kupffer cells in host defense and liver disease. *Liver Int.* 26, 1175–1186.

Block, M. L., and Hong, J. S. (2005). Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* 76, 77–98.

Block, M. L., Zecca, L., and Hong, J. S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69.

Boillée, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., et al. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 2, 1389–1392.

Boster, A., Edan, G., Frohman, E., Javed, A., Stuve, O., Tselis, A., et al. (2008). Intense immunosuppression in patients with rapidly worsening multiple sclerosis: treatment guidelines for the clinician. *Lancet Neurol.* 7, 173–183.

Burgio, V. L., Ballardini, G., Artini, M., Caratozzolo, M., Bianchi, F. B., and Levrero, M. (1998). Expression of co-stimulatory molecules by Kupffer cells in chronic hepatitis of hepatitis C virus etiology. *Hepatology* 27, 1600–1606.

Butovsky, O., Hauben, E., and Schwartz, M. (2001). Morphological aspects of spinal cord autoimmune neuroprotection: colocalization of T cells with B7–2 (CD86) and prevention of cyst formation. *FASEB J.* 15, 1065–1067.

Butovsky, O., Koronyo-Hamaoui, M., Kunis, G., Ophir, E., Landa, G., Cohen, H., et al. (2006a). Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11784–11789.

Butovsky, O., Landa, G., Kunis, G., Ziv, Y., Avidan, H., Greenberg, N., et al. (2006b). Induction and blockage of oligodendrogenesis by differently activated microglia in an animal model of multiple sclerosis. *J. Clin. Invest.* 116, 905–915.

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A. E., Pluchino, S., et al. (2006c). Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31, 149–160.

Butovsky, O., Kunis, G., Koronyo-Hamaoui, M., and Schwartz, M. (2007). Selective ablation of bone marrow-derived dendritic cells increases amyloid plaques in a mouse Alzheimer's disease model. *Eur. J. Neurosci.* 26, 413–416.

Butovsky, O., Siddiqui, S., Gabriely, G., Lanser, A. J., Dake, B., Murugaiyan, G., et al. (2012). Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. *J. Clin. Invest.* 122, 3063–3087.

Butovsky, O., Talpalar, A. E., Ben-Yaakov, K., and Schwartz, M. (2005). Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. *Mol. Cell. Neurosci.* 29, 381–393.

Cajal, S. R. (1913). Contribución al conocimiento de la neuroglia del cerebro humano. *Trab. Lab. Invest. Biol.* 11, 255–345.

Callery, M. P., Mangino, M. J., and Flye, M. W. (1991). Arginine-specific suppression of mixed lymphocyte culture reactivity by Kupffer cells – a basis of portal venous tolerance. *Transplantation* 51, 1076–1080.

Carson, M. J., Bilousova, T. V., Puntambekar, S. S., Melchior, B., Doose, J. M., and Ethell, I. M. (2007). A rose by any other name? The potential consequences of microglial heterogeneity during CNS health and disease. *Neurotherapeutics* 4, 571–579.

Centonze, D., Muzio, L., Rossi, S., Cavasinni, F., De Chiara, V., Bergami, A., et al. (2009). Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J. Neurosci.* 29, 3442–3452.

Choi, S. H., Veeraraghavalu, K., Lazarov, O., Marler, S., Ransohoff, R. M., Ramirez, J. M., et al. (2008). Non-cell-autonomous effects of presenilin 1 variants on enrichment-mediated hippocampal progenitor cell proliferation and differentiation. *Neuron* 59, 568–580.

Chorro, L., and Geissmann, F. (2010). Development and homeostasis of 'resident' myeloid cells: the case of the Langerhans cell. *Trends Immunol.* 31, 438–445.

Chorro, L., Sarde, A., Li, M., Woollard, K. J., Chambon, P., Malissen, B., et al. (2009). Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. *J. Exp. Med.* 206, 3089–3100.

Chung, H., Brazil, M. I., Soe, T. T., and Maxfield, F. R. (1999). Uptake, degradation, and release of fibrillar and soluble forms of Alzheimer's amyloid beta-peptide by microglial cells. *J. Biol. Chem.* 274, 32301–32308.

Colton, C. A. (2013). Immune heterogeneity in neuroinflammation: dendritic cells in the brain. *J. Neuroimmune Pharmacol.* 8, 145–162.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* 8, 752–758.

de Jong, M. A., and Geijtenbeek, T. B. (2010). Langerhans cells in innate defense against pathogens. *Trends Immunol.* 31, 452–459.

Del Rio-Hortega, P. (1919). El tercer elemento de los centros nerviosos I La microglia en estado normal II Intervencíon de la microglia en los procesos patológicos III Naturaleza probable de la microglia. *Bol. de la Soc. Esp. de Biol.* 9, 69–120.

Derecki, N. C., Cronk, J. C., and Kipnis, J. (2013). The role of microglia in brain maintenance: implications for Rett syndrome. *Trends Immunol.* 34, 144–150.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

de Witte, L., Nabatov, A., Pion, M., Fluitsma, D., De Jong, M. A., De Gruijl, T., et al. (2007). Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat. Med.* 13, 367–371.

Eglitis, M. A., and Mezey, E. (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 4080–4085.

Ekdahl, C. T., Claasen, J. H., Bonde, S., Kokaia, Z., and Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13632–13637.

Elkabes, S., Dicicco-Bloom, E. M., and Black, I. B. (1996). Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J. Neurosci.* 16, 2508–2521.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Gomez Perdiguero, E., Schulz, C., and Geissmann, F. (2013). Development and homeostasis of "resident" myeloid cells: the case of the microglia. *Glia* 61, 112–120.

Gordon, S. (2003). Alternative activation of macrophages. *Nat. Rev. Immunol.* 3, 23–35.

Gordon, S., and Martinez, F. O. (2010). Alternative activation of macrophages: mechanism and functions. *Immunity* 32, 593–604.

Gordon, S., and Taylor, P. R. (2005). Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* 5, 953–964.

Gris, D., Marsh, D. R., Oatway, M. A., Chen, Y., Hamilton, E. F., Dekaban, G. A., et al. (2004). Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function. *J. Neurosci.* 24, 4043–4051.

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394.

Hausler, K. G., Prinz, M., Nolte, C., Weber, J. R., Schumann, R. R., Kettenmann, H., et al. (2002). Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages. *Eur. J. Neurosci.* 16, 2113–2122.

Heppner, F. L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hovelmeyer, N., et al. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat. Med.* 11, 146–152.

Hirasawa, T., Ohsawa, K., Imai, Y., Ondo, Y., Akazawa, C., Uchino, S., et al. (2005). Visualization of microglia in living tissues using Iba1-EGFP transgenic mice. *J. Neurosci. Res.* 81, 357–362.

Hirotani, T., Lee, P. Y., Kuwata, H., Yamamoto, M., Matsumoto, M., Kawase, I., et al. (2005). The nuclear IkappaB protein

IkappaBNS selectively inhibits lipopolysaccharide-induced IL-6 production in macrophages of the colonic lamina propria. *J. Immunol.* 174, 3650–3657.

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., et al. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* 209, 1167–1181.

Hsieh, J., Aimone, J. B., Kaspar, B. K., Kuwabara, T., Nakashima, K., and Gage, F. H. (2004). IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. *J. Cell Biol.* 164, 111–122.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M. J., Kreutzberg, G. W., Sher, A., et al. (2000). Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol. Cell. Biol.* 20, 4106–4114.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Klein, I., Cornejo, J. C., Polakos, N. K., John, B., Wuensch, S. A., Topham, D. J., et al. (2007). Kupffer cell heterogeneity: functional properties of bone marrow derived and sessile hepatic macrophages. *Blood* 110, 4077–4085.

Knolle, P., Schlaak, J., Uhrig, A., Kempf, P., Meyer Zum Buschenfelde, K. H., and Gerken, G. (1995). Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. *J. Hepatol.* 22, 226–229.

Kotter, M. R., Setzu, A., Sim, F. J., Van Rooijen, N., and Franklin, R. J. (2001). Macrophage depletion impairs oligodendrocyte remyelination following lysolecithin-induced demyelination. *Glia* 35, 204–212.

Langerhans, P. (1868). Über die Nerven der menschlichen Haut. *Virchows Arch.* 44, 325–337.

Lee, J. L., Everitt, B. J., and Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science* 304, 839–843.

Liu, Y., Hao, W., Letiembre, M., Walter, S., Kulanga, M., Neumann, H., et al. (2006). Suppression of microglial inflammatory activity by myelin phagocytosis: role of p47-PHOX-mediated generation of reactive oxygen species. *J. Neurosci.* 26, 12904–12913.

London, A., Itskovich, E., Benhar, I., Kalchenko, V., Mack, M., Jung, S., et al. (2011). Neuroprotection and progenitor cell renewal in the injured adult murine retina requires healing monocyte-derived macrophages. *J. Exp. Med.* 208, 23–39.

Maezawa, I., and Jin, L. W. (2010). Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J. Neurosci.* 30, 5346–5356.

Magnus, T., Chan, A., Grauer, O., Toyka, K. V., and Gold, R. (2001). Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation. *J. Immunol.* 167, 5004–5010.

Majumdar, A., Cruz, D., Asamoah, N., Buxbaum, A., Sohar, I., Lobel, P., et al. (2007). Activation of microglia acidifies lysosomes and leads to degradation of Alzheimer amyloid fibrils. *Mol. Biol. Cell* 18, 1490–1496.

Mantovani, A., Sica, A., and Locati, M. (2005). Macrophage polarization comes of age. *Immunity* 23, 344–346.

Martinez, F. O., Helming, L., and Gordon, S. (2009). Alternative activation of macrophages: an immunologic functional perspective. *Annu. Rev. Immunol.* 27, 451–483.

McGeer, P. L., McGeer, E., Rogers, J., and Sibley, J. (1990). Anti-inflammatory drugs and Alzheimer disease. *Lancet* 335, 1037.

McKercher, S. R., Torbett, B. E., Anderson, K. L., Henkel, G. W., Vestal, D. J., Baribault, H., et al. (1996). Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J.* 15, 5647–5658.

Merad, M., Ginhoux, F., and Collin, M. (2008). Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat. Rev. Immunol.* 8, 935–947.

Merad, M., Manz, M. G., Karsunky, H., Wagers, A., Peters, W., Charo, I., et al. (2002). Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat. Immunol.* 3, 1135–1141.

Mildner, A., Schlevogt, B., Kierdorf, K., Bottcher, C., Erny, D., Kummer, M. P., et al. (2011). Distinct and non-redundant roles of microglia and myeloid subsets in mouse models of Alzheimer's disease. *J. Neurosci.* 31, 11159–11171.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., et al. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* 10, 1544–1553.

Miyagawa-Hayashino, A., Tsuruyama, T., Egawa, H., Haga, H., Sakashita, H., Okuno, T., et al. (2007). FasL expression in hepatic antigen-presenting cells and phagocytosis of apoptotic T cells by FasL+ Kupffer cells are indicators of rejection activity in human liver allografts. *Am. J. Pathol.* 171, 1499–1508.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., and Nabeshima, T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *J. Neurosci.* 20, 7116–7121.

Monje, M. L., Toda, H., and Palmer, T. D. (2003). Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 302, 1760–1765.

Mosser, D. M., and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8, 958–969.

Mowat, A. M., and Bain, C. C. (2011). Mucosal macrophages in intestinal homeostasis and inflammation. *J. Innate Immun.* 3, 550–564.

Munn, N. A. (2000). Microglia dysfunction in schizophrenia: an integrative theory. *Med. Hypotheses* 54, 198–202.

Murabe, Y., and Sano, Y. (1982). Morphological studies on neuroglia. V. Microglial cells in the cerebral cortex of the rat, with special reference to their possible involvement in synaptic function. *Cell Tissue Res.* 223, 493–506.

Muzio, L., Martino, G., and Furlan, R. (2007). Multifaceted aspects of inflammation in multiple sclerosis: the role of microglia. *J. Neuroimmunol.* 191, 39–44.

Nahrendorf, M., Swirski, F. K., Aikawa, E., Stangenberg, L., Wurdinger, T., Figueiredo, J. L., et al. (2007). The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J. Exp. Med.* 204, 3037–3047.

Naito, M., Hasegawa, G., Ebe, Y., and Yamamoto, T. (2004). Differentiation and function of Kupffer cells. *Med. Electron Microsc.* 37, 16–28.

Nakajima, K., Honda, S., Tohyama, Y., Imai, Y., Kohsaka, S., and Kurihara, T. (2001). Neurotrophin secretion from cultured microglia. *J. Neurosci. Res.* 65, 322–331.

Neumann, J., Gunzer, M., Gutzeit, H. O., Ullrich, O., Reymann, K. G., and Dinkel, K. (2006). Microglia provide neuroprotection after ischemia. *FASEB J.* 20, 714–716.

Neumann, J., Sauerzweig, S., Ronicke, R., Gunzer, F., Dinkel, K., Ullrich, O., et al. (2008). Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. *J. Neurosci.* 28, 5965–5975.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314–1318.

Okamura, T., Katayama, T., Obinata, C., Iso, Y., Chiba, Y., Kobayashi, H., et al. (2012). Neuronal injury induces microglial production of macrophage inflammatory protein-1alpha in rat corticostriatal slice cultures. *J. Neurosci. Res.* 90, 2127–2133.

Ovanesov, M. V., Moldovan, K., Smith, K., Vogel, M. W., and Pletnikov, M. V. (2008). Persistent Borna Disease Virus (BDV) infection activates microglia prior to a detectable loss of granule cells in the hippocampus. *J. Neuroinflammation* 5, 16.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Parker, G. A., and Picut, C. A. (2012). Immune functioning in non lymphoid organs: the liver. *Toxicol. Pathol.* 40, 237–247.

Perry, V. H., Nicoll, J. A., and Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat. Rev. Neurol.* 6, 193–201.

Popovich, P. G., Guan, Z., Wei, P., Huitinga, I., Van Rooijen, N., and Stokes, B. T. (1999). Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. *Exp. Neurol.* 158, 351–365.

Popovich, P. G., and Hickey, W. F. (2001). Bone marrow chimeric rats reveal the unique distribution of resident and recruited macrophages in the contused rat spinal cord. *J. Neuropathol. Exp. Neurol.* 60, 676–685.

Prewitt, C. M., Niesman, I. R., Kane, C. J., and Houle, J. D. (1997). Activated macrophage/microglial cells can promote the regeneration of sensory axons into the injured spinal cord. *Exp. Neurol.* 148, 433–443.

Priller, J., Flugel, A., Wehner, T., Boentert, M., Haas, C. A., Prinz, M., et al. (2001). Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers

microglial engraftment. *Nat. Med.* 7, 1356–1361.

Prinz, M., Priller, J., Sisodia, S. S., and Ransohoff, R. M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nat. Neurosci.* 14, 1227–1235.

Rabchevsky, A. G., and Streit, W. J. (1997). Grafting of cultured microglial cells into the lesioned spinal cord of adult rats enhances neurite outgrowth. *J. Neurosci. Res.* 47, 34–48.

Ransohoff, R. M., and Engelhardt, B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat. Rev. Immunol.* 12, 623–635.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Ravichandran, K. S. (2003). "Recruitment signals" from apoptotic cells: invitation to a quiet meal. *Cell* 113, 817–820.

Rogler, G., Hausmann, M., Vogl, D., Aschenbrenner, E., Andus, T., Falk, W., et al. (1998). Isolation and phenotypic characterization of colonic macrophages. *Clin. Exp. Immunol.* 112, 205–215.

Rolls, A., Shechter, R., London, A., Segev, Y., Jacob-Hirsch, J., Amariglio, N., et al. (2008). Two faces of chondroitin sulfate proteoglycan in spinal cord repair: a role in microglia/macrophage activation. *PLoS Med.* 5:e171. doi: 10.1371/journal.pmed.0050171

Saederup, N., Cardona, A. E., Croft, K., Mizutani, M., Cotleur, A. C., Tsou, C. L., et al. (2010). Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. *PLoS ONE* 5:e13693. doi: 10.1371/journal.pone.0013693

Sargsyan, S. A., Blackburn, D. J., Barber, S. C., Monk, P. N., and Shaw, P. J. (2009). Mutant SOD1 G93A microglia have an inflammatory phenotype and elevated production of MCP-1. *Neuroreport* 20, 1450–1455.

Scheffel, J., Regen, T., Van Rossum, D., Seifert, S., Ribes, S., Nau, R., et al. (2012). Toll-like receptor activation reveals developmental reorganization and unmasks responder subsets of microglia. *Glia* 60, 1930–1943.

Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336, 86–90.

Schwartz, M., Butovsky, O., Bruck, W., and Hanisch, U. K. (2006). Microglial phenotype: is the commitment reversible? *Trends Neurosci.* 29, 68–74.

Shaked, I., Porat, Z., Gersner, R., Kipnis, J., and Schwartz, M. (2004). Early activation of microglia as antigen-presenting cells correlates with T cell-mediated protection and repair of the injured central nervous system. *J. Neuroimmunol.* 146, 84–93.

Shaked, I., Tchoresh, D., Gersner, R., Meiri, G., Mordechai, S., Xiao, X., et al. (2005). Protective autoimmunity: interferon-gamma enables microglia to remove glutamate without evoking inflammatory mediators. *J. Neurochem.* 92, 997–1009.

Shechter, R., London, A., Varol, C., Raposo, C., Cusimano, M., Yovel, G., et al. (2009). Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. *PLoS Med.* 6:e1000113. doi: 10.1371/journal.pmed.1000113

Shechter, R., Miller, O., Yovel, G., Rosenzweig, N., London, A., Ruckh, J., et al. (2013). Recruitment of beneficial M2 macrophages to injured spinal cord is orchestrated by remote brain choroid plexus. *Immunity* doi: 10.1016/j.immuni.2013.02.012 [Epub ahead of print].

Shechter, R., Raposo, C., London, A., Sagi, I., and Schwartz, M. (2011). The glial scar-monocyte interplay: a pivotal resolution phase in spinal cord repair. *PLoS ONE* 6:e27969. doi: 10.1371/journal.pone.0027969

Sica, A., and Mantovani, A. (2012). Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* 122, 787–795.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., and Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 49, 489–502.

Smith, P. D., Ochsenbauer-Jambor, C., and Smythies, L. E. (2005). Intestinal macrophages: unique effector cells of the innate immune system. *Immunol. Rev.* 206, 149–159.

Smith, P. D., Smythies, L. E., Shen, R., Greenwell-Wild, T., Gliozzi, M., and Wahl, S. M. (2011). Intestinal macrophages and response to microbial encroachment. *Mucosal Immunol.* 4, 31–42.

Stadelmann, C., Kerschensteiner, M., Misgeld, T., Bruck, W., Hohlfeld, R., and Lassmann, H. (2002). BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? *Brain* 125, 75–85.

Stalder, M., Deller, T., Staufenbiel, M., and Jucker, M. (2001). 3D-Reconstruction of microglia and amyloid in APP23 transgenic mice: no evidence of intracellular amyloid. *Neurobiol. Aging* 22, 427–434.

Stirling, D. P., Khodarahmi, K., Liu, J., Mcphail, L. T., Mcbride, C. B., Steeves, J. D., et al. (2004). Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J. Neurosci.* 24, 2182–2190.

Streit, W. J. (2002). Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia* 40, 133–139.

Thored, P., Heldmann, U., Gomes-Leal, W., Gisler, R., Darsalia, V., Taneera, J., et al. (2009). Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* 57, 835–849.

Tremblay, M. E. (2011). The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies. *Neuron Glia Biol.* 7, 67–76.

Turville, S. G., Cameron, P. U., Handley, A., Lin, G., Pohlmann, S., Doms, R. W., et al. (2002). Diversity of receptors binding HIV on dendritic cell subsets. *Nat. Immunol.* 3, 975–983.

Uematsu, S., Jang, M. H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., et al. (2006). Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. *Nat. Immunol.* 7, 868–874.

Varol, C., Zigmond, E., and Jung, S. (2010). Securing the immune tightrope: mononuclear phagocytes in the intestinal lamina propria. *Nat. Rev. Immunol.* 10, 415–426.

Vukovic, J., Colditz, M. J., Blackmore, D. G., Ruitenberg, M. J., and Bartlett, P. F. (2012). Microglia modulate hippocampal neural precursor activity in response to exercise and aging. *J. Neurosci.* 32, 6435–6443.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Walton, N. M., Sutter, B. M., Laywell, E. D., Levkoff, L. H., Kearns, S. M., Marshall, G. P. II, et al. (2006). Microglia instruct subventricular zone neurogenesis. *Glia* 54, 815–825.

Wang, Y., Zhang, T. Y., Xin, J., Li, T., Yu, H., Li, N., et al. (2012). Differential involvement of brain-derived neurotrophic factor in reconsolidation and consolidation of conditioned taste aversion memory. *PLoS ONE* 7:e49942. doi: 10.1371/journal.pone.0049942

Wegiel, J., Wang, K. C., Imaki, H., Rubenstein, R., Wronska, A., Osuchowski, M., et al. (2001). The role of microglial cells and astrocytes in fibrillar plaque evolution in transgenic APP(SW) mice. *Neurobiol. Aging* 22, 49–61.

Wilson, E. H., Weninger, W., and Hunter, C. A. (2010). Trafficking of immune cells in the central nervous system. *J. Clin. Invest.* 120, 1368–1379.

Wolf, S. A., Steiner, B., Akpinarli, A., Kammertoens, T., Nassenstein, C., Braun, A., et al. (2009). CD4-positive T lymphocytes provide a neuroimmunological link in the control of adult hippocampal neurogenesis. *J. Immunol.* 182, 3979–3984.

Wright, D. E., Wagers, A. J., Gulati, A. P., Johnson, F. L., and Weissman, I. L. (2001). Physiological migration of hematopoietic stem and progenitor cells. *Science* 294, 1933–1936.

Yin, Y., Henzl, M. T., Lorber, B., Nakazawa, T., Thomas, T. T., Jiang, F., et al. (2006). Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. *Nat. Neurosci.* 9, 843–852.

Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., et al. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat. Neurosci.* 9, 268–275.

Ziv, Y., and Schwartz, M. (2008). Orchestrating brain-cell renewal: the role of immune cells in adult neurogenesis in health and disease. *Trends Mol. Med.* 14, 471–478.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that

could be construed as a potential conflict of interest.

Received: 30 January 2013; paper pending published: 04 March 2013; accepted: 18 March 2013; published online: 08 April 2013.
Citation: London A, Cohen M and Schwartz M (2013) Microglia and monocyte-derived macrophages: functionally distinct populations that act in concert in CNS plasticity and repair. Front. Cell. Neurosci. 7:34. doi: 10.3389/fncel.2013.00034

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 30 January 2013
doi: 10.3389/fncel.2013.00006

Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis

Amanda Sierra*[1,2,3]*, *Oihane Abiega*[1,2], *Anahita Shahraz*[4] *and Harald Neumann*[4]

[1] *Achucarro—Basque Center for Neuroscience, Zamudio, Spain*
[2] *Department of Neuroscience, University of the Basque Country EHU/UPV, Leioa, Spain*
[3] *Ikerbasque—Basque Foundation for Science, Bilbao, Spain*
[4] *Neural Reconstruction Group, Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany*

Edited by:
Marie-Eve Tremblay, Université Laval, Canada

Reviewed by:
Marcel Leist, University of Konstanz, Germany
Rafael Linden, Federal University of Rio de Janeiro, Brazil

***Correspondence:**
Amanda Sierra, Achucarro—Basque Center for Neuroscience, Laida Bidea, Building 205, Bizkaia Technological Park, 48170 Zamudio, Spain.
e-mail: a.sierra@ikerbasque.org
Harald Neumann, Neural Reconstruction Group, Institute of Reconstructive Neurobiology, University Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany.
e-mail: hneuman1@uni-bonn.de

Microglia are the resident brain macrophages and they have been traditionally studied as orchestrators of the brain inflammatory response during infections and disease. In addition, microglia has a more benign, less explored role as the brain professional phagocytes. Phagocytosis is a term coined from the Greek to describe the receptor-mediated engulfment and degradation of dead cells and microbes. In addition, microglia phagocytoses brain-specific cargo, such as axonal and myelin debris in spinal cord injury or multiple sclerosis, amyloid-β deposits in Alzheimer's disease, and supernumerary synapses in postnatal development. Common mechanisms of recognition, engulfment, and degradation of the different types of cargo are assumed, but very little is known about the shared and specific molecules involved in the phagocytosis of each target by microglia. More importantly, the functional consequences of microglial phagocytosis remain largely unexplored. Overall, phagocytosis is considered a beneficial phenomenon, since it eliminates dead cells and induces an anti-inflammatory response. However, phagocytosis can also activate the respiratory burst, which produces toxic reactive oxygen species (ROS). Phagocytosis has been traditionally studied in pathological conditions, leading to the assumption that microglia have to be activated in order to become efficient phagocytes. Recent data, however, has shown that unchallenged microglia phagocytose apoptotic cells during development and in adult neurogenic niches, suggesting an overlooked role in brain remodeling throughout the normal lifespan. The present review will summarize the current state of the literature regarding the role of microglial phagocytosis in maintaining tissue homeostasis in health as in disease.

Keywords: microglia, phagocytosis, apoptosis, synapses, debris, myelin, amyloid, inflammation

Abbreviations: ACAMPs, apoptotic cells-associated cellular patterns; AD, Alzheimer's disease; AIF, apoptosis-inducing factor; APCs, antigen presenting cells; APP, amyloid precursor protein; ASD, autism spectrum disorders; ATP, adenosine trinucleotide phosphate; Aβ, amyloid beta; BAI-1, brain-specific angiogenesis inhibitor 1; CAD, caspase-activated DNAse; CNS, central nervous system; CR1, complement receptor 1; DAP12, DNAX-activation protein X; DCs, dendritic cells; DOCK-180, dedicator of cytokinesis 180; EAE, experimental acute encephalomyelitis; ELMO, engulfing and cell motility protein; HMGB1, high mobility group 1 protein; Hsp60, heat shock protein 60; IFNγ, interferon gamma; IGF-1, insulin-like growth factor 1; IL-10, interleukin 10; IL-1β, interleukin 1beta; IL-4, interleukin 4; LPS, bacterial lipopolysaccharides; MBP, myelin basic protein; MCAO, medial cerebral artery occlusion; MFG-E8, milk fat globule-epidermal growth factor; MHC-II, major histocompatibility complex class II; MS, multiple sclerosis; NADPH, nicotinamide adenine dinucleotide phosphate; NGF, neural growth factor; PAMPS, pathogen-associated molecular patterns; PCD, programmed cell death; PD, Parkinson's disease; PLOSL, polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy; PNS, peripheral nervous system; PS, phosphatidylserine; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; RPE, retinal pigment epithelium; SIRPα, signal regulatory protein alpha; SVZ, subventricular zone; TAMs, tumor-associated macrophages; TGFβ, transforming growth factor beta; TLRs, Toll-like receptors; TNFα, tumor necrosis factor alpha; TREM2, triggering receptor expressed on myeloid cells-2; TRIF, TIR domain-containing adapter inducing interferon beta; UDP, urydil dinucleotide phosphate; UTP, urydil trinucleotide phosphate; WD, Wallerian degeneration.

DEFINITION OF PHAGOCYTOSIS

Phagocytosis is a Greek-derived term which literally means the cellular process of eating. It describes the recognition, engulfment, and degradation of large (>0.5 μm), particulated organisms or structures (Mukherjee et al., 1997). Phagocytosis of invading microorganisms by immune cells was first discovered by the father of cellular immunology Ilya Metchnikoff in 1882, for which he was awarded the Nobel Prize. Most cell types, including unicellular organisms, have the capacity to phagocytose. In multicellular organisms, and more particularly in animals with a well-developed immune system, phagocytosis is mostly performed by specialized, professional phagocytes: macrophages, dendritic cells (DCs), and neutrophils. Together with inflammation, phagocytosis composes the first line of defense against multicellular organisms by the innate immune system. In jawed vertebrates, including mammals, phagocytosis also helps to initiate the more specific adaptive immune response through antigen-presentation to T lymphocytes (Litman et al., 2005). In the mammalian central nervous system (CNS), the innate immune response is orchestrated by microglia.

Microglia are the brain resident macrophages. They are of myeloid origin and share many properties with other well-studied tissue macrophages. However, they have a different origin and are a unique macrophage cell type in the adult organism. Microglia are yolk sac-derived, invade the brain during early embryonic development and then locally proliferate in the brain (Ginhoux et al., 2010; Schulz et al., 2012). In contrast to other yolk sac-derived macrophages, they are not replaced during the postnatal period and later life by liver- or bone marrow-derived macrophages (Hoeffel et al., 2012). Cell biology of phagocytosis has been mainly established on bone marrow-derived tissue macrophages. In this review we directly extrapolate basic phagocytic mechanisms from bone marrow-derived tissue macrophages to the less-known processes of microglia, but would like to point out that these assumed similarities might not fully hold true to the yolk sac-derived microglia. Particularly, yolk sac-derived macrophages including microglia may possibly have different tasks since they are confronted with different target structures, mainly apoptotic cells during developmental tissue remodeling, while bone marrow-derived macrophages have a higher chance to be confronted with pathogens, mainly during defense against invading microbes. Overall, microglial phagocytosis is a relatively unknown process in terms of the receptors involved, the mechanisms of its execution, its beneficial or detrimental consequences, and its ultimate impact in maintaining tissue homeostasis. To further complicate the issue, macrophage phagocytosis has two main targets: dead resident cells (apoptotic or necrotic) and live invading microorganisms; whereas microglial phagocytosis appears to be adapted to the brain environment for remodeling tasks such as engulfment of synapses, axonal and myelin debris, or clearance of proteins with very high turnover such as amyloid beta (Aβ) protein.

One major limitation is that in most studies, microglial phagocytosis is assessed exclusively *in vitro*, utilizing a model of phagocytosis in which primary or transformed microglial cells are "fed" with a variety of targets, ranging from latex beads to primary or transformed apoptotic cells, cell debris, or Aβ. These models have proved to be extremely valuable to study the molecular pathways involved in the recognition, engulfment, and degradation of the targets. However, the lack of molecular tools to specifically block these three steps has precluded our understanding of the global impact of microglial phagocytosis *in vivo*. In fact, many *in vivo* experiments rely on the use of phagocytosis "markers" such as CD68 (ED1 or macrosialin) as a proxy. One major problem is that the function of CD68 is unknown. While located in the lysosomal compartment, anti-CD68 antibodies do not block macrophage phagocytosis *in vitro* (Damoiseaux et al., 1994), and macrophages from mice deficient in CD68 have no defects in phagocytosis of bacteria (Song et al., 2011). In the adult hippocampus, the expression of ED1 in microglia phagocytosing apoptotic cells is similar to non-phagocytic microglia and much lower than the expression induced by inflammatory challenge (Sierra et al., 2010). However, a higher expression of ED1 seems to correlate with the capacity of postnatal microglia to engulf synapses (Schafer et al., 2012). Furthermore, few studies have attempted to quantify the extent of microglial phagocytosis *in vivo* (Ashwell, 1990; Dalmau et al., 2003; Parnaik et al., 2000; Sierra et al., 2010) and many rely on a qualitative observation of microglial engulfment to determine whether microglia is phagocytosing or not.

The rapid clearance time of dead cells is likely behind this qualitative rather than quantitative assessment. *In vitro*, live imaging has determined that the clearance time to completely eliminate an apoptotic cell by microglia is 25–95 min in a co-culture system (Parnaik et al., 2000); *in vivo*, the microglial clearance time of apoptotic cells in physiological conditions has been estimated to be 70–90 min (Sierra et al., 2010). These numbers are in agreement with a general 1–2 h clearance time for macrophages (Henson and Hume, 2006). Thus, the number of apoptotic cells observed in particular time point represents only a small fraction of the actual number of apoptotic cells generated (or total number of cells which disappear). For example, if 400 cells die in a 24 h period, a clearance time of 1.5 h would mean that only 25 dead cells can be observed at any time point [for more details on estimation of the clearance time, see Barres et al. (1992); Sierra et al. (2010)]. The phagocytosis of smaller particles (synapses, axons, protein aggregates) is likely to be even faster. The promptness of microglia to eliminate cell debris is therefore an important parameter to take into account when analyzing the dynamics of cell death and phagocytosis.

In addition to the clearance time, two main parameters to quantify microglial phagocytosis of apoptotic cells *in vivo* using immunofluorescence and confocal microscopy have been established: the phagocytic index, or proportion of apoptotic cells which are three-dimensionally engulfed by microglia over the total number of apoptotic cells; and the phagocytic capacity, or proportion of phagocytosing microglia multiplied by the number of phagocytic pouches (i.e., number of apoptotic cells engulfed) over the total number of microglia (Sierra et al., 2010). More recently, a similar parameter was described to quantify microglial phagocytosis of synaptic inputs (allegedly, presynaptic terminals) using high resolution confocal microscopy and three-dimensional rendering to estimate the volume of internalized inputs over the total volume of microglia (Schafer et al., 2012). Similarly, microglial phagocytosis of axonal or myelin debris can be quantified using confocal microscopy to determine the percentage of microglia containing neurofilament-positive axonal material (Hosmane et al., 2012) or myelin basic protein (MBP) (Nielsen et al., 2009). The utilization of these or similar parameters is a necessary starting point to obtain a quantitative picture of microglial phagocytosis across a range of physiological and pathological conditions, which will help us to address many of the open questions. Over the next sections, we will provide an overall description of the mechanical process of phagocytosis; its beneficial and detrimental consequences; and the particular details of phagocytosis of different targets: cells and cell debris, microorganisms, tumor cells, spines, and Aβ deposits.

THE MECHANICS OF PHAGOCYTOSIS

Our current understanding of the mechanical process of phagocytosis is summarized in the three-step model: find-me, eat-me, and digest-me (Savill et al., 2002), which addresses the engulfment and degradation of apoptotic cells and microorganisms by macrophages and DCs. While it is likely that microglia utilizes

similar mechanisms, direct evidence of the molecular details of these events in microglia, beyond the receptors involved in the recognition of the targets, is largely missing. In this section, we will address the featured molecular mechanisms in the process of phagocytosis by microglia (**Figure 1**).

FIND-ME

The process is initiated when the phagocyte encounters a target, either randomly or triggered by signals from the target. Microglial processes are highly motile (Davalos et al., 2005; Nimmerjahn et al., 2005) and their constant surveillance of the brain parenchyma supports their capacity to fortuitously encounter targets to engulf. In addition, apoptotic cells can release signals to attract macrophages and DCs, such as the extracellular nucleotides ATP and UTP (Elliott et al., 2009). In microglia, UDP, the product of degradation of UTP by extracellular ectonucleotidases, acts on $P2Y_6$ receptors to facilitate phagocytosis (Koizumi et al., 2007). Another important chemotactic signal released by apoptotic cells is fractalkine/CX3CL1 (Truman et al., 2008; Noda et al., 2011). Microglia express the fractalkine receptor (CX3CR1), which promotes phagocytosis of apoptotic cells (Noda et al., 2011) but shifts microglia toward a phenotype with less capacity to phagocytose Aβ (Lee et al., 2010; Liu et al., 2010). Once the phagocyte has reached the target a direct cell membrane contact is established via a receptor-ligand interaction and phagocytosis takes place.

EAT-ME

The process of recognition and engulfment of the targets is, arguably, the most important step in phagocytosis and where most research is concentrated. Phagocytes are equipped with a likely complementary array of receptors which enable them to recognize their targets (the so-called "eat-me" signals) and discriminate them from the remaining parenchyma, particularly from living cells (which express "don't-eat-me" signals) (Savill et al., 2002; Ravichandran, 2010). Some of these receptors serve to tether the phagocyte and the target together; others actually trigger internalization (Underhill and Goodridge, 2012). These receptors can be classified into two main types depending on their targets. Detection of pathogen-associated molecular patterns (PAMPS) is mediated through scavenger receptors in conjunction with Toll-like receptors (TLRs) such as the CD14/TLR4 complex, or receptors of the immunoglobulin superfamily (e.g., c-type lectins). Detection of apoptotic cells-associated cellular patterns (ACAMPs), of which exposure of phosphatidylserine (PS) in the outer leaflet of the cell membrane is the main exponent, is mediated directly by several receptors, including brain-specific angiogenesis inhibitor 1 [BAI-1; reviewed in Armstrong and Ravichandran (2011)], and by bridging molecules such as milk fat globule-epidermal growth factor (MFG-E8). Another important receptor that signals internalization is triggering receptor expressed on myeloid cells-2 (TREM2), whose loss of function prevents microglial phagocytosis (Takahashi et al., 2005; Hsieh et al., 2009). TREM2 is known to interact with the signaling adapter protein named DNAX-activation protein of 12 kD (DAP12) (Paloneva et al., 2002), however its ligand in apoptotic cells remains elusive. Several candidates have been proposed, namely, anionic oligosaccharides such as bacterial lipopolysaccharides (Daws et al., 2003; Quan et al., 2008) and heat shock protein 60 (Hsp60) (Stefano et al., 2009). Hsp60 is exposed in the surface of apoptotic cells (Goh et al., 2011) and increases the phagocytic activity of microglia (Stefano et al., 2009). In addition to the direct recognition of the targets by microglial cell membrane receptors, engulfment can also triggered by soluble opsonins that bind to receptors signaling internalization in the microglia. Antibodies such as IgG and proteins of the complement system such as C3b, bind to phagocyte Fc receptors and complement receptor 3 (CR3), respectively, and mediate phagocytosis (Underhill and Goodridge, 2012). Further examples of receptors and their targets are summarized in **Table 1** and **Figure 2** and will be provided in the following sections. The level of expression of the receptors involved in phagocytosis may

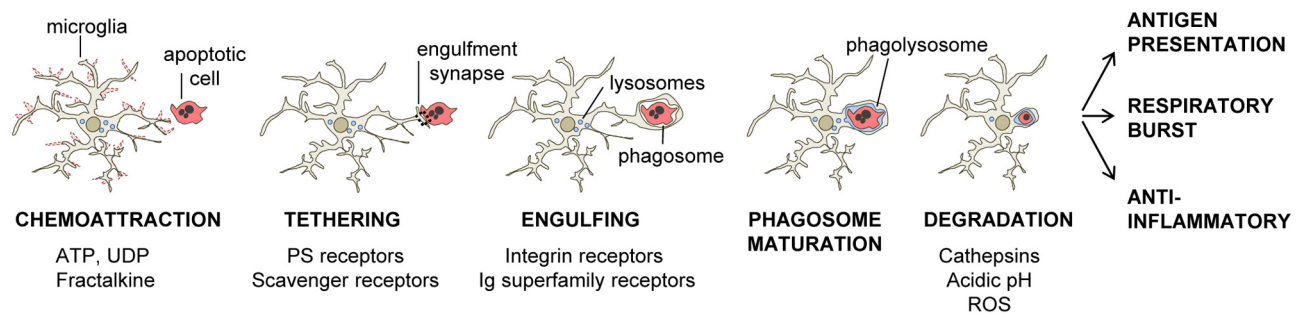


FIGURE 1 | Three-step model of microglial phagocytosis. In physiological conditions, microglial processes are highly motile and respond to chemoattractant molecules released by damaged or apoptotic cells ("find-me" signals) such as fractalkine and extracellular nucleotides (ATP, UDP). Next, an engulfment synapse is formed between a series of microglial receptors and their ligands in the membrane of the apoptotic cell ("eat-me" signals), leading to the tethering and engulfing of the apoptotic cell in a phagosome. The phagosome becomes mature by fusing with lysosomes and other organelles, and the apoptotic cell is fully degraded in the phagolysosome in less than 2 h (see text for details).

Table 1 | Summary of receptors involved in macrophage and microglial phagocytosis.

Receptors	Ligands	Macrophages function	References	Microglia function	References
PS RECEPTORS					
BAI-1	PS	Phagocytosis of apoptotic cells	Flannagan et al., 2012; Kim et al., 2012	Phagocytosis of apoptotic cells	Park et al., 2008
MER	PS [Gas 6, protein S]	Phagocytosis of apoptotic cells	Ravichandran, 2010	Phagocytosis of apoptotic cells	Grommes et al., 2008
PSR	PS	Phagocytosis of apoptotic cells	Taylor et al., 2005	Phagocytosis of apoptotic cells	De Simone et al., 2003
Stabilin-2	PS	Phagocytosis of apoptotic cells	Ravichandran, 2010; Flannagan et al., 2012	Function in phagocytosis unreported	N/R
TIM-1	PS	Phagocytosis of apoptotic cells	Flannagan et al., 2012	Phagocytosis of apoptotic cells	Noda and Suzumura, 2012
TIM-4	PS	Phagocytosis of apoptotic cells	Freeman et al., 2010; Flannagan et al., 2012	Phagocytosis of apoptotic cells	Mizuno, 2012; Noda and Suzumura, 2012
INTEGRIN RECEPTORS					
avβ5	PS, vitronectin [MFG-E8, thrombospondin]	Phagocytosis of apoptotic cells	Dupuy and Caron, 2008	Expressed. Function in phagocytosis unreported	Welser-Alves et al., 2011
CR1	MBL, C1q, C4b, C3b, C3bi	Adhesion to bacteria/pathogens	Fallman et al., 1993; Flannagan et al., 2012	Adhesion to opsonized erythrocytes	Ulvestad et al., 1994; Linnartz and Neumann, 2012
CR3	C3 and C1q [DAP12]	Adhesion to opsonized yeast particles; phagocytosis of bacteria; opsonized apoptotic cells; degenerated myelin and neurites	Fallman et al., 1993; Rotshenker, 2009; Linnartz and Neumann, 2012	Adhesion to opsonized yeast particles; phagocytosis of bacteria; opsonized apoptotic cells; degenerated myelin and neurites	Rotshenker, 2009; Linnartz and Neumann, 2012; Schafer et al., 2012
CR4	iC3b	Phagocytosis of opsonized apoptotic cells	Flannagan et al., 2012	Phagocytosis of opsonized apoptotic cells	Crehan et al., 2012
VnR	PS, vitronectin [MFG-E8, thrombospondin]	Adhesion to apoptotic cells; phagocytosis of apoptotic cells	Dupuy and Caron, 2008	Phagoptosis (killing of viable neurons)	Neher et al., 2011; Welser-Alves et al., 2011
IG SUPERFAMILY RECEPTORS					
FcγRI	IgG1 = IgG3 > IgG4	Phagocytosis of degenerated myelin	Rotshenker, 2009	Phagocytosis of degenerated myelin	Noda and Suzumura, 2012
FcγRIIa	IgG3 ≥ IgG1 = IgG2	Phagocytosis of pathogens and apoptotic cells	Hart et al., 2004	Phagocytosis of pathogens and apoptotic cells	Linnartz and Neumann, 2012
RAGE	Aβ, AGEs, PS, and HMGB1, C1q	Phagocytosis of apoptotic cells	He et al., 2011	Mediates pro-inflammatory effect of Aβ	Block et al., 2007; Noda and Suzumura, 2012
Siglec11	α2,8-linked polysialic acids	Reduced phagocytosis of apoptotic cells	Linnartz and Neumann, 2012	Reduced phagocytosis of apoptotic cells	Wang and Neumann, 2010; Linnartz and Neumann, 2012
SIRPα	Myelin [SP-A, D; CD47]	Recognition and downregulation of phagocytosis of myelin	Linnartz and Neumann, 2012	Recognition and downregulation of phagocytosis of myelin	Ransohoff and Perry, 2009; Noda and Suzumura, 2012
SIRPβ1	Unknown ligand [DAP12]	Increase of phagocytosis of opsonized red blood cells	Hayashi et al., 2004; Gaikwad et al., 2009	Phagocytosis of neuronal debris, fibrillary Aβ, latex beads	Gaikwad et al., 2009; Linnartz and Neumann, 2012
TREM2	Hsp60, oligosaccharides [DAP12]	Phagocytosis of apoptotic cells	Klesney-Tait et al., 2006	Phagocytosis of apoptotic cells	Klesney-Tait et al., 2006; Ransohoff and Perry, 2009

(Continued)

Table 1 | Continued

Receptors	Ligands	Macrophages function	References	Microglia function	References
SCAVENGER AND RELATED RECEPTORS					
CD36	Ox-LDL, Ox-PS [thrombospondin]	Adhesion to apoptotic cells; phagocytosis of apoptotic cells	Greenberg et al., 2006; Flannagan et al., 2012	Phagocytosis of apoptotic cells	Noda and Suzumura, 2012
CD68	Ox-LDL	Adhesion to erythrocytes	Hoffmann et al., 2001	Phagocytic marker. Function in phagocytosis unreported	Fulci et al., 2007
LOX-1	LDL, Ox-LDL, Hsp70	Phagocytosis of aged/apoptotic cells	Taylor et al., 2005	Expressed. Pro-inflammatory response. Function in phagocytosis unreported	Zhang et al., 2012
MARCO	Ac-LDL, bacteria	Adhesion to unopsonized particles and bacteria; phagocytosis of bacteria, apoptotic cells, and unopsonized latex beads	van der Laan et al., 1999; Rogers et al., 2009	Decrease of antigen internalization capacity; adhesion to Aβ, bacteria; decreased bead phagocytosis	Granucci et al., 2003; Block et al., 2007
SR-A	LPS, lipotheicoic acid, Ac-LDL	Adhesion to apoptotic thymocytes; phagocytosis of bacteria, apoptotic cells, and degenerated myelin	Savill et al., 2002; Taylor et al., 2005; Flannagan et al., 2012	Phagocytosis of bacteria, apoptotic cells, degenerated myelin and Aβ	Block et al., 2007; Wilkinson and El Khoury, 2012
SR-B1	HDL, LDL, Ox-HDL, Ox-LDL, advanced glycosylation end products	Phagocytosis of bacteria and apoptotic cells	Boullier et al., 2001	Adhesion to Aβ, phagocytosis of apoptotic cells, and endocytosis of fibrillar Aβ	Block et al., 2007
TLR2	Pam3Cys/Glycolipids, Hsp70, HMGB1	Function in phagocytosis unreported	N/R	Phagocytosis of Aβ	Landreth and Reed-Geaghan, 2009; Noda and Suzumura, 2012
TLR4	LPS, lipotheicoic acid, Hsp60,70; co-receptor CD14	Phagocytosis of bacteria	Anand et al., 2007; McCoy and O'Neill, 2008	Bacterial recognition; phagocytosis of Aβ	Block et al., 2007; Noda and Suzumura, 2012
C-TYPE LECTIN RECEPTORS					
Dectin-1	β glucans	Phagocytosis of yeast, fungus	Lowell, 2011; Flannagan et al., 2012	Phagocytosis of yeast, fungus	Shah et al., 2008
MR	Mannose, fucose	Phagocytosis of pathogens	Flannagan et al., 2012	Phagocytosis of yeast	Marzolo et al., 1999; Zimmer et al., 2003
OTHER RECEPTORS					
β2-GPI receptor (unidentified)	PS [β2-GPI]	Phagocytosis of apoptotic cells	Lauber et al., 2004; Taylor et al., 2005	Function in phagocytosis unreported	N/R
CD91	Multiprotein complex (calreticulin, MBL, C1q), HSPs	Initiate engulfment of apoptotic cells	Ogden et al., 2001	Function in phagocytosis unreported	Pais et al., 2008

Receptors have been classified into major functional/structural groups: PS receptors, integrin receptors, Ig superfamily receptors, scavenger and related receptors, C-type lectin receptors, and others. Bridging molecules are indicated in brackets.

Abbreviations: Aβ, amyloid beta protein; Ac-LDL, acetylated low density lipoprotein; AGEs, advanced glycation end products; BAI-1, brain angiogenesis inhibitor 1; CR, complement receptor; DAP12, DNAX-activation protein of 12 KDa; HDL, high density lipoprotein; HMGB1, high-mobility group box 1 protein; Hsp, heat-shock protein; LDL, low density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; LPS, bacterial lipopolysaccharides; MAC-1, macrophage antigen complex 1; MARCO, macrophage receptor with collagenous structure; MBL, mannan-binding lectin; Mer-TK, Mer tyrosine kinase; MFG-E8, milk fat globule-epidermal growth factor; MR, mannose receptor; N/R, non-reported; Ox-HDL, high density lipoprotein; Ox-LDL, oxidized low density lipoprotein; Ox-PS, oxidized phosphatydilserine; PS, phosphatydilserine; PSR, phosphatydilserine receptor; RAGE, receptor for advanced glycation endproducts; Siglec11, sialic acid binding immunoglobulin-like lectin 11; SIRPα, signal regulatory protein alpha; SIRPβ1, signal regulatory protein beta 1; SP, surfactant protein; SR, scavenger receptor; TIM, T-cell-immunoglobulin-mucin; TLR, toll-like receptor; TREM2, triggering receptor expressed on myeloid cells 2; VnR, vitronectin receptor.

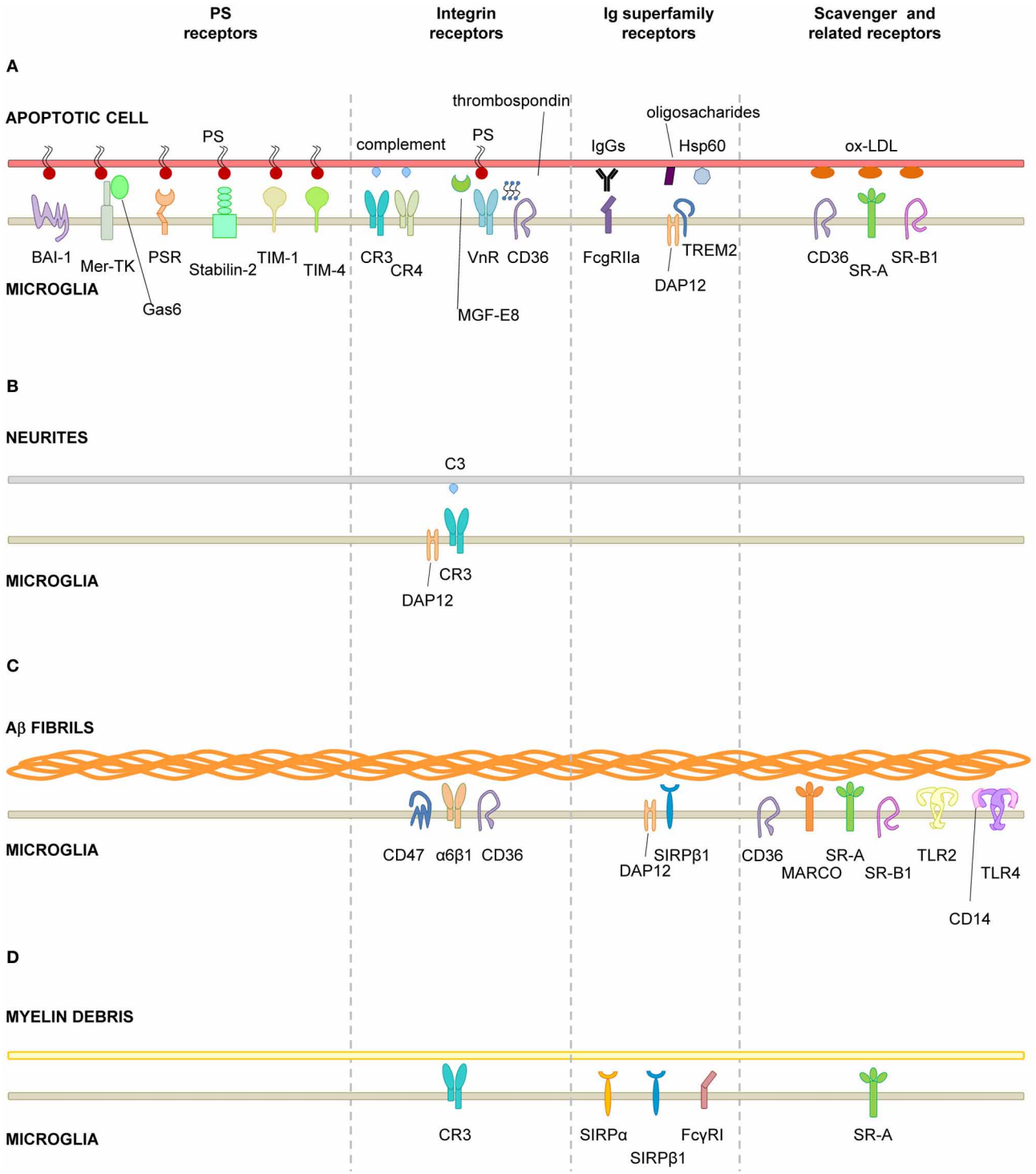


FIGURE 2 | Receptors involved in microglial phagocytosis of endogenous structures. (A), Apoptotic cells; **(B)**, Neurites; **(C)**, Aβ aggregates/fibrils; **(D)**, Myelin debris. Receptors have been classified into major functional/structural groups: phosphatidylserine (PS) receptors, integrin receptors, Ig superfamily-receptors, and scavenger and related receptors. For more details on the receptors, refer to **Table 1**. For receptors involved in the recognition of necrotic cells and microbes, refer to the main text. Abbreviations: Aβ, β-amyloid protein; BAI-1, brain angiogenesis inhibitor 1; CR, complement receptor; DAP12, DNAX-activation protein of 12 KDa; Hsp60, heat-shock protein of 60 KDa; MARCO, macrophage receptor with collagenous structure; Mer-TK, Mer tyrosine kinase; MFG-E8, milk fat globule-epidermal growth factor; Ox-LDL, oxidized low density lipoprotein; PS, phosphatidylserine; PSR, phosphatidylserine receptor; SIRPβ1, signal regulatory protein β1; SR-A, scavenger receptor class A; SR-B1, scavenger receptor class B1; TIM, T-cell-immunoglobulin-mucin; TLR, toll-like receptor; TREM2, triggering receptor expressed on myeloid cells 2; VnR, vitronectin receptor.

change under different stimuli such as inflammation (Falsig et al., 2008), but it is not known whether they ultimately impact the efficiency of microglial phagocytosis.

These receptors and their targets closely interact in what has been termed "engulfment synapse" or "phagocytic synapse" (Ravichandran, 2010; Dustin, 2012; Underhill and Goodridge, 2012). Similar in size (0.5 μm in diameter) and purpose (close cell–cell contact) to its immunological and neural counterparts, phagocytic synapses are specialized regions of the membrane where the apoptotic cell and the phagocyte interact through microclusters of receptors. Like the immunological synapse formed between antigen presenting cells (APCs) and T lymphocytes, and in contrast to the synapses formed between neurons, phagocytic synapses are short-lived and last only a few minutes (Dustin, 2012). Phagocytic synapses are also characterized by exclusion of phosphatases (Dustin, 2012), so that once the contact is established the signal is transduced by a variety of intracellular kinases (Syk kinase, phosphatidylinositol 3-kinase) and small GTPases (Rac, Rho) and adapter molecules such as engulfing and cell motility (ELMO) family (Gumienny et al., 2001; Lee et al., 2007). An emerging pivotal complex is that formed by ELMO-1 and dedicator of cytokinesis 180 (DOCK-180), which is activated among other receptors by BAI-1 and act as a guanine nucleotide exchange factor for Rac (Park et al., 2008; Patel et al., 2011). ELMO-1 function is regulated by phosphorylation by the hematopoietic cell kinase (HCK) (Yokoyama et al., 2005), a member of the Src family whose deficiency impairs macrophage phagocytosis (Lowell et al., 1994). These pathways lead to the remodeling of the phagocyte cytoskeleton through actin polymerization and membrane composition, triggering the formation of pseudopodia that form a phagocytic cup engulfing the target (Lee et al., 2007). Of the complex process of signal transduction and formation of the phagocytic cup, very little is known in microglia.

DIGEST-ME

The phagocytic cup closes and thus forms the phagosome around the target. To execute the degradation of the target, phagosomes go through a process of maturation in which they fuse sequentially with early and late endosomes, and lysosomes, to form phagolysosomes (Desjardins et al., 1994). These phagolysosomes contain hundreds of proteins, including hydrolases such as cathepsins to digest the target; and proton pumps such as vacuolar ATPases to acidify the medium (Garin et al., 2001). The acidic pH found in phagolysosomes (pH $\leq$ 5) is essential for the lysosomal degradation capabilities, as it is optimal for most hydrolases. Interestingly, the signaling associated with the acidic pH of lysosomes deactivates the generation of radicals from the oxidative burst (Li et al., 2010). Again, the process of phagosome formation and cargo degradation has been barely addressed in microglia. A recent study in *C. elegans* using live imaging showed that in this nematode microglial vATPases are required for phagosome–lysosome fusions and consequently to degrade cargo (Peri and Nusslein-Volhard, 2008). Further research is necessary to delineate the mechanisms of degradation of structurally different cargo, ranging from Aβ deposits to whole apoptotic cells, in microglia. Moreover, because phagocytosis is not only performed by ameboid, activated cells but also by terminal or en passant branches of ramified, resting microglia (Peri and Nusslein-Volhard, 2008; Sierra et al., 2010), the location where the actual degradation occur remains unexplored. Live imaging experiments show that resembling phagosomal cups are retrogradely transported to the cell soma in the mouse neocortex (Nimmerjahn et al., 2005); small puncta of apoptotic cell material are observed within branches of ramified microglia in fixed adult hippocampus, indirectly suggesting their transport (Sierra et al., 2010). Direct evidence of retrograde transport of cargo-containing phagosomes was recently found in *C. elegans* (Peri and Nusslein-Volhard, 2008). Collectively, these data indirectly suggest a yet unexplored role of the cytoskeleton in transporting the phagosome for the degradation of the engulfed material.

Overall, phagocytosis is considered to be essential for maintaining brain tissue homeostasis. The rapid clearance of apoptotic cells prevents their transformation into secondary necrotic cells (as it readily occurs *in vitro*), with the subsequent loss of permeability of the cell membranes and spillover of intracellular contents. For instance, the phagocytosis of apoptotic neutrophils and T cells during the resolution of the inflammatory response prevents the release of intracellular granules containing lysosomal enzymes and possibly acidification of the extracellular space (Magnus et al., 2001). Further, blockade of microglial phagocytosis of polymorphonuclear neutrophils, which infiltrate the brain parenchyma after focal ischemia, decreases neuronal viability in organotypic slices (Neumann et al., 2008). Phagocytosis of apoptotic neurons is also beneficial for neuronal survival. For instance, neurons can be rescued from excitotoxic death when phagocytosis is promoted by the chemokine fractalkine in neuron-microglia co-cultures, but not in neuronal cultures alone (Noda et al., 2011). In addition, myelin debris contains inhibitory molecules which inhibit axonal regeneration (Gitik et al., 2011); Aβ plaques cause physical damage of the neural tissue and lead to functional deficits if they are not removed (Wang et al., 2011); and removal of axonal debris by microglia increases axonal regeneration *in vitro* (Tanaka et al., 2009; Hosmane et al., 2012).

In the living brain, however, the beneficial effects of microglial phagocytosis have been harder to prove, possibly due to the lack of molecular tools to specifically interfere with microglial phagocytosis. Enhancing brain phagocytosis via intravenous delivery of TREM2-transduced bone marrow-derived precursor cells potentiates the clearance of degenerated myelin, decreases the inflammatory response and ameliorates the course of the disease in the mouse model of multiple sclerosis (MS), experimental acute encephalomyelitis (EAE) (Takahashi et al., 2007). A method recently proposed is the use of annexin V, a PS binding protein, to block phagocytosis. When administered systemically, annexin V reaches the brain and, as expected, leads to accumulation of apoptotic debris, although it has not been quantified whether it actually prevents phagocytosis (Lu et al., 2011). Treatment with annexin V impaires the generation of newborn neurons in adult neurogenic niches without affecting the proliferation of neuronal precursors, suggesting that phagocytosis is important for the differentiation and/or survival of the newborn cells (Lu et al., 2011). Direct evidence

of the beneficial effects of microglial phagocytosis was shown recently in a mouse model of Rett syndrome by knocking out the methyl-CpG-binding protein Mecp2 (Derecki et al., 2012). While Rett syndrome is mostly considered a primary neuronal disease, defects in phagocytosis and inflammatory response are found in Mecp2-deficient microglia (Derecki et al., 2012). Further, bone marrow cells transplanted into irradiated Rett mice differentiate into microglia and partially arrest the pathology of the disease (Derecki et al., 2012). Interestingly, the disease is mimicked by microglial-specific depletion of Mecp2 and phagocytosis is blocked by intravenous injection of annexin V, but not by either manipulation independently. These data suggest that a failure in microglial phagocytosis does not underlie the pathology of the Rett syndrome but, nonetheless, increasing phagocytosis may be beneficial (Derecki et al., 2012).

FUNCTIONAL CONSEQUENCES OF PHAGOCYTOSIS

Clearance of microbes or debris is a direct intervention of microglia to remove invading pathogens or to clean up the tissue from unwanted material. The direct consequences of this clearance function were already described in the chapter above. Furthermore, ingestion and degradation of targets has several indirect consequences for the phagocyte as well as the surrounding tissue. Phagocytosis triggers activation of several intracellular signaling pathways and remodels the cytoskeleton and cell membrane (Lee et al., 2007). In addition, microglia receive a tremendous metabolic load from the lipids, carbohydrates, proteins, and other components from the digested target (particularly in the case of apoptotic cells and microorganisms), leading to direct changes in the phagocyte's lipid and cholesterol, and possibly glucose metabolism (Han and Ravichandran, 2011). In addition, whether phagocytosis impacts proliferation, survival, or differentiation of the phagocyte remains unexplored. In contrast, three major functional consequences of microglial phagocytosis have been well-described: antigen presentation, activation of the respiratory burst, and modulation of inflammatory responses.

ANTIGEN PRESENTATION

Professional APCs (macrophages, DCs, and B lymphocytes) engulf their targets locally; next, they travel to lymph nodes, where the digested exogenous proteins (antigens) are expressed in their membrane attached to specific receptors (major histocompatibility complex class II, or MHC-II), which enable their recognition by naïve T lymphocytes, initiating the cellular arm of the adaptive immune response. Antigen presentation is a complex process beyond the scope of this review. Nonetheless, it is important to note that antigen presentation in the CNS has been a matter of hot debate in the last few years [reviewed by Ransohoff and Engelhardt (2012)]. In normal conditions, microglia do not express MHC-II and only a subpopulation expresses co-stimulatory molecules such as CD11c (Bulloch et al., 2008), and in agreement they have poor antigen presenting activity *in vivo* (Ford et al., 1995). Upon local inflammatory challenge, microglia show increased expression of MHC-II and CD11c *in vivo* and increased antigen presentation *in vitro*, albeit with much lower efficiency than professional APCs (Gottfried-Blackmore et al., 2009). While it is clear that they have the capacity of presenting antigens under some particular conditions, the microglial contribution to antigen presentation *in vivo* is thought to be mostly irrelevant (Galea et al., 2007; Ransohoff and Engelhardt, 2012). A major unresolved issue is whether microglia would have the capability of abandoning the brain parenchyma and reach the lymph nodes. In fact, antigen presentation in the CNS is thought to occur not in the parenchyma, but in the meninges and choroid plexus, which contain perivascular APCs, or by direct drainage of CNS antigens in the cerebrospinal fluid from the subarachnoidal space to channels in the cribiform plate and ultimately into deep cervical lymph nodes (Galea et al., 2007; Ransohoff and Engelhardt, 2012). Self-antigens, including CNS antigens, do not produce a response since self-reactive T cells are eliminated by clonal deletion in the thymus. In the autoimmune disease MS, however, myelin-specific T cells may escape from this tolerance mechanism and enter the brain under some circumstances, leading to demyelination (Goverman, 2011). Microglia seems to play a negative role in the disease progression of EAE, the animal model of MS, as its depletion reduces the severity of the disease (Heppner et al., 2005). Whether this potentially detrimental role of microglia in EAE is related to phagocytosis and/or antigen presentation remains unknown (Ransohoff and Engelhardt, 2012).

RESPIRATORY BURST

During phagosome maturation the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is assembled in the phagosome. NADPH oxidase catalyzes the reaction of NADPH and oxygen to form $NADP^+$, protons and the superoxide anion (O_2^-), in a process known as the respiratory burst (Minakami and Sumimotoa, 2006). In the acidic pH of the phagosome, the superoxide anion is dismutated into hydrogen peroxide, H_2O_2, and later transformed into other reactive oxygen species (ROS) which contribute to killing of engulfed microorganisms and degradation of other cargo (Minakami and Sumimotoa, 2006). ROS are extremely aggressive oxidants and can induce both apoptosis and necrosis (Pourova et al., 2010). Potentially toxic for the phagocyte itself and the surrounding tissue, they are thought to be released exclusively within the phagosome. In fact, NADPH assembly is a tightly controlled process in phagocytes and includes the recruitment to the phagosome of the catalytic core $gp92^{phox}$ and $p22^{phox}$, and the phosphorylation and membrane translocation of the regulatory subunit $p47^{phox}$ (Quinn and Gauss, 2004). To ensure further regulation, the enzyme is rapidly deactivated, resulting in transient activity (Decoursey and Ligeti, 2005). Most of our understanding about the molecular mechanisms involved in activation or the respiratory burst comes from studies performed in neutrophils (Minakami and Sumimotoa, 2006), one of the most important components of the immune system in the defense against invading microorganisms. In microglia, the respiratory burst appears to be activated by phagocytosis through similar mechanisms (Ueyama et al., 2004) after phagocytosis of zymosan (a yeast cell wall preparation) (Newell et al., 2007) or myelin (Williams et al., 1994), although it remains to be studied whether it is also activated after phagocytosis of other types of cargo.

Recently, it has been suggested that the NADPH oxidase can be assembled in alternative locations, driving the respiratory burst in the absence of phagocytosis (Bylund et al., 2010). In microglia, the respiratory burst is triggered by hypoxia/reoxygenation (Spranger et al., 1998) and LPS (Qin et al., 2004) and results in the extracellular release of ROS. In turn, inflammation-induced respiratory burst leads to the release of glutamate by microglia, further contributing to neuronal damage (Barger et al., 2007). Interestingly, the microglial respiratory burst has been linked to induction of neuronal apoptosis in the developing cerebellum *in vitro* (Marin-Teva et al., 2004) and in the developing hippocampus *in vivo* (Wakselman et al., 2008). In both studies, ROS-trapping by free radical scavengers resulted in a significant rescue of neurons from apoptosis. However, it remains unclear whether the activation of the respiratory burst was activated as a consequence of phagocytosis or by independent mechanisms. While microglia were clearly identified as the only source of ROS, the actual intracellular location (i.e., in the phagolysosome or otherwise) was not addressed (Marin-Teva et al., 2004; Wakselman et al., 2008). The hippocampal study suggested indirectly that phagocytosis was indeed involved in the activation of the respiratory burst, because mice lacking the complement receptor CR3 or expressing mutant DAP12 showed reduced ROS production and apoptosis (Wakselman et al., 2008). In spite of the pro-phagocytic role of CR3 and DAP12, whether phagocytosis was disrupted in these transgenic mice was not addressed. Two possible scenarios remain open: in the first one, microglia would attack live (possibly healthy) neurons, phagocytose them via CR3/DAP12, and kill them intracellularly through the respiratory burst. In the second scenario, an undetermined stimulus would activate microglia to produce ROS via CR3/DAP12, which would be released extracellularly and kill neurons, subsequently phagocytosed by microglia. Clearly, more precise methods to quantify ROS (extracellular, intracellular, phagolysosomal, etc.) (Bylund et al., 2010) and to quantify phagocytosis and the nature of the cargo (Sierra et al., 2010) are necessary to unravel the role of microglial phagocytosis and respiratory burst in the developing hippocampus and cerebellum.

MODULATION OF INFLAMMATORY RESPONSES

Historically, it was believed that phagocytosis of apoptotic cells was a neutral immune event because it does not initiate an inflammatory response [reviewed by Savill et al. (2002)]. Over the past 20 years, it has been made clear that the phagocytosis of apoptotic cells is largely anti-inflammatory (Stern et al., 1996; Voll et al., 1997; Fadok et al., 1998). The inhibition of pro-inflammatory cytokines synthesis requires tethering of the phagocyte and the apoptotic cell, but not actual engulfment, followed by paracrinal release of the anti-inflammatory cytokine transforming growth factor beta (TGFβ), although the mechanism is not well-understood (Lucas et al., 2006). In addition, phagocytosis of other types of cargo such as myelin can trigger an anti-inflammatory response (Liu et al., 2006). On the contrary, phagocytosis of microbes associated with TLR stimulation is pro-inflammatory (Erdman et al., 2009). The anti-phlogistic response to phagocytosis of apoptotic cells also occurs in microglia. Microglia phagocytosing apoptotic T cells produce less tumor necrosis factor alpha (TNFα), a major pro-inflammatory cytokine, than naïve microglia after LPS challenge *in vitro* (Magnus et al., 2001). Similarly, phagocytosis of an apoptotic neuronal line induces an increased production of TGFβ and neural growth factor (NGF) in basal conditions; as well as a decreased production of TNFα, nitrite, and prostaglandin E2 during LPS challenge *in vitro* (De Simone et al., 2003). These anti-inflammatory effects are mediated by microglial PS receptor (De Simone et al., 2002), as well as by TREM2 (Takahashi et al., 2005). More recently it has been described that the immunosuppression induced by phagocytosis of apoptotic cells depends on the presence of the complement protein C1q bound to apoptotic cells, without which phagocytosis is pro-inflammatory (Fraser et al., 2010). It is important to note that complement proteins are present in the fetal bovine serum commonly used in primary cultures. Nonetheless, it remains to be determined whether the opsonin C1q is produced in the normal brain and binds to apoptotic cells phagocytosed by microglia *in vivo*. As a matter of fact, there is a big gap in our knowledge of the type of inflammatory response resulting from phagocytosis *in vivo*. The only *in vivo* data derives from a model of prion disease, where phagocytic microglia have been qualitatively observed not to express the pro-inflammatory cytokine interleukin 1beta (IL-1β) (Hughes et al., 2010). Because inflammation has severe effects in the brain, including neurotoxicity (Pickering et al., 2005) and epileptogenesis (Galic et al., 2012), more research is necessary to conclusively determine whether microglial phagocytosis of apoptotic cells is anti-inflammatory *in vivo*.

MICROGLIAL PHAGOCYTOSIS IN PHYSIOLOGICAL CONDITIONS

The widely accepted theory of linear activation of microglia proposed by Gennadij Raivitch, William Streit, and collaborators (Raivich et al., 1999; Streit et al., 1999) leads to the generalized assumption that microglia have to be preactivated or primed by inflammatory challenge in order to become efficient phagocytes (Kettenmann, 2007). More current are the terms M1 and M2 to describe a spectrum of states of microglial activation, similar to the polarization of macrophages responses (Mantovani et al., 2002). The M1 phenotype or classic activation, is induced by PAMPs and pro-inflammatory cytokines, and is characterized by high expression of TLRs, TNFα, co-regulatory molecules for antigen presentation, and increased release of ROS. On the contrary, the M2 phenotype, or alternative activation, is driven by stimuli such as interleukin 4 (IL-4) or interleukin 13 (IL-13), and is characterized by the production of anti-inflammatory interleukin 10 (IL-10) and TGFβ, and higher expression of scavenger receptors. The M1 phenotype is basically neurotoxic, while the M2 phenotype is more neuroprotective (Durafourt et al., 2012). However it is not very clear what is the relationship between the M1/M2 phenotypes and phagocytosis. For instance, classic activation paradigms driving a M1 phenotype lead to increased enhanced engulfment of apoptotic cells *in vitro* (Chan et al., 2001; McArthur et al., 2010). In addition, expression of phagocytic receptors is triggered by many inflammatory and neurodegenerative paradigms *in vivo*

assumedly inducing an M1 phenotype. On the other hand, a M2 phenotype driven by IL-4 and IL-13 increases the phagocytosis of myelin (Durafourt et al., 2012), whereas a M2 phenotype induced by glioma cancer stem cells reduces phagocytosis (Wu et al., 2010). Regardless of the nomenclature used to describe microglial phenotype after activation, the notion that microglia require to be preactivated to phagocytose efficiently is challenged by a number of recent papers showing that "resting" or "steady-state" microglia are efficient phagocytes of spines and apoptotic cells during physiological conditions in the adult and developing brain (Dalmau et al., 2003; Sierra et al., 2010; Schafer et al., 2012) (**Figure 3**).

MICROGLIAL PHAGOCYTOSIS OF SPINES

The capacity for microglia to phagocytose synapses has just begun to be unraveled. In the juvenile visual cortex, most microglia interact with synaptic elements (spines, axon terminals, perisynaptic astrocytes, and synaptic clefts) (Tremblay et al., 2010). Furthermore, live imaging indirectly supported a role for microglia in pruning synapses, as spines contacted by microglia where more frequently eliminated than non-contacted spines (Tremblay et al., 2010). Qualitative examples of microglia containing pre- and postsynaptic material labeled respectively with SNAP25, a protein associated with presynaptic vesicles, and with PSD95, a marker of the excitatory postsynaptic density, have been shown in the developing postnatal hippocampus (Paolicelli et al., 2011). Interestingly, a transient increase in dendritic spines and immature synapses was observed in CX3CR1 knock-out mice compared to wild-type mice, possibly due to a reduction in microglial density (Paolicelli et al., 2011). Unfortunately, the authors did not carry out a quantification of microglial phagocytosis of spines in CX3CR1-KO mice and thus the role of the fractalkine receptor as an opsonin for microglial phagocytosis of spines cannot be ruled out. Direct, quantified evidence of microglia phagocytosing synapses (mostly, presynaptic elements) in the developing visual system has been recently provided using confocal and electron microscopy (Schafer et al., 2012). In the dorsal lateral geniculate nucleus (dLGN), active pruning of synaptic inputs from the retinal ganglion cells (RGCs) produces well-established ipsi- and contralateral innervations territories. This pruning is at least partially mediated by complement-mediated microglial phagocytosis, because mice deficient either for the receptor CR3 or its ligand, C3, have decreased phagocytosis of synaptic inputs, a sustained increase in synaptic density, and deficits in segregation of the eye-specific territories compared to wild-type mice (Schafer et al., 2012). Thus, unwanted synapses seem to be tagged for removal by deposition of complement proteins but—who is the decision maker? Do microglia actively instruct which synapses must be removed? *In vitro*, microglia produce several components of the complement cascade, including C1q and C3, which are involved in the phagocytosis of neurites via CR3 (Linnartz et al., 2012). Interestingly, removal of the monosaccharide sialic acid from the neural glycocalyx is essential for C1q binding to neurites and subsequent

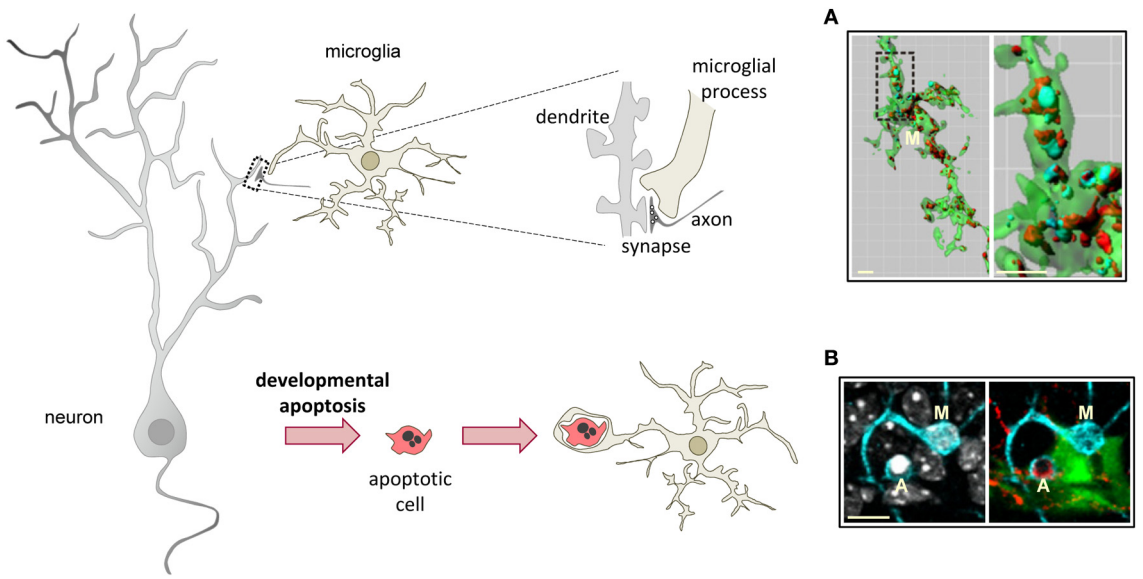


FIGURE 3 | Microglial phagocytosis in health. Motile, ramified, unchallenged microglia phagocytose neurites (**A**, dendritic spines, axons) and developmentally apoptotic neurons **(B)** in physiological conditions in the adult and developing brain. **(A)** Surface-rendered CX3CR1^{+}/EGFP microglia (M; green) from the dorsolateral geniculate nucleus engulfing ipsi- (blue, labeled with cholera-toxin **(B)** conjugated to Alexa 647) and contralateral (red, labeled labeled with cholera-toxin **(B)** conjugated to Alexa 594) inputs from retinal ganglion cells at postnatal day 5, when robust pruning occurs. The insert is shown at higher magnification on the right panel. **(B)** Iba-1 labeled microglia (M; cyan) branching a phagocytic pouch which engulfs a newborn apoptotic/pycnotic cell (A; labeled with the DNA dye DAPI, white), containing immunoreactivity for the neuroblast marker polysialic acid neural cell adhesion molecule (red). The cell is located in the subgranular zone of the hippocampus, where neural stem cells (visualized in nestin-GFP mice) are located and proliferate throughout adulthood. Images **(A)** and **(B)** are reprinted with permission from Elsevier. **(A)** is reprinted from Schafer et al. (2012). **(B)** is reprinted from Sierra et al. (2010).

microglial phagocytosis in cultured neurons (Linnartz et al., 2012). It seems plausible that synapse pruning is regulated by a combination of mechanisms involving activity-dependent control of synaptic strength, alterations in the neuronal glycocalyx, and tagging of altered synapses by microglial-release of complement proteins, followed by removal of pre- and/or postsynaptic components by microglial phagocytosis. In contrast to the more established mechanism of synaptic pruning by simple retraction or degeneration of the input axon (Luo and O'Leary, 2005), microglia-dependent pruning is likely to require more time and energy, while perhaps adding more levels of control to a phenomenon which is essential to determine brain connectivity. A possible similar combination of mechanisms has been observed in the developmental pruning of dendrites in Drosophila neurons, where branch retraction and local fragmentation of dendrites are observed together with phagocytosis of caspase-labeled dendrites by blood phagocytes (Williams and Truman, 2005; Williams et al., 2006).

Overall, these data support a novel role for microglia in monitoring synapses in the healthy developing brain, with potentially profound consequences for diseases in which neural connectivity and microglial activation concur. One such disease is transient ischemia, in which the turnover of synapses (Zhang et al., 2005) as well as microglial activation and inflammation increase (Lambertsen et al., 2012). In the ischemic cerebral cortex microglial contacts with synaptic boutons last-longer (1 h) than in the control cortex (5 min) (Wake et al., 2009). While it is tempting to speculate that extended contacts are related to synapse pruning, only a minority of boutons disappeared after being contacted by microglia and phagocytosis was not observed (Wake et al., 2009). In autism spectrum disorders (ASD), a complex set of diseases with unknown pathophysiology, both neural hyperconnectivity (Testa-Silva et al., 2012) and inflammation (Vargas et al., 2005) develop. In MECP2-deficient mice, a model of Rett syndrome, microglia has defective phagocytosis *in vitro*, and preventing phagocytosis in the mice exacerbates the symptoms (Derecki et al., 2012). The potential defects in spine and/or apoptotic cell phagocytosis by microglia in Rett syndrome and other ASDs remain to be elucidated.

MICROGLIAL PHAGOCYTOSIS OF APOPTOTIC CELLS DURING BRAIN DEVELOPMENT AND IN NEUROGENIC NICHES

A detailed map estimating microglial phagocytosis during development showed an overall high efficiency of microglia-removing apoptotic cells (Dalmau et al., 2003). At time points where apoptosis is maximal, microglia phagocytose 97% of apoptotic cells in the fimbria (embryonic day E18), 88 and 100% in the CA region of the hippocampus (E16 and postnatal day 0, respectively), 18 and 86% in the cerebral cortex (E16 and P0, respectively), and 93% in the dentate gyrus (P0). These estimates match well with the phagocytic efficiency of microglia in the dentate gyrus throughout adulthood, where new neurons are continuously produced. From 1 to 12 months of age in mice, microglia phagocytose over 90% of the cells undergoing apoptosis (Sierra et al., 2010). Nonetheless, the phagocytic capacity is region-specific, as microglia only phagocytose 50% of the apoptotic cells at the maximum period of cell death (P3) in the developing cerebellum (Ashwell, 1990; Marin-Teva et al., 2004; Wakselman et al., 2008).

But, what happens to the remaining apoptotic cells? A possible interpretation is that they will be engulfed by microglia at a later time point, so that the percentage of non-phagocytosed apoptotic cells is an indirect representation of the "find-me" time. Alternatively, these cells may be disposed of through other mechanisms, such as phagocytosis by other cell types. Microglia are considered the professional brain phagocytes. Non-professional phagocytes such as astrocytes delay phagocytosis for several hours (Parnaik et al., 2000) and engulf with much lower capacity (Magnus et al., 2002), at least *in vitro*. Nonetheless, other cell types mediate phagocytosis of apoptotic cells *in vivo* during development. For instance, in the developing cerebellum (P7) phagocytosis of apoptotic oligodendrocytes is carried out by specialized astrocytes, the Bergmann glia, albeit at a relative low efficiency (55% of apoptotic cells engulfed) (Parnaik et al., 2000). In the developing retina, phagocytosis is executed by microglia and Müller cells (Egensperger et al., 1996), and throughout lifetime the excess of photoreceptors membrane shredded is phagocytosed by retinal pigment epithelium (RPE) cells (Kevany and Palczewski, 2010). While considered non-professional phagocytes, RPE cells have an enormous phagocytic capacity, as they engulf up to 10% of the photoreceptors volume on a daily basis (Kevany and Palczewski, 2010). In the adult hippocampal neurogenic cascade, only microglia have been observed to phagocytose apoptotic newborn cells (Sierra et al., 2010). Nonetheless, neural-committed neuroprogenitors (neuroblasts, labeled with doublecortin) have some phagocytosing capabilities which contribute to the maintenance of the neurogenic cascade, although what they actually take in remains to be determined (Lu et al., 2011). In peripheral ganglia, the apoptotic neurons are phagocytosed by satellite glial cell precursors (Wu et al., 2009). It has been speculated that low microglial density in some regions may be related to the recruitment of non-professional phagocytes (Parnaik et al., 2000) but the reasons behind this promiscuous phagocytosis during brain development are not known. Assumedly, microglial phagocytosis contributes to the maintenance of tissue homeostasis during development and in adult neurogenic niches by rapidly removing cellular debris. It can also be speculated that microglial phagocytosis takes a more active role in regulating neurogenesis. For instance, after phagocytosis, cultured microglia produce higher levels of TGFβ and NGF (De Simone et al., 2003), which are negative and positive regulators of hippocampal neurogenesis, respectively (Buckwalter et al., 2006; Frielingsdorf et al., 2007). After stroke, microglia in the subventricular zone (SVZ) show a pro-neurogenic phenotype which includes the expression of insulin-like growth factor 1 (IGF-1) (Thored et al., 2009), another well-known inducer of neurogenesis (O'Kusky et al., 2000). Stroke-responding microglia were labeled with ED1 but unfortunately phagocytosis was not quantified. Future research will delineate the contribution of microglia to neurogenesis.

The high efficiency of phagocytosis, that is, the high coupling between apoptosis and phagocytosis, has also suggested the idea that phagocytosis executes the final stages of apoptotic cell

death. Apoptosis can be initiated by two major pathways: the extrinsic pathway, driven by activation of membrane receptors such as CD95, or the TNFα receptor; and the intrinsic pathway, initiated by cellular stress (DNA mutations, deprivation of survival factors, Ca^{2+} overload, etc.) (Reubold and Eschenburg, 2012). While the signaling cascades are complex and varied, a common mechanism of execution of most forms of apoptosis is the formation of the apoptosome, a macromolecular complex which activates the effector caspase 3, a cystein protease responsible for DNA fragmentation, membrane blebbing, cytoskeleton degradation, and the other major hallmarks of apoptosis (Blank and Shiloh, 2007). In addition to the cell-autonomous degradation mediated by caspases, phagocytes may also contribute to carry out death. For instance, mutations in *C. elegans* engulfment genes, such as ced-1, permit the survival of cells that would normally die (Reddien et al., 2001). In addition, DNA fragmentation is not only mediated by cell-autonomous caspase-activated DNase (CAD) but can also be partly attributed to postengulfment degradation by lysosomal enzymes (McIlroy et al., 2000). The lysosomal DNase II of macrophages contributes to thymocyte DNA degradation during thymus development (Kawane et al., 2003). Whether this mechanism is universal to macrophages and microglia is unknown, because DNase II deficient mice embryos show severe defects in the thymus and kidney, but not in the brain (Kawane et al., 2003). Nevertheless, these evidences suggest that, since there are no stop points in apoptosis analog to the check points found in mitosis, phagocytosis is the de facto mechanism to discriminate between moribund and dead cells.

In some circumstances this effective coupling may have detrimental consequences. It has been suggested that if phagocytosis is too effective, it may lead to the removal of cells which will otherwise have time to repair themselves (Kao et al., 2011). For example, the macrophages of mice and worms deficient in the secreted glycoprotein progranulin have an enhanced phagocytic efficiency, which is perhaps related to the neurodegeneration found in human patients of familial frontotemporal lobar degeneration, mostly caused by mutations in progranulin (Kao et al., 2011). Going further, microglial phagocytosis may be the primary cause of cell death under some circumstances. Macrophages are known to interact with live cells but disengage quickly because of the "don't eat-me" signals (Ravichandran, 2010). Under some circumstances, however, phagocytes kill live cells. For instance, macrophages induce apoptosis of normal vascular endothelial cells of the hyaloid vascular system as well as papillary cells in the developing mouse eye (Lang and Bishop, 1993; Diez-Roux and Lang, 1997). Similarly, microglial phagocytosis has been reported to induce the death of viable, motile non-apoptotic polymorphonuclear neutrophils in organotypic hippocampal cultures in which ischemia was induced by oxygen and glucose deprivation (Neumann et al., 2008). Further, in inflammatory conditions driven by activation of TLR2 or TLR4, microglial phagocytosis induces cerebellar granule cell death by a complex mechanism involving the microglial release of peroxynitrite which leads to a transient exposure of PS in the neurons and opsonization with MFG-E8, followed by recognition through the vitronectin receptor and phagocytosis by microglia *in vitro* (Neher et al., 2011) and in the striatum *in vivo* (Fricker et al., 2012). However, high concentrations of peroxynitrite lead to increased intracellular calcium, exposure of PS, activation of caspases and, ultimately, apoptotic cell death (Leist et al., 1997). Neurons dying from primary phagocytosis do not express features of apoptosis or necrosis, and the actual mechanism executing death is an open area of research (Brown and Neher, 2012). Thus, phagocytosis represents a wide range of responses, from the mere passive clearing of apoptotic cells and the active execution of final stages of apoptosis during development to the aberrant killing of live, healthy neurons during inflammation.

MICROGLIAL PHAGOCYTOSIS IN PATHOLOGICAL CONDITIONS

Overall, phagocytosis is considered a beneficial phenomenon and its alteration has been linked to autoimmune diseases (Nagata et al., 2010). In addition, the best-known case of a phagocytic system disease is the relatively rare but lethal Nasu–Hakola disease or polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), due to loss-off-function mutations of TREM2 and/or DAP12 (Paloneva et al., 2002). PLOSL is characterized by defects in bone resorption by osteoclasts leading to the formation of cysts, as well as dementia (Bianchin et al., 2004). The contribution of microglia to the pathology of PLOSL remains obscure because DAP12-deficient mice do not reflect the human neurodegenerative pathology. Initial reports showed altered synaptogenesis and hypomyelination as well as behavioral impairments in mice deficient or expressing loss-off-function mutations of DAP12 (Kaifu et al., 2003; Roumier et al., 2004). In the mouse brain, DAP12 is exclusively expressed in microglia, suggesting an interesting link between microglia and synaptic plasticity through a yet unknown mechanism (Roumier et al., 2004). More recent studies have shown a strong demyelination in the brain of DAP12-deficient human patients; however this demyelination did not seem to correlate with changes in microglial density or activation (Satoh et al., 2011). Therefore, the exact pathophysiology of microglia in PLOSL patients remains unclear. Nonetheless, there are many other pathological conditions related to the microglial phagocytosis of apoptotic cells, viral and bacterial pathogens, tumor cells, Aβ, myelin, and axonal debris (**Figure 4**).

MICROGLIAL PHAGOCYTOSIS OF DEAD CELLS IN PATHOLOGICAL CONDITIONS

Neurodegeneration by apoptosis is a major part of several brain diseases such as stroke, epilepsy, prion disease, Alzheimer's disease (AD), and Parkinson's disease (PD). Microglial phagocytosis of apoptotic cells in pathological conditions is assumed to be highly efficient, possibly because of an extrapolation of the physiological phagocytosis during brain development, however this may not be always the case. For instance, there is qualitative evidence that apoptotic neurons in a mouse model of neonatal stroke by medial cerebral artery occlusion (MCAO) are poorly phagocytosed by activated microglia compared to the contralateral healthy hemisphere, where less apoptosis occurs (Faustino et al., 2011). The reasons behind this low phagocytosis in stroke

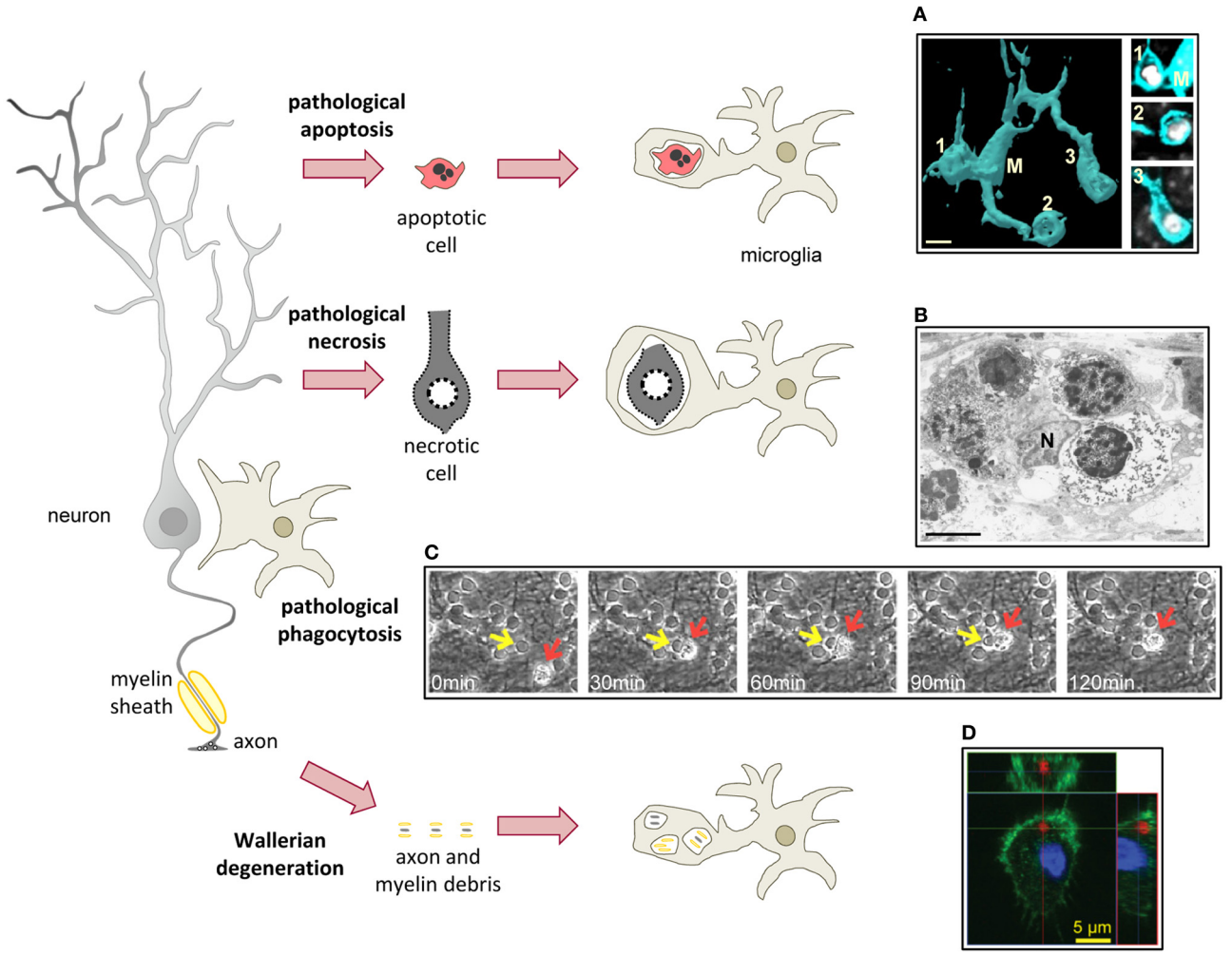


FIGURE 4 | Microglial phagocytosis in disease. In pathological conditions microglia is challenged and usually assumes a hypertrophic morphology. Challenged microglia phagocytose apoptotic and necrotic cells **(A,B)**. In some inflammatory conditions, microglial phagocytosis can kill healthy neurons **(C)**. Microglia also phagocytose axonal and myelin debris resulting from Wallerian degeneration of severed axons **(D)**. **(A)** Surface-rendered fms-EGFP microglia (M; cyan) from the adult hippocampus phagocytosing three apoptotic/pycnotic cells (labeled with DAPI, white), in a mouse systemically challenged with bacterial lipopolysaccharides (5 mg/kg). **(B)** Electron microscopy microphotograph of embryonic (E20) microglia (N, microglial nucleus) from the rat cingulate cortex following maternal hypoxia (1 day) containing four engulfed dead cells. In the developing brain, hypoxia induces a whole range of death mechanisms, including apoptosis, pathological apoptosis, and necrosis (Blomgren et al., 2007). **(C)** Time-lapse imaging of microglia (red arrow) engulfing a live neuron (yellow arrow) in a co-culture challenged with the TLR2 agonist lipoteichoich acid (50 µg/ml). **(D)** Orthogonal projection of a confocal z-stack showing the engulfment of degenerated axons (labeled with tdTomato, red) by microglia (labeled with Iba1, green; nucleus labeled with DAPI, blue) in a co-culture model. Scale bars, 5 µm. Image **(A)** is reprinted with permission from Elsevier from Sierra et al. (2010). Image **(B)** is reprinted with permission from Elsevier from Li et al. (1998). Image **(C)** is reprinted with permission from Neher et al. (2011), Copyright 2011. The American Association of Immunologists., Inc. Image **(D)** is reprinted with permission from Hosmane et al. (2012).

are unknown, but it has been speculated that high levels of pro-inflammatory cytokines produced by astrocytes and vessels may be the culprit (Faustino et al., 2011). However, microglial phagocytosis of apoptotic cells *in vitro* is increased by the pro-inflammatory interferon gamma (IFNγ), but it remains unaltered by TNFβ or TGFβ (Chan et al., 2001). Prion disease is another condition where microglial phagocytosis is impaired. *In vitro*, the pathogenic form of prion protein (PrP^{Sc}), reduces the phagocytosis of latex beads (Ciesielski-Treska et al., 2004); however *in vivo* (ME7 model of prion disease) microglia show increased phagocytosis of beads, and enhanced expression of the phagocytic machinery genes, including scavenger receptors, cathepsins, and proteins of the respiratory burst. In addition, qualitative phagocytosis of neuronal debris by microglia suggests that the microglial phagocytic machinery in prion disease is not disrupted (Hughes et al., 2010), but microglia do fail to phagocytose prion protein (PrP^{Sc}), which accumulates in the brain and leads to neurodegeneration (Hughes et al., 2010). PD is one more example in which the phagocytosis behavior of microglia is altered. Here the phagocytosis of latex beads is prevented by the aggregated

form, and enhanced by the monomeric form of alpha synuclein, which are found in the parenchyma and the CSF of parkinsonian patients, respectively (Park et al., 2008). Moreover, peripheral blood monocytes from PD patients, compared to age-matched controls, exhibit decreased phagocytosis of latex beads *ex vivo* (Salman et al., 1999), however defects in microglial phagocytosis have not been reported *in vivo* so far. Again, the lack of a standardized approach to quantify microglial phagocytosis hampers our understanding of its role in brain disorders where it is expected to play a significant role in removing cellular debris.

Death by necrosis is also an important component of many brain diseases. Contrary to apoptosis, necrosis is characterized by bursting of the cell membrane and spillover of cellular contents (Savill et al., 2002). Necrosis remains an obscure process, and molecular details of its execution are not well-known. Two main forms have been recognized: accidental necrosis, or cell lysis, by exposure of toxins, physical damage, freezing, etc.; and necrosis-like programmed cell death (PCD), which involves specialized caspase-independent signaling pathways such as the apoptosis-inducing factor (AIF) or cathepsins, and evolves without chromatin condensation (Leist and Jaattela, 2001). While accidental necrosis is unavoidable unless the stimulus is removed, cells can be rescued from necrotic PCD. The mechanisms of necrotic cells recognition by macrophages and microglia are not fully understood but may involve similar signaling pathways to the recognition of apoptotic cells. For instance, in some forms of necrotic PCD, exposure of PS by calcium-independent signals triggers recognition of necrotic cells by macrophages and microglia (Hirt et al., 2000; Hirt and Leist, 2003). One alternative mechanism could be the passive release of high mobility group 1 protein (HMGB1) by necrotic cells, which binds to the receptor for advanced glycation end products (RAGE) in phagocytes and triggers inflammation (Scaffidi et al., 2002). It is worth noting that HMGB1 is not released by apoptotic cells even when they fail to be phagocytosed and transform into secondary necrotic cells (Scaffidi et al., 2002). HMGB1 is also released during ischemia (assumedly, from the necrotic core) in stroke patients and in mice subjected to MCAO, launched an inflammatory response, which is detrimental for neuronal survival (Kim et al., 2006; Muhammad et al., 2008). Initial reports suggested that microglia does not mount an inflammatory response when co-cultured with necrotic PC12 neurons (De Simone et al., 2003). Others have shown that the phagocytosis of necrotic PCD Jurkat cells reduces the release of TNFα by microglia (Hirt and Leist, 2003). More recently, it has been shown that when co-cultured with necrotic HT22 neurons microglia do indeed express higher levels of pro-inflammatory cytokines (TNFα, IL-6), pro-inflammatory enzymes (nitric oxide synthase, 2-cyclooxygenase), MHC-II, and the integrin CD11b through a mechanism involving MYD88, a TLR adapter protein (Pais et al., 2008). In summary, very little is known about the efficiency and consequences of microglial phagocytosis of necrotic cells.

Death of living cells by phagocytosis, recently termed "phagoptosis," might contribute to the pathophysiology of some diseases where inflammation, the main trigger for phagoptosis, occurs. For instance AD, which characterized at the histological level by plaques of Aβ as well as neurofibrillary tangles, has a strong inflammatory component (Johnston et al., 2011). *In vitro*, low concentrations of Aβ (1–42) (nanomolar range) lead to activation of microglia and an inflammatory response, which is partly responsible for neuronal damage (Maezawa et al., 2011). In this situation, either depleting microglia, blocking vitronectin receptors, inhibiting cytoskeleton polymerization, or preventing recognition of PS with annexin V, all prevent neuronal death induced by nanomolar Aβ (Neniskyte et al., 2011). These data suggest that phagoptosis might be partly responsible for neuronal death induced by Aβ, perhaps providing an explanation for the low numbers of dead neurons found in AD (Neniskyte et al., 2011). Nonetheless, death by apoptosis has been documented in AD patients and in animal models of AD, although it is assumed to occur at low levels over a long period of time (Shimohama, 2000). We argue that the clearance time for phagoptotic neurons is likely to be longer than for phagocytosed apoptotic neurons, because phagoptotic cells fail to activate caspases and other mechanisms of self-destruction and rely exclusively on microglia for degradation (Brown and Neher, 2012). Time-lapse imaging experiments suggest that engulfing a live neuron may take microglia under 2 h (Neher et al., 2011) (**Figure 4**), however the time to fully degrade it has not been estimated. As a longer clearance time implies a higher probability of phagocytosis detection, it should then be possible to visualize and quantify microglial engulfment of live cells in AD or in other inflammatory diseases to ultimately determine the contribution of phagoptosis to brain pathologies *in vivo*.

MICROGLIAL PHAGOCYTOSIS OF INVADING MICROORGANISMS

In the adult and developing brain, microglia are capable of phagocytosing many pathogens, including bacteria (*Escherichia coli, Streptococcus pneumonia, Staphylococcus aureus, Enterococcus faecalis*), yeast (*Saccharomyces cerevisiae*), and fungus (*Candida albicans*) (Kaur et al., 2004; Falsig et al., 2008; Shah et al., 2008; Hadas et al., 2010; Ribes et al., 2010; Peppoloni et al., 2011; Kochan et al., 2012). Different PAMPs are recognized by different receptors: for instance, recognition of *S. aureus* peptidoglycan is mediated by TLR2 (Kielian et al., 2005); recognition of *E. coli* LPS is mediated by TLR4 (Sivagnanam et al., 2010); and recognition of *S. cerevisiae* β-glucans (such as zymosan) is mediated by Dectin1, CR3, and the mannose receptor (MR, CD206) (Hadas et al., 2010). TLR2 and 4 are constitutively expressed by microglia (Bsibsi et al., 2002) and recognition of their ligands enhances phagocytosis (Ribes et al., 2010; Kochan et al., 2012), supporting the capacity of microglia to efficiently engulf and degrade infecting microorganisms. For instance, *E. coli* bacteria injected into the corpus callosum of early postnatal rats are engulfed in large numbers 1–3 h after injection, and are completely eliminated in 24 h (Kaur et al., 2004). In contrast, phagosomes were found within microglial cells up to 7 days after (Kaur et al., 2004), suggesting that degradation and killing of living microorganisms is a time-consuming process. Phagocytosis of bacteria activates the inflammatory cascade in microglia, inducing the expression of pro-inflammatory cytokines (TNFα), TLRs (TLR4, TLR9), complement receptors (CR3), scavenger receptors (SRA), and MHC-II (Sivagnanam et al., 2010). Although the data on

microglial phagocytosis of microorganisms is not abundant, it seems to suggest a high capacity to fight against brain infections such as meningitis.

MICROGLIAL PHAGOCYTOSIS OF TUMOR CELLS

Brain tumors are very aggressive and have extremely poor prognosis possibly due to a failure of the innate and adaptive immune response to efficiently eliminate them. Tumor-associated microglia and macrophages (TAMs) are found in large numbers in human glioblastoma and have a complex and bidirectional relationship with glioma cells which has been reviewed in detail elsewhere (Watters et al., 2005). On one hand, glioma-initiating cells contribute to the recruitment of TAMs into the tumor mass (Yi et al., 2011) and polarize them toward a M2 phenotype (Wu et al., 2010). On the other hand, TAMs contribute to glioma progression by enhancing tumor migration and proliferation through growth factors, angiogenic molecules, and enzymes degrading the extracellular matrix (Watters et al., 2005). Unfortunately, glioma cells are rarely phagocytosed by TAMs in mouse models of glioma (Galarneau et al., 2007). Microglia from human glioblastoma patients do have the capability of phagocytosing latex microbeads *ex vivo* (Hussain et al., 2006), however *in vitro* studies have shown that co-culture with tumor cells decreases bead phagocytosis after a transient increase (Voisin et al., 2010). Further, it seems that tumor cells evade phagocytosis by lacking in their membrane the appropriate "eat-me" signals. In fact, microglia do phagocytose tumor cells which have been previously induced apoptosis with the cytotoxic agent etoposide (Chang et al., 2000) or UV light (Kulprathipanja and Kruse, 2004), although many tumor cells decrease their sensitivity to apoptosis inducers during oncogenic transformation (Maher et al., 2001). This data has suggested the use of alternative therapies to promote tumor cell apoptosis, such as oncolytic viruses, which selectively replicate in tumor cells, leading to their destruction (Zeyaullah et al., 2012). This therapy, while safe, has been proven ineffectual because TAMs clear the viruses away from the tumor (Fulci et al., 2007). While the literature confirms that TAM phagocytosis of tumor cells is not very effective, others have suggested that some tumor cells, particularly in highly invasive tumors such as glioblastoma, phagocytose neighboring cells perhaps as a way to fuel their constant growing [reviewed by Huysentruyt and Seyfried (2010)].

MICROGLIAL PHAGOCYTOSIS OF Aβ DEPOSITS

Aβ is a small peptide produced by proteolytic cleavage from amyloid precursor protein (APP) by β- and γ-secretases. The most pathogenic form is Aβ (1–42), which forms fibrils, insoluble aggregates found in amyloid plaques in the brains of AD patients, as well as human immunodeficiency virus (HIV)-associated neurocognitive disorders (Xu and Ikezu, 2009). A major effort has been put into developing therapies to lower the amyloid burden. Aβ reduction can occur either by decreasing its synthesis rate (e.g., with secretase inhibitors); or by increasing its elimination rate. The particular location of microglia surrounding plaques in human patients and mouse models of AD lead to the early suggestion that they could be responsible for releasing Aβ and forming the plaques (Lai and McLaurin, 2012). While microglia can synthesize Aβ *in vitro* (Banati et al., 1993), APP mRNA is not found in microglia of human AD brains (Scott et al., 1993). It was alternatively proposed that microglia could be responsible for phagocytosing Aβ and contribute to its clearance (Paresce et al., 1996). *In vitro*, microglia recognizes and engulfs fluorescently labeled fibrillary Aβ through a variety of receptors, including scavenger receptors (Paresce et al., 1996), TLR2 (Liu et al., 2012), and the TLR4-interacting molecule, CD14 (Liu et al., 2005). Further, Aβ induces a positive chemotaxis of microglia via TGFβ (Huang et al., 2010), possibly explaining their location around plaques. Recent live imaging experiments have shown that microglia is rapidly attracted to already formed plaques in a mouse model of AD with mutated APP and presenilin 1 (a protein of the γ-secretase complex) (Meyer-Luehmann et al., 2008), strongly suggesting that microglia does not participate in the initial stages of plaque formation and confirming the chemotactic nature of Aβ. Importantly, the size of the plaques remained constant and no evidence of phagocytosis or plaque clearance was obtained (Meyer-Luehmann et al., 2008). In agreement, a detailed 3D reconstruction of microglia and amyloid fibrils in APP mutated mice showed that microglial processes and amyloid fibrils were interlaced forming a network, but Aβ was not found within microglia, further suggesting that microglia does not phagocytose Aβ *in vivo* (Stalder et al., 2001). The failure of microglia to clear Aβ plaques of AD patients is unclear. Cultured microglia engulf and partially degrade Aβ *in vitro* over the first 3 days, but no additional degradation and a slow release of intact Aβ is found afterwards (Chung et al., 1999). Furthermore, microglia from old mutant APP/presenilin 1 mice have a decreased expression of phagocytic genes (SRA, CD36, RAGE, etc.) and increased expression of pro-inflammatory cytokines (TNFα, IL-1β), compared with age-matched wild type mice (Hickman et al., 2008), but both findings cannot explain the failure of microglia to sufficiently clear Aβ plaque *in vivo*. The role of inflammation in Aβ clearance is also not resolved. *In vitro*, inflammatory challenge by LPS, TNFα, IL-1β, or IFNγ inhibits fibrillary Aβ phagocytosis, whereas anti-inflammatory cytokines had no effect (Koenigsknecht-Talboo and Landreth, 2005). Aβ phagocytosis is blocked by the anti-inflammatory celecoxib (Persaud-Sawin et al., 2009), but not by minocyline (Familian et al., 2007). *In vivo*, LPS administered locally either transiently reduced (Herber et al., 2004), or enhanced (Qiao et al., 2001) Aβ load in mouse models of AD. The fractalkine receptor, a regulator of phagocytosis, has been found to contribute to the Aβ burden by some (Lee et al., 2010; Liu et al., 2010) but not others (Fuhrmann et al., 2010). Importantly, common treatments for HIV and AD may be detrimental for Aβ clearance, at least *in vitro*. HIV protease inhibitors either block Aβ degradation or enhance secretion of non-degraded Aβ by macrophages (Lan et al., 2012). Similarly, γ-secretase inhibitors prevent microglial Aβ engulfment (Farfara et al., 2011). Another point of controversy is the participation of invading macrophages. The blood-brain barrier (BBB) is partially disturbed in AD patients, facilitating the extravasation of circulating monocytes (Lai and McLaurin, 2012). Macrophages have higher Aβ capacity intake than microglia *in vitro* (Lai and McLaurin, 2012), and

ablation experiments have suggested that the Aβ burden is cleared by blood-borne macrophages, but not resident microglia (Simard et al., 2006). The issue is further complicated by the fact that experimental interventions such as irradiation of mice lead to invasion of blood-monocytes into the brain parenchyma and that macrophages and microglia are phenotypically indistinguishable by surface markers.

MICROGLIAL PHAGOCYTOSIS OF MYELIN AND AXONAL DEBRIS

The only available data on microglial phagocytosis of axonal debris comes from *in vitro* models. In cortical explants where growing neurites were sectioned, the debris was cleared by added microglia (Tanaka et al., 2009). In a compartmentalized co-culture model where neurons were grown in a chamber and their axons extended in bundles through microchannels, axotomy, or nitric oxide treatment-induced axonal degeneration and microglia rapidly cleared the axonal debris (Hosmane et al., 2012). Interestingly, the mechanisms of recognition of axonal debris seem to be different from those of apoptotic cells (Tanaka et al., 2009). A candidate receptor is TIR domain-containing adapter inducing interferon beta (TRIF). The clearance of axonal debris is prevented by blocking TRIF signaling *in vitro*, and TRIF-deficient mice have a smaller percentage of microglia containing neurofilament-positive axonal material after dorsal root axotomy (Hosmane et al., 2012). In both set ups, axonal debris (lacking myelin) had a detrimental effect on axon regrown, which was prevented by microglial phagocytosis (Tanaka et al., 2009; Hosmane et al., 2012), suggesting that enhancing microglial phagocytosis is a novel therapeutical tool in traumatic brain injuries.

More attention has been put into the mechanisms of myelin debris clearance, particularly during MS and in spinal cord and nerve injuries. In spinal cord injury, the degeneration of the severed axons through anterograde or Wallerian degeneration (WD) is followed by degradation of myelin and apoptosis of myelinating cells, oligodendrocytes (Crowe et al., 1997). The etiology of MS remains to be fully elucidated and several hypotheses have been proposed to explain the demyelination found in patients, from an autoimmune attack to myelin followed by axonal degeneration, to the developmental, environmental, or virus-induced degeneration of axons by WD followed by myelin degradation; in both cases, myelin debris accumulates (Stys et al., 2012). Myelin proteins such as Nogo are well-known to interfere with axonal regeneration and repair, therefore an efficient myelin clearance is an absolute requirement for recovery (Wang et al., 2002). Furthermore, myelin inhibits its own phagocytosis through myelin CD47 binding to microglia and macrophages signal regulatory protein α (SIRPα) (Gitik et al., 2011). Contrary to the peripheral nervous system (PNS), myelin clearance after WD is very inefficient in the CNS [reviewed in Gaudet et al. (2011)]. In PNS lesions, the myelinating Schwann cells are the major phagocytic population in the first few days, followed by invading macrophages (Hirata and Kawabuchi, 2002). Importantly, macrophages express Nogo receptors NgR1 and NgR2, which facilitate their migration out of the healing nerve, thus resulting in the resolution of the inflammatory response (Fry et al., 2007). In culture, myelin-phagocytosing macrophages inhibit T cell proliferation, further containing the immune response (Bogie et al., 2011). The rapid clearance of myelin and resolution of the immune response is greatly responsible for regeneration after nerve injury.

In contrast, CNS regeneration after trauma does not occur, and microglial poor phagocytosing capabilities together with poor or slow recruitment of macrophages are partly to blame (Gaudet et al., 2011). *In vitro*, microglia recognize and phagocytose myelin through CR3, SRA, and FcRs among other receptors (Smith, 2001). Microglia do phagocytose myelin to some extent in mouse models of MS, a phenomenon which is stimulated by the presence of MBP-reactive T cells (Nielsen et al., 2009). However, myelin debris is still observed in the human spinal cord years after the injury (Buss et al., 2004). Together with insufficient activation of microglia, the absence of autoreactive antibodies, and the subsequent lack of activation of FcRs in traumatic brain injury have been suggested to explain why microglia phagocytose myelin in MS but not in spinal cord injury (Rotshenker, 2003). Microglial phagocytosis of myelin after hemisection of the ascending sensory tract is increased after treating the mice with LPS, resulting in decreased myelin debris, but, unfortunately, no axonal regeneration (Vallieres et al., 2006), possibly because of the pro-inflammatory phenotype induced by LPS. More recently, a microglial phenotype supportive of remyelination has been described (Olah et al., 2012). During the remyelination phase after cuprizone-induced demyelination in the corpus callosum, a mouse model of MS, myelin-phagocytosing microglia express genes involved not only in phagocytosis but also in the activation, migration, proliferation, and differentiation of oligodendrocytes precursor cells (Olah et al., 2012). While it remains to be directly assessed whether myelin phagocytosis triggers this remyelination-supportive phenotype, this data suggests that the beneficial consequences of enhancing microglial phagocytosis of myelin may be two-fold: clearing myelin and facilitating remyelination.

CONCLUSION

In conclusion, microglial phagocytosis is a pivotal mechanism of clearance of cellular debris in health and disease. Like Janus, the roman god of war and peace, microglial phagocytosis has beneficial (e.g., anti-inflammatory) and detrimental (e.g., respiratory burst) consequences for tissue homeostasis which remain largely unexplored *in vivo*. In particular, the establishment of standardized methods to quantify microglial phagocytosis of different types of cargo, as well as the development of novel tools to specifically block recognition, engulfment, and degradation of cargo, will undoubtedly delineate the ultimate impact of microglial phagocytosis *in vivo*.

ACKNOWLEDGMENTS

Research of Anahita Shahraz and Harald Neumann was supported by the Hertie-Foundation and the Deutsche Forschungsgemeinschaft (FOR1336, SFB704, KFO177). Research of Oihane Abiega and Amanda Sierra was supported by Ikerbasque, the Basque Foundation of Science.

REFERENCES

Anand, R. J., Kohler, J. W., Cavallo, J. A., Li, J., Dubowski, T., and Hackam, D. J. (2007). Toll-like receptor 4 plays a role in macrophage phagocytosis during peritoneal sepsis. *J. Pediatr. Surg.* 42, 927–932. discussion: 933.

Armstrong, A., and Ravichandran, K. S. (2011). Phosphatidylserine receptors: what is the new RAGE? *EMBO Rep.* 12, 287–288.

Ashwell, K. (1990). Microglia and cell death in the developing mouse cerebellum. *Brain Res. Dev. Brain Res.* 55, 219–230.

Banati, R. B., Gehrmann, J., Czech, C., Monning, U., Jones, L. L., Konig, G., et al. (1993). Early and rapid *de novo* synthesis of Alzheimer beta A4-amyloid precursor protein (APP) in activated microglia. *Glia* 9, 199–210.

Barger, S. W., Goodwin, M. E., Porter, M. M., and Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J. Neurochem.* 101, 1205–1213.

Barres, B. A., Hart, I. K., Coles, H. S., Burne, J. F., Voyvodic, J. T., Richardson, W. D., et al. (1992). Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* 70, 31–46.

Bianchin, M. M., Capella, H. M., Chaves, D. L., Steindel, M., Grisard, E. C., Ganev, G. G., et al. (2004). Nasu-Hakola disease (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy–PLOSL): a dementia associated with bone cystic lesions. From clinical to genetic and molecular aspects. *Cell. Mol. Neurobiol.* 24, 1–24.

Blank, M., and Shiloh, Y. (2007). Programs for cell death: apoptosis is only one way to go. *Cell Cycle* 6, 686–695.

Block, M. L., Zecca, L., and Hong, J. S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69.

Blomgren, K., Leist, M., and Groc, L. (2007). Pathological apoptosis in the developing brain. *Apoptosis* 12, 993–1010.

Bogie, J. F., Stinissen, P., Hellings, N., and Hendriks, J. J. (2011). Myelin-phagocytosing macrophages modulate autoreactive T cell proliferation. *J. Neuroinflammation* 8:85. doi: 10.1186/1742-2094-8-85

Boullier, A., Bird, D. A., Chang, M. K., Dennis, E. A., Friedman, P., Gillotre-Taylor, K., et al. (2001). Scavenger receptors, oxidized LDL, and atherosclerosis. *Ann. N.Y. Acad. Sci.* 947, 214–222. discussion: 222–223.

Brown, G. C., and Neher, J. J. (2012). Eaten alive! Cell death by primary phagocytosis: 'phagoptosis'. *Trends Biochem. Sci.* 37, 325–332.

Bsibsi, M., Ravid, R., Gveric, D., and van Noort, J. M. (2002). Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol.* 61, 1013–1021.

Buckwalter, M. S., Yamane, M., Coleman, B. S., Ormerod, B. K., Chin, J. T., Palmer, T., et al. (2006). Chronically increased transforming growth factor-beta1 strongly inhibits hippocampal neurogenesis in aged mice. *Am. J. Pathol.* 169, 154–164.

Bulloch, K., Miller, M. M., Gal-Toth, J., Milner, T. A., Gottfried-Blackmore, A., Waters, E. M., et al. (2008). CD11c/EYFP transgene illuminates a discrete network of dendritic cells within the embryonic, neonatal, adult, and injured mouse brain. *J. Comp. Neurol.* 508, 687–710.

Buss, A., Brook, G. A., Kakulas, B., Martin, D., Franzen, R., Schoenen, J., et al. (2004). Gradual loss of myelin and formation of an astrocytic scar during Wallerian degeneration in the human spinal cord. *Brain* 127, 34–44.

Bylund, J., Brown, K. L., Movitz, C., Dahlgren, C., and Karlsson, A. (2010). Intracellular generation of superoxide by the phagocyte NADPH oxidase: how, where, and what for? *Free Radic. Biol. Med.* 49, 1834–1845.

Ciesielski-Treska, J., Grant, N. J., Ulrich, G., Corrotte, M., Bailly, Y., Haeberle, A. M., et al. (2004). Fibrillar prion peptide (106-126) and scrapie prion protein hamper phagocytosis in microglia. *Glia* 46, 101–115.

Crehan, H., Hardy, J., and Pocock, J. (2012). Microglia, Alzheimer's disease, and complement. *Int. J. Alzheimers Dis.* 2012:983640. doi: 10.1155/2012/983640

Crowe, M. J., Bresnahan, J. C., Shuman, S. L., Masters, J. N., and Beattie, M. S. (1997). Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. *Nat. Med.* 3, 73–76.

Chan, A., Magnus, T., and Gold, R. (2001). Phagocytosis of apoptotic inflammatory cells by microglia and modulation by different cytokines: mechanism for removal of apoptotic cells in the inflamed nervous system. *Glia* 33, 87–95.

Chang, G. H., Barbaro, N. M., and Pieper, R. O. (2000). Phosphatidylserine-dependent phagocytosis of apoptotic glioma cells by normal human microglia, astrocytes, and glioma cells. *Neuro Oncol.* 2, 174–183.

Chung, H., Brazil, M. I., Soe, T. T., and Maxfield, F. R. (1999). Uptake, degradation, and release of fibrillar and soluble forms of Alzheimer's amyloid beta-peptide by microglial cells. *J. Biol. Chem.* 274, 32301–32308.

Dalmau, I., Vela, J. M., Gonzalez, B., Finsen, B., and Castellano, B. (2003). Dynamics of microglia in the developing rat brain. *J. Comp. Neurol.* 458, 144–157.

Damoiseaux, J. G., Dopp, E. A., Calame, W., Chao, D., MacPherson, G. G., and Dijkstra, C. D. (1994). Rat macrophage lysosomal membrane antigen recognized by monoclonal antibody ED1. *Immunology* 83, 140–147.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758.

Daws, M. R., Sullam, P. M., Niemi, E. C., Chen, T. T., Tchao, N. K., and Seaman, W. E. (2003). Pattern recognition by TREM-2: binding of anionic ligands. *J. Immunol.* 171, 594–599.

Decoursey, T. E., and Ligeti, E. (2005). Regulation and termination of NADPH oxidase activity. *Cell. Mol. Life Sci.* 62, 2173–2193.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

De Simone, R., Ajmone-Cat, M. A., Nicolini, A., and Minghetti, L. (2002). Expression of phosphatidylserine receptor and down-regulation of pro-inflammatory molecule production by its natural ligand in rat microglial cultures. *J. Neuropathol. Exp. Neurol.* 61, 237–244.

De Simone, R., Ajmone-Cat, M. A., Tirassa, P., and Minghetti, L. (2003). Apoptotic PC12 cells exposing phosphatidylserine promote the production of anti-inflammatory and neuroprotective molecules by microglial cells. *J. Neuropathol. Exp. Neurol.* 62, 208–216.

Desjardins, M., Huber, L. A., Parton, R. G., and Griffiths, G. (1994). Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. *J. Cell Biol.* 124, 677–688.

Diez-Roux, G., and Lang, R. A. (1997). Macrophages induce apoptosis in normal cells *in vivo*. *Development* 124, 3633–3638.

Dupuy, A. G., and Caron, E. (2008). Integrin-dependent phagocytosis: spreading from microadhesion to new concepts. *J. Cell Sci.* 121, 1773–1783.

Durafourt, B. A., Moore, C. S., Zammit, D. A., Johnson, T. A., Zaguia, F., Guiot, M. C., et al. (2012). Comparison of polarization properties of human adult microglia and blood-derived macrophages. *Glia* 60, 717–727.

Dustin, M. L. (2012). Signaling at neuro/immune synapses. *J. Clin. Invest.* 122, 1149–1155.

Egensperger, R., Maslim, J., Bisti, S., Hollander, H., and Stone, J. (1996). Fate of DNA from retinal cells dying during development: uptake by microglia and macroglia (Muller cells). *Brain Res. Dev. Brain Res.* 97, 1–8.

Elliott, M. R., Chekeni, F. B., Trampont, P. C., Lazarowski, E. R., Kadl, A., Walk, S. F., et al. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 461, 282–286.

Erdman, L. K., Cosio, G., Helmers, A. J., Gowda, D. C., Grinstein, S., and Kain, K. C. (2009). CD36 and TLR interactions in inflammation and phagocytosis: implications for malaria. *J. Immunol.* 183, 6452–6459.

Fadok, V. A., Bratton, D. L., Konowal, A., Freed, P. W., Westcott, J. Y., and Henson, P. M. (1998). Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J. Clin. Invest.* 101, 890–898.

Fallman, M., Andersson, R., and Andersson, T. (1993). Signaling properties of CR3 (CD11b/CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles. *J. Immunol.* 151, 330–338.

Falsig, J., van Beek, J., Hermann, C., and Leist, M. (2008). Molecular basis for detection of invading pathogens in the brain. *J. Neurosci. Res.* 86, 1434–1447.

Familian, A., Eikelenboom, P., and Veerhuis, R. (2007). Minocycline does not affect amyloid beta phagocytosis by human microglial cells. *Neurosci. Lett.* 416, 87–91.

Farfara, D., Trudler, D., Segev-Amzaleg, N., Galron, R., Stein, R., and

Frenkel, D. (2011). gamma-Secretase component presenilin is important for microglia beta-amyloid clearance. *Ann. Neurol.* 69, 170–180.

Faustino, J. V., Wang, X., Johnson, C. E., Klibanov, A., Derugin, N., Wendland, M. F., et al. (2011). Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. *J. Neurosci.* 31, 12992–13001.

Flannagan, R. S., Jaumouille, V., and Grinstein, S. (2012). The cell biology of phagocytosis. *Annu. Rev. Pathol.* 7, 61–98.

Ford, A. L., Goodsall, A. L., Hickey, W. F., and Sedgwick, J. D. (1995). Normal adult ramified microglia separated from other central nervous system macrophages by flow cytometric sorting. Phenotypic differences defined and direct *ex vivo* antigen presentation to myelin basic protein-reactive CD4+ T cells compared. *J. Immunol.* 154, 4309–4321.

Fraser, D. A., Pisalyaput, K., and Tenner, A. J. (2010). C1q enhances microglial clearance of apoptotic neurons and neuronal blebs, and modulates subsequent inflammatory cytokine production. *J. Neurochem.* 112, 733–743.

Freeman, G. J., Casasnovas, J. M., Umetsu, D. T., and DeKruyff, R. H. (2010). TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. *Immunol. Rev.* 235, 172–189.

Fricker, M., Neher, J. J., Zhao, J. W., Thery, C., Tolkovsky, A. M., and Brown, G. C. (2012). MFG-E8 mediates primary phagocytosis of viable neurons during neuroinflammation. *J. Neurosci.* 32, 2657–2666.

Frielingsdorf, H., Simpson, D. R., Thal, L. J., and Pizzo, D. P. (2007). Nerve growth factor promotes survival of new neurons in the adult hippocampus. *Neurobiol. Dis.* 26, 47–55.

Fry, E. J., Ho, C., and David, S. (2007). A role for Nogo receptor in macrophage clearance from injured peripheral nerve. *Neuron* 53, 649–662.

Fuhrmann, M., Bittner, T., Jung, C. K., Burgold, S., Page, R. M., Mitteregger, G., et al. (2010). Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nat. Neurosci.* 13, 411–413.

Fulci, G., Dmitrieva, N., Gianni, D., Fontana, E. J., Pan, X., Lu, Y., et al. (2007). Depletion of peripheral macrophages and brain microglia increases brain tumor titers of oncolytic viruses. *Cancer Res.* 67, 9398–9406.

Gaikwad, S., Larionov, S., Wang, Y., Dannenberg, H., Matozaki, T., Monsonego, A., et al. (2009). Signal regulatory protein-beta1: a microglial modulator of phagocytosis in Alzheimer's disease. *Am. J. Pathol.* 175, 2528–2539.

Galarneau, H., Villeneuve, J., Gowing, G., Julien, J. P., and Vallieres, L. (2007). Increased glioma growth in mice depleted of macrophages. *Cancer Res.* 67, 8874–8881.

Galea, I., Bechmann, I., and Perry, V. H. (2007). What is immune privilege (not)? *Trends Immunol.* 28, 12–18.

Galic, M. A., Riazi, K., and Pittman, Q. J. (2012). Cytokines and brain excitability. *Front. Neuroendocrinol.* 33:116–125. doi: 10.1016/j.yfrne.2011.12.002

Garin, J., Diez, R., Kieffer, S., Dermine, J. F., Duclos, S., Gagnon, E., et al. (2001). The phagosome proteome: insight into phagosome functions. *J. Cell Biol.* 152, 165–180.

Gaudet, A. D., Popovich, P. G., and Ramer, M. S. (2011). Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J. Neuroinflammation* 8:110. doi: 10.1186/1742-2094-8-110

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Gitik, M., Liraz-Zaltsman, S., Oldenborg, P. A., Reichert, F., and Rotshenker, S. (2011). Myelin down-regulates myelin phagocytosis by microglia and macrophages through interactions between CD47 on myelin and SIRPalpha (signal regulatory protein-alpha) on phagocytes. *J. Neuroinflammation* 8:24. doi: 10.1186/1742-2094-8-24

Goh, Y. C., Yap, C. T., Huang, B. H., Cronshaw, A. D., Leung, B. P., Lai, P. B., et al. (2011). Heat-shock protein 60 translocates to the surface of apoptotic cells and differentiated megakaryocytes and stimulates phagocytosis. *Cell. Mol. Life Sci.* 68, 1581–1592.

Gottfried-Blackmore, A., Kaunzner, U. W., Idoyaga, J., Felger, J. C., McEwen, B. S., and Bulloch, K. (2009). Acute *in vivo* exposure to interferon-gamma enables resident brain dendritic cells to become effective antigen presenting cells. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20918–20923.

Goverman, J. M. (2011). Immune tolerance in multiple sclerosis. *Immunol. Rev.* 241, 228–240.

Granucci, F., Petralia, F., Urbano, M., Citterio, S., Di Tota, F., Santambrogio, L., et al. (2003). The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. *Blood* 102, 2940–2947.

Greenberg, M. E., Sun, M., Zhang, R., Febbraio, M., Silverstein, R., and Hazen, S. L. (2006). Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J. Exp. Med.* 203, 2613–2625.

Grommes, C., Lee, C. Y., Wilkinson, B. L., Jiang, Q., Koenigsknecht-Talboo, J. L., Varnum, B., et al. (2008). Regulation of microglial phagocytosis and inflammatory gene expression by Gas6 acting on the Axl/Mer family of tyrosine kinases. *J. Neuroimmune Pharmacol.* 3, 130–140.

Gumienny, T. L., Brugnera, E., Tosello-Trampont, A. C., Kinchen, J. M., Haney, L. B., Nishiwaki, K., et al. (2001). CED-12/ELMO, a novel member of the CrkII/Dock180/Rac pathway, is required for phagocytosis and cell migration. *Cell* 107, 27–41.

Hadas, S., Reichert, F., and Rotshenker, S. (2010). Dissimilar and similar functional properties of complement receptor-3 in microglia and macrophages in combating yeast pathogens by phagocytosis. *Glia* 58, 823–830.

Han, C. Z., and Ravichandran, K. S. (2011). Metabolic connections during apoptotic cell engulfment. *Cell* 147, 1442–1445.

Hart, S. P., Alexander, K. M., and Dransfield, I. (2004). Immune complexes bind preferentially to Fc gamma RIIA (CD32) on apoptotic neutrophils, leading to augmented phagocytosis by macrophages and release of proinflammatory cytokines. *J. Immunol.* 172, 1882–1887.

Hayashi, A., Ohnishi, H., Okazawa, H., Nakazawa, S., Ikeda, H., Motegi, S., et al. (2004). Positive regulation of phagocytosis by SIRPbeta and its signaling mechanism in macrophages. *J. Biol. Chem.* 279, 29450–29460.

He, M., Kubo, H., Morimoto, K., Fujino, N., Suzuki, T., Takahasi, T., et al. (2011). Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells. *EMBO Rep.* 12, 358–364.

Henson, P. M., and Hume, D. A. (2006). Apoptotic cell removal in development and tissue homeostasis. *Trends Immunol.* 27, 244–250.

Heppner, F. L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hovelmeyer, N., et al. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nat. Med.* 11, 146–152.

Herber, D. L., Roth, L. M., Wilson, D., Wilson, N., Mason, J. E., Morgan, D., et al. (2004). Time-dependent reduction in Abeta levels after intracranial LPS administration in APP transgenic mice. *Exp. Neurol.* 190, 245–253.

Hickman, S. E., Allison, E. K., and El Khoury, J. (2008). Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J. Neurosci.* 28, 8354–8360.

Hirata, K., and Kawabuchi, M. (2002). Myelin phagocytosis by macrophages and nonmacrophages during Wallerian degeneration. *Microsc. Res. Tech.* 57, 541–547.

Hirt, U. A., Gantner, F., and Leist, M. (2000). Phagocytosis of nonapoptotic cells dying by caspase-independent mechanisms. *J. Immunol.* 164, 6520–6529.

Hirt, U. A., and Leist, M. (2003). Rapid, noninflammatory and PS-dependent phagocytic clearance of necrotic cells. *Cell Death Differ.* 10, 1156–1164.

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., et al. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* 209, 1167–1181.

Hoffmann, P. R., deCathelineau, A. M., Ogden, C. A., Leverrier, Y., Bratton, D. L., Daleke, D. L., et al. (2001). Phosphatidylserine (PS) induces PS receptor-mediated macropinocytosis and promotes clearance of apoptotic cells. *J. Cell Biol.* 155, 649–659.

Hosmane, S., Tegenge, M. A., Rajbhandari, L., Uapinyoying, P., Kumar, N. G., Thakor, N., et al. (2012). Toll/interleukin-1 receptor domain-containing adapter inducing interferon-beta mediates microglial phagocytosis of degenerating axons. *J. Neurosci.* 32, 7745–7757.

Hsieh, C. L., Koike, M., Spusta, S. C., Niemi, E. C., Yenari, M., Nakamura, M. C., et al. (2009). A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. *J. Neurochem.* 109, 1144–1156.

Huang, W. C., Yen, F. C., Shie, F. S., Pan, C. M., Shiao, Y. J., Yang, C. N., et al. (2010). TGF-beta1 blockade of microglial chemotaxis toward Abeta aggregates involves SMAD signaling and down-regulation of CCL5. *J. Neuroinflammation* 7:28. doi: 10.1186/1742-2094-7-28

Hughes, M. M., Field, R. H., Perry, V. H., Murray, C. L., and Cunningham, C. (2010). Microglia in the degenerating brain are capable of phagocytosis of beads and of apoptotic cells, but do not efficiently remove PrPSc, even upon LPS stimulation. *Glia* 58, 2017–2030.

Hussain, S. F., Yang, D., Suki, D., Aldape, K., Grimm, E., and Heimberger, A. B. (2006). The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. *Neuro Oncol.* 8, 261–279.

Huysentruyt, L. C., and Seyfried, T. N. (2010). Perspectives on the mesenchymal origin of metastatic cancer. *Cancer Metastasis Rev.* 29, 695–707.

Johnston, H., Boutin, H., and Allan, S. M. (2011). Assessing the contribution of inflammation in models of Alzheimer's disease. *Biochem. Soc. Trans.* 39, 886–890.

Kaifu, T., Nakahara, J., Inui, M., Mishima, K., Momiyama, T., Kaji, M., et al. (2003). Osteopetrosis and thalamic hypomyelinosis with synaptic degeneration in DAP12-deficient mice. *J. Clin. Invest.* 111, 323–332.

Kao, A. W., Eisenhut, R. J., Martens, L. H., Nakamura, A., Huang, A., Bagley, J. A., et al. (2011). A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4441–4446.

Kaur, C., Too, H. F., and Ling, E. A. (2004). Phagocytosis of *Escherichia coli* by amoeboid microglial cells in the developing brain. *Acta Neuropathol.* 107, 204–208.

Kawane, K., Fukuyama, H., Yoshida, H., Nagase, H., Ohsawa, Y., Uchiyama, Y., et al. (2003). Impaired thymic development in mouse embryos deficient in apoptotic DNA degradation. *Nat. Immunol.* 4, 138–144.

Kettenmann, H. (2007). Neuroscience: the brain's garbage men. *Nature* 446, 987–989.

Kevany, B. M., and Palczewski, K. (2010). Phagocytosis of retinal rod and cone photoreceptors. *Physiology (Bethesda)* 25, 8–15.

Kielian, T., Esen, N., and Bearden, E. D. (2005). Toll-like receptor 2 (TLR2) is pivotal for recognition of *S. aureus* peptidoglycan but not intact bacteria by microglia. *Glia* 49, 567–576.

Kim, J. B., Sig Choi, J., Yu, Y. M., Nam, K., Piao, C. S., Kim, S. W., et al. (2006). HMGB1, a novel cytokine-like mediator linking acute neuronal death and delayed neuroinflammation in the postischemic brain. *J. Neurosci.* 26, 6413–6421.

Kim, S., Park, S. Y., Kim, S. Y., Bae, D. J., Pyo, J. H., Hong, M., et al. (2012). Cross talk between engulfment receptors stabilin-2 and integrin alphavbeta5 orchestrates engulfment of phosphatidylserine-exposed erythrocytes. *Mol. Cell. Biol.* 32, 2698–2708.

Klesney-Tait, J., Turnbull, I. R., and Colonna, M. (2006). The TREM receptor family and signal integration. *Nat. Immunol.* 7, 1266–1273.

Kochan, T., Singla, A., Tosi, J., and Kumar, A. (2012). Toll-like receptor 2 ligand pretreatment attenuates retinal microglial inflammatory response but enhances phagocytic activity toward *Staphylococcus aureus*. *Infect. Immun.* 80, 2076–2088.

Koenigsknecht-Talboo, J., and Landreth, G. E. (2005). Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. *J. Neurosci.* 25, 8240–8249.

Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., et al. (2007). UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. *Nature* 446, 1091–1095.

Kulprathipanja, N. V., and Kruse, C. A. (2004). Microglia phagocytose alloreactive CTL-damaged 9L gliosarcoma cells. *J. Neuroimmunol.* 153, 76–82.

Lai, A. Y., and McLaurin, J. (2012). Clearance of amyloid-beta peptides by microglia and macrophages: the issue of what, when and where. *Future Neurol.* 7, 165–176.

Lambertsen, K. L., Biber, K., and Finsen, B. (2012). Inflammatory cytokines in experimental and human stroke. *J. Cereb. Blood Flow Metab.* 32, 1677–1698.

Lan, X., Kiyota, T., Hanamsagar, R., Huang, Y., Andrews, S., Peng, H., et al. (2012). The effect of HIV protease inhibitors on amyloid-beta peptide degradation and synthesis in human cells and Alzheimer's disease animal model. *J. Neuroimmune Pharmacol.* 7, 412–423.

Landreth, G. E., and Reed-Geaghan, E. G. (2009). Toll-like receptors in Alzheimer's disease. *Curr. Top. Microbiol. Immunol.* 336, 137–153.

Lang, R. A., and Bishop, J. M. (1993). Macrophages are required for cell death and tissue remodeling in the developing mouse eye. *Cell* 74, 453–462.

Lauber, K., Blumenthal, S. G., Waibel, M., and Wesselborg, S. (2004). Clearance of apoptotic cells: getting rid of the corpses. *Mol. Cell* 14, 277–287.

Lee, S., Varvel, N. H., Konerth, M. E., Xu, G., Cardona, A. E., Ransohoff, R. M., et al. (2010). CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two Alzheimer's disease mouse models. *Am. J. Pathol.* 177, 2549–2562.

Lee, W. L., Mason, D., Schreiber, A. D., and Grinstein, S. (2007). Quantitative analysis of membrane remodeling at the phagocytic cup. *Mol. Biol. Cell* 18, 2883–2892.

Leist, M., and Jaattela, M. (2001). Four deaths and a funeral: from caspases to alternative mechanisms. *Nat. Rev. Mol. Cell Biol.* 2, 589–598.

Leist, M., Volbracht, C., Kuhnle, S., Fava, E., Ferrando-May, E., and Nicotera, P. (1997). Caspase-mediated apoptosis in neuronal excitotoxicity triggered by nitric oxide. *Mol. Med.* 3, 750–764.

Li, Q., Jagannath, C., Rao, P. K., Singh, C. R., and Lostumbo, G. (2010). Analysis of phagosomal proteomes: from latex-bead to bacterial phagosomes. *Proteomics* 10, 4098–4116.

Li, Y. B., Kaur, C., and Ling, E. A. (1998). Neuronal degeneration and microglial reaction in the fetal and postnatal rat brain after transient maternal hypoxia. *Neurosci. Res.* 32, 137–148.

Linnartz, B., Kopatz, J., Tenner, A. J., and Neumann, H. (2012). Sialic acid on the neuronal glycocalyx prevents complement C1 binding and complement receptor-3-mediated removal by microglia. *J. Neurosci.* 32, 946–952.

Linnartz, B., and Neumann, H. (2012). Microglial activatory (immunoreceptor tyrosine-based activation motif)- and inhibitory (immunoreceptor tyrosine-based inhibition motif)-signaling receptors for recognition of the neuronal glycocalyx. *Glia* 61, 37–46.

Litman, G. W., Cannon, J. P., and Dishaw, L. J. (2005). Reconstructing immune phylogeny: new perspectives. *Nat. Rev. Immunol.* 5, 866–879.

Liu, S., Liu, Y., Hao, W., Wolf, L., Kiliaan, A. J., Penke, B., et al. (2012). TLR2 is a primary receptor for Alzheimer's amyloid beta peptide to trigger neuroinflammatory activation. *J. Immunol.* 188, 1098–1107.

Liu, Y., Hao, W., Letiembre, M., Walter, S., Kulanga, M., Neumann, H., et al. (2006). Suppression of microglial inflammatory activity by myelin phagocytosis: role of p47-PHOX-mediated generation of reactive oxygen species. *J. Neurosci.* 26, 12904–12913.

Liu, Y., Walter, S., Stagi, M., Cherny, D., Letiembre, M., Schulz-Schaeffer, W., et al. (2005). LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. *Brain* 128, 1778–1789.

Liu, Z., Condello, C., Schain, A., Harb, R., and Grutzendler, J. (2010). CX3CR1 in microglia regulates brain amyloid deposition through selective protofibrillar amyloid-beta phagocytosis. *J. Neurosci.* 30, 17091–17101.

Lowell, C. A. (2011). Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: signaling cross talk. *Cold Spring Harb. Perspect. Biol.* 3:pii: a002352. doi: 10.1101/cshperspect.a002352

Lowell, C. A., Soriano, P., and Varmus, H. E. (1994). Functional overlap in the src gene family: inactivation of hck and fgr impairs natural immunity. *Genes Dev.* 8, 387–398.

Lu, Z., Elliott, M. R., Chen, Y., Walsh, J. T., Klibanov, A. L., Ravichandran, K. S., et al. (2011). Phagocytic activity of neuronal progenitors regulates adult neurogenesis. *Nat. Cell Biol.* 13, 1076–1083.

Lucas, M., Stuart, L. M., Zhang, A., Hodivala-Dilke, K., Febbraio, M., Silverstein, R., et al. (2006). Requirements for apoptotic cell contact in regulation of macrophage responses. *J. Immunol.* 177, 4047–4054.

Luo, L., and O'Leary, D. D. (2005). Axon retraction and degeneration in development and disease. *Annu. Rev. Neurosci.* 28, 127–156.

Maezawa, I., Zimin, P. I., Wulff, H., and Jin, L. W. (2011). Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity. *J. Biol. Chem.* 286, 3693–3706.

Magnus, T., Chan, A., Grauer, O., Toyka, K. V., and Gold, R. (2001). Microglial phagocytosis of apoptotic inflammatory T cells leads to down-regulation of microglial immune activation. *J. Immunol.* 167, 5004–5010.

Magnus, T., Chan, A., Linker, R. A., Toyka, K. V., and Gold, R. (2002). Astrocytes are less efficient in the removal of apoptotic lymphocytes

than microglia cells: implications for the role of glial cells in the inflamed central nervous system. *J. Neuropathol. Exp. Neurol.* 61, 760–766.

Maher, E. A., Furnari, F. B., Bachoo, R. M., Rowitch, D. H., Louis, D. N., Cavenee, W. K., et al. (2001). Malignant glioma: genetics and biology of a grave matter. *Genes Dev.* 15, 1311–1333.

Mantovani, A., Sozzani, S., Locati, M., Allavena, P., and Sica, A. (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* 23, 549–555.

Marin-Teva, J. L., Dusart, I., Colin, C., Gervais, A., van Rooijen, N., and Mallat, M. (2004). Microglia promote the death of developing Purkinje cells. *Neuron* 41, 535–547.

Marzolo, M. P., von Bernhardi, R., and Inestrosa, N. C. (1999). Mannose receptor is present in a functional state in rat microglial cells. *J. Neurosci. Res.* 58, 387–395.

McArthur, S., Cristante, E., Paterno, M., Christian, H., Roncaroli, F., Gillies, G. E., et al. (2010). Annexin A1: a central player in the anti-inflammatory and neuroprotective role of microglia. *J. Immunol.* 185, 6317–6328.

McCoy, C. E., and O'Neill, L. A. (2008). The role of toll-like receptors in macrophages. *Front. Biosci.* 13, 62–70.

McIlroy, D., Tanaka, M., Sakahira, H., Fukuyama, H., Suzuki, M., Yamamura, K., et al. (2000). An auxiliary mode of apoptotic DNA fragmentation provided by phagocytes. *Genes Dev.* 14, 549–558.

Meyer-Luehmann, M., Spires-Jones, T. L., Prada, C., Garcia-Alloza, M., de Calignon, A., Rozkalne, A., et al. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature* 451, 720–724.

Minakami, R., and Sumimotoa, H. (2006). Phagocytosis-coupled activation of the superoxide-producing phagocyte oxidase, a member of the NADPH oxidase (nox) family. *Int. J. Hematol.* 84, 193–198.

Mizuno, T. (2012). The biphasic role of microglia in Alzheimer's disease. *Int. J. Alzheimers Dis.* 2012:737846. doi: 10.1155/2012/737846

Muhammad, S., Barakat, W., Stoyanov, S., Murikinati, S., Yang, H., Tracey, K. J., et al. (2008). The HMGB1 receptor RAGE mediates ischemic brain damage. *J. Neurosci.* 28, 12023–12031.

Mukherjee, S., Ghosh, R. N., and Maxfield, F. R. (1997). Endocytosis. *Physiol. Rev.* 77, 759–803.

Nagata, S., Hanayama, R., and Kawane, K. (2010). Autoimmunity and the clearance of dead cells. *Cell* 140, 619–630.

Neher, J. J., Neniskyte, U., Zhao, J. W., Bal-Price, A., Tolkovsky, A. M., and Brown, G. C. (2011). Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. *J. Immunol.* 186, 4973–4983.

Neniskyte, U., Neher, J. J., and Brown, G. C. (2011). Neuronal death induced by nanomolar amyloid beta is mediated by primary phagocytosis of neurons by microglia. *J. Biol. Chem.* 286, 39904–39913.

Neumann, J., Sauerzweig, S., Ronicke, R., Gunzer, F., Dinkel, K., Ullrich, O., et al. (2008). Microglia cells protect neurons by direct engulfment of invading neutrophil granulocytes: a new mechanism of CNS immune privilege. *J. Neurosci.* 28, 5965–5975.

Newell, E. W., Stanley, E. F., and Schlichter, L. C. (2007). Reversed Na+/Ca2+ exchange contributes to Ca2+ influx and respiratory burst in microglia. *Channels (Austin)* 1, 366–376.

Nielsen, H. H., Ladeby, R., Fenger, C., Toft-Hansen, H., Babcock, A. A., Owens, T., et al. (2009). Enhanced microglial clearance of myelin debris in T cell-infiltrated central nervous system. *J. Neuropathol. Exp. Neurol.* 68, 845–856.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318.

Noda, M., Doi, Y., Liang, J., Kawanokuchi, J., Sonobe, Y., Takeuchi, H., et al. (2011). Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression. *J. Biol. Chem.* 286, 2308–2319.

Noda, M., and Suzumura, A. (2012). Sweepers in the CNS: microglial migration and phagocytosis in the alzheimer disease pathogenesis. *Int. J. Alzheimers Dis.* 2012:891087. doi: 10.1155/2012/891087

Ogden, C. A., deCathelineau, A., Hoffmann, P. R., Bratton, D., Ghebrehiwet, B., Fadok, V. A., et al. (2001). C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J. Exp. Med.* 194, 781–795.

O'Kusky, J. R., Ye, P., and D'Ercole, A. J. (2000). Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. *J. Neurosci.* 20, 8435–8442.

Olah, M., Amor, S., Brouwer, N., Vinet, J., Eggen, B., Biber, K., et al. (2012). Identification of a microglia phenotype supportive of remyelination. *Glia* 60, 306–321.

Pais, T. F., Figueiredo, C., Peixoto, R., Braz, M. H., and Chatterjee, S. (2008). Necrotic neurons enhance microglial neurotoxicity through induction of glutaminase by a MyD88-dependent pathway. *J. Neuroinflammation* 5:43. doi: 10.1186/1742-2094-5-43

Paloneva, J., Manninen, T., Christman, G., Hovanes, K., Mandelin, J., Adolfsson, R., et al. (2002). Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. *Am. J. Hum. Genet.* 71, 656–662.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Paresce, D. M., Ghosh, R. N., and Maxfield, F. R. (1996). Microglial cells internalize aggregates of the Alzheimer's disease amyloid beta-protein via a scavenger receptor. *Neuron* 17, 553–565.

Park, J. Y., Paik, S. R., Jou, I., and Park, S. M. (2008). Microglial phagocytosis is enhanced by monomeric alpha-synuclein, not aggregated alpha-synuclein: implications for Parkinson's disease. *Glia* 56, 1215–1223.

Parnaik, R., Raff, M. C., and Scholes, J. (2000). Differences between the clearance of apoptotic cells by professional and non-professional phagocytes. *Curr. Biol.* 10, 857–860.

Patel, M., Pelletier, A., and Cote, J. F. (2011). Opening up on ELMO regulation: new insights into the control of Rac signaling by the DOCK180/ELMO complex. *Small GTPases* 2, 268–275.

Peppoloni, S., Posteraro, B., Colombari, B., Manca, L., Hartke, A., Giard, J. C., et al. (2011). Role of the (Mn)superoxide dismutase of *Enterococcus faecalis* in the *in vitro* interaction with microglia. *Microbiology* 157, 1816–1822.

Peri, F., and Nusslein-Volhard, C. (2008). Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion *in vivo*. *Cell* 133, 916–927.

Persaud-Sawin, D. A., Banach, L., and Harry, G. J. (2009). Raft aggregation with specific receptor recruitment is required for microglial phagocytosis of Abeta42. *Glia* 57, 320–335.

Pickering, M., Cumiskey, D., and O'Connor, J. J. (2005). Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. *Exp. Physiol.* 90, 663–670.

Pourova, J., Kottova, M., Voprsalova, M., and Pour, M. (2010). Reactive oxygen and nitrogen species in normal physiological processes. *Acta Physiol. (Oxf.)* 198, 15–35.

Qiao, X., Cummins, D. J., and Paul, S. M. (2001). Neuroinflammation-induced acceleration of amyloid deposition in the APPV717F transgenic mouse. *Eur. J. Neurosci.* 14, 474–482.

Qin, L., Liu, Y., Wang, T., Wei, S. J., Block, M. L., Wilson, B., et al. (2004). NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. *J. Biol. Chem.* 279, 1415–1421.

Quan, D. N., Cooper, M. D., Potter, J. L., Roberts, M. H., Cheng, H., and Jarvis, G. A. (2008). TREM-2 binds to lipooligosaccharides of *Neisseria gonorrhoeae* and is expressed on reproductive tract epithelial cells. *Mucosal Immunol.* 1, 229–238.

Quinn, M. T., and Gauss, K. A. (2004). Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. *J. Leukoc. Biol.* 76, 760–781.

Raivich, G., Bohatschek, M., Kloss, C. U., Werner, A., Jones, L. L., and Kreutzberg, G. W. (1999). Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Res. Brain Res. Rev.* 30, 77–105.

Ransohoff, R. M., and Engelhardt, B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat. Rev. Immunol.* 12, 623–635.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Ravichandran, K. S. (2010). Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. *J. Exp. Med.* 207, 1807–1817.

Reddien, P. W., Cameron, S., and Horvitz, H. R. (2001). Phagocytosis

promotes programmed cell death in *C. elegans*. *Nature* 412, 198–202.

Reubold, T. F., and Eschenburg, S. (2012). A molecular view on signal transduction by the apoptosome. *Cell. Signal.* 24, 1420–1425.

Ribes, S., Ebert, S., Regen, T., Agarwal, A., Tauber, S. C., Czesnik, D., et al. (2010). Toll-like receptor stimulation enhances phagocytosis and intracellular killing of nonencapsulated and encapsulated *Streptococcus pneumoniae* by murine microglia. *Infect. Immun.* 78, 865–871.

Rogers, N. J., Lees, M. J., Gabriel, L., Maniati, E., Rose, S. J., Potter, P. K., et al. (2009). A defect in Marco expression contributes to systemic lupus erythematosus development via failure to clear apoptotic cells. *J. Immunol.* 182, 1982–1990.

Rotshenker, S. (2003). Microglia and macrophage activation and the regulation of complement-receptor-3 (CR3/MAC-1)-mediated myelin phagocytosis in injury and disease. *J. Mol. Neurosci.* 21, 65–72.

Rotshenker, S. (2009). The role of Galectin-3/MAC-2 in the activation of the innate-immune function of phagocytosis in microglia in injury and disease. *J. Mol. Neurosci.* 39, 99–103.

Roumier, A., Bechade, C., Poncer, J. C., Smalla, K. H., Tomasello, E., Vivier, E., et al. (2004). Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. *J. Neurosci.* 24, 11421–11428.

Salman, H., Bergman, M., Djaldetti, R., Bessler, H., and Djaldetti, M. (1999). Decreased phagocytic function in patients with Parkinson's disease. *Biomed. Pharmacother.* 53, 146–148.

Satoh, J., Tabunoki, H., Ishida, T., Yagishita, S., Jinnai, K., Futamura, N., et al. (2011). Immunohistochemical characterization of microglia in Nasu-Hakola disease brains. *Neuropathology* 31, 363–375.

Savill, J., Dransfield, I., Gregory, C., and Haslett, C. (2002). A blast from the past: clearance of apoptotic cells regulates immune responses. *Nat. Rev. Immunol.* 2, 965–975.

Scaffidi, P., Misteli, T., and Bianchi, M. E. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 418, 191–195.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., et al. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336, 86–90.

Scott, S. A., Johnson, S. A., Zarow, C., and Perlmutter, L. S. (1993). Inability to detect beta-amyloid protein precursor mRNA in Alzheimer plaque-associated microglia. *Exp. Neurol.* 121, 113–118.

Shah, V. B., Huang, Y., Keshwara, R., Ozment-Skelton, T., Williams, D. L., and Keshvara, L. (2008). Beta-glucan activates microglia without inducing cytokine production in Dectin-1-dependent manner. *J. Immunol.* 180, 2777–2785.

Shimohama, S. (2000). Apoptosis in Alzheimer's disease–an update. *Apoptosis* 5, 9–16.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Simard, A. R., Soulet, D., Gowing, G., Julien, J. P., and Rivest, S. (2006). Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 49, 489–502.

Sivagnanam, V., Zhu, X., and Schlichter, L. C. (2010). Dominance of *E.* coli phagocytosis over LPS in the inflammatory response of microglia. *J. Neuroimmunol.* 227, 111–119.

Smith, M. E. (2001). Phagocytic properties of microglia *in vitro*: implications for a role in multiple sclerosis and EAE. *Microsc. Res. Tech.* 54, 81–94.

Song, L., Lee, C., and Schindler, C. (2011). Deletion of the murine scavenger receptor CD68. *J. Lipid Res.* 52, 1542–1550.

Spranger, M., Kiprianova, I., Krempien, S., and Schwab, S. (1998). Reoxygenation increases the release of reactive oxygen intermediates in murine microglia. *J. Cereb. Blood Flow Metab.* 18, 670–674.

Stalder, M., Deller, T., Staufenbiel, M., and Jucker, M. (2001). 3D-Reconstruction of microglia and amyloid in APP23 transgenic mice: no evidence of intracellular amyloid. *Neurobiol. Aging* 22, 427–434.

Stefano, L., Racchetti, G., Bianco, F., Passini, N., Gupta, R. S., Panina Bordignon, P., et al. (2009). The surface-exposed chaperone, Hsp60, is an agonist of the microglial TREM2 receptor. *J. Neurochem.* 110, 284–294.

Stern, M., Savill, J., and Haslett, C. (1996). Human monocyte-derived macrophage phagocytosis of senescent eosinophils undergoing apoptosis. Mediation by alpha v beta 3/CD36/thrombospondin recognition mechanism and lack of phlogistic response. *Am. J. Pathol.* 149, 911–921.

Streit, W. J., Walter, S. A., and Pennell, N. A. (1999). Reactive microgliosis. *Prog. Neurobiol.* 57, 563–581.

Stys, P. K., Zamponi, G. W., van Minnen, J., and Geurts, J. J. (2012). Will the real multiple sclerosis please stand up? *Nat. Rev. Neurosci.* 13, 507–514.

Takahashi, K., Prinz, M., Stagi, M., Chechneva, O., and Neumann, H. (2007). TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. *PLoS Med.* 4:e124. doi: 10.1371/journal.pmed.0040124

Takahashi, K., Rochford, C. D., and Neumann, H. (2005). Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J. Exp. Med.* 201, 647–657.

Tanaka, T., Ueno, M., and Yamashita, T. (2009). Engulfment of axon debris by microglia requires p38 MAPK activity. *J. Biol. Chem.* 284, 21626–21636.

Taylor, P. R., Martinez-Pomares, L., Stacey, M., Lin, H. H., Brown, G. D., and Gordon, S. (2005). Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* 23, 901–944.

Testa-Silva, G., Loebel, A., Giugliano, M., de Kock, C. P., Mansvelder, H. D., and Meredith, R. M. (2012). Hyperconnectivity and slow synapses during early development of medial prefrontal cortex in a mouse model for mental retardation and autism. *Cereb. Cortex* 22, 1333–1342.

Thored, P., Heldmann, U., Gomes-Leal, W., Gisler, R., Darsalia, V., Taneera, J., et al. (2009). Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* 57, 835–849.

Tremblay, M. E., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Truman, L. A., Ford, C. A., Pasikowska, M., Pound, J. D., Wilkinson, S. J., Dumitriu, I. E., et al. (2008). CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood* 112, 5026–5036.

Ueyama, T., Lennartz, M. R., Noda, Y., Kobayashi, T., Shirai, Y., Rikitake, K., et al. (2004). Superoxide production at phagosomal cup/phagosome through beta I protein kinase C during Fc gamma R-mediated phagocytosis in microglia. *J. Immunol.* 173, 4582–4589.

Ulvestad, E., Williams, K., Bjerkvig, R., Tiekotter, K., Antel, J., and Matre, R. (1994). Human microglial cells have phenotypic and functional characteristics in common with both macrophages and dendritic antigen-presenting cells. *J. Leukoc. Biol.* 56, 732–740.

Underhill, D. M., and Goodridge, H. S. (2012). Information processing during phagocytosis. *Nat. Rev. Immunol.* 12, 492–502.

Vallieres, N., Berard, J. L., David, S., and Lacroix, S. (2006). Systemic injections of lipopolysaccharide accelerates myelin phagocytosis during Wallerian degeneration in the injured mouse spinal cord. *Glia* 53, 103–113.

van der Laan, L. J., Dopp, E. A., Haworth, R., Pikkarainen, T., Kangas, M., Elomaa, O., et al. (1999). Regulation and functional involvement of macrophage scavenger receptor MARCO in clearance of bacteria *in vivo*. *J. Immunol.* 162, 939–947.

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., and Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann. Neurol.* 57, 67–81.

Voisin, P., Bouchaud, V., Merle, M., Diolez, P., Duffy, L., Flint, K., et al. (2010). Microglia in close vicinity of glioma cells: correlation between phenotype and metabolic alterations. *Front. Neuroenergetics* 2:131. doi: 10.3389/fnene.2010.00131

Voll, R. E., Herrmann, M., Roth, E. A., Stach, C., Kalden, J. R., and Girkontaite, I. (1997). Immunosuppressive effects of apoptotic cells. *Nature* 390, 350–351.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly

monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Wakselman, S., Bechade, C., Roumier, A., Bernard, D., Triller, A., and Bessis, A. (2008). Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. *J. Neurosci.* 28, 8138–8143.

Wang, A., Das, P., Switzer, R. C. 3rd., Golde, T. E., and Jankowsky, J. L. (2011). Robust amyloid clearance in a mouse model of Alzheimer's disease provides novel insights into the mechanism of amyloid-beta immunotherapy. *J. Neurosci.* 31, 4124–4136.

Wang, K. C., Koprivica, V., Kim, J. A., Sivasankaran, R., Guo, Y., Neve, R. L., et al. (2002). Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* 417, 941–944.

Wang, Y., and Neumann, H. (2010). Alleviation of neurotoxicity by microglial human Siglec-11. *J. Neurosci.* 30, 3482–3488.

Watters, J. J., Schartner, J. M., and Badie, B. (2005). Microglia function in brain tumors. *J. Neurosci. Res.* 81, 447–455.

Welser-Alves, J. V., Boroujerdi, A., Tigges, U., and Milner, R. (2011). Microglia use multiple mechanisms to mediate interactions with vitronectin; non-essential roles for the highly-expressed alphavbeta3 and alphavbeta5 integrins. *J. Neuroinflammation* 8:157. doi: 10.1186/1742-2094-8-157

Wilkinson, K., and El Khoury, J. (2012). Microglial scavenger receptors and their roles in the pathogenesis of Alzheimer's disease. *Int. J. Alzheimers Dis.* 2012:489456. doi: 10.1155/2012/489456

Williams, D. W., Kondo, S., Krzyzanowska, A., Hiromi, Y., and Truman, J. W. (2006). Local caspase activity directs engulfment of dendrites during pruning. *Nat. Neurosci.* 9, 1234–1236.

Williams, D. W., and Truman, J. W. (2005). Cellular mechanisms of dendrite pruning in Drosophila: insights from *in vivo* time-lapse of remodeling dendritic arborizing sensory neurons. *Development* 132, 3631–3642.

Williams, K., Ulvestad, E., Waage, A., Antel, J. P., and McLaurin, J. (1994). Activation of adult human derived microglia by myelin phagocytosis *in vitro*. *J. Neurosci. Res.* 38, 433–443.

Wu, A., Wei, J., Kong, L. Y., Wang, Y., Priebe, W., Qiao, W., et al. (2010). Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol.* 12, 1113–1125.

Wu, H. H., Bellmunt, E., Scheib, J. L., Venegas, V., Burkert, C., Reichardt, L. F., et al. (2009). Glial precursors clear sensory neuron corpses during development via Jedi-1, an engulfment receptor. *Nat. Neurosci.* 12, 1534–1541.

Xu, J., and Ikezu, T. (2009). The comorbidity of HIV-associated neurocognitive disorders and Alzheimer's disease: a foreseeable medical challenge in post-HAART era. *J. Neuroimmune Pharmacol.* 4, 200–212.

Yi, L., Xiao, H., Xu, M., Ye, X., Hu, J., Li, F., et al. (2011). Glioma-initiating cells: a predominant role in microglia/macrophages tropism to glioma. *J. Neuroimmunol.* 232, 75–82.

Yokoyama, N., deBakker, C. D., Zappacosta, F., Huddleston, M. J., Annan, R. S., Ravichandran, K. S., et al. (2005). Identification of tyrosine residues on ELMO1 that are phosphorylated by the Src-family kinase Hck. *Biochemistry* 44, 8841–8849.

Zeyaullah, M., Patro, M., Ahmad, I., Ibraheem, K., Sultan, P., Nehal, M., et al. (2012). Oncolytic viruses in the treatment of cancer: a review of current strategies. *Pathol. Oncol. Res.* 18, 771–781.

Zhang, D., Sun, L., Zhu, H., Wang, L., Wu, W., Xie, J., et al. (2012). Microglial LOX-1 reacts with extracellular HSP60 to bridge neuroinflammation and neurotoxicity. *Neurochem. Int.* 61, 1021–1035.

Zhang, S., Boyd, J., Delaney, K., and Murphy, T. H. (2005). Rapid reversible changes in dendritic spine structure *in vivo* gated by the degree of ischemia. *J. Neurosci.* 25, 5333–5338.

Zimmer, H., Riese, S., and Regnier-Vigouroux, A. (2003). Functional characterization of mannose receptor expressed by immunocompetent mouse microglia. *Glia* 42, 89–100.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 October 2012; paper pending published: 11 November 2012; accepted: 09 January 2013; published online: 30 January 2013.

Citation: Sierra A, Abiega O, Shahraz A and Neumann H (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. Front. Cell. Neurosci. 7:6. doi: 10.3389/fncel.2013.00006

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 15 May 2013
doi: 10.3389/fncel.2013.00070

Microglia and synapse interactions: fine tuning neural circuits and candidate molecules

Akiko Miyamoto[1,2], Hiroaki Wake[3]*, Andrew J. Moorhouse[4] and Junichi Nabekura[1,2,5]

[1] *Division of Homeostatic Development, National Institute for Physiological Sciences, Okazaki, Japan*
[2] *Department of Physiological Sciences, The Graduate School for Advanced Study, Hayama, Japan*
[3] *Division of Brain Circuits, National Institute for Basic Biology, Okazaki, Japan*
[4] *School of Medical Sciences, The University of New South Wales, Sydney, NSW Australia*
[5] *Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Saitama, Japan*

Edited by:
Marie-Eve Tremblay, Université Laval, Canada

Reviewed by:
Etienne Audinat, Université Paris Descartes, France
Rosa C. Paolicelli, University of Zurich, Switzerland

****Correspondence:***
Hiroaki Wake, Assistant Professor, Division of Brain Circuits, National Institute for Basic Biology, 38 Nishigonaka, Myodaijicho, Okazaki, 444-8585, Japan.
e-mail: hirowake@nibb.ac.jp

Brain function depends critically on the interactions among the underlying components that comprise neural circuits. This includes coordinated activity in pre-synaptic and postsynaptic neuronal elements, but also in the non-neuronal elements such as glial cells. Microglia are glial cells in the central nervous system (CNS) that have well-known roles in neuronal immune function, responding to infections and brain injury and influencing the progress of neurodegenerative disorders. However, microglia are also surveyors of the healthy brain, continuously extending and retracting their processes and making contacts with pre- and postsynaptic elements of neural circuits, a process that clearly consumes considerable energy. Pruning of synapses during development and in response to injury has also been documented, and we propose that this extensive surveillance of the brain parenchyma in adult healthy brain results in similar "fine-tuning" of neural circuits. A reasonable extension is that a dysfunction of such a homeostatic role of microglia could be a primary cause of neuronal disease. Indeed, neuronal functions including cognition, personality, and information processing are affected by immune status. In this review we focus on the interactions between microglia and synapses, the possible cellular and molecular mechanisms that mediate such contacts, and the possible implications these interactions may have in the fine tuning of neural circuits that is so important for physiological brain function.

Keywords: microglia, synapse, plasticity, elimination

INTRODUCTION

Microglia are hematopoietic-cell derived glial cells in the central nervous system (CNS) that function as the only resident immune cells of the CNS (Ginhoux et al., 2010; Prinz and Mildner, 2011). Consistent with their immune cell status, microglia combat brain infections and diseases. This includes playing a significant role in the pathological progression of some of the major neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and chronic pain (Finsen et al., 1993; Coull et al., 2005; Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009; Graeber and Streit, 2010; Kettenmann et al., 2011). Microglia in these disease states are generally secondary responders; in that they undergo a change in morphology and cytokine expression profile in response to the initial insult, through a process defined as an "activation." Indeed most studies of microglia and disease have focused on the role of these activated microglia in disease progression (Lassmann et al., 1993; Perry et al., 2010). A key remaining question is whether microglia can also be the primary cause of neuronal and psychiatric diseases. To address this, one needs to first characterize their physiological functions in healthy brain and then hypothesis that dysfunction of such physiological roles could result in specific neurological and psychiatric disease.

MICROGLIA—SYNAPSE CONTACTS AND FUNCTIONAL CONSEQUENCES

Non-"activated" microglia in the healthy brain are highly motile cells, extending and retracting their processes as they survey the microenvironment in the CNS (Nimmerjahn et al., 2005). The multiple components of the synapse represent a major target of this extensive surveillance by the ramified microglia's processes (Wake et al., 2009; Tremblay et al., 2010). In somatosensory cortex of young mice, microglia made brief (≈5 min) contacts with synapses with a frequency of about 1 contact/hour (Wake et al., 2009). Both pre-synaptic boutons and postsynaptic spines were contacted by microglial processes. In visual cortex, the microglia-synapse contacts were examined in closer resolution using 3D reconstruction serial electron microscopy (Tremblay et al., 2010). This study revealed that, in addition to pre- and postsynaptic specializations, microglial processes also contacted peri-synaptic astrocytes and the synaptic cleft (Tremblay et al., 2010).

Such a thorough surveillance of all components of the synapse poses the question about what are the functional consequences of this surveillance. In developing and injured brain, a critical function of microglia-synapse contacts appears to be in shaping, or re-wiring, neuronal circuits by phagocytosis (**Figure 1**).

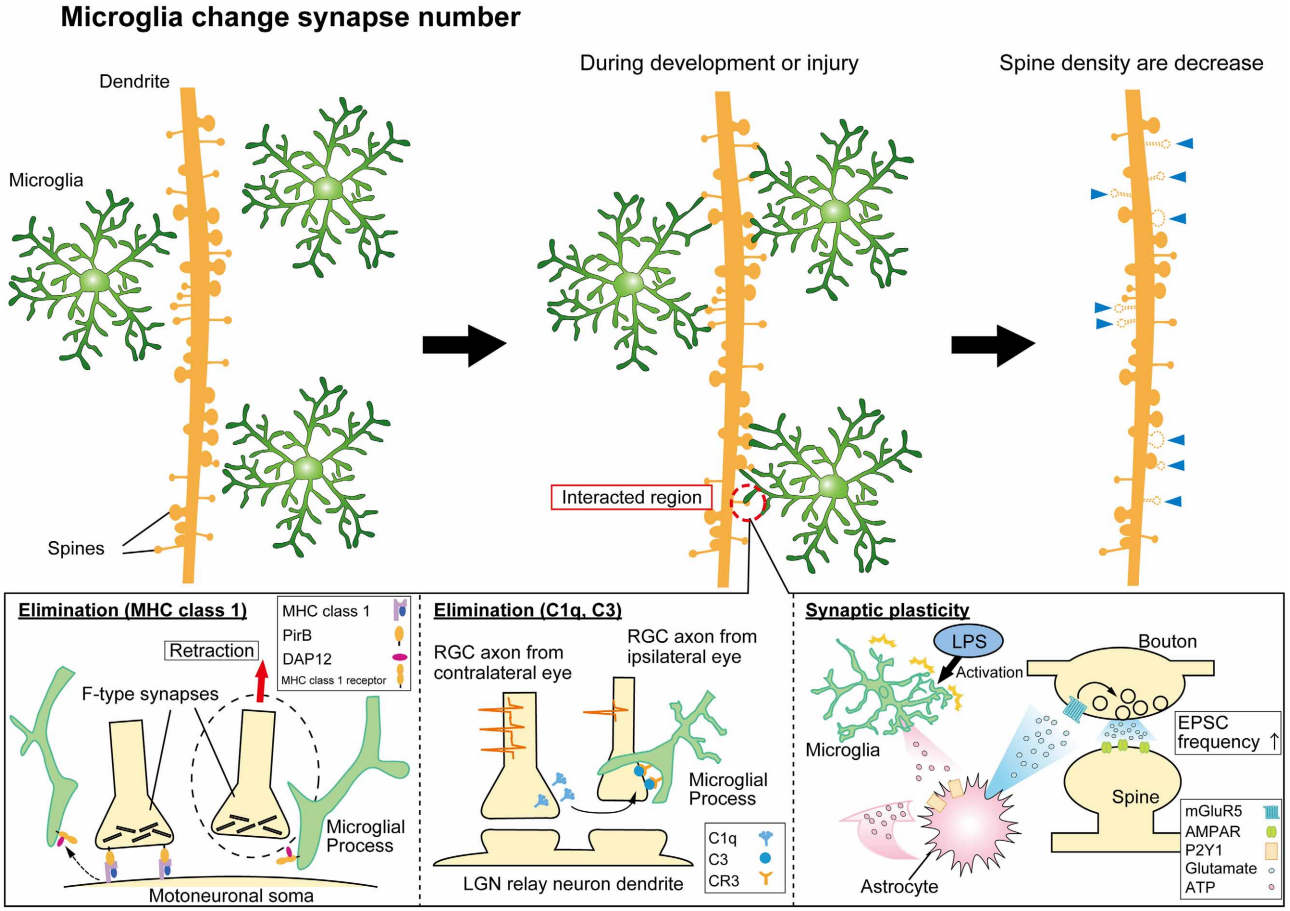


FIGURE 1 | Synaptic modification by microglial cells. Microglia monitor and interact with synapses to modulate neural circuit formation and function. Microglia can phagocytose "weak" synapses during development and injury to modify synapse numbers (**top** panels). The lower panels indicate mechanisms mediating functional microglia-synapse interactions. Candidate molecules such as MHC class 1 proteins (**lower left** panel) and complement cascade proteins (**lower center** panel) have been suggested to contribute to the phagocytosis of synapses by microglia. Activated microglia can also modify functional transmission at synapses via signaling pathways involving ATP, astrocytes, and glutamate receptors as indicated (**lower right** panel).

Specific presynaptic (SNAP25) and postsynaptic (PSD95) proteins have been identified inside microglial processes following synaptic contacts, by confocal or immune-gold electron microscopy, respectively (Paolicelli et al., 2011). The synaptic pruning that accompanies developmental refinements of neural circuits coincides with a period of increased density of resident CNS microglia and involves microglial phagocytosis of synapses. This has been most elegantly demonstrated in the developing visual system, where excessive synapses from each retina into the lateral geniculate nuclei (LGN) in the thalamus become pruned by microglia as appropriate binocular visual maps are formed. Genetic deletion of key components of the complement signaling pathway (discussed below) decreased microglial phagocytosis and disrupts this developmental pruning, with LGN neurons inappropriately retaining innervation from both eyes (Schafer et al., 2012). Similarly, conditions which result in enhanced developmental plasticity in V1 cortex of the visual system (dark adaptation during critical period) are associated with evidence for increased phagocytosis of synaptic debris by microglia (Tremblay et al., 2010). Interestingly, microglial contacts with postsynaptic spines appeared to alter their growth and/or morphology (**Figure 1**), perhaps as part of phagocytosis and remodeling of these elements. In aged animals with reduced auditory function there was also an increase in phagocytotic synaptic debris in microglia in auditory cortex (Tremblay et al., 2012). Hence, developmental and experience-dependent plasticity may involve microglial interactions with synapses and physical remodeling of this component of neural circuits. Experiments to disrupt microglia-synapse contacts in a well-defined synaptic plasticity paradigm and to determine the functional consequences at the ultra-structural and behavioral level will be important in substantiating these exciting hypotheses.

Microglia have long been known to phagocytose neuronal debris as a result of traumatic injury or infections (Perry and Gordon, 1988; Graeber and Streit, 2010), but microglia also can more selectively remove specific neuronal structures, such as neurite extensions in cultured neurons (Linnartz et al., 2012)

or axonal terminals and synapses projecting on to injured and degenerating neurons (Oliveira et al., 2004; Yamada et al., 2008). This occurs in the facial injury model, where transection of the motor neurons results in removal of the afferent inputs on to the axotomized neurons (Blinzinger and Kreutzberg, 1968; Graeber et al., 1993). The density of microglia (and astrocytes) increase at the synaptic cleft and appear to tear off the afferent terminals by phagocytosis—the so-called "synaptic stripping" model (Blinzinger and Kreutzberg, 1968). Such synaptic stripping has also been observed in cerebral cortex following microglia activation by bacterial fragments (Trapp et al., 2007). However, as noted by Perry and O'Connor (2010), the close apposition of microglia and synapses in these studies does not constitute evidence for an active role of microglia in "synapse stripping." In cortex ischemic penumbra, microglia-synapse contacts become markedly prolonged, from ≈5 min to ≈60 min, and this correlates to an increased turnover of presynaptic boutons and postsynaptic spines (Brown et al., 2007; Wake et al., 2009). With prolonged time-lapse imaging, some synapses that had experienced this prolonged contact were indeed observed to disappear, suggesting a possible direct link between microglia-synapse contact and post-ischemic synapse remodeling. Again, experiments designed to selectively modulate these contacts and then to test the effects on post-injury recovery and rewiring will be insightful in determining the functional consequences of such injury induced phagocytosis.

Microglia- synapse interactions are also observed in several neurodegenerative diseases. A reduction in the function and number of synapses, along with an activation of microglia, is an early event in the pathogenesis of Alzheimer's disease, Huntington's disease, and other neurodegenerative diseases (Perry and O'Connor, 2010). Whether microglia initiate such synapse defects is unclear, but two examples of potential mechanisms will be briefly highlighted. In a neuron—microglia coculture model of Alzheimer's disease, microglia activation releases interleukin-1 that leads to a loss of synaptophysin via phosphorylation of tau (Li et al., 2003). Loss of synaptic proteins like synaptophysin apparently strongly correlates with impaired cognitive function (Coleman et al., 2003; Li et al., 2003). Secondly, the activation of microglial CB2 receptors in a Huntington's disease mouse model (the R6/2 mouse) attenuates neurodegeneration and cognitive decline by reducing microglia activation (Palazuelos et al., 2009). These and similar studies hint at a relationship between microglia activation and synaptic and cognitive defects in neurodegenerative diseases, but further investigation is needed to demonstrate a direct link and underlying mechanisms.

MECHANISMS OF MICROGLIA-SYNAPSE CONTACTS

General observations indicate that there may exist some specific signaling mechanism to direct microglial processes to synapses. Firstly, the surveillance in healthy brain does not seem completely random but is directed toward synaptic compartments (Wake et al., 2009; Tremblay et al., 2012). Similarly in certain situations, such as in developmental pruning and synaptic stripping, microglial phagocytosis is focused on synapses although neuronal soma and other CNS constituents can be frequently phagocytosed by microglia during injury or apoptosis (MaríN-Teva et al., 2004; Sierra et al., 2010). A number of features of synapses—high metabolic rates and activity per unit area, high transmitter and ion turnover, close appositions with astrocytes—may underlie these signals although recent evidence has focused on the importance of neuronal activity. The mechanisms that may mediate microglial-synapse contacts can be broadly classed into "find me" and "eat me" signals, partly based on analogies with the peripheral immune system, and neuronal activity appears to act as a "find-me" signal by both increasing microglial process motility and/or contact frequency (Nimmerjahn et al., 2005; Wake et al., 2009; Tremblay et al., 2010). A recent report from zebrafish larval optic tectum neurons directly correlated a higher relative microglial contact frequency with neurons with higher electrical activity (Li et al., 2012). The model adopted readily enabled the authors to directly visualize neurons and combine imaging of GFP labeled microglia with Ca^{2+} fluorescence as a measure of neuronal activity. More active neurons received more frequent contacts. Transfection of an inward rectifying K^+ channel (Kir2.1) to reduce global activity (Hua et al., 2005), reduced microglial contact frequency. Furthermore, following contact neuronal activity was decreased, as if microglia were turning down hyperactive neurons (Li et al., 2012). Whether microglial processes are specifically targeted to more active synapses in mammalian CNS has yet to be directly determined and if similar approaches can be applied to mammalian CNS synapses the question may be resolved. The answer is bound to be striking, as the exact opposite is observed for synapse pruning in developing visual system of mice. In developmental synapse elimination, weaker synapses are eliminated while stronger ones are maintained or strengthened, a classic rule that also applies to Hebbian plasticity and learning (Penn et al., 1998; Lichtman and Colman, 2000; Hooks and Chen, 2006; Kano and Hashimoto, 2009). When the activity of afferent inputs from each eyes are decreased by tetrodotoxin, the extent of microglial phagocytosis of their terminals in the LGN is increased. On the contrary: when afferent nerve activity is increased by forskolin injections the extent of microglial phagocytosis is reduced (Schafer et al., 2012). Hence, although neuronal activity may increase microglial motility and/or synapse contact frequency (and neuronal soma contacts in zebrafish), it decreases the extent of phagocytosis of presynaptic terminals at least. How neuronal activity, microglial process dynamics and phagocytosis are linked, and how weaker and stronger synapses are detected and marked for phagocytosis by microglia, are important and fascinating questions that still need to be answered.

Adenosine triphosphate (ATP) has been revealed as a clear "find me" signal, with surveillent microglia strongly attracted to the source of ATP, and undergo activation toward a more phagocytic phenotype, via a signaling pathway involving P2Y purinoceptors (Davalos et al., 2005; Koizumi et al., 2007; Ohsawa and Kohsaka, 2011). ATP is a likely candidate mediating activity-dependent recruitment of microglia (Fontainhas et al., 2011; Li et al., 2012). Glutamate has also been considered a potential candidate "find-me" signal mediating activity dependent microglia migration, although its ability to attract microglia may only hold for a more activated phenotype (Fontainhas et al., 2011).

Furthermore, the effects of glutamate may be largely mediated by inducing ATP release from astrocytes, to then recruit and activate microglia (Fontainhas et al., 2011; Wong et al., 2011).

Once microglia are recruited to synapses via a "find-me" signal, one of the potential consequences can be phagocytosis in response to an "eat-me" signal. A number of molecules with analogy to the peripheral immune system have been shown to play some role in triggering phagocytosis of synapses. These include the major histocompatibility complex class 1 group of proteins (MHC-1) and the complement cascade proteins, including C1q and C3 (**Figure 1**). Genetic disruption of proteins involved in the MHC-1 complex reduces synaptic pruning in development of the LGN synapses (Huh et al., 2000) while increasing the extent of synaptic stripping in the facial nerve axotomy injury model (Oliveira et al., 2004; Cullheim and Thams, 2007). Interestingly, the excess loss of synapses in the injury model is largely accounted for by inhibitory inputs. Investigating how microglia-synapse interactions differ between excitatory and inhibitory synapses is an area that has received very little attention beyond this particular study (Oliveira et al., 2004).

Recent studies showed that the immune complement molecules C1q, C3 and the receptor CR3 (CD11b/CD18) are key molecules contributing to microglial phagocytosis of neurites and synapses (Stevens et al., 2007; Schafer et al., 2012). Retinal ganglion cell (RGC) neurons express C1q, the upstream signaling molecule of C3, at P5 (Stevens et al., 2007). The receptor molecule for C3, CR3 is expressed in microglia (Schafer et al., 2012). Genetic deletion of either the C3 ligand, or the CR3 receptor, reduced inclusions of presynaptic terminals in microglia, indicating a decreased microglial synapse phagocytosis (Schafer et al., 2012). Further details of this interaction were revealed in a hippocampal neuron—microglia co culture model. Removal of the sialic acid cap of the glycocalyx enabled C1q to bind to neurites, "tagging" them for phagocytotic clearance by microglia via C3R (Linnartz et al., 2012). Identifying further these "find me" and "eat me" signals, that undoubtably act in combination with other signaling pathways (including "don't eat me" signals), and whether their role generalizes to other models of plasticity are important areas for future experiments.

EFFECTS OF MICROGLIA ON SYNAPTIC TRANSMISSION

Interactions between microglia and synapses extends beyond the structural effects in phagocytosing and shaping synapses as described above. Although functional effects have been less well studied, microglia can also clearly influence synaptic transmission and also the functional maturation of synapses.

Pascual et al. (2011) activated microglia in hippocampal slices using bath application of lipopolysaccharide (LPS) and observed an increase in frequency of mEPSPs. A signaling pathway was proposed for this effect based on pharmacological evidence. ATP released by activated microglia then activate astrocytes via P2Y receptors, triggering the release of glutamate which in turn acts on presynaptic metabotropic glutamate receptors on neurons enhancing neurotransmitter release (**Figure 1**) (Pascual et al., 2011). Peripheral nerve injury resulting in chronic pain also induces microglia to alter synaptic transmission, but via a different signaling pathway. ATP release (likely from both neurons and astrocytes) in the spinal cord stimulates BDNF release from microglia that in turn changes neuronal Cl^- homeostasis by decreasing the Cl^- efflux transporter, KCC2, and thereby reduces the efficacy of inhibition mediated via GABAergic transmission (Coull et al., 2005; Tsuda et al., 2010).

Microglia may also play some role in maturation of synaptic properties, and this has been suggested by recent studies. Activation of the CX3 chemokine receptor (CX3CR1) by its ligand fractaline (CX3CL1) reduces AMPA-mediated postsynaptic currents via a postsynaptic mechanism (Ragozzino et al., 2006). Recent studies investigating chemokine signaling between neurons and microglia. Paolicelli et al. (2011) examined synaptic activity in the acute hippocampal slice isolated from juvenile mouse lacking the chemokine receptor CX3CR1. In the CA1 of these mice, a transient decrease in microglia density was associated with an impaired phagocytosis of developing synapses, as supported by the increased dendritic spine density. This was associated with altered synaptic properties (enhanced LTD, increased mEPSC frequency in the knockout mouse) consistent with a delayed maturation of excitatory transmission (Paolicelli et al., 2011). A deficiency in LTP in the hippocampal slice has also been recently reported for this CX3CR1 knockout mouse (Rogers et al., 2011). Hoshiko et al. (2012) examined synaptic transmission at thalamo-cortical synapses. Typically, excitatory transmission at these synapses changes its kinetics over development, from a slow EPSC mediated by kainate receptors to a faster EPSC involving AMPA receptors (Kidd and Isaac, 1999; Daw et al., 2007). Changes in NMDA receptor subtypes also contributes to these developmental changes in EPSC kinetics (Carmignoto and Vicini, 1992; Barth and Malenka, 2001; Lu et al., 2001). Hoshiko et al. also used the CX3CR1 knockout mice, reporting that there was a delayed migration of microglia into the cortical layer 4 where these synapses are found, and this was associated with a corresponding delay in the maturation of the AMPA/NMDA ratio. The suggestion was that microglia are also involved in maturation of the postsynaptic receptor subtype at these synapses (Hoshiko et al., 2012).

The above indicate a number of examples by which microglia can interact with synapses, either directly or via associated astrocytes, during developmental plasticity and in response to injury. It will be important to now similarly identify the mechanisms and function of microglia synapses interactions in the healthy adult brain.

CONCLUDING REMARKS

In this brief review, we have highlighted some of the recent examples of synapse-microglia interactions and outstanding issues. Microglia play a role in shaping structural features of synaptic connections within neural circuits during development and following injury by phagocytosing pre- and post-synaptic components. Microglial interactions with synapses can also affect the functional maturation of pre- and postsynaptic properties, and influence synaptic transmission in response to injury. The extensive surveillance of all components of synapses by microglia in healthy adult brain, and the modification of this activity by sensory experience and neuronal activity, alludes to key roles of microglia-synapse interactions beyond that just in development

and injury. Elucidating the molecular mechanisms and functional significance of these interactions in healthy brain will be crucial for determining whether defects in microglia's physiological function could trigger disease. Already some recent exciting results have implicated microglia as the primary site of defects in Rett's syndrome, a model of autism spectrum disorders (ASD), and Hoxb8-deficiency, a model of obsessive compulsive disorder (Chen et al., 2010; Derecki et al., 2012). Infections and compromised immune system are known risk factors associated with schizophrenia and other psychiatric diseases, which are characterized by alterations in synapse number and function (Glausier and Lewis, 2012). Hence it seems highly plausible to propose alterations in microglia-synapse interactions as a possible cause for a range of neurological disease and we eagerly await further studies to allow this hypothesis to move beyond mere speculation.

ACKNOWLEDGMENTS

This work was supported by The Uehara memorial foundation (to Hiroaki Wake) and the Japan Science and Technology Agency for Core Research for Evolutional Science and Technology (to Junichi Nabekura) and Research Fellow of the Japan Society for the Promotion of Science (to Akiko Miyamoto).

REFERENCES

Barth, A. L., and Malenka, R. C. (2001). Nmdar epsc kinetics do not regulate the critical period for ltp at thalamocortical synapses. *Nat. Neurosci.* 4, 235–236.

Blinzinger, K., and Kreutzberg, G. (1968). Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Z. Zellforsch. Mikrosk. Anat.* 85, 145–157.

Brown, C. E., Li, P., Boyd, J. D., Delaney, K. R., and Murphy, T. H. (2007). Extensive turnover of dendritic spines and vascular remodeling in cortical tissues recovering from stroke. *J. Neurosci.* 27, 4101–4109.

Carmignoto, G., and Vicini, S. (1992). Activity-dependent decrease in nmda receptor responses during development of the visual cortex. *Nature* 258, 1007–1011.

Chen, S.-K., Tvrdik, P., Peden, E., Cho, S., Wu, S., Spangrude, G., et al. (2010). Hematopoietic origin of pathological grooming in hoxb8 mutant mice. *Cell* 141, 775–785.

Coleman, P., Federoff, H., and Kurlan, R. (2003). A focus on the synapse for neuroprotection in alzheimer disease and other dementias. *Neurology* 63, 1155–1162.

Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., et al. (2005). Bdnf from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021.

Cullheim, S., and Thams, S. (2007). The microglial networks of the brain and their role in neuronal network plasticity after lesion. *Brain Res. Rev.* 55, 89–96.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). Atp mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758.

Daw, M. I., Scott, H. L., and Isaac, J. T. R. (2007). Developmental synaptic plasticity at the thalamocortical input to barrel cortex: mechanisms and roles. *Mol. Cel. Neurosci.* 34, 493–502.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B. G., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of rett syndrome. *Nature* 484, 105–109.

Finsen, B. R., Jørgensen, M. B., Diemer, N. H., and Zimmer, J. (1993). Microglial mhc antigen expression after ischemic and kainic acid lesions of the adult rat hippocampus. *Glia* 7, 41–49.

Fontainhas, A. M., Wang, M., Liang, K. J., Chen, S., Mettu, P., Damani, M., et al. (2011). Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and gabaergic neurotransmission. *PLoS ONE* 6:e15973. doi: 10.1371/journal.pone.0015973

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845.

Glausier, J. R., and Lewis, D. A. (2012). Dendritic spine pathology in schizophrenia. *Neuroscience*. doi: 10.1016/j.neuroscience.2012.04.044. [Epub ahead of print].

Graeber, M., Bise, K., and Mehraein, P. (1993). Synaptic stripping in the human facial nucleus. *Acta Neuropathol.* 86, 179–181.

Graeber, M. B., and Streit, W. J. (2010). Microglia: biology and pathology. *Acta Neuropathol.* 119, 89–105.

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

Hooks, B. M., and Chen, C. (2006). Distinct roles for spontaneous and visual activity in remodeling of the retinogeniculate synapse. *Neuron* 52, 281–291.

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012). Deficiency of the microglial receptor cx3cr1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J. Neurosci.* 32, 15106–15111.

Hua, J. Y., Smear, M. C., Baier, H., and Smith, S. J. (2005). Regulation of axon growth *in vivo* by activity-based competition. *Nature* 434, 1022–1026.

Huh, G. S., Boulanger, L. M., Du, H., Riquelme, P. A., Brotz, T. M., and Shatz, C. J. (2000). Functional requirement for class 1 mhc in cns development and plasticity. *Science* 290, 2155–2159.

Kano, M., and Hashimoto, K. (2009). Synapse elimination in the central nervous system. *Curr. Opin. Neurobiol.* 19, 154–161.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Kidd, F. L., and Isaac, J. T. R. (1999). Developmental and activity- dependent regulation of kainate receptors at thalamocortical synapses. *Nature* 400, 569–573.

Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., et al. (2007). Udp acting at p2y6 receptors is a mediator of microglial phagocytosis. *Nature* 446, 1091–1095.

Lassmann, H., Schmied, M., Vass, K., and Hickey, W. F. (1993). Bone marrow derived elements and resident microglia in brain inflammation. *Glia* 7, 19–24.

Li, Y., Du, X.-F., Liu, C.-S., Wen, Z.-L., and Du, J.-L. (2012). Reciprocal regulation between resting microglial dynamics and neuronal activity *in vivo*. *Dev. Cell* 23, 1189–1202.

Li, Y., Liu, L., Barger, S. W., and Griffin, W. S. T. (2003). Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-mapk pathway. *J. Neurosci.* 23, 1605–1611.

Lichtman, J. W., and Colman, H. (2000). Synapse elimination and indelible memory. *Neuron* 25, 269–278.

Linnartz, B., Kopatz, J., Tenner, A. J., and Neumann, H. (2012). Sialic acid on the neuronal glycocalyx prevents complement c1 binding and complement receptor-3-mediated removal by microglia. *J. Neurosci.* 32, 946–952.

Lu, H.-C., Gonzalez, E., and Crair, M. C. (2001). Barrel cortex critical period plasticity is independent of changes in nmda receptor subunit composition. *Neuron* 32, 619–634.

MarínN-Teva, J. L., Dusart, I., Colin, C., Gervais, A., Rooijen, N. V., and Mallat, M. (2004). Microglia promote the death of developing purkinje cells. *Neuron* 41, 535–547.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318.

Ohsawa, K., and Kohsaka, S. (2011). Dynamic motility of microglia: Purinergic modulation of microglial movement in the normal and pathological brain. *Glia* 59, 1793–1799.

Oliveira, A. L. R., Thams, S., Lidman, O., Piehl, F., Kfelt, T. H., Rre, K. K., et al. (2004). A role for mhc class 1 molecules in synaptic plasticity and regeneration of neurons after axotomy. *Proc. Natl. Acad. Sci. U.S.A.* 101, 17843–17848.

Palazuelos, J., Aguado, T., Pazos, M. R., Julien, B., Carrasco, C., Resel, E., et al. (2009). Microglial cb2 cannabinoid receptors are neuroprotective in huntington's disease excitotoxicity. *Brain* 132, 3152–3164.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning

by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2011). Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U.S.A.* 109, E197–E205.

Penn, A. A., Riquelme, P. A., Feller, M. B., and Shatz, C. J. (1998). Competition in retinogeniculate patterning driven by spontaneous activity. *Science* 279, 2108–2112.

Perry, V. H., and Gordon, S. (1988). Macrophages and microglia in the nervous system. *Trends Neurosci.* 11, 6273–6277.

Perry, V. H., Nicoll, J. A., and Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat. Rev. Neurol.* 6, 193–201.

Perry, V. H., and O'Connor, V. (2010). The role of microglia in synaptic stripping and synaptic degeneration: a revised perspective. *ASN Neuro* 2:e00047. doi: 10.1042/AN20100024

Prinz, M., and Mildner, A. (2011). Microglia in the cns: immigrants from another world. *Glia* 59, 177–187.

Ragozzino, D., Di Angelantonio, S., Trettel, F., Bertollini, C., Maggi, L., Gross, C., et al. (2006). Chemokine fractalkine/CX3CL1 negatively modulates active glutamatergic synapses in rat hippocampal neurons. *J. Neurosci.* 26, 10488–10498.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., et al. (2011). Cx3cr1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J. Neurosci.* 31, 16241–16250.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Sierra, A., Encinas, J. M., Deudero, J. J. P., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., et al. (2007). The classical complement cascade mediates cns synapse elimination. *Cell* 131, 1164–1178.

Trapp, B. D., Wujek, J. R., Criste, G. A., Jalabi, W., Yin, X., Kidd, G. J., et al. (2007). Evidence for synaptic stripping by cortical microglia. *Glia* 55, 360–368.

Tremblay, M.-È., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Tremblay, M.-È., Zettel, M. L., Ison, J. R., Allen, P. D., and Majewska, A. K. (2012). Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. *Glia* 60, 541–558.

Tsuda, M., Tozaki-Saitoh, H., and Inoue, K. (2010). Pain and purinergic signaling. *Brain Res. Rev.* 63, 222–232.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Wong, W. T., Wang, M., and Lia, W. (2011). Regulation of microglia by ionotropic glutamatergic and gabaergic neurotransmission. *Neuron Glia Biol.* 7, 41–46.

Yamada, J., Hayashi, Y., Jinno, S., Wu, Z., Inoue, K., Kohsaka, S., et al. (2008). Reduced synaptic activity precedes synaptic stripping in vagal motoneurons after axotomy. *Glia* 56, 1448–1462.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2013; accepted: 26 April 2013; published online: 15 May 2013.

Citation: Miyamoto A, Wake H, Moorhouse AJ and Nabekura J (2013) Microglia and synapse interactions: fine tuning neural circuits and candidate molecules. Front. Cell. Neurosci. 7:70. doi: 10.3389/fncel.2013.00070

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 19 April 2013
doi: 10.3389/fncel.2013.00049

Neurotransmitter signaling in the pathophysiology of microglia

María Domercq*[1,2,3], *Nuria Vázquez-Villoldo*[1,2,3] *and Carlos Matute*[1,2,3]

[1] *Departamento de Neurociencias, Universidad del País Vasco-UPV/EHU, Leioa, Spain*
[2] *Achucarro Basque Center for Neuroscience-UPV/EHU, Zamudio, Spain*
[3] *Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Leioa, Spain*

Edited by:
Amanda Sierra, Universidad del País Vasco UPV/EHU, Spain

Reviewed by:
Uwe-Karsten Hanisch, Univesrity of Göttingen, Germany
Hiroaki Wake, National Institute for Basic Biology, Japan

****Correspondence:***
Carlos Matute, Laboratorio de Neurobiología, Departamento de Neurociencias, Facultad de Medicina y Odontología, Universidad del País Vasco (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Vizcaya, Spain.
e-mail: carlos.matute@ehu.es

Microglial cells are the resident immune cells of the central nervous system. In the resting state, microglia are highly dynamic and control the environment by rapidly extending and retracting motile processes. Microglia are closely associated with astrocytes and neurons, particularly at the synapses, and more recent data indicate that neurotransmission plays a role in regulating the morphology and function of surveying/resting microglia, as they are endowed with receptors for most known neurotransmitters. In particular, microglia express receptors for ATP and glutamate, which regulate microglial motility. After local damage, the release of ATP induces microgliosis and activated microglial cells migrate to the site of injury, proliferate, and phagocytose cells, and cellular compartments. However, excessive activation of microglia could contribute to the progression of chronic neurodegenerative diseases, though the underlying mechanisms are still unclear. Microglia have the capacity to release a large number of substances that can be detrimental to the surrounding neurons, including glutamate, ATP, and reactive oxygen species. However, how altered neurotransmission following acute insults or chronic neurodegenerative conditions modulates microglial functions is still poorly understood. This review summarizes the relevant data regarding the role of neurotransmitter receptors in microglial physiology and pathology.

Keywords: microglia, ATP, glutamate, purinergic and glutamatergic receptors

INTRODUCTION

Microglial cells constitute the resident immune cell population of the mammalian central nervous system (CNS). Postnatally, microglia are present in all regions of the CNS in a non-overlapping territorial manner and comprise a large proportion of the total cellular makeup of the CNS, which is estimated to be as high as 12% (Lawson et al., 1990). Similar to macrophages, their peripheral counterparts, microglia display remarkable ranges of morphology and activity depending, in part, on the state of the surrounding tissue (Lynch, 2009; Ransohoff and Perry, 2009). "Resting" microglia are not functionally silent cells, but extremely dynamic *in vivo*, perpetually changing their morphology by extending and retracting highly motile processes on a time scale of minutes (Davalos et al., 2005; Nimmerjahn et al., 2005). In response to local damage (few micron lesions), microglial processes rapidly and automatically converge on the site of injury without cell body movement. The microglial branching response mediated by ATP release aims to shield and/or scavenge the affected side. In addition, part of the dynamic motility of surveying microglial processes *in vivo* is directed toward synapses, suggesting that microglia vigilantly monitor and respond to the functional status of synapses (Wake et al., 2009). In addition, microglia have been reported to be capable of sensing defunct synapses and phagocytose them in normal brain (Wake et al., 2009; Tremblay et al., 2010). Synaptic pruning by microglia is essential during development for the remodeling of synaptic circuits [Paolicelli et al., 2011; see also the reviews by Tremblay (2011) and Wake et al. (2013)]. Microglia also efficiently phagocytose apoptotic neurons in the neurogenic niche (Sierra et al., 2010).

In addition to its functions in normal brain, microglia are involved in most, if not all, known CNS pathologies. More than a decade ago, Georg Kreutzberg coined the term "microglial sensor of pathology" (Kreutzberg, 1996), which captures the essence of microglial cell function. Microglia are the brain's intrinsic immune cells and serve as damage sensors within the brain. Any type of injury or pathological process leads to the activation of these cells from their surveillant/resting state. In response to injury, microglia change their highly branched and ramified morphology by retracting their processes and taking on an ameboid appearance. Activated microglial cells can then migrate to the site of injury, proliferate, and release substances that affect pathological processes. These substances include pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, and interleukin (IL)-6 or IL-12, which signal the invading T lymphocytes.

Multiple signals converge on microglial cells to actively maintain or alter their functional state and orchestrate the specific repertoire of microglial functions. Transitions between surveillance and activated states are triggered when microglia perceive a sudden appearance, abnormal concentration, or unusual molecular format of certain factors (Hanisch and Kettenmann, 2007). This review focuses on the role of neurotransmitter receptors, particular ATP and glutamate receptors, in the control of microglial physiology and pathology. For the role of other

receptors or channels, see these other excellent reviews (Pocock and Kettenmann, 2007; Kettenmann et al., 2011).

ATP RECEPTORS

EXPRESSION OF ATP RECEPTORS IN MICROGLIA

Purines and pyrimidines act as widespread extracellular signaling molecules. The physiological effects of purines and pyrimidines are mediated through an extended family of purinoceptors activated by adenosine, classified as P1 receptors, or by ATP, classified as P2 receptors (Ralevic and Burnstock, 1998; North, 2002). Purinergic receptors are expressed in the majority of living cells and are particularly abundant in glia (Pocock and Kettenmann, 2007; Kettenmann et al., 2011). ATP activates a family of metabotropic P2Y, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, and ionotropic P2X1-7 receptors. Extracellular ATP is degraded to adenosine by ectonucleotidases, such as CD39 and CD73, which are known to be present in microglial cells (Braun et al., 2000) and adenosine activates G protein-coupled adenosine receptors A_1, A_{2A}, A_{2B}, and A_3. The A_1 and A_3 receptors can inhibit adenylyl cyclase or activate phospholipase C, whereas A_{2A} and A_{2B} receptors activate cyclic AMP production (Fredholm et al., 2001). Collectively, the actions of ATP and its degradation products produce responses that last from milliseconds to minutes, and even longer time scales through changes in gene regulation via second messengers (Khakh and North, 2012). P2X receptors are non-selective cation channels with high Ca^{2+} permeability that carry a depolarizing current under standard physiological conditions. In some cells, P2X channels are also significantly permeable to anions, such as the full-length P2X5 receptor (P2X5R), which is permeable to Cl^- (North, 2002). Functional homomeric P2X1R and P2X3Rs have fast desensitization properties. The other receptor types have slow desensitization properties, except P2X7R, which does not desensitize (Khakh and North, 2012). After prolonged activation, P2X7Rs open a large pore, causing cytolytic cell death (Surprenant et al., 1996). Signaling diversity is increased by the broad range of ATP sensitivities exhibited by ATP receptors, ranging from nanomoles in the case of P2Y receptors to hundreds of micromoles for P2X7Rs (North, 2002). Thus, ATP receptors respond over remarkably broad spatiotemporal scales, making ATP signaling highly dynamic.

P2X-mediated currents were identified in microglial cultures prepared from human and rodent brains more than two decades ago (Walz et al., 1993). Patch-clamp recordings have shown that cultured microglial cells respond to extracellular ATP (100 μM) with the activation of a transient inward non-selective cationic current, which is followed, in some cases, by an outward K^+ current (Walz et al., 1993; Nörenberg et al., 1994; McLarnon et al., 1999). These results were recently corroborated and extended in acute slices. Thus, ATP triggers a non-selective cationic inward current in association with the activation of P2X7Rs, and an outward K^+ current associated with the activation of P2Y6 and P2Y12 metabotropic receptors (Boucsein et al., 2003; Avignone et al., 2008). Importantly, these studies describe diverse electrophysiological properties for microglial cell types, one subtype with a lower resting membrane potential (between −50 mV and −60 mV) and another subtype with a higher membrane potential (−20 to −30 mV), and on the basis of different responses to ATP. These differences may be associated with the different functional roles of microglia.

Immunohistochemical studies have shown the expression of low levels of P2X4 (Ulmann et al., 2008) and P2X7 (Matute et al., 2007) and high levels of P2Y12 (Haynes et al., 2006) in microglia in the adult normal brain. The expression of purinergic receptors changes during development (Xiang and Burnstock, 2005). Thus, at embryonic day 16, the majority of microglial cells express P2X1 and P2X4 subunits, whereas only 30% of these cells express P2X7. From postnatal day 7, P2X4-positive microglia locate preferentially around blood vessels. At postnatal P30, the cells expressing P2X1 virtually disappear, the P2X7-positive cells are distributed widely through the forebrain, whereas cells bearing P2X4Rs are mainly localized around blood vessels and lining the subarachnoid space (Xiang and Burnstock, 2005). Constitutive expression of P2X4 in pervivascular cells was also described in the adult spinal cord (Guo and Schluesener, 2005). Pervivascular $P2X4^+$ cells are $ED1^+/OX42^+$, indicating that correspond to infiltrating monocytes/microglia, but not to lymphocytes.

From postnatal day 7, many microglial cells with P2X4 receptor-immunoreactivity were seen around the blood vessels. At postnatal day 30, microglial cells with P2X1 receptor-immunoreactivity disappeared and the cells with P2X4 receptor-immunoreactivity were mainly localized around blood vessels and lining the subarachnoid space. From postnatal day 30, the microglial cells with P2X7 receptor-immunoreactivity were found to be distributed widely in the forebrain. Cells with P2X7 receptor-immunoreactivity from P30 were not labeled by ED1, but some were labeled by isolectin B4. The expression of P2X1, P2X4, and P2X7 receptor mRNA and protein on primary cultures of rat microglial cells and on the N9 microglial cell line was demonstrated with immunocytochemistry and RT-PCR. This is the first report that the P2X1 receptor is expressed on microglial cells, at least in early development, before postnatal day 30.

Regarding adenosine receptors, *in vitro* functional studies have identified the expression of all P1 adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3 receptors in microglia (Haskó et al., 2005; Abbracchio and Ceruti, 2007). *In vivo*, the A_{2A} receptor appears to be expressed only in activated microglia after systemic lipopolysaccharide (LPS) injection (Orr et al., 2009). In contrast, adult healthy brains express relatively higher levels of A_1 and A_3 receptors (Koizumi et al., 2013).

FUNCTIONS OF ATP RECEPTORS IN MICROGLIA

The initial microglial responses that occur after brain injury and in various neurological diseases are characterized by microglial accumulation in the affected sites of the brain as a result of the migration and proliferation of these cells. The early-phase signal responsible for this accumulation is likely to be transduced by rapidly diffusible factors, such as ATP. Purinergic receptors control several microglial functions, including the motility of their fine processes, migration, cytokine release, and phagocytosis (**Figure 1**). Low ATP concentrations almost exclusively activate chemotaxis in order to recruit cells at the site of injury or inflammation. When the ATP concentration increases, additional

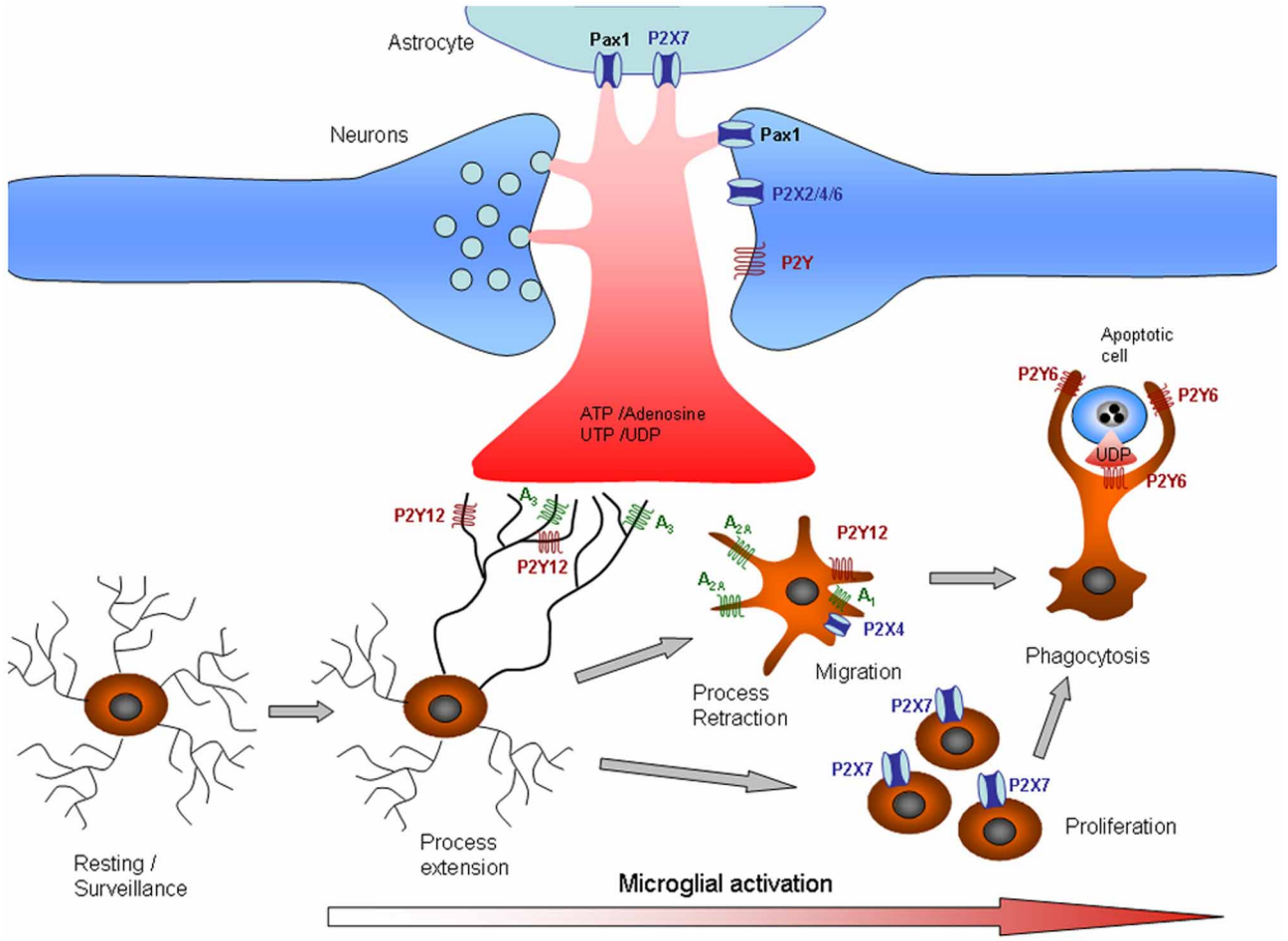


FIGURE 1 | Purinergic signaling in microglia. Release or leakage of nucleotides/nucleosides from injured neurons, astrocytes, or microglial cells induces phenotypic alterations in microglia. Microglial processes exhibit constitutive motility, which is dependent on ATP signaling. Microglial processes are rapidly recruited to sites of CNS tissue damage by P2Y12 and A_3 receptor activation. As the damage progresses, microglia undergo progressive changes, including altered expression of cell surface markers and inflammation-related genes, process retraction and the acquisition of an ameboid morphology, cell body migration, and increasing phagocytic ability. The changes in microglial functions are partly associated with changes in purinergic receptors that determine different responses to ATP. Thus, process retraction is mainly due to upregulation of A_{2A} and downregulation of P2Y12 receptors, whereas migration is mediated by A_1 and P2X4 receptors and proliferation by P2X7 receptors. Phagocytosis signaling is also unmasked by the upregulation of P2Y6, which is activated by the release of UTP by dying cells. See also **Table 1**.

effector functions, such as phagocytosis and cytokine secretion, are also triggered (**Table 1**).

Movement of the fine microglial processes is controlled primarily through the activation of $P2Y_{12}$ receptors, which are expressed at high levels in microglia in normal brain. However, $P2Y_{12}$ receptors are downregulated in microglia after stroke or activation by LPS (Haynes et al., 2006). Microglial chemotaxis is characterized by cell body movement, as well as process movement, and is mediated through the activation of P2X4 and P2Y12 receptors (Honda et al., 2001; Ohsawa et al., 2007). Haynes et al. (2006) showed that, in $P2Y_{12}$ receptor-null mice undergoing focal laser cortical ablation, the chemotactic response of microglia was markedly impaired in the first 40 min of the observation period compared to wild-type animals. However, when microglia from mutant mice were examined 2 h after injury, the degree of chemotaxis approached that observed in wild-type animals. Moreover, P2Y12 receptor expression on microglia was barely observable 24 h after injury. The loss of P2Y12 expression accompanied microglial transformation from highly ramified to an amoeboid state. These observations indicate that P2Y12 receptors are involved in the early, rather than late, responses of microglia to injury (Haynes et al., 2006). In contrast, P2X4 has been shown to be markedly upregulated after microglial activation; thus, chemotaxis after injury could be mediated by this receptor (Ohsawa et al., 2007).

Phagocytosis is the terminal removal of cellular debris by phagocytes. In vertebrates, phagocytosis is performed mostly by macrophages and other specialized innate immune cells engulfing the cellular debris in phagosomes, membrane protrusions that fuse with lysosomes for terminal degradation. Although phagocytosis is activated primarily by the expression of "eat-me" signals on the surface of damaged or dead cells, injured neurons

Table 1 | Expression and function of purinergic receptors on microglia.

Receptor type	Presence	Function	References
P1			
A_1	+	Migration/Neuropathic pain/Antiinflammatory properties	Färber et al., 2008; Haselkorn et al., 2010; Luongo et al., 2012
A_{2A}	+	Process retraction/Microglial activation	Orr et al., 2009; Yao et al., 2012
A_{2B}	+	Anti-inflammatory properties; release of IL-10	Koscsó et al., 2012
A_3	+	Process extension and migration	Ohsawa et al., 2012
P2			
Ionotropic			
P2X1	–	–	Cavaliere et al., 2005; Xiang and Burnstock, 2005
P2X2	–	–	Unpublished observation
P2X3	+	–	Unpublished observaton
P2X4	+	Migration/Neuropathic pain	Ohsawa et al., 2007; Beggs et al., 2012
P2X5	?	–	
P2X6	–	–	Cavaliere et al., 2005
P2X7	+	Microglial proliferation/Inflammasome signaling	Rigato et al., 2012; Volonte et al., 2012
Metabotropic			
P2Y1	?	Purine release/Activation of a K^+ current	Boucsein et al., 2003; Ballerini et al., 2005
P2Y2	+	Aβ(1–42) degradation and uptake	Kim et al., 2012
P2Y4	+	Not determined	–
P2Y6	+	Phagocytosis	Koizumi et al., 2007
P2Y11	?	Microglial activation	Brandenburg et al., 2010
P2Y12	+	Process extension/Migration	Haynes et al., 2006
P2Y13	+	Neuropathic pain	Kobayashi et al., 2012
P2Y14	+	Neuropathic pain	Kobayashi et al., 2012

?, Not determined

leak diffusible UDP signals that activate $P2Y_6$-dependent phagocytic signaling in microglia (Koizumi et al., 2007). UDP, which also acts on G_q-coupled P2Y6 receptors, induces the expression of chemokines (Kim et al., 2011). In contrast to the positive effect of P2Y6, the stimulation of P2X7 or P1 receptors attenuates microglial phagocytosis (Fang et al., 2009; Bulavina et al., 2012). Phagocytosis by microglia is also regulated by the ratio of P2/P1 activation. The interplay between P2 and P1 receptor activation is controlled by a cascade of extracellular enzymes that dephosphorylate purines, resulting in the formation of adenosine. In microglia, the capacity to degrade ATP and ADP depends on the expression of ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1, also called CD39). Deletion of CD39 practically abolishes ATP degradation and increases microglial phagocytic activity (Bulavina et al., 2012). Interestingly, the P2X7 receptor has also been detected in phagosomes. Lipids stimulate both actin assembly and the transport of ADP across the phagosomal membrane into the lumen. In the lumen, ADP is converted to ATP by adenylate kinase activity and activates P2X7 receptors in phagosomes, triggering actin assembly on the cytoplasmic membrane surface (Kuehnel et al., 2009a,b). These data indicate a distinct role of intracellular P2X7 receptors in phagocytosis.

In addition to its role in normal CNS function, ATP signaling is involved in neuroinflammation in a broad range of CNS pathologies (Di Virgilio et al., 2009). The extracellular concentration of ATP increases dramatically during inflammation (Idzko et al., 2007; Pellegatti et al., 2008), and P2X7 receptors are overexpressed in microglial cells in the neuroinflammatory foci of numerous neurodegenerative conditions (Weisman et al., 2012). The overexpression of P2X7 receptors in microglia, in the absence of a pathological insult, is sufficient to drive the activation and proliferation of microglia (**Figure 1**), which depends on the pore-forming capacity of this receptor (Monif et al., 2009). The activation of P2X7 receptors is coupled to the maturation and secretion of the key pro-inflammatory cytokine IL-1β [Ferrari et al., 1996; reviewed in Di Virgilio et al. (2009)], a signaling pathway that depends on P2X7 coupling with pannexin-1 and subsequent casapse-1 activation (Pelegrin and Surprenant, 2006). Activation of the large-pore P2X7 receptor also leads to the release of TNFα (Suzuki et al., 2004), the endocannabinoid 2-arachidonylglycerol (Witting et al., 2004), and superoxide (Parvathenani et al., 2003). However, whether the pore capacity of P2X7 depends on its coupling with pannexin-1 is a matter of debate. In neurons and astrocytes, pannexin1 appears to be the molecular substrate for the permeabilization pore (or death receptor channel) recruited into the P2X7 receptor signaling complex (Locovei et al., 2007; Silverman et al., 2009). In innate immune cells, including microglia and macrophages, P2X7 signaling appears to be independent of pannexin-1 (Hanley et al., 2012; Rigato et al., 2012). Thus, microglia proliferate and phagocytose dying motor neurons during early embryonic spinal cord invasion (Rigato et al., 2011). Notably, microglial invasion and proliferation are controlled by P2X7 receptor signaling in a pannexin-1-independent manner (Rigato et al., 2012). The pore dilation capacity of P2X7 receptors in microglia does not depend on the expression of pannexin-1 at this embryonic stage (Rigato et al., 2012).

The main function of adenosine receptors is the control of innate immune function. Adenosine receptors A_1 and A_3 block TNFα release by microglia, whereas A_{2B} stimulates the production and secretion of anti-inflammatory cytokine IL-10 (Koscsó et al., 2012), indicating an anti-inflammatory role of P1 receptor activation in the brain. Signaling through adenosine receptor A_{2A} drives the proliferation of spinal microglia after nerve injury (Bura et al., 2008), and intrathecal blockade of this receptor has been shown to abolish neuropathic pain in the same model (Loram et al., 2009). Finally, adenosine can suppress inflammation and aid in tissue restitution, in part, by promoting alternative macrophage activation. Alternative activation occurs in a Th2 cytokine environment and promotes the immunomodulatory and anti-inflammatory, rather than pro-inflammatory, properties of macrophages/microglia. Adenosine treatment of IL-4- or IL-13-activated macrophages augments the expression of alternative macrophage markers, primarily through the activation of A_{2B} receptors, though A_{2A} receptors also contribute to the effect (Csóka et al., 2012). Acting through A_3 receptors, adenosine is also involved in the extension and migration of microglial processes (Ohsawa et al., 2012). Interestingly, simultaneous stimulation of P2Y12 and A_3 receptors is required for microglial process extension, suggesting that intimate crosstalk occurs between P2Y12 and A_3 receptors.

CHANGES IN ATP RECEPTOR EXPRESSION IN MICROGLIA

Microglia adopt an appropriate stimulus modality–dependent phenotype in response to injury or disease. The phenotypic catalog of microglia includes proliferative, migrational, and phagocytic responses, though how distinct the discrete molecular fingerprints of the phenotypes are is not clear (Hanisch and Kettenmann, 2007). Microglia undergo dramatic changes in shape and gene expression patterns within hours of *in vivo* activation, including modulation of the expression and function of purinergic receptors (**Figure 1**). Remodeling of purinoceptor expression has been observed *in situ* in various pathological models. Thus, epileptic seizures induced by kainate injections trigger an activation of microglia in hippocampal slices (Avignone et al., 2008), accompanied by an upregulation of the expression of mRNA specific for P2X1, P2X4, P2X7, P2Y6, P2Y12, and P2Y13 receptors. Functionally, this upregulation manifests as an increase in ATP-induced membrane currents and ATP-induced microglial motility (Avignone et al., 2008). Depending on the microglial stage, changes in purinergic expression determine the responses, sometimes with opposite effects, to extracellular purines. For example, the microglial chemotactic response to ATP is reversed following microglial activation. The switch from process attraction to repulsion is driven by upregulation of the G_s-coupled A_{2A} receptor (Orr et al., 2009) concomitant with downregulation of the G_i-coupled $P2Y_{12}$ receptor (Haynes et al., 2006).

The extended and divergent time course of microglial activation suggests that the activation process is regulated by complex mechanisms, which may differ significantly depending on the initiating stimulus. *In vivo*, P2Y12 receptor expression decreases as microglia become activated after LPS injection in the striatum (Haynes et al., 2006). In contrast, facial-nerve axotomy, a classical model of microglial activation, induces an upregulation of microglial $P2Y_{12}$ mRNA (Sasaki et al., 2003). Other stimuli, such as epileptic seizures or trauma, also lead to rapid upregulation of P2Y12 mRNA and protein in microglia (Franke et al., 2007; Avignone et al., 2008; Tozaki-Saitoh et al., 2008).

The P2X4R has been reported to be associated with the activation of microglia/macrophages after CNS injury and may play roles in inflammatory cascades involved in secondary brain damage. The development of mechanical allodynia temporally correlates with an increase in spinal P2X4R expression in microglia (Ulmann et al., 2008). Microglial P2X4R upregulation, the $P2X4R^+$ state of microglia, seems to be common in most acute and chronic neurodegenerative diseases associated with inflammation. Microglial activation after traumatic brain injury also parallels a significant increase in P2X4R expression in microglia, which is suppressed by systemic treatment with dexamethasone (Zhang et al., 2007). The upregulation of microglial P2X4Rs has also been observed in animals expressing superoxide dismutase 1 mutant, an animal model of amyotrophic lateral sclerosis (D'Ambrosi et al., 2009), in the acute experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) (Guo and Schluesener, 2005), after spinal cord injury (Schwab et al., 2005), and in cerebral ischemia (Cavaliere et al., 2003). Different regulators of P2X4R expression in microglia have been described, such as the chemokine CCL2 (also known as monocyte chemoattractant protein, MCP-1; Biber et al., 2011; Toyomitsu et al., 2012), interferon-γ (Tsuda et al., 2009), and the extracellular matrix molecule fibronectin acting through Lyn kinase (Tsuda et al., 2008, 2009).

The role of P2X4R in microglial activation and how its expression affects microglial functions is unclear. In neurons, P2X4R influences inflammasome activation after spinal cord injury (de Rivero Vaccari et al., 2012). The inflammasome is a multiprotein complex that promotes the activation of caspase-1 and release of mature inflammatory cytokines, such as IL-1β and IL-18. This complex likely controls many aspects of neuroinflammatory processes. P2X4 knock-out mice exhibit impaired inflammasome signaling in the spinal cord, resulting in decreased IL-1β levels and reduced infiltration of neutrophils and monocyte-derived M1 macrophages, resulting in significant tissue sparing and improved functional outcomes (de Rivero Vaccari et al., 2012).

The metabotropic P2Y6R that controls microglial phagocytosis is highly expressed in surveying microglia, but it is also slightly upregulated in microglia following neuronal damage induced by kainic acid injection in the hippocampus (Koizumi et al., 2007), by trauma (Franke et al., 2007), or in animals expressing dismutase 1 mutant (D'Ambrosi et al., 2009). These data point to a role of this receptor in controlling the phagocytosis of necrotic cells after damage (Koizumi et al., 2007). In contrast, the phagocytosis of apoptotic cells prevents the spillover of cellular contents. Thus, whether UDP levels are sufficient to activate the phagocytosis of apoptotic cells or debris by microglia under normal physiological conditions remains to be determined.

Finally, the expression of adenosine receptors varies depending on microglial activation. Thus, surveying microglia express high levels of A_1 and A_3 receptors, and activation leads to their downregulation. In contrast, the expression level of the adenosine A_{2A} receptor in surveying/resting microglia is relatively low, but LPS

dramatically increases its expression (Orr et al., 2009). The upregulation of A_{2A} receptor expression is also observed in pathological states, such as Parkinson disease and ischemia (Pedata et al., 2001; Schwarzschild et al., 2006).

GLUTAMATE RECEPTORS

EXPRESSION OF GLUTAMATE RECEPTORS IN MICROGLIA

Glutamate is the major excitatory neurotransmitter of the CNS and perturbations in this transmitter's homeostasis have been reported in most neurodegenerative diseases. Glutamate activates both ionotropic and metabotropic receptors. Ionotropic receptors are classified into a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-d-aspartate (NMDA) subtypes according to their preferred agonist. Molecular cloning has revealed that each receptor subtype is composed of several subunits with high homology within each receptor class. Thus, AMPA receptors are formed by GluR1-4, kainate receptors by GluR5-7 and KA1-2, and NMDA receptors by NMDAR1, NMDAR2A-D, and NMDAR3A-B subunits (Cull-Candy and Leszkiewicz, 2004). AMPA receptors are activated by AMPA and kainate, whereas kainate receptors are activated by kainate and are best functionally isolated in the presence of GYKI53655, a selective AMPA receptor antagonist (Lerma, 2003). Similarly, metabotropic GluRs (mGluRs) can be classified as group I (mGluR1, mGluR5), group II (mGluR2, mGluR3), and group III (mGluR4, mGluR6-8) seven transmembrane receptors (Swanson et al., 2005). The individual mGluR groups are coupled to various G-proteins that activating phospholipase C (PLC, group I) or inhibit adenylate cyclase (groups II and III).

Few studies have characterized the functional expression of ionotropic glutamate receptors in microglial cells. An early study showed inward currents corresponding to the activation of low-Ca^{2+} permeability AMPA-type glutamate receptors (expressing the GluR2 subunit) in cultured microglia, the activation of which leads to TNF-α release [Noda et al., 2000; reviewed by Pocock and Kettenmann (2007)]. AMPA-type glutamate receptor activation also leads to *c-fos* expression (Eun et al., 2004). In contrast, no direct evidence has been found for the functional expression of kainate receptors in microglial cells. In contrast to *in vitro* conditions, electrophysiological recordings of microglial cells in retina or hippocampus slices clamped at −50 mV failed to detect any inward or outward current in response to glutamate or AMPA (Wu and Zhuo, 2008; Fontainhas et al., 2011). The expression of functional NMDA receptors in microglia in normal brain has not been reported, but the activation of microglia after the induction of transient forebrain ischemia leads to NMDAR1 subunit upregulation (Gottlieb and Matute, 1997). The functional significance of NMDA receptor upregulation in microglia is still unknown. However, NMDA injection into the somatosensory cortex of newborn rats triggers transient microglial activation (Acarin et al., 1996), whereas systemic administration of MK-801 prevents rapid microglial activation in the hippocampus, secondary to ischemic insults (Streit et al., 1992) or LPS treatment (Thomas and Kuhn, 2005). Whether NMDA receptor activation controls microglial activation directly or indirectly remains to be determined. Therefore, additional studies are necessary to characterize the existence of functional ionotropic glutamate receptors in the resident and activated microglia of slices, which could respond to glutamate release during synaptic activity or damage.

Regarding metabotropic glutamate receptors, different subunits of metabotropic groups I (mGluR5), II (mGluR2 and 3), and III (mGluR4, 6, and 8, but not mGluR7) are expressed by microglia and regulate microglial transformation into neuroprotective (via group III mGluRs) or neurotoxic (via group II mGluRs) phenotypes (Biber et al., 1999; Taylor et al., 2002, 2003, 2005; Pocock and Kettenmann, 2007). Microglial activation of group II mGluRs, particularly mGluR2, induces TNF-α and Fas ligand release, which trigger neuronal caspase-3 activation via TNFR1 (also known as p55) and Fas receptor, leading to neuronal death (Taylor et al., 2005). However, an agonist of mGluR3, a member of group II, has been shown to inhibit the toxicity of microglia toward oligodendrocytes (Pinteaux-Jones et al., 2008). Activation of groups II and III metabotropic glutamate receptors also modulates LPS-induced glutamate release by the xCT antiporter in microglia (McMullan et al., 2012), suggesting a neuroprotective role of its activation. Groups I and III metabotropic glutamate receptors also modulate the activity of NADPH oxidases, the main source of superoxide anions (Mead et al., 2012).

FUNCTIONS OF GLUTAMATE RECEPTORS IN MICROGLIA

Similar to ATP, glutamate is a chemotactic neurotransmitter for microglia. Microglia stimulated by kainate, via either AMPA or kainate receptors, undergo dramatic morphological and cytoskeletal changes characterized by the condensation of cytoplasmic actin filaments, rapid depolymerization and repolymerization, and cytoplasmic redistribution of condensed actin bundles (Christensen et al., 2006). Actin filament rearrangement is thought to be involved in locomotion and phagocytosis and to indicate an increased level of activation. Microglia cells exposed to glutamate exhibit increased cell membrane ruffling and migrate to a source of glutamate in cell culture and spinal cord slices. This chemotaxis is mediated by AMPA and metabotropic glutamate receptors on the microglia, and is dependent on the redistribution of actin filaments and tubulin following receptor activation (Liu et al., 2009).

However, the role of glutamate in regulating baseline motility remains controversial. Initial studies showed that neuronal neurotransmission and activity-dependent synaptic plasticity does not affect surveying microglial motility in the hippocampus (Wu and Zhuo, 2008). However, other studies have suggested that endogenous glutamatergic neurotransmission positively regulates the dendritic morphology and process motility of surveying microglia (Fontainhas et al., 2011). The processes of surveying microglia have been demonstrated to make brief (∼5 min) and direct contacts with neuronal synapses at a frequency of approximately once per hour. These contacts are activity-dependent and reduce in frequency with reduced neuronal activity (Wake et al., 2009; Li et al., 2012). Neuronal activity affects the direction but not the basal level of microglial process motility (Li et al., 2012), which could explain the previous discrepancy with the article by Wu and Zhuo. Thus, neuronal activity steers surveying/resting microglia and facilitates their contacts with highly active neurons. This effect is not direct, as microglia do not express glutamate

receptors in processes and lack direct responses to glutamatergic agonists *in situ*. Instead, these influences are mediated indirectly via extracellular ATP, which is released in response to glutamatergic neurotransmission through probenecid-sensitive pannexin hemichannels (Fontainhas et al., 2011; Li et al., 2012). The consequences of these microglia-synapse contacts depend probably on the nature and intensity of the stimulus. After transient cerebral ischemia, the duration of these microglia–synapse contacts is markedly prolonged (∼1 h) and is frequently followed by the disappearance of the presynaptic bouton (Wake et al., 2009). However, an increase in spontaneous neuronal activity (i.e., by glutamate uncaging or kir channels overexpression in neurons) leads to the formation of microglial bulbous endings contacts with neurons that, surprisingly, reduce the activity of contacted neurons. This study suggests a role of surveying microglial in homeostatic regulation of neuronal activity (Li et al., 2012). These results suggest that microglia vigilantly monitor and respond to the functional status of synapses either to eliminate dysfunctional synapses or to silence them. Additional studies are needed to explore the mechanism and neurotransmitter involved in microglial detection of the functional status of synapses.

Glutamate is involved in the transmission of the death signal to microglia. Using the optically transparent larval zebrafish brain, rapidly propagating Ca^{2+} waves have been shown to determine the range of microglial responses to neuronal cell death. Though Ca^{2+}-mediated microglial responses require ATP, the spreading of intercellular Ca^{2+} waves is ATP independent, and glutamate has been identified as a potent inducer of Ca^{2+}-transmitted microglial attraction. Thus, the real-time analysis revealed the existence of a mechanism controlling microglial-targeted migration to neuronal injuries initiated by glutamate and proceeding across the brain in the form of a Ca^{2+} wave (Sieger et al., 2012).

ROLE OF MICROGLIAL NEUROTRANSMITTER RECEPTORS IN PATHOLOGY

Early during a pathological process, microglia may be stimulated by either non-self pathogens (stranger signals) or injured-self components (danger signals). Both stimuli can activate pattern-recognition receptors, such as the Toll-like receptors, scavenger receptors, and NOD system. The effector outputs of these stimuli focus on the clearance of tissue debris, generation of cues for tissue restoration, and resistance to pathogens. Together, these reactions comprise innate immune responses. Subsequent events may require the establishment of responses, including lymphocyte effector functions, antibodies, and immunological memory—collectively termed adaptive immunity. Microglial cells contribute to this process with antigen presentation, including the instruction of T cells to adopt varied effector programs (Th1, Th2, Th17) and, in some cases, directing them to the tissue from which the pathogenic material originated.

Although the innate immune response is beneficial in principal, an excessive and sustained activation of microglial cells is detrimental to neurons and oligodendrocytes (Merrill et al., 1993; Bezzi et al., 2001). Microglia activation has been described extensively in most pathological conditions in the CNS (Block and Hong, 2005), though its role is still debated (Schwartz et al., 2006). The outcome of microglial activation is complex and likely dependent on context. Microglia and macrophages can be activated by the cytokines interferon-g (IFN-g), IL-17, or LPS to a pro-inflammatory phenotype (M1), whereas IL-4 or IL-13 induce a state of alternative activation (M2) that is associated with neuroprotective functions that promote repair (Butovsky et al., 2006; Ponomarev et al., 2007; Kawanokuchi et al., 2008). Understanding the different processes and regulators of microglial activation will be important to unraveling their many complex functions. The thinking behind this dichotomization is that understanding these two microglial responses may minimize the harmful effects and capitalize on the beneficial effects (Popovich et al., 2011). In addition, a given facet of microglial activation that is beneficial in principle, such as phagocytosis, could turn detrimental under other circumstances. For example, phagocytosis under inflammatory conditions actively induces neuronal death (Neher et al., 2011) because inflammation causes viable neurons to express the "eat-me" signal, phosphatidylserine, on their surface, leading to their death through phagocytosis.

ROLE OF PURINERGIC RECEPTORS IN CNS INJURY

Purinergic signaling regulates both innate and adaptive immune responses and is involved in numerous acute insults and chronic neurodegenerative diseases of the CNS (Burnstock, 2008) because purine homeostasis is compromised in most diseases. However, relatively few studies have described the specific contribution of purinoceptor signaling pathways in microglia to neuropathology. In this section we summarize the data demonstrating the beneficial role of blocking P2 and P1 receptors, particularly P2X4R and P2X7R, in different CNS pathologies.

P2 RECEPTORS IN NEUROPATHIC PAIN: P2X4

Spinal microglia react and undergo a series of changes that directly influence the establishment of neuropathic pain states. Purinergic signaling via P2X4R is at the center of this reactivity (Beggs et al., 2012). Microglial P2X4 upregulation determines the behavioral manifestations of neuropathic pain arising from peripheral nerve injury and is sufficient to convert the response to normal non-painful peripheral inputs, from innocuous to nociceptive (review in Beggs et al., 2012). These findings are the basis for the concept that the microglial phenotype characterized by dramatic upregulation of P2X4Rs. The $P2X4R^{+}$ state, is critical in the etiology of neuropathic pain (Beggs et al., 2012). However, other microglial purinergic receptors, such as P2X7R, P2Y12R, and P1 receptors, are also involved in neuropathic pain. The blockage of P2X7Rs has been shown to alleviate chronic pain in three different models of neuropathic pain (Honore et al., 2009). Microglial P2X7Rs might participate in the neuronal hyperexcitability of dorsal horn neurons and development of neuropathic pain through the production of pro-inflammatory cytokines and chemokines (Tsuda et al., 2013). P2X7R also participates in microglial P2X4R trafficking and assembly (Boumechache et al., 2009), which could indirectly modulate P2X4R-regulated neuropathic pain. In macrophages and microglia, P2X4 and P2X7 form homotrimers that interact. P2X7Rs are found predominantly at the cell surface, whereas P2X4Rs are primarily intracellular. However, microglial activation induces a rapid translocation of

P2X4R to the surface, which is an efficient means of enhancing the function of P2X4R (Boumechache et al., 2009).

P2Y12R expression levels are also dramatically increased in microglial cells in the spinal cord after peripheral nerve injury, and the blockage of P2Y12Rs alleviates neuropathic pain (Kobayashi et al., 2008; Tozaki-Saitoh et al., 2008). After nerve injury, microglia are more abundant in layers II-III of the dorsal horn than in other areas, and some of them adhere to and engulf both injured and uninjured myelinated axons. This microglial engulfment is controlled by P2Y12R activation and directly involved in tactile allodynia (Maeda et al., 2010). The upregulation of other metabotropic P2 receptors, such as P2Y6R, P2Y13R, and P2Y14R, after nerve injury was reported recently (Kobayashi et al., 2012), and the concomitant block of the three receptors results in a longer suppressive effect on pain behavior (Kobayashi et al., 2012). In view of the crucial role played by different purinergic receptors in neuropathic pain, investigating the mechanisms of ATP release and how to modulate it as a means of attenuating neuropathic pain will be important.

Interestingly, a recent paper describes the involvement of microglial P2X4Rs in hyperalgesia produced by the gold-standard opiate analgesics. Morphine and other opiates are indispensable in the treatment of moderate-to-severe postoperative and chronic pain, but the use of these drugs is plagued by the development of two major problems: hyperalgesia and tolerance. Hyperalgesia is a sensitization process in which opioids, paradoxically, cause pain hypersensitivity. The spinal dorsal horn lamina I neurons are central targets for the analgesic effects of morphine and other opiates, and mediate morphine-induced hyperalgesia and tolerance. In particular, morphine induces analgesia via inhibition in lamina I neurons. In contrast, morphine induces hyperalgesia via the P2X4R-BDNF-KCC2 disinhibition cascade between microglia and lamina I neurons. Thus, BDNF release by activation of P2X4Rs in microglia impairs Cl^- homeostasis by downregulating K^+–Cl^- co-transporter KCC2 in those neurons (Ferrini et al., 2013).

P2X4Rs are also involved in other acute insults. Thus, the activation of microglia after hypoxia in the neonatal rat brain, a model of periventricular white matter damage, is mediated by P2X4R signaling (Li et al., 2011). P2X4 is also upregulated in microglial cells in the CA1 and transition zone to CA2 regions of the hippocampus after ischemia (Cavaliere et al., 2003), and its blockade confers neuronal protection (Cavaliere et al., 2005). Conversely, activation of P2X4 purinergic signaling in glia after traumatic injury stimulates the synthesis and release of thrombospondin-1, an extracellular matrix molecule that induces synapse formation during development, and it may play a role in CNS repair and remodeling after injury (Tran and Neary, 2006).

P2X7 RECEPTORS: A PROMISING TARGET FOR NEUROPROTECTION

In immune cells, P2X7R activation promotes assembly of the inflammasome, and caspase-1-dependent cleavage and release of biologically active IL-1β and IL-18 *in vitro* and *in vivo*, ultimately leading to a rapid form of cell death called pyroptosis (Miao et al., 2011). P2X7R antagonists improve neuronal viability by inhibiting P2X7R-activated NLRP3 inflammasome formation and the subsequent IL-1β release from glia (Murphy et al., 2012). P2X7R stimulation in neurons also induces inflammasome activation in these cells (Silverman et al., 2009). In addition, P2X7R activation in neurons and oligodendrocytes leads to a massive calcium influx that induces mitochondrial damage and initiates the apoptotic cascade (Matute et al., 2007; Díaz-Hernández et al., 2009; Arbeloa et al., 2012). P2X7Rs have unique properties that could be relevant to pathological conditions. First, P2X7Rs have high Ca^{2+} permeability, similar to that of NMDA receptors. Second, in contrast to NMDA receptors, P2X7Rs can be activated at resting membrane potentials and do not require membrane depolarization. Finally, P2X7Rs do not desensitize and open a large pore that causes cytolytic cell death after prolonged activation (Surprenant et al., 1996). Prolonged activation of P2X7Rs kills all CNS cells, including neurons (Jun et al., 2007; Díaz-Hernández et al., 2009; Arbeloa et al., 2012), oligodendrocytes (Matute et al., 2007), astrocytes (Kim et al., 2011), and microglia (Harada et al., 2011).

Acute insults, such as trauma and ischemia, lead to a massive release of nucleotides from disrupted cells at a level sufficient to activate low-affinity P2X7Rs in neighboring neurons and oligodendrocytes, leading to their death by excitotoxicity (Wang et al., 2004; Domercq et al., 2010; Arbeloa et al., 2012). Both insults induced the activation of microglia and dramatic remodeling of purinoceptors in microglia, which could influence microglial functions with beneficial or detrimental consequences. P2X7R antagonists modulate microglial activation and the inflammatory response after ischemia, which could contribute to the therapeutic value of these antagonists (Melani et al., 2006; Chu et al., 2012). Cerebral microvascular occlusion elicits microvascular injury, mimicking the different degrees of stroke severity observed in patients. Recently, a role of microglial P2X7R in this type of injury has been proposed. After inducing focal microsphere embolism to microvessels, microglia are recruited to the lesion site through a P2X7R-dependent mechanism and release FasL contributing to neuroinflammation (Lu et al., 2012). The microglial response to P2X7R activation appears to be region specific. Thus, in the status epilepticus (SE), microglia appear amoeboid or phagocytic in the dentate gyrus (DG) and piriform cortex due to P2X7R activation, but elongated in the CA1 hippocampal regions and frontoparietal cortex (Choi et al., 2012).

P2X7R antagonists have also been protective in animal models of MS, amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, and Alzheimer's disease (**Table 2**). Whether protection is mediated by blocking neuronal or oligodendroglial excitotoxicity, inflammation, or both remains to be determined in most neurodegenerative diseases. In Alzheimer's disease, different mechanisms determine the beneficial effects of P2X7R antagonists. *In vivo* administration of P2X7R antagonists reduce amyloid plaque formation through a signaling cascade involving the activation of GSK-3 kinase and increased non-amyloidogenic amyloid precursor protein processing by α-secretase (Diaz-Hernandez et al., 2012). However, P2X7Rs are necessary for β-amyloid-induced microglial activation (Sanz et al., 2009). The treatment of chronic EAE, the animal model of MS, with P2X7R antagonists reduces demyelination and ameliorates associated neurological symptoms (Matute et al., 2007). Because ATP signaling can trigger oligodendrocyte excitotoxicity (Matute et al.,

2007), the beneficial effect of P2X7R antagonists in this pathology may be due its protective role in oligodendrocytes and axons, more than the possible interference of the immune system. Importantly, sustained activation of P2X7Rs *in vivo* causes lesions that are reminiscent of the major features of MS plaques, and P2X7 RNA and protein levels are elevated in normal-appearing axon tracts in MS patients, suggesting that oligodendroglial signaling through P2X7Rs is enhanced in MS, which may render this cell type more vulnerable to ATP dysregulation (Matute et al., 2007).

P2X7Rs have a low affinity for ATP (100 μM-10 mM), and ATP levels in the extracellular space are in the low nanomolar range due to its rapid inactivation by powerful ubiquitous ecto-ATPases, whether this receptor is activated under physiological conditions is unclear. However, ATP is available at high concentrations within the cytoplasm (1–3 mM) and quickly released in sufficient quantities to activate P2X7Rs following cell damage in acute insults and chronic neurodegenerative diseases. These characteristics indicate an almost exclusive activation of P2X7Rs in pathological states and a low or negligible interference with

Table 2 | Neuroprotective properties of P2X7 receptor antagonists.

Disease	P2X7 receptor involvement	References
CHRONIC NEURODEGENERATION		
Alzheimer's disease	P2X7 mediates microglial neuroinflammatory reaction in different models of Alzheimer's disease	Parvathenani et al., 2003; Rampe et al., 2004; McLarnon et al., 2006
	P2X7 receptor blocks α-secretase activity/P2X7 triggers α-secretase activity	Delarasse et al., 2011; León-Otegui et al., 2011
	In vivo P2X7 inhibition reduces amyloid plaques in Alzheimer's disease	Diaz-Hernandez et al., 2012
	Upregulation of P2X7 in microglia in the cerebral cortex of the APPswe/PS1dE9 mice, a mouse model of AD	Lee et al., 2011
Amyotrophic lateral sclerosis	P2X7 receptor activation in spinal cord SOD1(G93A) astrocytes leads to motor neuron death	Gandelman et al., 2010
	P2X7 is overexpressed in activated microglial in ALS	Yiangou et al., 2006
Parkinson's disease	ATP mediates necrotic cell death in SN4741 dopaminergic neurons though P2X7 receptors	Jun et al., 2007
	P2X7 increases in astrocytes in the rotenone Parkinson's disease model	Gao et al., 2011
Huntington's disease	P2X7 antagonists prevented neuronal apoptosis in HD mice	Díaz-Hernández et al., 2009
Multiple sclerosis	$P2X7^{-/-}$ mice are more susceptble to EAE, the MS model	Chen and Brosnan, 2006; Witting et al., 2006
	P2X7 mediates ATP excitotoxicity to oligodendrocytes and P2X7 blockage improves neurological damage in EAE	Matute et al., 2007
	Association of gain of function P2X7 variants with MS	Oyanguren-Desez et al., 2011
ACUTE INSULTS		
Epilepsy	Enhanced purinergic signaling in microglia in status epilepticus	Avignone et al., 2008
	$P2X7^{-/-}$mice and Panx1 gene silencing showed greater susceptibility to pilocarpine-induced seizures	Kim and Kang, 2011
	P2X7 antagonists as well Panx1 gene silencing blocked status epilepticus induced by kainic acid	Santiago et al., 2011; Engel et al., 2012
	P2X7 antagonists prevented astroglial apoptosis in status epilepticus	Kim et al., 2009
Ischemia	P2X7 receptors is overexpressed in activated microglia and in neurons in different models of *in vitro* and *in vivo* ischemia	Cavaliere et al., 2004, 2005; Franke et al., 2004
	P2X7 antagonists reduces neuronal damage and infarct size after transient focal ischemia	Le Feuvre et al., 2003; Melani et al., 2006; Arbeloa et al., 2012
	P2X7 blockage amielorates oligodendroglial and axonal damage after white matter ischemia	Domercq et al., 2010
Trauma	P2X7 receptor inhibition improves recovery after spinal cord injury	Wang et al., 2004; Peng et al., 2009

normal brain functionality. Therefore, P2X7R could be an ideal therapeutic target for neurodegenerative diseases.

ADENOSINE RECEPTORS

Adenosine plays a relevant role as a neuromodulator and, thus, contributes to MS, a chronic disease with an autoimmune and inflammatory basis. A_1 receptor-null mice have been shown to develop more severe demyelination and motor symptoms in chronic EAE compared to their wild-type counterparts (Tsutsui et al., 2004). The aggravation of EAE is mostly mediated by cells in the microglial lineage and involves the release of toxic factors by macrophages/microglia lacking A_1 receptors (Tsutsui et al., 2004). More recently, a protective role of adenosine A_{2A} receptors was reported in this model. Genetic inactivation of the A_{2A} receptor exacerbates EAE pathology in mice. In addition, A_{2A} receptor knockout mice display increased inflammatory cell infiltration and enhanced microglial cell activation in the cortex, brainstem, and spinal cord (Mills et al., 2012; Yao et al., 2012).

Inflammation also contributes to post-ischemic delayed cerebral damage. A_3 adenosine receptor expression is modulated by the activation state of inflammatory cells and, in turn, its activation regulates the inflammatory activity of immune cells (Bar-Yehuda et al., 2007; Ochaion et al., 2009). The administration of A_3 agonists before or immediately after ischemic insults has been shown to significantly protect the brain in rodent ischemia models (Von Lubitz et al., 1994, 2001; Chen et al., 2006). Importantly, A_3 agonists protect against ischemic brain injury when applied 7 h after the ischemic insult (5.5 h after starting reperfusion) (Choi et al., 2011). The effect could be due to an inhibitory effect of the A_3 agonist on microglial/monocyte migration through the regulation of Rho GTPases (Choi et al., 2011).

ROLE OF GLUTAMATE RECEPTORS IN CNS INJURY

The possible contribution of microglia glutamate signaling to pathology has not been analyzed thoroughly. Few studies have demonstrated that the activation of ionotropic glutamate receptors in microglia has deleterious consequences to neurons and oligodendrocytes. NMDA receptor expression is upregulated in activated microglia following ischemia (Gottlieb and Matute, 1997; Kaur et al., 2006), which contributes to oligodendrocyte damage in hypoxic postnatal rats, a model of periventricular white matter damage. The activation of NMDA receptors in microglia leads to NO release in response to NF-kb signaling, which is known to induce oligodendrocyte cell death (Li et al., 2005). Pharmacological inhibition of NMDA receptors (MK801), NF-kb (BAY), and iNOS (1400w) prevents oligodendrocyte cell death (Murugan et al., 2011). Thus, NMDA receptor blockade protects oligodendrocytes by reducing the release of NO from microglia (Tahraoui et al., 2001; Murugan et al., 2011), in addition to the effect of direct blockage of NMDA receptors in these cells (Manning et al., 2008). In contrast, kainate-activated microglia induce IL-1β and TNF-α release, which mediate increased excitability of hippocampal CA3 neurons (Zheng et al., 2010; Zhu et al., 2010), an effect that could be relevant in acute insults.

The activation of microglial metabotropic receptors has been reported to regulate superoxide production by modulating NADPH oxidase (Nox) activity. Nox enzymes are major generators of ROS, which contribute to the progression of CNS disorders as diverse as amyotrophic lateral sclerosis, schizophrenia, Alzheimer's disease, Parkinson's disease, and stroke. Microglia are the predominant cells expressing Nox enzymes (Harrigan et al., 2008). Nox activation is elicited by agonists of metabotropic mGlu3 receptors, promotes neurotoxicity, and is inhibited by antagonists of mGluR5 receptors (Mead et al., 2012). For example, the regulation of Nox activity by mGluRs could contribute to limiting microglial activation after traumatic brain injury, improving motor and cognitive recovery (Byrnes et al., 2012).

ROLE OF MICROGLIA IN GLUTAMATE AND ATP HOMEOSTASIS AND ITS CONTRIBUTION TO NEURODEGENERATIVE DISEASES

ATP HOMEOSTASIS

ATP homeostasis is compromised in most CNS pathologies. Immediately after acute CNS injury, astrocytes and damaged cells release ATP, resulting in rapid activation of microglia. ATP and UTP are released by apoptotic cells as a "find-me" signal in the earliest stages of death to recruit phagocytes to the plasma membrane channel pannexin 1 (PANX1). Pharmacological inhibition and siRNA-mediated knockdown of PANX1 leads to decreased nucleotide release and monocyte recruitment by apoptotic cells (Chekeni et al., 2010). Pannexins also open following ischemic insult (Thompson et al., 2006; Domercq et al., 2010; MacVicar and Thompson, 2010), in response to high extracellular K^+ (Silverman et al., 2009), after NMDA receptor stimulation (Thompson et al., 2008), and, surprisingly, in response to caspase cleavage (Chekeni et al., 2010), which suggests that pannexins may open in most pathological contexts. However, the expression of pannexins in microglia and their possible influence on ATP release under normal conditions and after microglial activation has not been characterized. Finally, microglia are able to release ATP after activation with LPS, leading to an increase in excitatory neurotransmission (Pascual et al., 2012). Different mechanisms have been proposed, including microglial release of ATP via zinc uptake by zinc transporters (Higashi et al., 2011). In addition, lysosomes in microglia contain abundant ATP and exhibit Ca^{2+}-dependent exocytosis in response to various stimuli (Dou et al., 2012).

GLUTAMATE HOMEOSTASIS

Since the discovery of excitotoxicity and its contribution to neuronal cell death in neuropathology, numerous studies have aimed to understand the origin of alterations in glutamate homeostasis, which determines lethal overactivation of glutamate receptors. *In vitro* studies have demonstrated that activated microglia play a deleterious role by releasing glutamate or altering its homeostasis. Surprisingly, a recent study demonstrated a neuroprotective role of surveying microglia in excitotoxicity-induced neurodegeneration in the hippocampus (Vinet et al., 2012). This region exhibits differential sensitivity to excitotoxicity, with the CA1 region more vulnerable than CA3 or DG neurons. However, ablation of ramified microglia in the latter two areas exacerbates neuronal cell death, suggesting a protective role of surveying microglia in these

areas (Vinet et al., 2012). The mechanism by which surveying microglia confer protection remains to be determined.

Microglia, mainly in the activated state, contribute to alterations in neurotransmitter homeostasis via three main mechanisms: (1) release of excitotoxins, including glutamate (Piani et al., 1992), quinolinate (Heyes et al., 1996), D-serine (Wu et al., 2004), and ATP; (2) interfering with glutamate uptake, which is mainly carried out by astrocytes, leading to extracellular glutamate accumulation; or (3) altering astrocyte gliotransmitter release (including glutamate) or synaptic transmission.

A key determinant of microglial neurotoxicity is the release of excitotoxins, such as glutamate. The vast majority of glutamate exported from activated microglia can be attributed to the x_c^- exchange mechanism (a.k.a., SLC7A11 or CCBR1). This antiporter is a membrane-bound, Cl^--dependent, Na^+-independent antiporter that mediates the cellular uptake of cystine in exchange for glutamate at a 1:1 ratio, primarily following the relative concentration gradients of each of these amino acids (Bridges et al., 2012). This mechanism becomes extremely active in microglia because it is the primary route for internalizing cystine, which is converted to cysteine intracellularly, the rate-limiting substrate in glutathione synthesis (Bridges et al., 2012). As activated microglia produce ROS, they place themselves under severe oxidative stress. Although the bulk of superoxide produced by Nox is released from microglia, some remains intracellular. Thus, the microglial oxidative burst creates a GSH shortage that is alleviated by cystine influx through the x_c^- antiporter, extruding glutamate in the balance (Barger et al., 2007). The obligate exchange of glutamate could be deleterious to neuronal cells and other tissues that are susceptible to excitotoxic damage. Accordingly, the cystine/glutamate exchanger has been implicated in glutamate-associated disorders, such as glioma-derived epileptic seizures (Buckingham et al., 2011), oxidative glutamate toxicity (Oka et al., 1993; Albrecht et al., 2010), Alzheimer's disease (Barger and Basile, 2001; Qin et al., 2006), bacterial infection/LPS (Taguchi et al., 2007), MS (Domercq et al., 2007; Pampliega et al., 2011), Parkinson's disease, AIDS (Zeng et al., 2010), virally-induced encephalopathy (Espey et al., 1998; Qin et al., 2010), tumor proliferation (Ogunrinu and Sontheimer, 2010), antigen presentation (D'Angelo et al., 2010), and hypoxia (Fogal et al., 2007; Jackman et al., 2010).

Both surveying and activated microglia release glutamate through the cystine/glutamate antiporter (Domercq et al., 2007) (**Figure 2**). However, glutamate released through surveying microglia is rapidly and efficiently removed by glutamate transporters in other glial cells, mainly astrocytes, though also oligodendrocytes (Domercq et al., 1999). In contrast, the activation of microglia induces the release of factors, such as ROS, TNF-α, and IL-1β, that impair the function of EAATs, resulting in an increase in the extracellular levels of glutamate (Domercq et al., 2007). In addition, autoantigen-activated myelin basic protein-specific T cells also inhibit EAATs (Korn et al., 2005), suggesting that these mechanisms could contribute to alterations in glutamate homeostasis in the plasma and cerebrospinal fluid of MS patients. Gliomas also achieve excitotoxic levels of glutamate through high levels of system x_c^- activity coupled with a relative absence of sodium-dependent transport (Ye and Sontheimer, 1999; Ye et al., 1999; Kim et al., 2001; Takano et al., 2001; Rothstein, 2002; Chung et al., 2005). Glutamate released by system x_c^- in gliomas triggers excitotoxic cell death in the regions surrounding the tumor, allowing the tumor cells to migrate and invade (Lyons et al., 2007). Interestingly, glutamate export by the microglial cystine/glutamate antiporter is inhibited by mGluRII and III metabotropic glutamate receptor activation (McMullan et al., 2012).

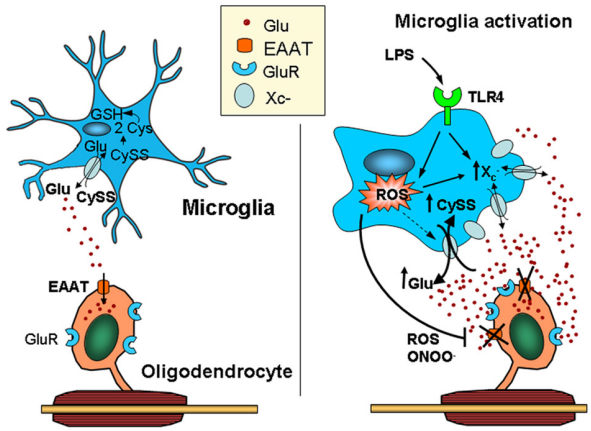


FIGURE 2 | Activated microglia can kill oligodendrocytes via a dual mechanism leading to glutamate excitotoxicity. Microglia release glutamate, primarily through the cystine/glutamate exchange system (system x_c^-), which is highly active in these cells due to its high rate of reactive oxygen species (ROS) production. Thus, cystine is intracellularly converted into cysteine, the rate-limiting substrate in glutathione synthesis. Under physiological conditions, glutamate released by the exchanger is efficiently taken up by glutamate transporters expressed in surrounding cells, including astrocytes and oligodendrocytes. In contrast, microglia activated by pro-inflammatory stimuli (e.g., LPS acting at TLR4) release ROS and pro-inflammatory cytokines that impair the function of glutamate transporters, such as TNF-α and IL-1β, elevating extracellular glutamate levels. In addition, the over-expression of system x_c^- in activated microglia increases ambient glutamate concentrations. Together, these deleterious effects on glutamate homeostasis can result in excitotoxicity (Domercq et al., 2007). CySS, cystine; Glu, glutamate; LPS, lipopolysaccharide; TLR4, Toll-like receptor.

Finally, microglia may regulate astrocyte-dependent synaptic modulation, called gliotransmission (Perea et al., 2009; Pascual et al., 2012). The first evidence of gliotransmission came from the seminal observation that glutamate release from cultured astrocytes (in this case stimulated via CXCR4 chemokine receptors) is dramatically amplified by the presence of activated microglia in the astrocytic microenvironment (Bezzi et al., 2001). Constitutive TNFα was reported to control astrocyte gliotransmission under physiological conditions (Stellwagen and Malenka, 2006; Santello et al., 2011). However, microglia activated by LPS release massive concentrations of TNF-α (approximately 10-fold); at these high concentrations, TNF-α changes its mode of action, not only gating, but also directly causing glutamate release from astrocytes. This alternative TNF-α action is mediated by prostaglandin E_2 (PGE_2) and was found to induce slow excitotoxic neuronal damage, both in cell culture and *in vivo* (Bezzi et al., 2001).

CONCLUSIONS

Neurotransmitter signaling is relevant to microglial functions. Microglia express receptors for neurotransmitters and contribute to neurotransmitter homeostasis. In particular, microglia are endowed with virtually all types of purinergic receptors that are differentially expressed at different stages and mediate process extension and retraction, cytokine release, migration, proliferation, and phagocytosis. In turn, activated microglia can release ATP and modulate synaptic activity. In addition, microglia also have an ample variety of glutamate receptors that mediate chemotaxis, ATP release, and surveillance of the functional status of synapses. Notably, microglia are critical controllers of glutamate homeostasis via the cystine/glutamate Xc- exchanger. Both ATP and glutamate receptors in microglia are relevant to neuroinflammation in various pathological conditions, including neurodegenerative diseases, and can be valuable targets for drug development in neuropathic pain and neuroprotection.

ACKNOWLEDGMENTS

Our work is funded by Fundación Mutua Madrileña, MINECO, ERANET-Neuron (Nanostroke), CIBERNED, Gobierno Vasco and Universidad del País Vasco. Nuria Vázquez-Villoldo holds a fellowship from Gobierno Vasco.

REFERENCES

Abbracchio, M. P., and Ceruti, S. (2007). P1 receptors and cytokine secretion. *Purinergic Signal* 3, 13–25.

Acarin, L., González, B., Castellano, B., and Castro, A. J. (1996). Microglial response to N-methyl-D-aspartate-mediated excitotoxicity in the immature rat brain. *J. Comp. Neurol.* 367, 361–374.

Albrecht, P., Lewerenz, J., Dittmer, S., Noack, R., Maher, P., and Methner, A. (2010). Mechanisms of oxidative glutamate toxicity: the glutamate/cystine antiporter system xc- as a neuroprotective drug target. *CNS Neurol. Disord. Drug Targets* 9, 373–382.

Arbeloa, J., Pérez-Samartín, A., Gottlieb, M., and Matute, C. (2012). P2X7 receptor blockade prevents ATP excitotoxicity in neurons and reduces brain damage after ischemia. *Neurobiol. Dis.* 45, 954–961.

Avignone, E., Ulmann, L., Levavasseur, F., Rassendren, F., and Audinat, E. (2008). Status epilepticus induces a particular microglial activation state characterized by enhanced purinergic signaling. *J. Neurosci.* 28, 9133–9144.

Ballerini, P., Di Iorio, P., Ciccarelli, R., Caciagli, F., Poli, A., Beraudi, A., et al. (2005). P2Y1 and cysteinyl leukotriene receptors mediate purine and cysteinyl leukotriene co-release in primary cultures of rat microglia. *Int. J. Immunopathol. Pharmacol.* 18, 255–268.

Barger, S. W., and Basile, A. S. (2001). Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function. *J. Neurochem.* 76, 846–854.

Barger, S. W., Goodwin, M. E., Porter, M. M., and Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J. Neurochem.* 101, 1205–1213.

Bar-Yehuda, S., Silverman, M. H., Kerns, W. D., Ochaion, A., Cohen, S., and Fishman, P. (2007). The anti-inflammatory effect of A3 adenosine receptor agonists: a novel targeted therapy for rheumatoid arthritis. *Expert Opin. Investig. Drugs* 16, 1601–1613.

Beggs, S., Trang, T., and Salter, M. W. (2012). P2X4R+ microglia drive neuropathic pain. *Nat. Neurosci.* 15, 1068–1073.

Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., et al. (2001). CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. *Nat. Neurosci.* 4, 702–710.

Biber, K., Laurie, D. J., Berthele, A., Sommer, B., Tölle, T. R., Gebicke-Härter, P. J., et al. (1999). Expression and signaling of group I metabotropic glutamate receptors in astrocytes and microglia. *J. Neurochem.* 72, 1671–1680.

Biber, K., Tsuda, M., Tozaki-Saitoh, H., Tsukamoto, K., Toyomitsu, E., Masuda, T., et al. (2011). Neuronal CCL21 up-regulates microglia P2X4 expression and initiates neuropathic pain development. *EMBO J.* 30, 1864–1873.

Block, M. L., and Hong, J. S. (2005). Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* 76, 77–98.

Boucsein, C., Zacharias, R., Färber, K., Pavlovic, S., Hanisch, U. K., and Kettenmann, H. (2003). Purinergic receptors on microglial cells: functional expression in acute brain slices and modulation of microglial activation *in vitro*. *Eur. J. Neurosci.* 17, 2267–2276.

Boumechache, M., Masin, M., Edwardson, J. M., Górecki, D. C., and Murrell-Lagnado, R. (2009). Analysis of assembly and trafficking of native P2X4 and P2X7 receptor complexes in rodent immune cells. *J. Biol. Chem.* 284, 13446–13454.

Brandenburg, L. O., Jansen, S., Wruck, C. J., Lucius, R., and Pufe, T. (2010). Antimicrobial peptide rCRAMP induced glial cell activation through P2Y receptor signalling pathways. *Mol. Immunol.* 47, 1905–1913.

Braun, N., Sévigny, J., Robson, S. C., Enjyoji, K., Guckelberger, O., Hammer, K., et al. (2000). Assignment of ecto-nucleoside triphosphate diphosphohydrolase-1/cd39 expression to microglia and vasculature of the brain. *Eur. J. Neurosci.* 12, 4357–4366.

Bridges, R., Lutgen, V., Lobner, D., and Baker, D. A. (2012). Thinking outside the cleft to understand synaptic activity: contribution of the cystine-glutamate antiporter (system xc) to normal and pathological glutamatergic signaling. *Pharmacol. Rev.* 64, 780–802.

Buckingham, S. C., Campbell, S. L., Haas, B. R., Montana, V., Robel, S., Ogunrinu, T., et al. (2011). Glutamate release by primary brain tumors induces epileptic activity. *Nat. Med.* 17, 1269–1274.

Bulavina, L., Szulzewsky, F., Rocha, A., Krabbe, G., Robson, S. C., Matyash, V., et al. (2012). NTPDase1 activity attenuates microglial phagocytosis. *Purinergic Signal*. doi: 10.1007/s11302-012-9339-y. [Epub ahead of print].

Bura, S. A., Nadal, X., Ledent, C., Maldonado, R., and Valverde, O. (2008). A 2A adenosine receptor regulates glia proliferation and pain after peripheral nerve injury. *Pain* 140, 95–103.

Burnstock, G. (2008). Purinergic signalling and disorders of the central nervous system. *Nat. Rev. Drug Discov.* 7, 575–590.

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A. E., Pluchino, S., et al. (2006). Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31, 149–160.

Byrnes, K. R., Loane, D. J., Stoica, B. A., Zhang, J., and Faden, A. I. (2012). Delayed mGluR5 activation limits neuroinflammation and neurodegeneration after traumatic brain injury. *J. Neuroinflammation* 28, 9–43.

Cavaliere, F., Amadio, S., Sancesario, G., Bernardi, G., and Volonté, C. (2004). Synaptic P2X7 and oxygen/glucose deprivation in organotypic hippocampal cultures. *J. Cereb. Blood Flow Metab.* 24, 392–398.

Cavaliere, F., Dinkel, K., and Reymann, K. (2005). Microglia response and P2 receptor participation in oxygen/glucose deprivation-induced cortical damage. *Neuroscience* 136, 615–623.

Cavaliere, F., Florenzano, F., Amadio, S., Fusco, F. R., Viscomi, M. T., D'Ambrosi, N., et al. (2003). Up-regulation of P2X2, P2X4 receptor and ischemic cell death: prevention by P2 antagonists. *Neuroscience* 120, 85–98.

Chekeni, F. B., Elliott, M. R., Sandilos, J. K., Walk, S. F., Kinchen, J. M., Lazarowski, E. R., et al. (2010). Pannexin 1 channels mediate 'find-me' signal release and membrane permeability during apoptosis. *Nature* 467, 863–867.

Chen, G. J., Harvey, B. K., Shen, H., Chou, J., Victor, A., and Wang, Y. (2006). Activation of adenosine A3 receptors reduces ischemic brain injury in rodents. *J. Neurosci. Res.* 84, 1848–1855.

Chen, L., and Brosnan, C. F. (2006). Exacerbation of experimental autoimmune encephalomyelitis in P2X7R-/- mice: evidence for loss of apoptotic activity in lymphocytes. *J. Immunol.* 176, 3115–3126.

Choi, H. K., Ryu, H. J., Kim, J. E., Jo, S. M., Choi, H. C., Song, H. K., et al. (2012). The roles of P2X7 receptor in regional-specific microglial responses in the rat brain following status epilepticus. *Neurol. Sci.* 33, 515–525.

Choi, I. Y., Lee, J. C., Ju, C., Hwang, S., Cho, G. S., Lee, H. W., et al. (2011). A3 adenosine receptor agonist reduces brain ischemic injury and inhibits inflammatory cell migration in rats. *Am. J. Pathol.* 179, 2042–2052.

Christensen, R. N., Ha, B. K., Sun, F., Bresnahan, J. C., and Beattie, M. S. (2006). Kainate induces rapid redistribution of the actin cytoskeleton in ameboid microglia. *J. Neurosci. Res.* 84, 170–181.

Chu, K., Yin, B., Wang, J., Peng, G., Liang, H., Xu, Z., et al. (2012). Inhibition of P2X7 receptor ameliorates transient global cerebral ischemia/reperfusion injury via modulating inflammatory responses in the rat hippocampus. *J. Neuroinflammation* 9:69. doi: 10.1186/1742-2094-9-69

Chung, W. J., Lyons, S. A., Nelson, G. M., Hamza, H., Gladson, C. L., Gillespie, G. Y., et al. (2005). Inhibition of cystine uptake disrupts the growth of primary brain tumors. *J. Neurosci.* 25, 7101–7110.

Csóka, B., Selmeczy, Z., Koscsó, B., Németh, Z. H., Pacher, P., Murray, P. J., et al. (2012). Adenosine promotes alternative macrophage activation via A2A and A2B receptors. *FASEB J.* 26, 376–386.

Cull-Candy, S. G., and Leszkiewicz, D. N. (2004). Role of distinct NMDA receptor subtypes at central synapses. *Sci. STKE* 2004:re16. doi: 10.1126/stke.2552004re16

D'Ambrosi, N., Finocchi, P., Apolloni, S., Cozzolino, M., Ferri, A., Padovano, V., et al. (2009). The proinflammatory action of microglial P2 receptors is enhanced in SOD1 models for amyotrophic lateral sclerosis. *J. Immunol.* 183, 4648–4656.

D'Angelo, J. A., Dehlink, E., Platzer, B., Dwyer, P., Circu, M. L., Garay, J., et al. (2010). The cystine/glutamate antiporter regulates dendritic cell differentiation and antigen presentation. *J. Immunol.* 185, 3217–3226.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758.

Delarasse, C., Auger, R., Gonnord, P., Fontaine, B., and Kanellopoulos, J. M. (2011). The purinergic receptor P2X7 triggers alpha-secretase-dependent processing of the amyloid precursor protein. *J. Biol. Chem.* 286, 2596–2606.

de Rivero Vaccari, J. P., Bastien, D., Yurcisin, G., Pineau, I., Dietrich, W. D., De Koninck, Y., et al. (2012). P2X4 receptors influence inflammasome activation after spinal cord injury. *J. Neurosci.* 32, 3058–3066.

Di Virgilio, F., Ceruti, S., Bramanti, P., and Abbracchio, M. P. (2009). Purinergic signalling in inflammation of the central nervous system. *Trends Neurosci.* 32, 79–87.

Diaz-Hernandez, J. I., Gomez-Villafuertes, R., León-Otegui, M., Hontecillas-Prieto, L., Del Puerto, A., Trejo, J. L., et al. (2012). *In vivo* P2X7 inhibition reduces amyloid plaques in Alzheimer's disease through GSK3β and secretases. *Neurobiol. Aging* 33, 1816–1828.

Díaz-Hernández, M., Díez-Zaera, M., Sánchez-Nogueiro, J., Gómez-Villafuertes, R., Canals, J. M., Alberch, J., et al. (2009). Altered P2X7-receptor level and function in mouse models of Huntington's disease and therapeutic efficacy of antagonist administration. *FASEB J.* 23, 1893–1906.

Domercq, M., Perez-Samartin, A., Aparicio, D., Alberdi, E., Pampliega, O., and Matute, C. (2010). P2X7 receptors mediate ischemic damage to oligodendrocytes. *Glia* 58, 730–740.

Domercq, M., Sánchez-Gómez, M. V., Areso, P., and Matute, C. (1999). Expression of glutamate transporters in rat optic nerve oligodendrocytes. *Eur. J. Neurosci.* 11, 2226–2236.

Domercq, M., Sánchez-Gómez, M. V., Sherwin, C., Etxebarria, E., Fern, R., and Matute, C. (2007). System xc- and glutamate transporter inhibition mediates microglial toxicity to oligodendrocytes. *J. Immunol.* 178, 6549–6556.

Dou, Y., Wu, H. J., Li, H. Q., Qin, S., Wang, Y. E., Li, J., et al. (2012). Microglial migration mediated by ATP-induced ATP release from lysosomes. *Cell Res.* 22, 1022–1033.

Engel, T., Gomez-Villafuertes, R., Tanaka, K., Mesuret, G., Sanz-Rodriguez, A., Garcia-Huerta, P., et al. (2012). Seizure suppression and neuroprotection by targeting the purinergic P2X7 receptor during status epilepticus in mice. *FASEB J.* 26, 1616–1628.

Espey, M. G., Kustova, Y., Sei, Y., and Basile, A. S. (1998). Extracellular glutamate levels are chronically elevated in the brains of LP-BM5-infected mice: a mechanism of retrovirus-induced encephalopathy. *J. Neurochem.* 71, 2079–2087.

Eun, S. Y., Hong, Y. H., Kim, E. H., Jeon, H., Suh, Y. H., Lee, J. E., et al. (2004). Glutamate receptor-mediated regulation of c-fos expression in cultured microglia. *Biochem. Biophys. Res. Commun.* 325, 320–327.

Fang, K. M., Yang, C. S., Sun, S. H., and Tzeng, S. F. (2009). Microglial phagocytosis attenuated by short-term exposure to exogenous ATP through P2X receptor action. *J. Neurochem.* 111, 225–237.

Färber, K., Markworth, S., Pannasch, U., Nolte, C., Prinz, V., Kronenberg, G., et al. (2008). The ectonucleotidase cd39/ENTPDase1 modulates purinergic-mediated microglial migration. *Glia* 56, 331–341.

Ferrari, D., Villalba, M., Chiozzi, P., Falzoni, S., Ricciardi-Castagnoli, P., and Di Virgilio, F. (1996). Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. *J. Immunol.* 156, 1531–1539.

Ferrini, F., Trang, T., Mattioli, T. A., Laffray, S., Del'guidice, T., Lorenzo, L. E., et al. (2013). Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl(-) homeostasis. *Nat. Neurosci.* 16, 183–192.

Fogal, B., Li, J., Lobner, D., McCullough, L. D., and Hewett, S. J. (2007). System x(c)- activity and astrocytes are necessary for interleukin-1 beta-mediated hypoxic neuronal injury. *J. Neurosci.* 27, 10094–10105.

Fontainhas, A. M., Wang, M., Liang, K. J., Chen, S., Mettu, P., Damani, M., et al. (2011). Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and GABAergic neurotransmission. *PLoS ONE* 6:e15973. doi: 10.1371/journal.pone.0015973

Franke, H., Günther, A., Grosche, J., Schmidt, R., Rossner, S., Reinhardt, R., et al. (2004). P2X7 receptor expression after ischemia in the cerebral cortex of rats. *J. Neuropathol. Exp. Neurol.* 63, 686–699.

Franke, H., Schepper, C., Illes, P., and Krügel, U. (2007). Involvement of P2X and P2Y receptors in microglial activation *in vivo*. *Purinergic Signal.* 3, 435–445.

Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., Klotz, K. N., and Linden, J. (2001). International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53, 527–552.

Gandelman, M., Peluffo, H., Beckman, J. S., Cassina, P., and Barbeito, L. (2010). Extracellular ATP and the P2X7 receptor in astrocyte-mediated motor neuron death: implications for amyotrophic lateral sclerosis. *J. Neuroinflammation* 7:33. doi: 10.1186/1742-2094-7-33

Gao, X. F., Wang, W., Yu, Q., Burnstock, G., Xiang, Z. H., and He, C. (2011). Astroglial P2X7 receptor current density increased following long-term exposure to rotenone. *Purinergic Signal.* 7, 65–72.

Gottlieb, M., and Matute, C. (1997). Expression of ionotropic glutamate receptor subunits in glial cells of the hippocampal CA1 area following transient forebrain ischemia. *J. Cereb. Blood Flow Metab.* 17, 290–300.

Guo, L. H., and Schluesener, H. J. (2005). Lesional accumulation of P2X4 receptor-macrophages in rat CNS during experimental autoimmune encephalomyelitis. *Neuroscience* 134, 199–205.

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394.

Hanley, P. J., Kronlage, M., Kirschning, C., del Rey, A., Di Virgilio, F., Leipziger, J., et al. (2012). Transient P2X7 receptor activation triggers macrophage death independent of Toll-like receptors 2 and 4, caspase-1, and pannexin-1 proteins. *J. Biol. Chem.* 287, 10650–10663.

Harada, K., Hide, I., Seki, T., Tanaka, S., Nakata, Y., and Sakai, N. (2011). Extracellular ATP differentially modulates Toll-like receptor 4-mediated cell survival and death of microglia. *J. Neurochem.* 116, 1138–1147.

Harrigan, T. J., Abdullaev, I. F., Jourd'heuil, D., and Mongin, A. A. (2008). Activation of microglia with zymosan promotes excitatory amino acid release via volume-regulated anion channels: the role of NADPH oxidases. *J. Neurochem.* 106, 2449–2462.

Haselkorn, M. L., Shellington, D. K., Jackson, E. K., Vagni, V. A., Janesko-Feldman, K., Dubey, R. K., et al. (2010). Adenosine A1 receptor activation as a brake on the microglial response after experimental traumatic brain injury in mice. *J. Neurotrauma* 27, 901–910.

Haskó, G., Pacher, P., Vizi, E. S., and Illes, P. (2005). Adenosine receptor signaling in the brain immune

system. *Trends Pharmacol. Sci.* 26, 511–516.

Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W. B., et al. (2006). The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* 9, 1512–1519.

Heyes, M. P., Achim, C. L., Wiley, C. A., Major, E. O., Saito, K., and Markey, S. P. (1996). Human microglia convert l-tryptophan into the neurotoxin quinolinic acid. *Biochem. J.* 320, 595–597.

Higashi, Y., Segawa, S., Matsuo, T., Nakamura, S., Kikkawa, Y., Nishida, K., et al. (2011). Microglial zinc uptake via zinc transporters induces ATP release and the activation of microglia. *Glia* 59, 1933–1945.

Honda, S., Sasaki, Y., Ohsawa, K., Imai, Y., Nakamura, Y., Inoue, K., et al. (2001). Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. *J. Neurosci.* 21, 1975–1982.

Honore, P., Donnelly-Roberts, D., Namovic, M., Zhong, C., Wade, C., Chandran, P., et al. (2009). The antihyperalgesic activity of a selective P2X7 receptor antagonist, A-839977, is lost in IL-1alphabeta knockout mice. *Behav. Brain Res.* 204, 77–81.

Idzko, M., Hammad, H., van Nimwegen, M., Kool, M., Willart, M. A., Muskens, F., et al. (2007). Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat. Med.* 13, 913–919.

Jackman, N. A., Uliasz, T. F., Hewett, J. A., and Hewett, S. J. (2010). Regulation of system x(c)(-)activity and expression in astrocytes by interleukin-1β: implications for hypoxic neuronal injury. *Glia* 58, 1806–1815.

Jun, D. J., Kim, J., Jung, S. Y., Song, R., Noh, J. H., Park, Y. S., et al. (2007). Extracellular ATP mediates necrotic cell swelling in SN4741 dopaminergic neurons through P2X7 receptors. *J. Biol. Chem.* 282, 37350–37358.

Kaur, C., Sivakumar, V., Ang, L. S., and Sundaresan, A. (2006). Hypoxic damage to the periventricular white matter in neonatal brain: role of vascular endothelial growth factor, nitric oxide and excitotoxicity. *J. Neurochem.* 98, 1200–1216.

Kawanokuchi, J., Shimizu, K., Nitta, A., Yamada, K., Mizuno, T., Takeuchi, H., et al. (2008). Production and functions of IL-17 in microglia. *J. Neuroimmunol.* 194, 54–61.

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Khakh, B. S., and North, R. A. (2012). Neuromodulation by extracellular ATP and P2X receptors in the CNS. *Neuron* 76, 51–69.

Kim, B., Jeong, H. K., Kim, J. H., Lee, S. Y., Jou, I., and Joe, E. H. (2011). Uridine 5′-diphosphate induces chemokine expression in microglia and astrocytes through activation of the P2Y6 receptor. *J. Immunol.* 186, 3701–3709.

Kim, H. J., Ajit, D., Peterson, T. S., Wang, Y., Camden, J. M., Gibson Wood, W., et al. (2012). Nucleotides released from $A\beta_{1–42}$-treated microglial cells increase cell migration and $A\beta_{1–42}$ uptake through $P2Y_2$ receptor activation. *J. Neurochem.* 121, 228–238.

Kim, J. E., and Kang, T. C. (2011). The P2X7 receptor-pannexin-1 complex decreases muscarinic acetylcholine receptor-mediated seizure susceptibility in mice. *J. Clin. Invest.* 121, 2037–2047.

Kim, J. E., Kwak, S. E., Jo, S. M., and Kang, T. C. (2009). Blockade of P2X receptor prevents astroglial death in the dentate gyrus following pilocarpine-induced status epilepticus. *Neurol. Res.* 31, 982–988.

Kim, J. Y., Kanai, Y., Chairoungdua, A., Cha, S. H., Matsuo, H., Kim, D. K., et al. (2001). Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochim. Biophys. Acta* 1512, 335–3344.

Kobayashi, K., Yamanaka, H., Fukuoka, T., Dai, Y., Obata, K., and Noguchi, K. (2008). P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. *J. Neurosci.* 28, 2892–2902.

Kobayashi, K., Yamanaka, H., Yanamoto, F., Okubo, M., and Noguchi, K. (2012). Multiple P2Y subtypes in spinal microglia are involved in neuropathic pain after peripheral nerve injury. *Glia* 60, 1529–1539.

Koizumi, S., Ohsawa, K., Inoue, K., and Kohsaka, S. (2013). Purinergic receptors in microglia: functional modal shifts of microglia mediated by P2 and P1 receptors. *Glia* 61, 47–54.

Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., et al. (2007). UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. *Nature* 446, 1091–1095.

Korn, T., Magnus, T., and Jung, S. (2005). Autoantigen specific T cells inhibit glutamate uptake in astrocytes by decreasing expression of astrocytic glutamate transporter GLAST: a mechanism mediated by tumor necrosis factor-alpha. *FASEB J.* 19, 1878–1880.

Koscsó, B., Csóka, B., Selmeczy, Z., Himer, L., Pacher, P., Virág, L., et al. (2012). Adenosine augments IL-10 production by microglial cells through an A2B adenosine receptor-mediated process. *J. Immunol.* 188, 445–453.

Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312–318.

Kuehnel, M. P., Reiss, M., Anand, P. K., Treede, I., Holzerm, D., Hoffmann, E., et al. (2009a). Sphingosine-1-phosphate receptors stimulate macrophage plasma-membrane actin assembly via ADP release, ATP synthesis and P2X7R activation. *J. Cell Sci.* 122, 505–512.

Kuehnel, M. P., Rybin, V., Anand, P. K., Anes, E., and Griffiths, G. (2009b). Lipids regulate P2X7-receptor-dependent actin assembly by phagosomes via ADP translocation and ATP synthesis in the phagosome lumen. *J. Cell Sci.* 122, 499–504.

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170.

Le Feuvre, R. A., Brough, D., Touzani, O., and Rothwell, N. J. (2003). Role of P2X7 receptors in ischemic and excitotoxic brain injury *in vivo*. *J. Cereb. Blood Flow Metab.* 23, 381–384.

Lee, H. G., Won, S. M., Gwag, B. J., and Lee, Y. B. (2011). Microglial $P2X_7$ receptor expression is accompanied by neuronal damage in the cerebral cortex of the APPswe/PS1dE9 mouse model of Alzheimer's disease. *Exp. Mol. Med.* 43, 7–14.

León-Otegui, M., Gómez-Villafuertes, R., Díaz-Hernández, J. I., Díaz-Hernández, M., Miras-Portugal, M. T., and Gualix, J. (2011). Opposite effects of P2X7 and P2Y2 nucleotide receptors on α-secretase-dependent APP processing in Neuro-2a cells. *FEBS Lett.* 585, 2255–2262.

Lerma, J. (2003). Roles and rules of kainate receptors in synaptic transmission. *Nat. Rev. Neurosci.* 4, 481–495.

Li, F., Wang, L., Li, J. W., Gong, M., He, L., Feng, R., et al. (2011). Hypoxia induced amoeboid microglial cell activation in postnatal rat brain is mediated by ATP receptor P2X4. *BMC Neurosci.* 12:111. doi: 10.1186/1471-2202-12-111

Li, J., Baud, O., Vartanian, T., Volpe, J. J., and Rosenberg, P. A. (2005). Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9936–9941.

Li, Y., Du, X. F., Liu, C. S., Wen, Z. L., and Du, J. L. (2012). Reciprocal regulation between resting microglial dynamics and neuronal activity *in vivo*. *Dev. Cell* 23, 1189–1202.

Liu, G. J., Nagarajah, R., Banati, R. B., and Bennett, M. R. (2009). Glutamate induces directed chemotaxis of microglia. *Eur. J. Neurosci.* 29, 1108–1118.

Locovei, S., Scemes, E., Qiu, F., Spray, D. C., and Dahl, G. (2007). Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. *FEBS Lett.* 581, 483–488.

Loram, L. C., Harrison, J. A., Sloane, E. M., Hutchinson, M. R., Sholar, P., Taylor, F. R., et al. (2009). Enduring reversal of neuropathic pain by a single intrathecal injection of adenosine 2A receptor agonists: a novel therapy for neuropathic pain. *J. Neurosci.* 29, 14015–14025.

Lu, Y. M., Tao, R. R., Huang, J. Y., Li, L. T., Liao, M. H., Li, X. M., et al. (2012). P2X(7) signaling promotes microsphere embolism-triggered microglia activation by maintaining elevation of Fas ligand. *J. Neuroinflammation* 9:172. doi: 10.1186/1742-2094-9-172

Luongo, L., Petrelli, R., Gatta, L., Giordano, C., Guida, F., Vita, P., et al. (2012). 5′-Chloro-5′-deoxy-(and#177;)-ENBA, a potent and selective adenosine A1 receptor agonist, alleviates neuropathic pain in mice through functional glial and microglial changes without affecting motor or cardiovascular functions. *Molecules* 17, 13712–13726.

Lynch, M. A. (2009). The multifaceted profile of activated microglia. *Mol. Neurobiol.* 40, 139–156.

Lyons, S. A., Chung, W. J., Weaver, A. K., Ogunrinu, T., and Sontheimer, H. (2007). Autocrine glutamate signaling promotes glioma cell invasion. *Cancer Res.* 67, 9463–9471.

MacVicar, B. A., and Thompson, R. J. (2010). Non-junction functions of pannexin-1 channels. *Trends Neurosci.* 33, 93–102.

Maeda, M., Tsuda, M., Tozaki-Saitoh, H., Inoue, K., and Kiyama, H. (2010). Nerve injury-activated microglia engulf myelinated axons in a P2Y12 signaling-dependent

manner in the dorsal horn. *Glia* 58, 1838–1846.

Manning, S. M., Talos, D. M., Zhou, C., Selip, D. B., Park, H. K., Park, C. J., et al. (2008). NMDA receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. *J. Neurosci.* 28, 6670–6678.

Matute, C., Torre, I., Pérez-Cerdá, F., Pérez-Samartín, A., Alberdi, E., Etxebarria, E., et al. (2007). P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. *J. Neurosci.* 27, 9525–9533.

McLarnon, J. G., Ryu, J. K., Walker, D. G., and Choi, H. B. (2006). Upregulated expression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide-injected rat hippocampus. *J. Neuropathol. Exp. Neurol.* 65, 1090–1097.

McLarnon, J. G., Zhang, L., Goghari, V., Lee, Y. B., Walz, W., Krieger, C., et al. (1999). Effects of ATP and elevated K+ on K+ currents and intracellular Ca2+ in human microglia. *Neuroscience* 91, 343–352.

McMullan, S. M., Phanavanh, B., Li, G. G., and Barger, S. W. (2012). Metabotropic glutamate receptors inhibit microglial glutamate release. *ASN Neuro*. doi: 10.1042/AN20120044. [Epub ahead of print].

Mead, E. L., Mosley, A., Eaton, S., Dobson, L., Heales, S. J., and Pocock, J. M. (2012). Microglial neurotransmitter receptors trigger superoxide production in microglia; consequences for microglial-neuronal interactions. *J. Neurochem.* 121, 287–301.

Melani, A., Amadio, S., Gianfriddo, M., Vannucchi, M. G., Volontè, C., Bernardi, G., et al. (2006). P2X7 receptor modulation on microglial cells and reduction of brain infarct caused by middle cerebral artery occlusion in rat. *J. Cereb. Blood Flow Metab.* 26, 974–982.

Merrill, J. E., Ignarro, L. J., Sherman, M. P., Melinek, J., and Lane, T. E. (1993). Microglial cell cytotoxicity of oligodendrocytes is mediated through nitric oxide. *J. Immunol.* 151, 2132–2141.

Miao, E. A., Rajan, J. V., and Aderem, A. (2011). Caspase-1-induced pyroptotic cell death. *Immunol. Rev.* 243, 206–214.

Mills, J. H., Kim, D. G., Krenz, A., Chen, J. F., and Bynoe, M. S. (2012). A2A adenosine receptor signaling in lymphocytes and the central nervous system regulates inflammation during experimental autoimmune encephalomyelitis. *J. Immunol.* 188, 5713–5722.

Monif, M., Reid, C. A., Powell, K. L., Smart, M. L., and Williams, D. A. (2009). The P2X7 receptor drives microglial activation and proliferation: a trophic role for P2X7R pore. *J. Neurosci.* 29, 3781–3791.

Murphy, N., Cowley, T. R., Richardson, J. C., Virley, D., Upton, N., Walter, D., et al. (2012). The neuroprotective effect of a specific P2X7 receptor antagonist derives from its ability to inhibit assembly of the NLRP3 inflammasome in glial cells. *Brain Pathol.* 22, 295–306.

Murugan, M., Sivakumar, V., Lu, J., Ling, E. A., and Kaur, C. (2011). Expression of N-methyl D-aspartate receptor subunits in amoeboid microglia mediates production of nitric oxide via NF-κB signaling pathway and oligodendrocyte cell death in hypoxic postnatal rats. *Glia* 59, 521–539.

Neher, J. J., Neniskyte, U., Zhao, J. W., Bal-Price, A., Tolkovsky, A. M., and Brown, G. C. (2011). Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. *J. Immunol.* 186, 4973–4983.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318.

Noda, M., Nakanishi, H., Nabekura, J., and Akaike, N. (2000). AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. *J. Neurosci.* 20, 251–258.

Nörenberg, W., Langosch, J. M., Gebicke-Haerter, P. J., and Illes, P. (1994). Characterization and possible function of adenosine 5′-triphosphate receptors in activated rat microglia. *Br. J. Pharmacol.* 111, 942–950.

North, R. A. (2002). Molecular physiology of P2X receptors. *Physiol. Rev.* 82, 1013–1067.

Ochaion, A., Bar-Yehuda, S., Cohen, S., Barer, F., Patoka, R., Amital, H., et al. (2009). The anti-inflammatory target A(3) adenosine receptor is over-expressed in rheumatoid arthritis, psoriasis and Crohn's disease. *Cell. Immunol.* 258, 115–122.

Ogunrinu, T. A., and Sontheimer, H. (2010). Hypoxia increases the dependence of glioma cells on glutathione. *J. Biol. Chem.* 285, 37716–37724.

Ohsawa, K., Irino, Y., Nakamura, Y., Akazawa, C., Inoue, K., and Kohsaka, S. (2007). Involvement of P2X4 and P2Y12 receptors in ATP-induced microglial chemotaxis. *Glia* 55, 604–616.

Ohsawa, K., Sanagi, T., Nakamura, Y., Suzuki, E., Inoue, K., and Kohsaka, S. (2012). Adenosine A3 receptor is involved in ADP-induced microglial process extension and migration. *J. Neurochem.* 121, 217–227.

Oka, A., Belliveau, M. J., Rosenberg, P. A., and Volpe, J. J. (1993). Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. *J. Neurosci.* 13, 1441–1453.

Orr, A. G., Orr, A. L., Li, X.-J., Gross, R. E., and Traynelis, S. F. (2009). Adenosine A2A receptor mediates microglial process retraction. *Nat. Neurosci.* 12, 872–878.

Oyanguren-Desez, O., Rodríguez-Antigüedad, A., Villoslada, P., Domercq, M., Alberdi, E., and Matute, C. (2011). Gain-of-function of P2X7 receptor gene variants in multiple sclerosis. *Cell Calcium* 50, 468–472.

Pampliega, O., Domercq, M., Soria, F. N., Villoslada, P., Rodríguez-Antigüedad, A., and Matute, C. (2011). Increased expression of cystine/glutamate antiporter in multiple sclerosis. *J. Neuroinflammation* 8:63. doi: 10.1186/1742-2094-8-63

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Parvathenani, L. K., Tertyshnikova, S., Greco, C. R., Roberts, S. B., Robertson, B., and Posmantur, R. (2003). P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J. Biol. Chem.* 278, 13309–13317.

Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2012). Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U.S.A.* 109, 197–205.

Pedata, F., Corsi, C., Melani, A., Bordoni, F., and Latini, S. (2001). Adenosine extracellular brain concentrations and role of A2A receptors in ischemia. *Ann. N.Y. Acad. Sci.* 939, 74–84.

Pelegrin, P., and Surprenant, A. (2006). Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. *EMBO J.* 25, 5071–5082.

Pellegatti, P., Raffaghello, L., Bianchi, G., Piccardi, F., Pistoia, V., and Di Virgilio, F. (2008). Increased level of extracellular ATP at tumor sites: *in vivo* imaging with plasma membrane luciferase. *PLoS ONE* 3:2599. doi: 10.1371/journal.pone.0002599

Peng, W., Cotrina, M. L., Han, X., Yu, H., Bekar, L., Blum, L., et al. (2009). Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after spinal cord injury. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12489–12493.

Perea, G., Navarrete, M., and Araque, A. (2009). Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci.* 32, 421–431.

Piani, D., Spranger, M., Frei, K., Schaffner, A., and Fontana, A. (1992). Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines. *Eur. J. Immunol.* 22, 2429–2436.

Pinteaux-Jones, F., Sevastou, I. G., Fry, V. A., Heales, S., Baker, D., and Pocock, J. M. (2008). Myelin-induced microglial neurotoxicity can be controlled by microglial metabotropic glutamate receptors. *J. Neurochem.* 106, 442–454.

Pocock, J. M., and Kettenmann, H. (2007). Neurotransmitter receptors on microglia. *Trends Neurosci.* 30, 527–735.

Ponomarev, E. D., Maresz, K., Tan, Y., and Dittel, B. N. (2007). CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. *J. Neurosci.* 27, 10714–10721.

Popovich, P. G., Tovar, C. A., Wei, P., Fisher, L., Jakeman, L. B., and Basso, D. M. (2011). A reassessment of a classic neuroprotective combination therapy for spinal cord injured rats: LPS/pregnenolone/indomethacin. *Exp. Neurol.* 233, 677–685.

Qin, S., Colin, C., Hinners, I., Gervais, A., Cheret, C., and Mallat, M. (2006). System Xc- and apolipoprotein E expressed by microglia have opposite effects on the neurotoxicity of amyloid-beta peptide 1–40. *J. Neurosci.* 26, 3345–3356.

Qin, Z., Freitas, E., Sullivan, R., Mohan, S., Bacelieri, R., Branch, D., et al. (2010). Upregulation of xCT by KSHV-encoded microRNAs facilitates KSHV dissemination and persistence in an environment of oxidative stress. *PLoS Pathog.* 6:e1000742. doi: 10.1371/journal.ppat.1000742

Ralevic, V., and Burnstock, G. (1998). Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50, 413–492.

Rampe, D., Wang, L., and Ringheim, G. E. (2004). P2X7 receptor modulation of beta-amyloid- and LPS-induced cytokine secretion from human macrophages and microglia. *J. Neuroimmunol.* 147, 56–61.

Ransohoff, R. M., and Perry, V. H. (2009). Microglial physiology: unique stimuli, specialized responses. *Annu. Rev. Immunol.* 27, 119–145.

Rigato, C., Buckinx, R., Le-Corronc, H., Rigo, J. M., and Legendre, P. (2011). Pattern of invasion of the embryonic mouse spinal cord by microglial cells at the time of the onset of functional neuronal networks. *Glia* 59, 675–695.

Rigato, C., Swinnen, N., Buckinx, R., Couillin, I., Mangin, J. M., Rigo, J. M., et al. (2012). Microglia proliferation is controlled by P2X7 receptors in a Pannexin-1-independent manner during early embryonic spinal cord invasion. *J. Neurosci.* 32, 11559–11573.

Rothstein, J. D. (2002). Paving new pathways. *Nat. Med.* 8, 938–940.

Santello, M., Bezzi, P., and Volterra, A. (2011). TNFα controls glutamatergic gliotransmission in the hippocampal dentate gyrus. *Neuron* 69, 988–1001.

Santiago, M. F., Veliskova, J., Patel, N. K., Lutz, S. E., Caille, D., Charollais, A., et al. (2011). Targeting pannexin1 improves seizure outcome. *PLoS ONE* 6:e25178. doi: 10.1371/journal.pone.0025178

Sanz, J. M., Chiozzi, P., Ferrari, D., Colaianna, M., Idzko, M., Falzoni, S., et al. (2009). Activation of microglia by amyloid {beta} requires P2X7 receptor expression. *J. Immunol.* 182, 4378–4385.

Sasaki, Y., Hoshi, M., Akazawa, C., Nakamura, Y., Tsuzuki, H., Inoue, K., et al. (2003). Selective expression of Gi/o-coupled ATP receptor P2Y12 in microglia in rat brain. *Glia* 44, 242–250.

Schwab, J. M., Guo, L., and Schluesener, H. J. (2005). Spinal cord injury induces early and persistent lesional P2X4 receptor expression. *J. Neuroimmunol.* 163, 185–189.

Schwartz, M., Butovsky, O., Brück, W., and Hanisch, U. K. (2006). Microglial phenotype: is the commitment reversible? *Trends Neurosci.* 29, 68–74.

Schwarzschild, M. A., Agnati, L., Fuxe, K., Chen, J. F., and Morelli, M. (2006). Targeting adenosine A2A receptors in Parkinson's disease. *Trends Neurosci.* 29, 647–654.

Sieger, D., Moritz, C., Ziegenhals, T., Prykhozhij, S., and Peri, F. (2012). Long-range Ca2+ waves transmit brain-damage signals to microglia. *Dev. Cell* 22, 1138–1148.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Silverman, W. R., de Rivero Vaccari, J. P., Locovei, S., Qiu, F., Carlsson, S. K., Scemes, E., et al. (2009). The pannexin 1 channel activates the inflammasome in neurons and astrocytes. *J. Biol. Chem.* 284, 18143–18151.

Stellwagen, D., and Malenka, R. C. (2006). Synaptic scaling mediated by glial TNF-alpha. *Nature* 440, 1054–1059.

Streit, W. J., Morioka, T., and Kalehua, A. N. (1992). MK-801 prevents microglial reaction in rat hippocampus after forebrain ischemia. *Neuroreport* 3, 146–148.

Surprenant, A., Rassendren, F., Kawashima, E., North, R. A., and Buell, G. (1996). The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor. *Science* 272, 735–738.

Suzuki, T., Hide, I., Ido, K., Kohsaka, S., Inoue, K., and Nakata, Y. (2004). Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. *J. Neurosci.* 24, 1–7.

Swanson, C. J., Bures, M., Johnson, M. P., Linden, A. M., Monn, J. A., and Schoepp, D. D. (2005). Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. *Nat. Rev. Drug Discov.* 4, 131–144.

Taguchi, K., Tamba, M., Bannai, S., and Sato, H. (2007). Induction of cystine/glutamate transporter in bacterial lipopolysaccharide induced endotoxemia in mice. *J. Inflamm.* 4:20. doi: 10.1186/1476-9255-4-20

Tahraoui, S. L., Marret, S., Bodénant, C., Leroux, P., Dommergues, M. A., Evrard, P., et al. (2001). Central role of microglia in neonatal excitotoxic lesions of the murine periventricular white matter. *Brain Pathol.* 11, 56–71.

Takano, T., Lin, J. H., Arcuino, G., Gao, Q., Yang, J., and Nedergaard, M. (2001). Glutamate release promotes growth of malignant gliomas. *Nat. Med.* 7, 1010–1015.

Taylor, D. L., Diemel, L. T., Cuzner, M. L., and Pocock, J. M. (2002). Activation of group II metabotropic glutamate receptors underlies microglial reactivity and neurotoxicity following stimulation with chromogranin A, a peptide up-regulated in Alzheimer's disease. *J. Neurochem.* 82, 1179–1191.

Taylor, D. L., Diemel, L. T., and Pocock, J. M. (2003). Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. *J. Neurosci.* 23, 2150–2160.

Taylor, D. L., Jones, F., Kubota, E. S., and Pocock, J. M. (2005). Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. *J. Neurosci.* 25, 2952–2964.

Thomas, D. M., and Kuhn, D. M. (2005). MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity. *Brain Res.* 1050, 190–198.

Thompson, R. J., Jackson, M. F., Olah, M. E., Rungta, R. L., Hines, D. J., Beazely, M. A., et al. (2008). Activation of pannexin-1 hemichannels augments aberrant bursting in the hippocampus. *Science* 322, 1555–1559.

Thompson, R. J., Zhou, N., and MacVicar, B. A. (2006). Ischemia opens neuronal gap junction hemichannels. *Science* 312, 924–927.

Toyomitsu, E., Tsuda, M., Yamashita, T., Tozaki-Saitoh, H., Tanaka, Y., and Inoue, K. (2012). CCL2 promotes P2X4 receptor trafficking to the cell surface of microglia. *Purinergic Signal.* 8, 301–310.

Tozaki-Saitoh, H., Tsuda, M., Miyata, H., Ueda, K., Kohsaka, S., and Inoue, K. (2008). P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. *J. Neurosci.* 28, 4949–4956.

Tran, M. D., and Neary, J. T. (2006). Purinergic signaling induces thrombospondin-1 expression in astrocytes. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9321–9326.

Tremblay, M. È. (2011). The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies. *Neuron Glia Biol.* 7, 67–76.

Tremblay, M. È., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Tsuda, M., Beggs, S., Salter, M. W., and Inoue, K. (2013). Microglia and intractable chronic pain. *Glia* 61, 55–61.

Tsuda, M., Masuda, T., Kitano, J., Shimoyama, H., Tozaki-Saitoh, H., and Inoue, K. (2009). IFN-gamma receptor signaling mediates spinal microglia activation driving neuropathic pain. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8032–8037.

Tsuda, M., Tozaki-Saitoh, H., Masuda, T., Toyomitsu, E., Tezuka, T., Yamamoto, T., et al. (2008). Lyn tyrosine kinase is required for P2X(4) receptor upregulation and neuropathic pain after peripheral nerve injury. *Glia* 56, 50–58.

Tsutsui, S., Schnermann, J., Noorbakhsh, F., Henry, S., Yong, V. W., Winston, B. W., et al. (2004). A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J. Neurosci.* 24, 1521–1529.

Ulmann, L., Hatcher, J. P., Hughes, J. P., Chaumont, S., Green, P. J., Conquet, F., et al. (2008). Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J. Neurosci.* 28, 11263–11268.

Vinet, J., Weering, H. R., Heinrich, A., Kälin, R. E., Wegner, A., Brouwer, N., et al. (2012). Neuroprotective function for ramified microglia in hippocampal excitotoxicity. *J. Neuroinflammation* 9, 27.

Volonte, C., Apolloni, S., Skaper, S. D., and Burnstock, G. (2012). P2X7 receptors: channels, pores and more. *CNS Neurol. Disord. Drug Targets* 11, 705–721.

Von Lubitz, D. K., Lin, R. C., Popik, P., Carter, M. F., and Jacobson, K. A. (1994). Adenosine A3 receptor stimulation and cerebral ischemia. *Eur. J. Pharmacol.* 263, 59–67.

Von Lubitz, D. K., Simpson, K. L., and Lin, R. C. (2001). Right thing at a wrong time? Adenosine A3 receptors and cerebroprotection in stroke. *Ann. N.Y. Acad. Sci.* 939, 85–96.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Wake, H., Moorhouse, A. J., Miyamoto, A., and Nabekura, J. (2013). Microglia: actively surveying and shaping neuronal circuit structure

and function. *Trends Neurosci*. 36, 209–217.

Walz, W., Ilschner, S., Ohlemeyer, C., Banati, R., and Kettenmann, H. (1993). Extracellular ATP activates a cation conductance and a K+ conductance in cultured microglial cells from mouse brain. *J. Neurosci.* 13, 4403–4411.

Wang, X., Arcuino, G., Takano, T., Lin, J., Peng, W. G., Wan, P., et al. (2004). P2X7 receptor inhibition improves recovery after spinal cord injury. *Nat. Med.* 10, 821–827.

Weisman, G. A., Camden, J. M., Peterson, T. S., Ajit, D., Woods, L. T., and Erb, L. (2012). P2 receptors for extracellular nucleotides in the central nervous system: role of P2X7 and P2Y2 receptor interactions in neuroinflammation. *Mol. Neurobiol.* 46, 96–113.

Witting, A., Walter, L., Wacker, J., Möller, T., and Stella, N. (2004). P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3214–3219.

Witting, A., Chen, L., Cudaback, E., Straiker, A., Walter, L., Rickman, B., et al. (2006). Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. *Proc. Natl. Acad. Sci. U.S.A.* 103, 6362–6367.

Wu, L. J., and Zhuo, M. (2008). Resting microglial motility is independent of synaptic plasticity in mammalian brain. *J. Neurophysiol.* 99, 2026–2032.

Wu, S. Z., Bodles, A. M., Porter, M. M., Griffin, W. S., Basile, A. S., and Barger, S. W. (2004). Induction of serine racemase expression and D-serine release from microglia by amyloid beta-peptide. *J. Neuroinflammation* 1:2. doi: 10.1186/1742-2094-1-2

Xiang, Z., and Burnstock, G. (2005). Expression of P2X receptors on rat microglial cells during early development. *Glia* 52, 119–126.

Yao, S. Q., Li, Z. Z., Huang, Q. Y., Li, F., Wang, Z. W., Augusto, E., et al. (2012). Genetic inactivation of the adenosine A(2A) receptor exacerbates brain damage in mice with experimental autoimmune encephalomyelitis. *J. Neurochem.* 123, 100–112.

Ye, Z. C., Rothstein, J. D., and Sontheimer, H. (1999). Compromised glutamate transport in human glioma cells: reduction-mislocalization of sodium-dependent glutamate transporters and enhanced activity of cystine-glutamate exchange. *J. Neurosci.* 19, 10767–10777.

Ye, Z. C., and Sontheimer, H. (1999). Glioma cells release excitotoxic concentrations of glutamate. *Cancer Res*. 59, 4383–4391.

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I. P., Naylor, A., Bountra, C., et al. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol.* 6:12. doi: 10.1186/1471-2377-6-12

Zeng, Y., Li, Y., Chen, R. S., He, X., Yang, L., and Li, W. (2010). Overexpression of xCT induces upregulation of 14-3-3beta in Kaposi's sarcoma. *Biosci. Rep.* 30, 277–283.

Zhang, Z., Zhang, Z., Artelt, M., Burnet, M., and Schluesener, H. J. (2007). Dexamethasone attenuates early expression of three molecules associated with microglia/macrophages activation following rat traumatic brain injury. *Acta Neuropathol.* 113, 675–682.

Zheng, H., Zhu, W., Zhao, H., Wang, X., Wang, W., and Li, Z. (2010). Kainic acid-activated microglia mediate increased excitability of rat hippocampal neurons *in vitro* and *in vivo*: crucial role of interleukin-1beta. *Neuroimmunomodulation* 17, 31–38.

Zhu, W., Zheng, H., Shao, X., Wang, W., Yao, Q., and Li, Z. (2010). Excitotoxicity of TNFalpha derived from KA activated microglia on hippocampal neurons *in vitro* and *in vivo*. *J. Neurochem.* 114, 386–396.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 January 2013; paper pending published: 06 February 2013; accepted: 05 April 2013; published online: 19 April 2013.

Citation: Domercq M, Vázquez-Villoldo N and Matute C (2013) Neurotransmitter signaling in the pathophysiology of microglia. Front. Cell. Neurosci. 7:49. doi: 10.3389/fncel.2013.00049

frontiers in
CELLULAR NEUROSCIENCE

GENERAL COMMENTARY
published: 09 July 2013
doi: 10.3389/fncel.2013.00107

Erratum: Neurotransmitter signaling in the pathophysiology of microglia

María Domercq, Nuria Vazquez-Villoldo and Carlos Matute*

Department of Neuroscience, University of the Basque Country, Leioa, Spain
**Correspondence: carlos.matute@ehu.es*

Edited by:
Amanda Sierra, University of the Basque Country EHU/UPV, Spain

A commentary on

Neurotransmitter signaling in the pathophysiology of microglia.
by Domercq, M., Vázquez-Villoldo, N., and Matute, C. (2013). Front. Cell. Neurosci. 7:49. doi: 10.3389/fncel.2013.00049

At the top of **page 5**, the following changes need to be made.

. . . regulators of P2X4R expression in microglia have been described, such as the chemokine CCL2 (also known as monocyte chemoattractant protein, MCP-1; Biber et al., 2011; Toyomitsu et al., 2012),. . ..

Leaving the corrected text as follows:

. . . regulators of P2X4R expression in microglia have been described, such as the chemokine CCL21 (Biber et al., 2011). . ..

Page 16. The reference by Toyomitsu et al., 2012 should be removed.

REFERENCES

Biber, K., Tsuda, M., Tozaki-Saitoh, H., Tsukamoto, K., Toyomitsu, E., Masuda, T., et al. (2011). Neuronal CCL21 up-regulates microglia P2X4 expression and initiates neuropathic pain development. *EMBO J.* 30, 1864–1873. doi: 10.1038/emboj.2011.89

Received: 20 June 2013; accepted: 21 June 2013; published online: 09 July 2013.
Citation: Domercq M, Vazquez-Villoldo N and Matute C (2013) Erratum: Neurotransmitter signaling in the pathophysiology of microglia. Front. Cell. Neurosci. 7:107. doi: 10.3389/fncel.2013.00107

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 28 March 2013
doi: 10.3389/fncel.2013.00032

Microglial control of neuronal activity

Catherine Béchade, Yasmine Cantaut-Belarif and Alain Bessis*

Institut de Biologie, Ecole Normale Supérieure, Inserm U1025, CNRS UMR8197, Paris, France

Edited by:
Marie-Eve Tremblay, Université Laval, Canada

Reviewed by:
Ania K. Majewska, University of Rochester, USA
Rafael Linden, Federal University of Rio de Janeiro, Brazil
Wai T. Wong, National Eye Institute, NIH, USA
Junichi Nabekura, National Institute for Physiological Sciences, Japan

***Correspondence:**
Alain Bessis, Ecole Normale Supérieure, Institut de Biologie, CNRS, UMR 8197, 46 rue d'Ulm, F-75005 Paris, France.
e-mail: alain.bessis@ens.fr

Fine-tuning of neuronal activity was thought to be a neuron-autonomous mechanism until the discovery that astrocytes are active players of synaptic transmission. The involvement of astrocytes has changed our understanding of the roles of non-neuronal cells and shed new light on the regulation of neuronal activity. Microglial cells are the macrophages of the brain and they have been mostly investigated as immune cells. However, recent data discussed in this review support the notion that, similarly to astrocytes, microglia are involved in the regulation of neuronal activity. For instance, in most, if not all, brain pathologies a strong temporal correlation has long been known to exist between the pathological activation of microglia and dysfunction of neuronal activity. Recent studies have convincingly shown that alteration of microglial function is responsible for pathological neuronal activity. This causal relationship has also been demonstrated in mice bearing loss-of-function mutations in genes specifically expressed by microglia. In addition to these long-term regulations of neuronal activity, recent data show that microglia can also rapidly regulate neuronal activity, thereby acting as partners of neurotransmission.

Keywords: microglia, neurotransmission, inflammation, synapse, glial cells

INTRODUCTION

Microglial cells are one of the glial cell populations of the brain. In contrast to other glial cell types such as oligodendrocytes or astrocytes, the role of microglia in the regulation of neuronal activity has been somewhat overlooked. Microglia are macrophages of the nervous tissue and as immune cells they can detect and react to infection, trauma, ischemia, degeneration, or any alterations in brain homeostasis. Actually, most brain pathologies, if not all, are associated with early microglial activation[1]. Thus, microglial activation was demonstrated based on histopathological data, *in vivo* brain imaging or cytokine expression upon axotomy (Blinzinger and Kreutzberg, 1968), during degenerative (Haga et al., 1989; Cagnin et al., 2001; reviewed in Cameron and Landreth, 2010) or neuropsychiatric diseases (review in Beumer et al., 2012). Of note, the above-described disorders are also associated with early synaptic dysfunction (Blinzinger and Kreutzberg, 1968; references in Selkoe, 2002; Penzes et al., 2011; Peça and Feng, 2012). Such a temporal correlation between microglial activation and synaptic dysfunction during brain pathologies suggests that regulatory interactions exist between the activation of microglia and neurotransmission. In addition, the functional properties of microglia are compatible with an involvement in the control of neuronal activity. They express receptors for most neurotransmitters (Kettenmann et al., 2011; Kaindl et al., 2012) and produce a large repertoire of molecules known to modulate neuronal activity and plasticity. In addition, microglia are highly ramified cells and their ramifications rapidly scan the local environment and react to its modification (Davalos et al., 2005). Finally, microglial processes physically contact synaptic elements (Wake et al., 2009; Tremblay et al., 2010; see also Schafer et al., 2012), allowing for an accurate control of synaptic function.

In this review, we will highlight recent studies suggesting or demonstrating the involvement of microglia in the control of neuronal activity. Firstly, we will describe how microglial dysfunction is primarily responsible for the alterations in neuronal activity under pathological situations. We will then show that in the healthy brain microglia can be described as partners of neurotransmission.

MICROGLIA DYSFUNCTION PERTURBS NEURONAL ACTIVITY

Microglia were initially described as sensors of pathological events (Kreutzberg, 1996). It is now widely accepted that microglia are not only sensors but also active players of pathological states in the brain. Understanding the consequences of microglial dysfunction on neuronal phenotype is important to understand the etiology of the disease state and to propose therapeutic strategies. In this first section we will review studies in which microglia are the primary cause of alterations in neuronal activity during non-physiological states. Importantly, the information gathered from pathological situations is relevant for the understanding microglial function in the absence of pathology, as will be discussed in the second section of this review.

Analyses of mice bearing loss-of-function mutations in genes involved in microglia-specific pathways exemplify the link between microglial dysfunction and neuronal activity. CX3CR1 is

[1]The notion of "activation" of microglia is quite a loosely-defined concept adapted from the well-defined concept of macrophage activation (Taylor et al., 2005; Perry et al., 2007). Activation is associated with pathology but is often used to describe the consequences of any stimulation of microglia. Because different stimulation can induce different responses in microglia, there is not one single parameter that characterizes an "activation." Therefore, various parameters have been used to establish microglial "activation" such as changes in density, morphology, or expression of proteins. Whenever possible, we have used the notion of stimulation instead of activation and described the nature of the stimuli.

the microglial receptor for the neuronal chemokine fractalkine (CX3CL1). This complementary expression of ligand and receptor on neurons and microglia respectively, suggests that their interaction may play a role in modulating neurotransmission. Mice with a CX3CR1 loss-of-function mutation exhibit an impairment of hippocampal long-term potentiation (LTP) as well as cognitive deficits (Rogers et al., 2011). The CX3CL1/R1 signaling pathway also appears to be involved in synaptic maturation since CX3CR1 deficiency leads to a delay in the maturation of glutamatergic thalamocortical synapses, as well as a transient immature connectivity in the developing hippocampus (Paolicelli et al., 2011; Hoshiko et al., 2012). Of note, these latter alterations might be secondary to a decreased recruitment of microglia and not to a direct involvement of CX3CR1 signaling in the regulation of neurotransmission (Paolicelli et al., 2011; Hoshiko et al., 2012). Another example of a neuronal-microglial interaction is provided by the analysis of CD200-deficient mice. CD200R is a membrane protein exclusively expressed by microglia. Its ligand, CD200 is expressed by neurons, oligodendrocytes and astrocytes (Costello et al., 2011). It was demonstrated that LTP is inhibited in CD200-deficient mice, further supporting the notion that the integrity of microglial signaling is crucial for neurotransmission homeostasis (Costello et al., 2011). Finally, synaptic alterations have also been demonstrated upon the loss-of-function mutation of DAP12, a transmembrane protein associated with various lymphoid and myeloid receptors such as TREM2 (Tomasello et al., 2000). In the brain, DAP12 and TREM2 are exclusively expressed by microglia and DAP12 loss-of-function results in an enhanced hippocampal LTP and major changes in glutamatergic transmission (Roumier et al., 2004, 2008). As for CX3CR1- and CD200-deficient mice, the molecular mechanisms linking microglial deficiency to synaptic alterations in DAP12KO mice are not known. Interestingly however, the DAP12-mutant mouse is a model for Nasu-Hakola disease in which patients display progressive presenile dementia associated with bone cysts (Hakola, 1972), together with leukodystrophy and astrogliosis in the brain (Satoh et al., 2011). Nasu-Hakola disease is caused by mutations in the genes encoding microglial DAP12 or TREM2 (Paloneva et al., 2000), and because of this restricted expression, it has been described as the first microgliopathy (Bianchin et al., 2010). Thus, dysfunction of DAP12 signaling, which is exclusively expressed by microglia impacts synaptic transmission (Roumier et al., 2004), mouse behavior (Kaifu et al., 2003), and higher brain functions in human (Paloneva et al., 2000).

A link between microglia and higher brain function has also been proposed in the case of the mouse model of obsessive-compulsive disorder. Disruption of the Hoxb8 gene, expressed by a subpopulation of microglia, caused mice to groom compulsively (Chen et al., 2010; see however Holstege et al., 2008). Transplantation of wild type bone-marrow cells into Hoxb8 mutant mice rescued the phenotype (Chen et al., 2010) leading to the hypothesis that the pathological grooming behavior observed in Hoxb8 mutant mice may result from deficient mutant microglia.

Rett syndrome is another example of microglial involvement in psychiatric disease. Rett syndrome is an autism spectrum disorder caused by mutations in the gene encoding the methyl CpG binding protein-2 (MeCP2). Rett syndrome patients exhibit dendritic and synaptic abnormalities in selected regions (references in Chahrour and Zoghbi, 2007). MeCP2 deficient mice mimic the human syndrome (Chen et al., 2001; Guy et al., 2001; Shahbazian et al., 2002). Transplantation of wild type bone marrow into irradiated MeCP2-null hosts was recently shown to lead to engraftment of MeCP2-expressing microglia in the brain parenchyma and to a rescue of the brain phenotype (Derecki et al., 2012). Involvement of microglia in Rett syndrome is strengthened by *in vitro* observations showing that MeCP2-null microglia release high levels of glutamate, which induced changes in dendritic morphology and a reduced number of postsynaptic densities (Maezawa and Jin, 2010). Thus, microglia have an active role in this disorder by a mechanism that remains to be described.

These examples of psychiatric phenotypes induced primarily by deficiencies of microglial function support the notion that microglia can actively modulate neuronal functions, including learning and memory (Blank and Prinz, 2013). Yet, it cannot be excluded that microglial dysfunctions induce a general change of brain homeostasis resulting in non-specific defects in neuronal activity. However, in some instances, such as chronic pain, it could be shown that pathological effects on neuronal activity are due a deregulation of local microglial mechanisms that might be dedicated to the control of neurotransmission. For instance, stimulation of microglial P2X4 receptors induces the release of pain mediators such as PGE2 (Ulmann et al., 2010) or BDNF (Coull et al., 2005) and is necessary for the induction of allodynia after nerve injury (Tsuda et al., 2003). In addition, upon neuropathic pain, the dorsal horn microglia produces BDNF, which stimulates the neuronal TrkB receptor and induces a shift in the chloride gradient in nociceptive neurons (Coull et al., 2005). Such shift increases the excitability of the neurons through $GABA_A$ receptor-mediated depolarization (Coull et al., 2003).

These examples show that local and specific interactions between microglia and neurons can be responsible for the altered neuronal activity observed in pathology. However, microglia and neurons functionally interact in healthy conditions (Tremblay et al., 2011) as well, and several studies have now established that microglia can rapidly modulate neuronal activity in basal conditions.

MICROGLIA AS GENUINE PARTNERS OF SYNAPTIC ACTIVITY

Under physiological conditions, microglia react rapidly to neuronal activity by modulating the physical contacts that their numerous processes continuously establish with synaptic elements (Wake et al., 2009; Tremblay et al., 2010). Microglia are thus potentially accurate sensors of neuronal activity and a reciprocal control of neurotransmission by microglia can be expected. The ability of microglia to rapidly modulate synaptic activity was initially exemplified by treating cultured neurons or acute brain slices with medium conditioned by cultured microglia. Microglia conditioned-medium was shown to increase both the amplitude and duration of the NMDA-receptor induced currents (Moriguchi et al., 2003; Hayashi et al., 2006). The nature of the signaling molecules involved in this process is still unknown and were proposed to be a secreted protein(s) (Moriguchi et al., 2003)

or glycine (Hayashi et al., 2006). In fact, microglia produce a broad spectrum of signaling molecules known to regulate synaptic function, including cytokines (Elkabes et al., 1996; Hanisch, 2002), neurotransmitters (Piani and Fontana, 1994; Hayashi et al., 2006; Flierl et al., 2007; Pascual et al., 2012), and extracellular matrix proteins (Chamak et al., 1994). A direct regulation of synaptic properties by microglia is therefore expected. Amongst the microglial molecules with a putative role in neurotransmission, TNFα deserves specific attention. This cytokine was shown to control basal synaptic functions (Santello et al., 2011) as well as plasticity (Stellwagen and Malenka, 2006; Kaneko et al., 2008; Costello et al., 2011; Santello et al., 2011), and this role was attributed to TNFα produced by astrocytes (Stellwagen and Malenka, 2006). However, the astrocytes have often been thought to express TNFα because cultures of astrocytes are consistently contaminated by microglia (Saura, 2007; Barres, 2008). In addition, the transcriptome analysis from purified astrocytes reveals no TNFα-encoding transcript in astrocytes (Sharma et al., 2007; Cahoy et al., 2008; Doyle et al., 2008; Meissner et al., 2008; Foo et al., 2011; Zamanian et al., 2012). Thus, the TNFα that controls several aspects of synaptic transmission might, in fact, be produced by microglial cells, but this has not yet been firmly established.

It has also been shown that microglia can shed microvesicles a few seconds after ATP stimulation, most probably by a P2X7-dependent mechanism (Bianco et al., 2005). When these vesicles were harvested from cultured microglia and applied to cultured hippocampal neurons, they induced an increased frequency of miniature excitatory post-synaptic currents (mEPSC), supposedly through presynaptic regulation (Antonucci et al., 2012). Analysis of the regulatory pathway between microglia and synaptic activity led the authors to propose that microglial micro-vesicles regulate mEPSCs through a phosphatydil-dependent regulation of presynaptic vesicle release (Antonucci et al., 2012). The functional relevance and specificity of this mechanism remains to be established but it raises the provocative hypothesis that physical contacts, or membrane exchange between microglia and neurons, could actively and rapidly regulate neurotransmission.

The above-described studies suggest, but do not demonstrate, that microglia can rapidly modulate synaptic function. Several studies have specifically stimulated microglia and analyzed the consequences on neuronal activity in a similar way to what was done to investigate the role of astrocytes in neurotransmission. Application of fractalkine onto neuron cultures was shown to induce a strong and rapid modulation of calcium currents in neurons (Meucci et al., 1998). Such modulation was actually the first demonstration that stimulation of microglia could rapidly modulate the activity of neurons (although it was at first incorrectly attributed to a direct stimulation of neurons by fractalkine). This modulation has also been confirmed in acute hippocampal slices, in which stimulation of microglia by fractalkine induces a significant and transient reduction of the amplitude of evoked EPSCs in CA1 pyramidal neurons (Ragozzino et al., 2006; see **Figure 1A**). It was further demonstrated that this reduction involves adenosine, supposedly acting on neuronal A3R receptors (Piccinin et al., 2010). The probable mechanism of regulation is that fractalkine induces the microglial release of adenosine, which in turn inhibits the presynaptic release of glutamate (**Figure 1A**). Alternatively, microglia could produce ATP that is rapidly degraded into adenosine by ectonucleotidases. The involvement of other cell types such as astrocytes has not yet been ruled out.

An alternative rapid regulation of neuronal activity by microglia has recently been established upon application of lipo-polysaccharide (LPS—Pascual et al., 2012; **Figure 1A**). LPS is a ligand of TLR4 that mimics bacterial infection and can reveal pathological pathways. TLR4 is exquisitely expressed by microglia and can also be stimulated by several endogenous ligands (Habich et al., 2005; Gondokaryono et al., 2007; Midwood et al., 2009; Milanski et al., 2009; references in Lucin and Wyss-Coray, 2009). Therefore, the mechanisms revealed by LPS application probably have a physiological relevance. Stimulation of microglia by addition of LPS onto acute hippocampal slices induces a rapid and transient increase in the frequency of spontaneous synaptic AMPAergic post-synaptic currents in CA1 neurons. This effect does not occur in slices prepared from Pu.1 deficient mice that lack microglia, showing that the effect of LPS requires microglia. It was then demonstrated that upon LPS stimulation, microglia rapidly produce ATP, which recruits astrocytes. Astrocytes subsequently release glutamate, and this leads to increased excitatory transmission via a metabotropic glutamate receptor-dependent mechanism (Pascual et al., 2012).

The above-described studies show that stimulation of microglia modulates neuronal activity *in vitro*. The occurrence of regulating interactions between microglia and neuronal activity has recently been demonstrated *in vivo* in the zebrafish larva (Li et al., 2012; **Figure 1B**). In this system, microglia monitor spontaneous or visually evoked neuronal activity, and send bulbous processes toward the most active neurons, as detected by their production of ATP. These contacts between microglial endings and active neurons induce a rapid decrease in both frequency and amplitude of neuronal calcium events (Li et al., 2012). This study confirms and extends the data obtained upon stimulation of microglia and further demonstrates that microglia are genuine partners of neuronal activity in the healthy brain.

THE RIGHT TOOLS TO TARGET THE RIGHT CELLS

The role of microglia in the regulation of neurotransmission is far less studied than that of astrocytes. This might be due to a lesser involvement of microglia in such regulation. Alternatively, this could also be due to the fact that the characterization of microglia as regulators of neurotransmission has been hindered by the lack of tools to specifically stimulate or block their function. Such tools are available for astrocytes and their function has been blocked by application of pharmacological inhibitors such as Fluoroacetate or calcium chelators (Henneberger et al., 2010). Stimulation of astrocytes has also been achieved, mechanically (see e.g., Liu et al., 2011) or by local application of synthetic agonists or local uncaging of calcium or glutamate (Pascual et al., 2005; Agulhon et al., 2010). Although the physiological relevance of such treatments is still debated (Hamilton and Attwell, 2010),

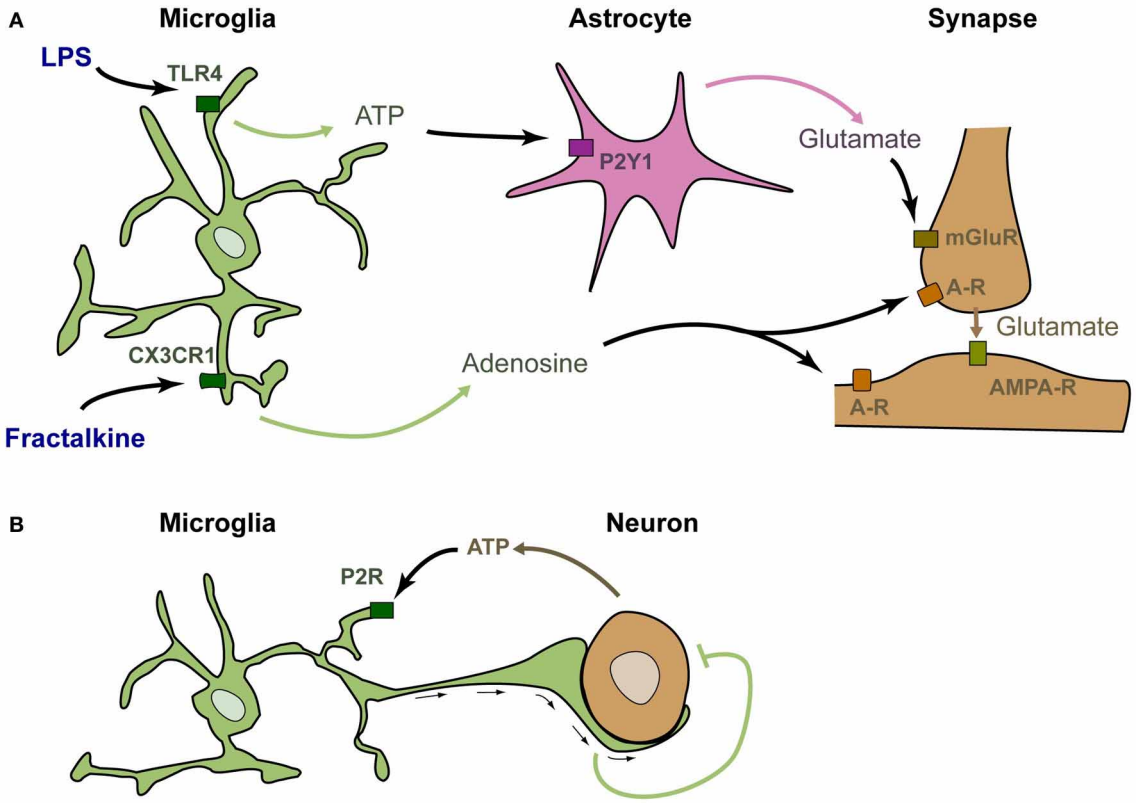


FIGURE 1 | Microglia are genuine partners of synaptic transmission. **(A)** In acute rodent brain slices, stimulation of microglia by LPS induces the rapid release of ATP, which recruits astrocytes. Upon purinergic stimulation, astrocytes release glutamate, inducing a mGluR-dependent release of presynaptic glutamate (Pascual et al., 2012). Stimulation of microglia by fractalkine induces the release of adenosine, which decreases neuronal activity (Meucci et al., 1998; Ragozzino et al., 2006; Piccinin et al., 2010). **(B)** In zebrafish larva, active neurons release the ATP that attracts microglial bulbous processes. These processes decrease neuronal activity by an as yet unknown mechanism (Li et al., 2012).

these protocols allowed the characterization of astrocytes as regulators of the normal function and plasticity of neural circuits *in vitro* and *in vivo*. Comparable tools to tune the function of microglia are lacking, mostly because of a specificity issue. For instance, minocycline is known to block microglial function (Yrjänheikki et al., 1998), but its molecular and cellular targets remain unidentified and its specificity remains to be firmly established. Moreover, microglia can be stimulated by a large variety of inflammatory molecules such as cytokines or interleukins, but their receptors have also been detected on neurons and astrocytes, preventing accurate interpretation of their putative effects. In addition, as mentioned previously in relation to the cellular origin of TNFα, the consistent contamination of neuronal and astrocyte cultures by microglia has made it difficult to address the correct expression of microglial molecules. For instance, CX3CR1, the fractalkine receptor that was initially thought to be expressed by neurons (Meucci et al., 1998, 2000; Hughes et al., 2002; Ragozzino et al., 2006), is now demonstrated to be exclusively expressed by microglia (Cardona et al., 2006; Lauro et al., 2008). Similarly, TLR4, the LPS receptor, was mistakenly detected in astrocytic (Bowman et al., 2003; Alfonso-Loeches et al., 2010) and neuronal cultures (Tang et al., 2007). Indeed, when microglia were efficiently depleted from astrocyte cultures, TLR4 was no longer detected (Lehnardt et al., 2002; Pascual et al., 2012). In addition, expression of TLR4 has never been found in healthy neurons or astrocytes. Finally, data mining of Gene Expression Omnibus DNA array experiments performed on purified cells confirmed that TLR4 is exclusively expressed by microglia (Pascual et al., 2012). Thus, CX3CR1 and TLR4 expression is limited to microglia and, as described above, can be used to specifically stimulate these cells and study their involvement in biological processes. We speculate that the future development tools to specifically block microglial function will also be instrumental to understand the involvement of these cells in wide variety of physiological processes.

CONCLUSION

The biological relevance of microglia as active sensors of brain parenchyma was until recently, principally recognized in pathological tissues. The role of microglia in the healthy brain is now acknowledged (Graeber, 2010; Pont-Lezica et al., 2011; Tremblay et al., 2011). Here we have reviewed studies indicating that microglia are able to control neuronal activity, from synaptic transmission to higher brain functions. Microglia have often been described as

"good" or "bad" cells (Kempermann and Neumann, 2003; Kettenmann, 2007; Watkins et al., 2007; Aguzzi et al., 2013). Considering microglia as partners of neuronal function will certainly help to provide a more accurate and integrated understanding of their roles, beyond the primary "beneficial vs. detrimental" dichotomy. It will also extend our understanding of non-cell autonomous regulation of neuronal activity and shed new light on the role of microglia in the pathological brain.

ACKNOWLEDGMENTS

We gratefully thank Lorena Pont-Lezica for critical reading of the manuscript. The work of our laboratory is supported by a grant from the 7th Framework Program Moodinflame (222963).

REFERENCES

Agulhon, C., Fiacco, T. A., and McCarthy, K. D. (2010). Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca2+ signaling. *Science* 327, 1250–1254.

Aguzzi, A., Barres, B. A., and Bennett, M. L. (2013). Microglia: scapegoat, saboteur, or something else? *Science* 339, 156–161.

Alfonso-Loeches, S., Pascual-Lucas, M., Blanco, A. M., Sanchez-Vera, I., and Guerri, C. (2010). Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. *J. Neurosci.* 30, 8285–8295.

Antonucci, F., Turola, E., Riganti, L., Caleo, M., Gabrielli, M., Perrotta, C., et al. (2012). Microvesicles released from microglia stimulate synaptic activity via enhanced sphingolipid metabolism. *EMBO J.* 31, 1231–1240.

Barres, B. A. (2008). The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron* 60, 430–440.

Beumer, W., Gibney, S. M., Drexhage, R. C., Pont-Lezica, L., Doorduin, J., Klein, H. C., et al. (2012). The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. *J. Leukoc. Biol.* 92, 959–975.

Bianchin, M. M., Martin, K. C., de Souza, A. C., de Oliveira, M. A., and Rieder, C. R. (2010). Nasu-Hakola disease and primary microglial dysfunction. *Nat. Rev. Neurol.* 6, 2 p following 523.

Bianco, F., Pravettoni, E., Colombo, A., Schenk, U., Möller, T., Matteoli, M., et al. (2005). Astrocyte-derived ATP induces vesicle shedding and IL-1 beta release from microglia. *J. Immunol.* 174, 7268–7277.

Blank, T., and Prinz, M. (2013). Microglia as modulators of cognition and neuropsychiatric disorders. *Glia* 61, 62–70.

Blinzinger, K., and Kreutzberg, G. (1968). Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Z. Zellforsch. Mikrosk. Anat.* 85, 145–157.

Bowman, C. C., Rasley, A., Tranguch, S. L., and Marriott, I. (2003). Cultured astrocytes express toll-like receptors for bacterial products. *Glia* 43, 281–291.

Cagnin, A., Brooks, D. J., Kennedy, A. M., Gunn, R. N., Myers, R., Turkheimer, F. E., et al. (2001). *In-vivo* measurement of activated microglia in dementia. *Lancet* 358, 461–467.

Cahoy, J. D., Emery, B., Kaushal, A., Foo, L. C., Zamanian, J. L., Christopherson, K. S., et al. (2008). A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* 28, 264–278.

Cameron, B., and Landreth, G. E. (2010). Inflammation, microglia, and Alzheimer's disease. *Neurobiol. Dis.* 37, 503–509.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., et al. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* 9, 917–924.

Chahrour, M., and Zoghbi, H. Y. (2007). The story of Rett syndrome: from clinic to neurobiology. *Neuron* 56, 422–437.

Chamak, B., Morandi, V., and Mallat, M. (1994). Brain macrophages stimulate neurite growth and regeneration by secreting thrombospondin. *J. Neurosci. Res.* 38, 221–233.

Chen, R. Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001). Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat. Genet.* 27, 327–331.

Chen, S.-K., Tvrdik, P., Peden, E., Cho, S., Wu, S., Spangrude, G., et al. (2010). Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* 141, 775–785.

Costello, D. A., Lyons, A., Denieffe, S., Browne, T. C., Cox, F. F., and Lynch, M. A. (2011). Long term potentiation is impaired in membrane glycoprotein CD200-deficient mice: a role for Toll-like receptor activation. *J. Biol. Chem.* 286, 34722–34732.

Coull, J. A. M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., et al. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021.

Coull, J. A. M., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., Sík, A., et al. (2003). Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* 424, 938–942.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B. G., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

Doyle, J. P., Dougherty, J. D., Heiman, M., Schmidt, E. F., Stevens, T. R., Ma, G., et al. (2008). Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell* 135, 749–762.

Elkabes, S., DiCicco-Bloom, E. M., and Black, I. B. (1996). Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J. Neurosci.* 16, 2508–2521.

Flierl, M. A., Rittirsch, D., Nadeau, B. A., Chen, A. J., Sarma, J. V., Zetoune, F. S., et al. (2007). Phagocyte-derived catecholamines enhance acute inflammatory injury. *Nature* 449, 721–725.

Foo, L. C., Allen, N. J., Bushong, E. A., Ventura, P. B., Chung, W.-S., Zhou, L., et al. (2011). Development of a method for the purification and culture of rodent astrocytes. *Neuron* 71, 799–811.

Gondokaryono, S. P., Ushio, H., Niyonsaba, F., Hara, M., Takenaka, H., Jayawardana, S. T. M., et al. (2007). The extra domain A of fibronectin stimulates murine mast cells via toll-like receptor 4. *J. Leukoc. Biol.* 82, 657–665.

Graeber, M. B. (2010). Changing face of microglia. *Science* 330, 783–788.

Guy, J., Hendrich, B., Holmes, M., Martin, J. E., and Bird, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* 27, 322–326.

Habich, C., Kempe, K., van der Zee, R., Rümenapf, R., Akiyama, H., Kolb, H., et al. (2005). Heat shock protein 60: specific binding of lipopolysaccharide. *J. Immunol.* 174, 1298–1305.

Haga, S., Akai, K., and Ishii, T. (1989). Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. An immunohistochemical study using a novel monoclonal antibody. *Acta Neuropathol.* 77, 569–575.

Hakola, H. P. (1972). Neuropsychiatric and genetic aspects of a new hereditary disease characterized by progressive dementia and lipomembranous polycystic osteodysplasia. *Acta Psychiatr. Scand. Suppl.* 232, 1–173.

Hamilton, N. B., and Attwell, D. (2010). Do astrocytes really exocytose neurotransmitters? *Nat. Rev. Neurosci.* 11, 227–238.

Hanisch, U.-K. (2002). Microglia as a source and target of cytokines. *Glia* 40, 140–155.

Hayashi, Y., Ishibashi, H., Hashimoto, K., and Nakanishi, H. (2006). Potentiation of the NMDA receptor-mediated responses through the activation of the glycine site by microglia secreting soluble factors. *Glia* 53, 660–668.

Henneberger, C., Papouin, T., Oliet, S. H. R., and Rusakov, D. A. (2010). Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463, 232–236.

Holstege, J. C., De Graaff, W., Hossaini, M., Cardona Cano, S., Jaarsma, D., Van den Akker, E., et al. (2008). Loss of Hoxb8 alters spinal dorsal laminae and sensory responses in mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6338–6343.

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012). Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J. Neurosci.* 32, 15106–15111.

Hughes, P. M., Botham, M. S., Frentzel, S., Mir, A., and Perry, V. H. (2002). Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. *Glia* 37, 314–327.

Kaifu, T., Nakahara, J., Inui, M., Mishima, K., Momiyama, T., Kaji, M., et al. (2003). Osteopetrosis and thalamic hypomyelinosis with synaptic degeneration in DAP12-deficient mice. *J. Clin. Invest.* 111, 323–332.

Kaindl, A. M., Degos, V., Peineau, S., Gouadon, E., Chhor, V., Loron, G., et al. (2012). Activation of microglial N-methyl-D-aspartate receptors triggers inflammation and neuronal cell death in the developing and mature brain. *Ann. Neurol.* 72, 536–549.

Kaneko, M., Stellwagen, D., Malenka, R. C., and Stryker, M. P. (2008). Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. *Neuron* 58, 673–680.

Kempermann, G., and Neumann, H. (2003). Neuroscience. Microglia: the enemy within? *Science* 302, 1689–1690.

Kettenmann, H. (2007). Neuroscience: the brain's garbage men. *Nature* 446, 987–989.

Kettenmann, H., Hanisch, U.-K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553.

Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312–318.

Lauro, C., Angelantonio, S. D., Cipriani, R., Sobrero, F., Antonilli, L., Brusadin, V., et al. (2008). Activity of adenosine receptors type 1 Is required for CX3CL1-mediated neuroprotection and neuromodulation in hippocampal neurons. *J. Immunol.* 180, 7590–7596.

Lehnardt, S., Lachance, C., Patrizi, S., Lefebvre, S., Follett, P. L., Jensen, F. E., et al. (2002). The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J. Neurosci.* 22, 2478–2486.

Li, Y., Du, X.-F., Liu, C.-S., Wen, Z.-L., and Du, J.-L. (2012). Reciprocal regulation between resting microglial dynamics and neuronal activity *in vivo*. *Dev. Cell* 23, 1189–1202.

Liu, T., Sun, L., Xiong, Y., Shang, S., Guo, N., Teng, S., et al. (2011). Calcium triggers exocytosis from two types of organelles in a single astrocyte. *J. Neurosci.* 31, 10593–10601.

Lucin, K. M., and Wyss-Coray, T. (2009). Immune activation in brain aging and neurodegeneration: too much or too little? *Neuron* 64, 110–122.

Maezawa, I., and Jin, L.-W. (2010). Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J. Neurosci.* 30, 5346–5356.

Meissner, A., Mikkelsen, T. S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., et al. (2008). Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* 454, 766–770.

Meucci, O., Fatatis, A., Simen, A. A., Bushell, T. J., Gray, P. W., and Miller, R. J. (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14500–14505.

Meucci, O., Fatatis, A., Simen, A. A., and Miller, R. J. (2000). Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8075–8080.

Midwood, K., Sacre, S., Piccinini, A. M., Inglis, J., Trebaul, A., Chan, E., et al. (2009). Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. *Nat. Med.* 15, 774–780.

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D. E., et al. (2009). Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. *J. Neurosci.* 29, 359–370.

Moriguchi, S., Mizoguchi, Y., Tomimatsu, Y., Hayashi, Y., Kadowaki, T., Kagamiishi, Y., et al. (2003). Potentiation of NMDA receptor-mediated synaptic responses by microglia. *Brain Res. Mol. Brain Res.* 119, 160–169.

Paloneva, J., Kestilä, M., Wu, J., Salminen, A., Böhling, T., Ruotsalainen, V., et al. (2000). Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. *Nat. Genet.* 25, 357–361.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Pascual, O., Achour, S. B., Rostaing, P., Triller, A., and Bessis, A. (2012). Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U.S.A.* 109, E197–E205.

Pascual, O., Casper, K. B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.-Y., et al. (2005). Astrocytic purinergic signaling coordinates synaptic networks. *Science* 310, 113–116.

Peça, J., and Feng, G. (2012). Cellular and synaptic network defects in autism. *Curr. Opin. Neurobiol.* 22, 866–872.

Penzes, P., Cahill, M. E., Jones, K. A., VanLeeuwen, J.-E., and Woolfrey, K. M. (2011). Dendritic spine pathology in neuropsychiatric disorders. *Nat. Neurosci.* 14, 285–293.

Perry, V. H., Cunningham, C., and Holmes, C. (2007). Systemic infections and inflammation affect chronic neurodegeneration. *Nat. Rev. Immunol.* 7, 161–167.

Piani, D., and Fontana, A. (1994). Involvement of the cystine transport system xc- in the macrophage-induced glutamate-dependent cytotoxicity to neurons. *J. Immunol.* 152, 3578–3585.

Piccinin, S., Di Angelantonio, S., Piccioni, A., Volpini, R., Cristalli, G., Fredholm, B. B., et al. (2010). CX3CL1-induced modulation at CA1 synapses reveals multiple mechanisms of EPSC modulation involving adenosine receptor subtypes. *J. Neuroimmunol.* 224, 85–92.

Pont-Lezica, L., Béchade, C., Belarif-Cantaut, Y., Pascual, O., and Bessis, A. (2011). Physiological roles of microglia during development. *J. Neurochem.* 119, 901–908.

Ragozzino, D., Angelantonio, S. D., Trettel, F., Bertollini, C., Maggi, L., Gross, C., et al. (2006). Chemokine fractalkine/CX3CL1 negatively modulates active glutamatergic synapses in rat hippocampal neurons. *J. Neurosci.* 26, 10488–10498.

Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., et al. (2011). CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J. Neurosci.* 31, 16241–16250.

Roumier, A., Béchade, C., Poncer, J.-C., Smalla, K.-H., Tomasello, E., Vivier, E., et al. (2004). Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. *J. Neurosci.* 24, 11421–11428.

Roumier, A., Pascual, O., Béchade, C., Wakselman, S., Poncer, J.-C., Réal, E., et al. (2008). Prenatal activation of microglia induces delayed impairment of glutamatergic synaptic function. *PLoS ONE* 3:e2595. doi: 10.1371/journal.pone.0002595

Santello, M., Bezzi, P., and Volterra, A. (2011). TNFα controls glutamatergic gliotransmission in the hippocampal dentate gyrus. *Neuron* 69, 988–1001.

Satoh, J.-I., Tabunoki, H., Ishida, T., Yagishita, S., Jinnai, K., Futamura, N., et al. (2011). Immunohistochemical characterization of microglia in Nasu-Hakola disease brains. *Neuropathology* 31, 363–375.

Saura, J. (2007). Microglial cells in astroglial cultures: a cautionary note. *J. Neuroinflammation* 4:26. doi: 10.1186/1742-2094-4-26

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science* 298, 789–791.

Shahbazian, M., Young, J., Yuva-Paylor, L., Spencer, C., Antalffy, B., Noebels, J., et al. (2002). Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* 35, 243–254.

Sharma, M. K., Mansur, D. B., Reifenberger, G., Perry, A., Leonard, J. R., Aldape, K. D., et al. (2007). Distinct genetic signatures among pilocytic astrocytomas relate to their brain region origin. *Cancer Res.* 67, 890–900.

Stellwagen, D., and Malenka, R. C. (2006). Synaptic scaling mediated by glial TNF-alpha. *Nature* 440, 1054–1059.

Tang, S.-C., Arumugam, T. V., Xu, X., Cheng, A., Mughal, M. R., Jo, D. G., et al. (2007). Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13798–13803.

Taylor, P. R., Martinez-Pomares, L., Stacey, M., Lin, H.-H., Brown, G. D., and Gordon, S. (2005). Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* 23, 901–944.

Tomasello, E., Desmoulins, P. O., Chemin, K., Guia, S., Cremer, H., Ortaldo, J., et al. (2000). Combined natural killer cell

and dendritic cell functional deficiency in KARAP/DAP12 loss-of-function mutant mice. *Immunity* 13, 355–364.

Tremblay, M.-È., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Tremblay, M.-È., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069.

Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M. W., et al. (2003). P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424, 778–783.

Ulmann, L., Hirbec, H., and Rassendren, F. (2010). P2X4 receptors mediate PGE2 release by tissue-resident macrophages and initiate inflammatory pain. *EMBO J.* 29, 2290–2300.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Watkins, L. R., Hutchinson, M. R., Ledeboer, A., Wieseler-Frank, J., Milligan, E. D., and Maier, S. F. (2007). Norman cousins lecture. Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids. *Brain Behav. Immun.* 21, 131–146.

Yrjänheikki, J., Keinänen, R., Pellikka, M., Hökfelt, T., and Koistinaho, J. (1998). Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15769–15774.

Zamanian, J. L., Xu, L., Foo, L. C., Nouri, N., Zhou, L., Giffard, R. G., et al. (2012). Genomic analysis of reactive astrogliosis. *J. Neurosci.* 32, 6391–6410.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 19 December 2012; accepted: 13 March 2013; published online: 28 March 2013.

Citation: Béchade C, Cantaut-Belarif Y and Bessis A (2013) Microglial control of neuronal activity. Front. Cell. Neurosci. 7:32. doi: 10.3389/fncel.2013.00032

frontiers in
CELLULAR NEUROSCIENCE

MINI REVIEW ARTICLE
published: 22 November 2013
doi: 10.3389/fncel.2013.00229

The role of microglia in adult hippocampal neurogenesis

***Carmelina Gemma*[1]* and *Adam D. Bachstetter*[2]**

[1] *Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA*
[2] *Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA*

Edited by:
Amanda Sierra, University of the Basque Country UPV/EHU, Spain

Reviewed by:
Juan M. Encinas, University of the Basque Country UPV/EHU, Spain
Eric D. Laywell, The Florida State University College of Medicine, USA

***Correspondence:**
Carmelina Gemma, Department of Anesthesiology and Pain Medicine, University of Washington, 908 Jefferson Street, Seattle, WA 98001, USA
e-mail: cgemma@u.washington.edu

Our view of microglia has dramatically changed in the last decade. From cells being "silent" in the healthy brain, microglia have emerged to be actively involved in several brain physiological functions including adult hippocampal neurogenesis, and cognitive and behavioral function. In light of recent discoveries revealing a role of microglia as important effectors of neuronal circuit reorganization, considerable attention has been focused on how microglia and hippocampal neurogenesis could be an interdependent phenomenon. In this review the role of microglia in the adult hippocampal neurogenesis under physiological condition is discussed.

Keywords: microglia, CX3CR1, neurogenesis, fractalkine, chemokines

ADULT NEUROGENESIS: THE GENERATION OF NEW NEURONS IN THE MATURE CNS

Neurogenesis occurs throughout life in the adult mammalian brain including humans (Eriksson et al., 1998; Roy et al., 2000; Wang et al., 2011). In rodents, neurogenesis occurs predominantly in the subgranular zone (SGZ) of the dentate gyrus in the hippocampal formation, and in the subventricular zone (SVZ) of the lateral ventricle (Gage, 2000). The process of generating new neurons consists of four phases: proliferation, migration, differentiation, and survival. Studies over the last decade have elegantly described each step in the neurogenic process (for review see Ming and Song, 2011). Adult hippocampal neurogenesis originates from a population of proliferating radial and non-radial precursor cells located in the SGZ, which give rise to neural progenitor cells (NPCs), which in turn generate neuroblasts (Ehninger and Kempermann, 2008). Immature neurons migrate into the granule cell layer and differentiate into dentate granule cell in the hippocampus. Although NPCs proliferation generates a vast number of neurons only a very small proportion survives for long period of time (Kempermann et al., 2003). Indeed, most of the newly born cells are eliminated by apoptosis during first few days following birth. The cells that do survive for the first two weeks are then stable and integrated into the network of the dentate gyrus throughout life. After this time point, only very small changes in cell number occur. There are only two critical periods for neural progeny survival: (1) during transition from amplifying neuroprogenitors to neuroblast (Platel et al., 2010; Sierra et al., 2010); and (2) during the integration stage of the immature neurons (Tashiro et al., 2006; Mouret et al., 2008). By two months the surviving neurons receive input from other neurons (van et al., 2002; Piatti et al., 2006), with some forming functional synapses, and possessing electrophysiological properties indistinguishable from those of mature neurons (Ge et al., 2008).

MICROGLIA HETEROGENEITY

Microglia are recognized as the resident brain immune cells and have important roles in the healthy central nervous system (CNS). For example as the brain's tissue macrophage, a primary function of microglia is to phagocytose dying cells and cellular debris silently (i.e., without producing inflammation). However, upon a pathologic insult, such as infection or brain injury, microglia respond rapidly. Part of this reactive response includes a morphological change in appearance. In the healthy CNS, microglia have highly *ramified* morphology with thin processes, which dynamically move in the brain parenchyma in what has been called a surveillance state (Nimmerjahn et al., 2005). We define microglia in this surveillance state as *ramified microglia*. In contrast, *reactive microglia*, (i.e., microglia that are no longer ramified microglia) can adopt a number of altered morphologies, including a hypertrophic cell with enlarged processes, or amoeboid macrophage like morphology.

MICROGLIA: ROLE OF RAMIFIED MICROGLIA IN ADULT NEUROGENESIS

Increasing evidence suggests that ramified microglia are an essential component of the neurogenic niche in the SGZ of the adult hippocampus. One of the critical roles of microglia in modulating hippocampal neurogenesis is pruning of newborn cells during the first critical period of survival. Sierra et al. (2010) demonstrated that ramified microglia have an important function in phagocytosis of apoptotic cells during the first days of their life. As the newborn cells are integrated into the existing circuits other cells become apoptotic. The apoptotic cells are subsequently removed by microglia in an immunologically silently process (i.e., without inflammation). In response to tissue damage, such as a traumatic brain injury, damage associated molecular patterns (DAMPs: e.g., Adenosine triphosphate (ATP), DNA) are released and cause microglia to become activated towards a proinflammatory state.

In the context of neurogenesis, the dying cells undergo a process of programmed cell death, and no DAMPs are released. As opposed to the phagocytosis by ameboid microglia observed during trauma or neurodegeneration, phagocytosis of apoptotic cells during neurogenesis is performed by ramified microglia. Ramified microglia remove apoptotic neurons by a special modification of the microglial process, which form phagocytic pouches that engulf the apoptotic cells. The phagocytic pouches, occurs independent from the cell body, in terminal or en passant branches, as opposed to engulfment of the soma by ameboid microglia. Furthermore, phagocytosis of apoptotic neurons by ramified microglia is highly efficient as demonstrated by the high proportion of apoptotic cells engulfed by ramified microglia, the proportion of microglia engaged in the engulfing process, and the time to completely eliminate apoptotic cells (Sierra et al., 2010).

MICROGLIA PROVIDE TROPHIC SUPPORT FOR ADULT HIPPOCAMPAL NEUROGENESIS

The role of microglia in neurogenesis is not limited to removal of cells. Evidence suggests that microglia secrete factors that can influence proliferation, differentiation into neurons or glia, and survival of the newborn cell. How could microglia-derived factors influence neurogenesis? Several different mechanisms have been proposed: (1) microglia could have a direct instructive role in dictating the commitment to a neuronal phenotype, (2) microglia could promote proliferation through secretion of neurotrophic factors, and (3) microglia could produce factors that regulate survival of neuronal cells. In favor of a direct instructive role of microglia to direct neuronal differentiation, *in vitro* studies demonstrate that microglia have the capacity to guide the differentiation of precursor cells isolated from embryonic brain as well as adult mouse neural precursor cells toward a neuronal phenotype (Aarum et al., 2003). In addition, precursor cell cultures grown in conditioned media from microglia cells contain a higher proportion of neurons than what would be expected from their spontaneous differentiation alone. Yet, microglia can affect proliferation and survival, in addition to neuron differentiation. For example, Morgan et al. (2004) further investigated the effect of non-stimulated microglia on neuronal proliferation and survival. The authors used microglia-conditioned medium collected from primary rat microglia to treat neurons *in vitro* for 7 days and found that the microglia-conditioned medium induced a 50% increase in neuronal survival compared to untreated neurons. A different study confirmed and extended these findings showing that addition of microglia-conditioned media in SVZ-derived culture increased neuroblast production (Walton et al., 2006). Furthermore, loss of inducible neurogenesis was paralleled by microglia depletion in proliferating culture. While a number of growth factors secreted by microglia could be responsible for such effect, evidence suggests that microglia are capable of producing growth factors, such as Insulin-like growth factor 1 (IGF-1) and Brain-derived neurotrophic factor (BDNF), which promote neurogenesis (Ziv and Schwartz, 2008). **Figure 1** highlights the known ways microglia are involved in the neurogenic process.

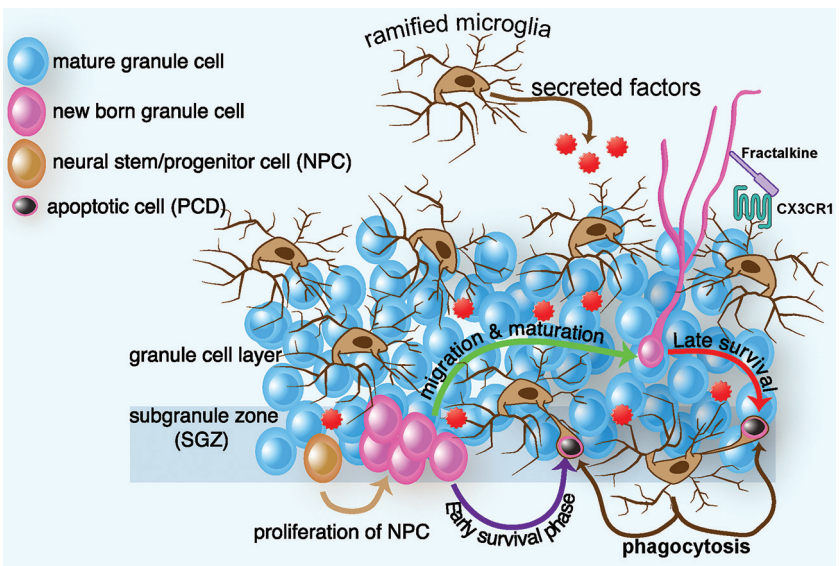


FIGURE 1 | Schematic diagram of ramified microglia and their effect on adult hippocampal neurogenesis. In intact brain, microglia regulate several steps of adult hippocampal neurogenesis. In the SGZ, progenitor cells migrate to the granule cell layer and differentiate into a neuronal phenotype, with most NPCs dying in the first few days of life. Within two months, the surviving neurons receive input, form functional synapses with their target cells, and exhibit electrophysiological properties indistinguishable from those of mature neurons. In intact brain, ramified microglia eliminate apoptotic newborn cells during the first few days of their life by phagocytosis. This phagocytosis occurs by a special modification of the microglial processes, which form phagocytic pouches that engulf the apoptotic cells. Microglia can also affect proliferation, differentiation, and survival, through the secretion of neurotrophic factors. Finally microglia communicate with nearby neurons through the CX3CR1/CX3CL1 signaling. Interactions between CX3CL1 and CX3CR1 contribute to the ability of microglia to maintain a surveillant/ramified phenotype. Disruption of this signaling results in a change in microglia phenotype and function, which leads to decreased hippocampal neurogenesis.

In rodents, environmental enrichment has been one of the most clear and reproducible ways to stimulate adult neurogenesis (van et al., 1999; Inokuchi, 2011). Environmental enrichment has many beneficial effects on CNS function; intriguingly, some of the effects of environmental enrichment may be mediated by microglia. A recent finding showed that when microglia extracted from the hippocampus of runner mice were added to the hippocampal preparation of sedentary mice, the number of NPCs in the hippocampal culture of sedentary mice increased (Vukovic et al., 2012), suggesting that microglia are intrinsically altered or "primed" by the enriching experience. Indeed, following an enriched environment or physical activity, beneficial microglia increase, and this increase correlates with an increase in hippocampal neurogenesis (Ziv et al., 2006; Choi et al., 2008). However, other studies have shown no correlation or an inverse correlation in the role of microglia in neurogenesis stimulated by environmental enrichment (Gebara et al., 2013).

NEURON–MICROGLIA DIALOGUE IN ADULT HIPPOCAMPAL NEUROGENESIS

Until recently, neurons were believed to be submissive to the effects of microglia. However, a number of neuronal signals were found that can regulate microglia activation (Biber et al., 2007), suggesting a neuron-microglia dialogue. Indeed, neurons may also deliver signals that keep microglia in their surveillance mode indicating normal function. Under physiological conditions several neuron-mediated signals have an anti-inflammatory action at the level of the microglia. Cluster of differentiation (CD) 200 (also called OX2), CD47, CD55, CX3CL1 (fractalkine), are all neuroimmunoregulatory proteins constitutively expressed in healthy neurons with a cognate receptor on microglia (Kierdorf and Prinz, 2013). In the context of adult hippocampal neurogenesis, the question to be considered is whether neurons in the neurogenic niche communicate with microglia to regulate neurogenesis. A recent report suggests that the NPCs could secrete factors that regulate microglia function. Using an *in vitro* assay, the conditioned medium from NPCs caused microglia to increase in numbers, migrate to the site of injury, and to become phagocytic (Mosher et al., 2012). As previously mentioned, microglia can regulate neurogenesis at a number of steps in the neurogenic process. Therefore, a bidirectional regulation of neurons/neurogenesis and microglia might provide a means to fine tune the neurogenic process.

FRACTALKINE/CX3CR1 AS AN EXAMPLE OF NEURON–MICROGLIA DIALOGUE

As noted, *ramified* microglia dynamically move their processes within a volume of parenchyma and, in normal conditions, target synaptic structure (Wake et al., 2009; Tremblay et al., 2010). How do microglia know where to move their processes? What signals do microglia receive when they survey the neurons? Are there different signals for synaptic pruning or neuron pruning? Some of these questions are beginning to be answered, but this is an exciting area of microglia biology that is largely unexplored.

One of the best-characterized examples of a neuronal signal that regulates microglia function is the chemokine fractalkine. The anatomical expression of fractalkine on neurons and CXRCR1 on microglia led to the hypothesis of a unique signaling whereby neurons may maintain microglia in a surveillant/ramified state through a repressive fractalkine signal. Fractalkine is constitutively expressed at high levels on healthy neurons. The receptor for fractalkine, CX3CR1, is more highly expressed on microglia, than macrophages. Over the past decade numerous investigators have provided strong support that fractalkine does suppress microglia activation (Cardona et al., 2006; Ransohoff et al., 2007; Bhaskar et al., 2010; Lee et al., 2010).

Interestingly, data suggest that fractalkine signaling may be involved in neuron-microglia dialogue in the neurogenic niche that regulates neurogenesis. First it was shown by genetic deletion or pharmacological antagonism of CX3CR1 in young adult rats that CX3CR1 was important for the maintenance of hippocampal neurogenesis, as animals with decreased CX3CR1 have less neurogenesis (Bachstetter et al., 2011). Furthermore, levels of fractalkine, which are abundantly expressed in young healthy rodent brains, were decreased in aged rodents (Bachstetter et al., 2011). It was suggested that the decrease in fractalkine signaling may contribute to the increased neuroinflammation and decreased hippocampal neurogenesis seen in the aged rodent brain. To test this hypothesis, aged rats were administered fractalkine, which reversed the age-related decrease in hippocampal neurogenesis and restored microglia to a ramified morphology (Bachstetter et al., 2011).

Loss of fractalkine/CX3CR1 signaling in a non-disease model, not only affects neurogenesis, it can cause impairments in motor learning, cognitive function, and synaptic plasticity through increased microglia activation and inflammation in the CNS (Rogers et al., 2011). Impairment of long-term potentiation (LTP) and neurogenesis likely represent the mechanism responsible for the defect observed in hippocampal-dependent associative and spatial memory formation. However, multiple mechanisms could account for the impairment in cognitive function and synaptic plasticity observed in the CX3CR1-deficient mice. Studies on CX3CR1/fractalkine signaling and neuronal activity have produced discordant results. For example, exogenous application of fractalkine inhibits LTP and LTP impairment failed to occur in CX3CR1 deficient mice (Maggi et al., 2009). Differences in experimental protocols, animal age and gender, and housing conditions etc. could explain the discordance between studies.

As noted earlier, environmental enrichment, including exercise can stimulate neurogenesis. Recent data suggest that fractalkine/CX3CR1 signaling may be involved in the exercise-induced increase in hippocampal neurogenesis. Exercise reversed the age-related decline in fractalkine hippocampal levels and increased hippocampal neurogenesis (Vukovic et al., 2012). Furthermore, pharmacological antagonism of CX3CR1 prevented the increase in hippocampal neurogenesis (Vukovic et al., 2012). Therefore, the aforementioned results suggest that fractalkine signaling is a way microglia function could be tuned by neurons. A previous interesting study (Maggi et al., 2011) showed that absence of CX3CR1 in female mice leads to an increase in hippocampal plasticity and learning performance and a decrease in hippocampal-dependent response to environmental stimulation. In addition, deficiency of CX3CR1 in female mice blunted the

positive effect of an enriched environment on neuronal plasticity. These controversial results are quite intriguing as they clearly show a gender difference effect of microglia in synaptic plasticity. Interesting, profound sex differences in the microglia colonization of the developing rodent brain were observed recently. At P4, male rats have significantly more microglia than females in many brain regions critical for cognition, learning, and memory including the hippocampus, parietal cortex, and amygdala (Schwarz and Bilbo, 2012). It would be interesting to know if a gender difference in microglia density exists during adulthood. Taken together these studies demonstrate that microglia through the CX3CR1 receptor play a physiological role in adult hippocampal neurogenesis and cognitive function.

CONCLUSION

Microglia are an essential component of the neurogenic niche and emerging evidence shows new and fundamental roles for microglia in the control of neuronal proliferation, differentiation, and survival of newborn neurons into the existing neuronal circuitry. However the specific molecular and cellular mechanisms through which microglia regulate different stages of neurogenesis are only beginning to be explored. A deeper understanding of the physiological function of microglia in the different steps of adult neurogenesis is needed.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Aging (5R21AG039739-03). We thank Paula Thomason for manuscript preparation and editing.

REFERENCES

Aarum, J., Sandberg, K., Haeberlein, S. L., and Persson, M. A. (2003). Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl. Acad. Sci. U S A* 100, 15983–15988. doi: 10.1073/pnas.2237050100

Bachstetter, A. D., Morganti, J. M., Jernberg, J., Schlunk, A., Mitchell, S. H., Brewster, K. W., et al. (2011). Fractalkine and CX(3)CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol. Aging* 32, 2030–2044. doi: 10.1016/j.neurobiolaging.2009.11.022

Bhaskar, K., Konerth, M., Kokiko-Cochran, O. N., Cardona, A., Ransohoff, R. M., and Lamb, B. T. (2010). Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* 68, 19–31. doi: 10.1016/j.neuron.2010.08.023

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci.* 30, 596–602. doi: 10.1016/j.tins.2007.08.007

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., et al. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nat. Neurosci.* 9, 917–924. doi: 10.1038/nn1715

Choi, S. H., Veeraraghavalu, K., Lazarov, O., Marler, S., Ransohoff, R. M., Ramirez, J. M., et al. (2008). Non-cell-autonomous effects of presenilin 1 variants on enrichment-mediated hippocampal progenitor cell proliferation and differentiation. *Neuron* 59, 568–580. doi: 10.1016/j.neuron.2008.07.033

Ehninger, D., and Kempermann, G. (2008). Neurogenesis in the adult hippocampus. *Cell Tissue Res.* 331, 243–250. doi: 10.1007/s00441-007-0478-3

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., et al. (1998). Neurogenesis in the adult human hippocampus. *Nat. Med.* 4, 1313–1317. doi: 10.1038/3305

Gage, F. H. (2000). Mammalian neural stem cells. *Science* 287, 1433–1438. doi: 10.1126/science.287.5457.1433

Ge, S., Sailor, K. A., Ming, G. L., and Song, H. (2008). Synaptic integration and plasticity of new neurons in the adult hippocampus. *J. Physiol.* 586, 3759–3765. doi: 10.1113/jphysiol.2008.155655

Gebara, E., Sultan, S., Kocher-Braissant, J., and Toni, N. (2013). Adult hippocampal neurogenesis inversely correlates with microglia in conditions of voluntary running and aging. *Front. Neurosci.* 7:145. doi: 10.3389/fnins.2013.00145

Inokuchi, K. (2011). Adult neurogenesis and modulation of neural circuit function. *Curr. Opin. Neurobiol.* 21, 360–364. doi: 10.1016/j.conb.2011.02.006

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130, 391–399. doi: 10.1242/dev.00203

Kierdorf, K., and Prinz, M. (2013). Factors regulating microglia activation. *Front. Cell. Neurosci.* 7:44. doi: 10.3389/fncel.2013.00044

Lee, S., Varvel, N. H., Konerth, M. E., Xu, G., Cardona, A. E., Ransohoff, R. M., et al. (2010). CX3CR1 deficiency alters microglial activation and reduces beta-amyloid deposition in two alzheimer's disease mouse models. *Am. J. Pathol.* 177, 2549–2562. doi: 10.2353/ajpath.2010.100265

Maggi, L., Scianni, M., Branchi, I., D'Andrea, I., Lauro, C., and Limatola, C. (2011). CX(3)CR1 deficiency alters hippocampal-dependent plasticity phenomena blunting the effects of enriched environment. *Front. Cell. Neurosci.* 5:22. doi: 10.3389/fncel.2011.00022

Maggi, L., Trettel, F., Scianni, M., Bertollini, C., Eusebi, F., Fredholm, B. B., et al. (2009). LTP impairment by fractalkine/CX3CL1 in mouse hippocampus is mediated through the activity of adenosine receptor type 3 (A3R). *J. Neuroimmunol.* 215, 36–42. doi: 10.1016/j.jneuroim.2009.07.016

Ming, G. L., and Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* 70, 687–702. doi: 10.1016/j.neuron.2011.05.001

Morgan, S. C., Taylor, D. L., and Pocock, J. M. (2004). Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. *J. Neurochem.* 90, 89–101. doi: 10.1111/j.1471-4159.2004.02461.x

Mosher, K. I., Andres, R. H., Fukuhara, T., Bieri, G., Hasegawa-Moriyama, M., He, Y., et al. (2012). Neural progenitor cells regulate microglia functions and activity. *Nat. Neurosci.* 15, 1485–1487. doi: 10.1038/nn.3233

Mouret, A., Gheusi, G., Gabellec, M. M., de, C. F., Olivo-Marin, J. C., and Lledo, P. M. (2008). Learning and survival of newly generated neurons: when time matters. *J. Neurosci.* 28, 11511–11516. doi: 10.1523/jneurosci.2954-08.2008

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

Piatti, V. C., Esposito, M. S., and Schinder, A. F. (2006). The timing of neuronal development in adult hippocampal neurogenesis. *Neuroscientist* 12, 463–468. doi: 10.1177/1073858406293538

Platel, J. C., Dave, K. A., Gordon, V., Lacar, B., Rubio, M. E., and Bordey, A. (2010). NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. *Neuron* 65, 859–872. doi: 10.1016/j.neuron.2010.03.009

Ransohoff, R. M., Liu, L., and Cardona, A. E. (2007). Chemokines and chemokine receptors: multipurpose players in neuroinflammation. *Int. Rev. Neurobiol.* 82, 187–204. doi: 10.1016/s0074-7742(07)82010-1

Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., et al. (2011). CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J. Neurosci.* 31, 16241–16250. doi: 10.1523/jneurosci.3667-11.2011

Roy, N. S., Wang, S., Jiang, L., Kang, J., Benraiss, A., Harrison-Restelli, C., et al. (2000). In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. *Nat. Med.* 6, 271–277. doi: 10.1038/73119

Schwarz, J. M., and Bilbo, S. D. (2012). Sex, glia, and development: interactions in health and disease. *Horm. Behav.* 62, 243–253. doi: 10.1016/j.yhbeh.2012.02.018

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495. doi: 10.1016/j.stem.2010.08.014

Tashiro, A., Sandler, V. M., Toni, N., Zhao, C., and Gage, F. H. (2006). NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* 442, 929–933. doi: 10.1038/nature05028

Tremblay, M. E., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

van, P. H., Christie, B. R., Sejnowski, T. J., and Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice.

Proc. Natl. Acad. Sci. U S A 96, 13427–13431. doi: 10.1073/pnas.96.23.13427

van, P. H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., and Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. *Nature* 415, 1030–1034. doi: 10.1038/4151030a

Vukovic, J., Colditz, M. J., Blackmore, D. G., Ruitenberg, M. J., and Bartlett, P. F. (2012). Microglia modulate hippocampal neural precursor activity in response to exercise and aging. *J. Neurosci.* 32, 6435–6443. doi: 10.1523/jneurosci.5925-11.2012

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). 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

Walton, N. M., Sutter, B. M., Laywell, E. D., Levkoff, L. H., Kearns, S. M., Marshall, G. P., et al. (2006). Microglia instruct subventricular zone neurogenesis. *Glia* 54, 815–825. doi: 10.1002/glia.20419

Wang, C., Liu, F., Liu, Y. Y., Zhao, C. H., You, Y., Wang, L., et al. (2011). Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. *Cell Res.* 21, 1534–1550. doi: 10.1038/cr.2011.83

Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., et al. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat. Neurosci.* 9, 268–275. doi: 10.1038/nn1629

Ziv, Y., and Schwartz, M. (2008). Immune-based regulation of adult neurogenesis: implications for learning and memory. *Brain Behav. Immun.* 22, 167–176. doi: 10.1016/j.bbi.2007.08.006

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 January 2013; accepted: 05 November 2013; published online: 22 November 2013.

Citation: Gemma C and Bachstetter AD (2013) The role of microglia in adult hippocampal neurogenesis. Front. Cell. Neurosci. 7:229. doi: 10.3389/fncel.2013.00229

This article was submitted to the journal Frontiers in Cellular Neuroscience.

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 06 September 2013
doi: 10.3389/fncel.2013.00145

Modulation of adult-born neurons in the inflamed hippocampus

Karim Belarbi [1,2] ***and Susanna Rosi*** [1,2,3] *

[1] *Brain and Spinal Injury Center, San Francisco General Hospital, University of California at San Francisco, San Francisco, CA, USA*
[2] *Department of Physical Therapy and Rehabilitation Science, University of California at San Francisco, San Francisco, CA, USA*
[3] *Department of Neurological Surgery, University of California at San Francisco, San Francisco, CA, USA*

Edited by:
Marie-Eve Tremblay, Université Laval, Canada

Reviewed by:
Valentin Nägerl, Institut Interdisciplinaire de NeuroSciences, Centre National de la Recherche Scientifique, France
John A. Olschowka, University of Rochester School of Medicine and Dentistry, USA

***Correspondence:**
Susanna Rosi, Brain and Spinal Injury Center, San Francisco General Hospital, University of California at San Francisco, 1001 Potrero Avenue, Building No. 1, Room No. 101, San Francisco, CA 94110, USA
e-mail: rosis@ptrehab.ucsf.edu

Throughout life new neurons are continuously added to the hippocampal circuitry involved with spatial learning and memory. These new cells originate from neural precursors in the subgranular zone of the dentate gyrus, migrate into the granule cell layer, and integrate into neural networks encoding spatial and contextual information. This process can be influenced by several environmental and endogenous factors and is modified in different animal models of neurological disorders. Neuroinflammation, as defined by the presence of activated microglia, is a common key factor to the progression of neurological disorders. Analysis of the literature shows that microglial activation impacts not only the production, but also the migration and the recruitment of new neurons. The impact of microglia on adult-born neurons appears much more multifaceted than ever envisioned before, combining both supportive and detrimental effects that are dependent upon the activation phenotype and the factors being released. The development of strategies aimed to change microglia toward states that promote functional neurogenesis could therefore offer novel therapeutic opportunities against neurological disorders associated with cognitive deficits and neuroinflammation. The present review summarizes the current knowledge on how production, distribution, and recruitment of new neurons into behaviorally relevant neural networks are modified in the inflamed hippocampus.

Keywords: adult neurogenesis, chemokines, cytokines, inflammation, microglia

INTRODUCTION

In the adult mammalian brain, the subgranular zone of the dentate gyrus (DG) is one of the brain regions where robust neurogenesis continues throughout life (Altman and Das, 1965; Eriksson et al., 1998; Spalding et al., 2013). Adult-born neurons have the capacity to migrate into the granule cell layer, to differentiate into mature granule neurons and to functionally integrate into hippocampal neural networks. This process is highly plastic, influenced by environmental and endogenous factors, and it appears to be altered during neuropathological conditions (Parent et al., 1997; Dash et al., 2001; Ekdahl et al., 2003). In this review, we summarize the current knowledge on the plasticity of adult-born neurons in animal models of brain injury associated with neuroinflammation and we discuss the role of activated microglia and the contribution of specific inflammatory factors.

FROM NEURAL PROGENITORS TO NEURONAL INTEGRATION INTO HIPPOCAMPAL NETWORKS

Hippocampal adult-born neurons originate from neural precursor cells located in the subgranular zone of the DG and these cells have limited self-renewal capacity (Kempermann et al., 2003). While most of the newly generated cells die shortly after generation (Kempermann et al., 1997; Biebl et al., 2000), some of the progeny gives rise to neuroblasts that migrate into the DG granule cell layer where they mature into fully functional granule neurons (Kempermann et al., 2003; Esposito et al., 2005). The new cells that become synaptically integrated, receive inputs from the entorhinal cortex, and send axonal projections to hilar neurons and CA3 pyramidal cells (Markakis and Gage, 1999; Laplagne et al., 2007; Toni et al., 2008) can be activated by various stimuli, including behavioral experience (Jessberger and Kempermann, 2003; Ramirez-Amaya et al., 2006; Kee et al., 2007; Belarbi et al., 2012a) or high-frequency electrical perforant path stimulation (Bruel-Jungerman et al., 2006; Jungenitz et al., 2013). During their maturation process, new neurons differ substantially from existing granule cells. Electrophysiological data show that they exhibit a decreased overall induction threshold for long-term potentiation and enhanced synaptic plasticity compared to older neurons (Schmidt-Hieber et al., 2004; Ge et al., 2007). In response to spatial exploration, new neurons are also more likely to express plasticity-related immediate-early genes (IEGs) such as *Arc* (activity-regulated cytoskeleton-associated protein) or IEGs encoding transcription factors such as *cfos* (Ramirez-Amaya et al., 2006; Kee et al., 2007). Furthermore, numerous studies ablating or enhancing adult neurogenesis have demonstrated that hippocampal adult-born neurons are required for hippocampus-dependent forms of spatial memory (Clelland et al., 2009; Trouche et al., 2009; Goodman et al., 2010; Nakashiba et al., 2012). Collectively, these data indicate that adult-born neurons are more likely than existing granule neurons to be recruited into hippocampal networks that process spatial and contextual information and exert a critical role in hippocampus-dependent functions.

THE INFLAMED HIPPOCAMPUS AND THE MULTIFACETED ROLE OF MICROGLIA ACTIVATION

Microglia derive from primitive myeloid progenitors and constitute the resident immune system in the brain (Ginhoux et al., 2010; Kierdorf et al., 2013). In the absence of pathological insult, microglia exist in a ramified morphological phenotype termed "resting microglia." Through their highly motile ramifications resting microglia continuously scan their territorial domain and communicate with the other surrounding cells by distinct signaling pathways (Davalos et al., 2005; Nimmerjahn et al., 2005; Hanisch and Kettenmann, 2007; Kettenmann et al., 2011). Furthermore, microglia transiently make contact with presynaptic boutons, postsynaptic spines, and the synaptic cleft (Wake et al., 2009; Tremblay et al., 2010) and facilitate synapses elimination and pruning, therefore likely contributing to the stability and organization of neural networks (Wake et al., 2009; Tremblay et al., 2010; Paolicelli et al., 2011). As a consequence of brain pathology, microglia respond to pathogen-associated or damage-associated molecules and acquire a reactive profile usually referred as "activated microglia." Typical morphological changes associated with microglia activation include thickening of ramifications and of cell bodies followed by acquisition of a rounded amoeboid shape (Kettenmann et al., 2011). This process is accompanied by expression of novel surface antigens and production of mediators that build up and maintain the inflammatory response of the brain parenchyma. This response is often associated with the recruitment of blood-born macrophages from the periphery which migrate into the injured brain parenchyma (Schilling et al., 2005; Schwartz and Shechter, 2010). Monocyte-derived macrophages are distinct in nature from resident microglia (for review, see London et al., 2013).

Activated microglia in the brain can operate as damage associated cells, producing a plethora of molecules that are essential for the elimination of pathogens, toxic factors (such as protein aggregates) and cellular debris (following neuronal death for example). By producing neurotrophic and growth factors that are pivotal for tissue repair and renewal they contribute to resolve infection or injury and to restore normal tissue homeostasis (Neumann et al., 2006; Lalancette-Hebert et al., 2007). On the other hand, through the release of proinflammatory cytokines, proteases, and reactive oxygen species they can induce neurotoxicity (Block et al., 2007; Hanisch and Kettenmann, 2007).

One of the brain regions most densely populated with microglia is the hippocampus (Lawson et al., 1990); microglia activation in this region is a common landmark following stimulation with the bacterial endotoxin lipopolysaccharide (LPS; Rosi et al., 2005; Belarbi et al., 2012a,b), ionizing irradiation (Monje et al., 2002, 2003; Rola et al., 2008; Rosi et al., 2008; Belarbi et al., 2013), traumatic brain injury (Piao et al., 2013), brain ischemia (Liu et al., 2007), and kainic acid-induced or pilocarpine-induced brain seizure (Andersson et al., 1991; Borges et al., 2003; Turrin and Rivest, 2004; Vezzani et al., 2008). Microglia activation is also present in various models of neurodegenerative diseases associated with abnormal protein aggregation such as in genetically modified mouse models mimicking Alzheimer's disease amyloid pathology (APP23: Stalder et al., 1999; Bornemann et al., 2001; PS/APP: Matsuoka et al., 2001; PS1 + APP: Gordon et al., 2002; Tg2576: Frautschy et al., 1998; Benzing et al., 1999; Sasaki et al., 2002) or tau pathology (P301S tau: Bellucci et al., 2004; Yoshiyama et al., 2007; TgTauP301L: Sasaki et al., 2008; Thy-Tau22: Belarbi et al., 2011). Normal aging is also characterized by chronic low-level of inflammation and increased microglia reactivity (Jurgens and Johnson, 2012).

Both macrophages (Porta et al., 2009) and microglia (Michelucci et al., 2009) can undergo different forms of polarized activation leading to a potentially neurotoxic "classic or M1 activation" (characterized by a release of pro-inflammatory factors) or a potentially neuroprotective "alternative or M2 activation" (characterized by anti-inflammatory cytokines). M1 activation is characterized by the release of several proinflammatory and neurotoxic factors including reactive oxygen species, nitric oxide, TNF-alpha, Il-6, Il-1beta, Il-12, and monocyte chemoattractant protein (MCP)-1 (Meda et al., 1996; Kettenmann et al., 2011; Qin et al., 2013). Polarization toward classic activation (M1) can be induced experimentally by exposure to pro-inflammatory cytokines such as interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha and interleukin (Il)-1beta, as well as bacterial-derived LPS (Lehnardt et al., 2003). Alternative M2 (protective) activation of microglia is characterized by increased expression of the anti-inflammatory cytokines Il-4, Il-10, and transforming growth factor (TGF)-beta, CD200, and growth factors such as insulin growth factor (IGF)-1, nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF; Butovsky et al., 2005; Yi et al., 2012). Alternative activation can be induced experimentally by anti-inflammatory cytokines such as Il-4 and Il-13 (Butovsky et al., 2006; Colton, 2009). The regulation of this functional polarization after brain injury is still not clear and evidence shows that it should be considered as a dynamic process (Colton, 2009). For example, following ischemia-induced injury in the striatum, microglia initially express the classic activation phenotype, but with time a portion of the cells acquire the alternative activation phenotype (Thored et al., 2009). Therefore, the link between activated microglia and neurogenesis is multifaceted, combining both supportive and detrimental effects dependent upon their phenotype and the factors being released (Butovsky et al., 2006; **Figure 1**).

PRODUCTION OF NEURONS IN THE INFLAMED HIPPOCAMPUS

Proliferation, differentiation, and survival of neurons in the adult brain has been shown to be modulated in pathological conditions associated with inflammation (Cho and Kim, 2010; Mu and Gage, 2011; Kohman and Rhodes, 2013). Animal models of brain irradiation typically display a significant loss of neural precursor cells that occurs within a few hours (Mizumatsu et al., 2003) and is still present several months after relatively low radiation doses (Tada et al., 2000; Raber et al., 2004a,b; Belarbi et al., 2013). Similarly, neuroinflammation induced by central or systemic administration of LPS significantly reduces basal neurogenesis (Ekdahl et al., 2003; Monje et al., 2003; Fujioka and Akema, 2010), although this is not observed when very low doses of LPS are chronically infused in the ventricular system (Belarbi et al., 2012a). In contrast, increased neuronal production has been

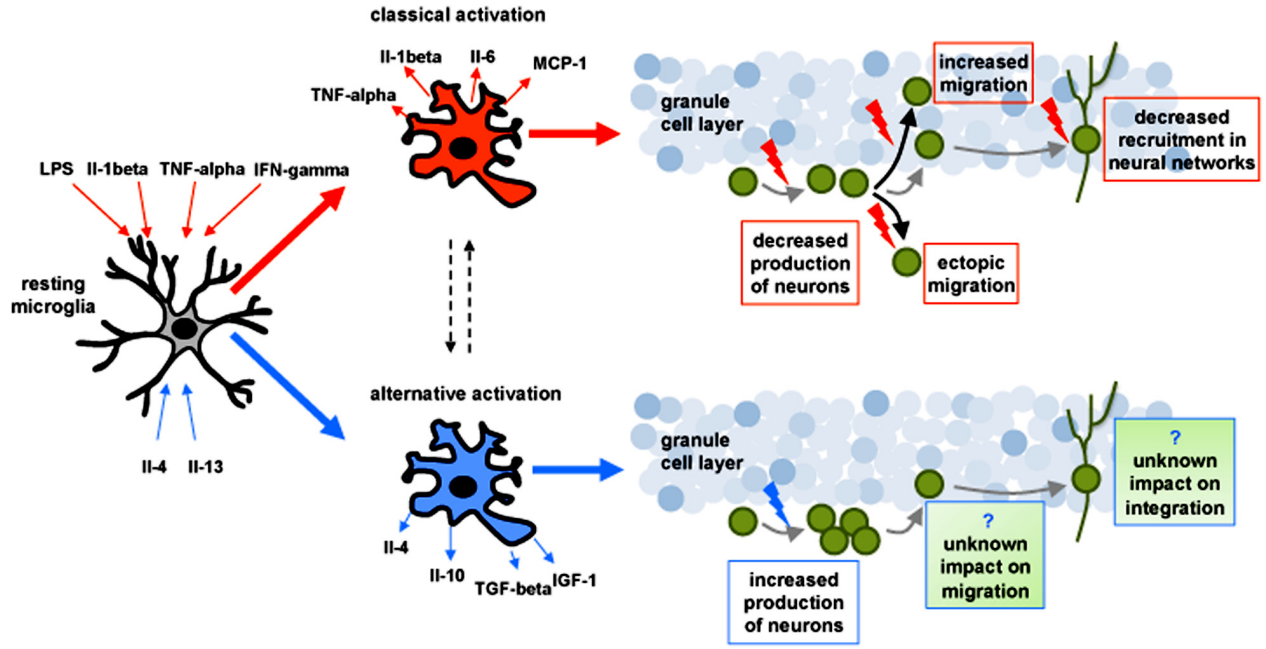


FIGURE 1 | Schematic drawing representing the impact of classical (depicted in red) and alternative (depicted in blue) activation of microglia on adult-born neurons (depicted in green) in the hippocampus. In response to changes in the microenvironment microglia can undergo to a potentially neurotoxic "classical activation" (characterized by the release of proinflammatory factors) or a potentially neuroprotective "alternative activation" (characterized by the release of anti-inflamatory cytokines). Polarization toward classical activation can be induced experimentally by exposure to pro-inflammatory cytokines such as (IFN)-gamma, tumor necrosis factor (TNF)-alpha and interleukin (Il)-1beta, as well as bacterial-derived LPS. Classically activated microglia has been shown to: (1) decrease the production of neurons, (2) alter their migration pattern, and (3) reduce their recruitment into neuronal networks (red arrows). Alternative activation of microglia can be induced experimentally by anti-inflammatory cytokines such as Il-4 and Il-13 and can increase the production of neurons (blue arrow). The impact of alternative activation of microglia on the migration and the integration of new neurons remains unknown.

reported in animal models of experimental traumatic brain injury (Dash et al., 2001; Kernie et al., 2001; Chirumamilla et al., 2002; Emery et al., 2005; Sun et al., 2007), brain ischemia (Liu et al., 1998; Kee et al., 2001; Yagita et al., 2001; Nakatomi et al., 2002; Choi et al., 2003), and kainic acid-induced or pilocarpine-induced status epilepticus (Parent et al., 1997; Choi et al., 2007). Different animal models of Alzheimer's disease, provided equivocal data, demonstrating both increased and decreased hippocampal neurogenesis (as reviewed in Mu and Gage, 2011). While differences in many parameters (bromodeoxyuridine administration, cell markers, etc.) could be the cause for these discrepancies, such data provide strong evidence that the modulation of hippocampal adult-born neurons is dependent on the nature of the injury and the time following injury. The initial work investigating the role of activated microglia on neurogenesis found an acute detrimental role for these cells. Classic activation of microglia induced through administration of LPS, either centrally or peripherally, has been shown to block hippocampal neurogenesis (Ekdahl et al., 2003; Monje et al., 2003; Butovsky et al., 2006). In addition, inhibition of microglial activation through administration of minocycline or indomethacin was shown to rescue hippocampal neurogenesis after LPS-induced inflammation (Monje et al., 2003), cranial irradiation (Ekdahl et al., 2003), or focal cerebral ischemia (Hoehn et al., 2005; Liu et al., 2007). In contrast, alternative microglia activation through Il-4 or low level of IFN-gamma could promote neurogenesis (Butovsky et al., 2006). Proinflammatory cytokines released by classically activated microglia can specifically inhibit neural precursor generation, neuronal differentiation, and survival. These include TNF-alpha (Cacci et al., 2005; Heldmann et al., 2005; Iosif et al., 2006), Il-1beta (Goshen et al., 2008; Koo and Duman, 2008; Kuzumaki et al., 2010; Wu et al., 2012), and Il-6 (Vallieres et al., 2002). Conversely, factors released by alternative activation of microglia seem to support the production of neurons as shown for Il-4 (Kiyota et al., 2010), Il-10 (Kiyota et al., 2012), TGF-beta (Battista et al., 2006; Mathieu et al., 2010), and IGF-1 (Choi et al., 2008; Annenkov, 2009). Taken together, these findings suggest that classically activated microglia generally impair neurogenesis whereas alternatively activated microglia promote it, and that these opposite effects are likely dependent upon the specific factors being released (**Figure 1**).

DISTRIBUTION OF ADULT-BORN NEURONS IN THE INFLAMED HIPPOCAMPUS

In the normal hippocampus neuronal precursors migrate a few micrometers into the granule cell layer where they differentiate into new neurons during the first 2 weeks after production (Kempermann et al., 2003; Seki et al., 2007; Sandoval et al., 2011; Belarbi et al., 2013). Comparative analyses of the distribution of adult-born neurons in different animal models of brain injury suggest

that the migration process is altered during pathological conditions. Parent and colleagues first reported ectopic destinations of neural progenitor cells after pilocarpine-induced seizure. Mature neurons were detected not only inside the granule cell layer but also in the molecular layer and inside the hilus of the DG (Parent et al., 1997, 2006). Altered distribution of new neurons within the hippocampus has been also reported in murine models of stroke (Kernie and Parent, 2010), traumatic brain injury (Rosi et al., 2012), cranial-irradiation (Belarbi et al., 2013), and LPS-induced chronic inflammation (Belarbi et al., 2012a). In these models, new neurons were distributed in average a longer distance from the subgranular zone into the granule cell layer. Additional evidence for modified migration of new neurons in the inflamed hippocampus comes from the work of Belmadani et al. (2006) who demonstrated that small cytokine signaling proteins, named chemokines, regulate the migration of neural progenitors to sites of neuroinflammation. In that study neural progenitor cells were grafted into the DG of cultured hippocampal slices and inflammation was achieved by injecting a solution, containing TNF-alpha, IFN-gamma, LPS, glycoprotein 120, or a beta-amyloid-expressing adenovirus, into the area of the fimbria. In control slices, neural progenitors showed little tendency to migrate, while in slices injected with inflammatory stimuli, neural progenitors migrated toward the site of the injection. However, when neural precursors from mice lacking the C–C chemokine receptor type 2 (CCR2 knock-out) were transplanted into slices, they exhibited a greatly reduced migration toward sites of inflammation (Belmadani et al., 2006). CCR2 and its primary ligand MCP-1 are considered to be critical for macrophage trafficking and activation in the brain (Prinz and Priller, 2010). CCR2 has also been shown to be expressed by neural progenitors (Tran et al., 2007). Therefore, these data further support a role for chemokines in the migration of neural progenitor during inflammation. In line with these findings, we recently reported that CCR2 deficiency, through genetic manipulation in mice, was sufficient to prevent the aberrant migration of new neurons observed *in vivo* following irradiation (Belarbi et al., 2013). Similarly, in the pilocarpine-induced status epilepticus rat model, the blockade of the MCP-1/CCR2 interaction with a selective CCR2 antagonist attenuated the ectopic migration of neuronal progenitors into the hilus (Hung et al., 2013). Collectively, these findings indicate that adult-born neurons have the capacity to migrate to the site of damage in response to the chemokine MCP-1/CCR2 signaling pathway. Currently, it is not known whether the change in migration induced by inflammation is beneficial, as, for example, increased migration would allow new neurons to replace dying or lost neurons, or deleterious, as altered migration could reflect the formation of aberrant circuits disrupting hippocampal functions.

RECRUITMENT OF ADULT-BORN NEURONS INTO BEHAVIORALLY RELEVANT NEURAL NETWORKS IN THE INFLAMED HIPPOCAMPUS

It is widely accepted that induction of effective synaptic plasticity associated with learning and memory requires *de novo* protein synthesis (Miyashita et al., 2008). The IEG *Arc* and its protein are dynamically regulated in response to neuronal activity, and are directly involved in plasticity processes that underlie memory consolidation (Guzowski et al., 2000). The expression of behaviorally induced Arc can be used to study the recruitment of adult-born mature neurons into functional neural networks. Using plasticity-related Arc expression, Ramirez-Amaya and coworkers demonstrated that the proportion of mature new neurons that expressed Arc in response to exploration was significantly higher than the proportion of cells that expressed Arc in the already existing population of granule cells. These data indicate that new neurons are preferentially recruited into hippocampal networks encoding spatial and contextual information (Ramirez-Amaya et al., 2006). In a rat model of LPS-induced chronic neuroinflammation 2-month-old neurons retained the capacity to express behaviorally induced Arc in response to spatial exploration. However, the proportion of new neurons that expressed behaviorally induced Arc was significantly lower than that from sham control animals, indicating that chronic inflammation decreased the recruitment of new neurons into hippocampal networks (Belarbi et al., 2012a). These findings are consistent with the work of Jakubs et al. (2008) that reported an increased inhibitory synaptic drive of new neurons that developed during LPS-induced neuroinflammation. Although adult-born neurons likely contribute to the encoding of recent spatial and contextual information, it is difficult to determine whether decreased excitability of new neurons is beneficial or deleterious to brain function during inflammatory conditions. Indeed, because neuroinflammation was shown to increase the proportion of granule cells expressing behaviorally induced Arc (Rosi et al., 2005), the decrease in new neurons expressing behaviorally induced Arc may be a compensatory mechanism to maintain an optimal level of neuronal activation and ensure the maintenance of pattern separation using a very sparse coding strategy (McNaughton et al., 1996; Rosi, 2011). Arc expression in new neurons as response to behavioral exploration was also reported in mice following exposure to low-dose irradiation combined or not with a subsequent traumatic brain injury in the presence of activated microglia (Rosi et al., 2012). Collectively, these findings show that while new neurons retain the capacity to be recruited into behaviorally relevant neural networks following brain injury, their recruitment is significantly decreased following classical microglia activation.

The chemokine receptor CX3CR1 is present in microglia and circulating monocytes and its unique ligand fractalkine (CX3CL1) is expressed in neurons and peripheral endothelia cells (Bazan et al., 1997; Mizoue et al., 1999). CX3CL1 signaling in the brain promotes microglial survival and controls microglial neurotoxicity through its receptor CX3CR1 under certain neurodegenerative and inflammatory conditions (Garcia et al., 2013). CX3CL1/CX3CR1 signaling is regulated in the inflamed brain, and CX3CR1 is a key regulator of microglia activation contributing to adaptive immune responses (Garcia et al., 2013). Recent evidence demonstrates that in the uninjured brain microglia play a critical role in monitoring and maintaining synapses by directly interacting with synaptic elements (Wake et al., 2009; Tremblay et al., 2010; Paolicelli et al., 2011). Using CX3CR1 knock-out mice, Paolicelli et al. (2011) reported a transient reduction in microglial numbers paralleled by a delay in synaptic pruning with consequent excess of dendritic spines and a delayed maturation of

excitatory transmission in the developing brain. These results, together with recent data (Rogers et al., 2011; Hoshiko et al., 2012) suggest that CX3CL1/CX3CR1 is an important neuron-microglia signaling pathway necessary for synaptic pruning and maturation (Paolicelli et al., 2011). In light of the role of CX3CL1/CX3CR1 signaling in synaptic maturation together with its involvement in inflammation, it is possible that in the inflamed hippocampus the alteration of this signaling pathway may lead to delayed maturation and/or integration of adult-born neurons. Further studies are needed to better understand how microglia may impact the maturation of adult-born neurons depending of their activation phenotype and the different signaling molecules.

PERSPECTIVES AND CONCLUDING REMARKS

Available data indicate that the generation, migration, and functional integration of adult-born neurons can be modulated in the inflamed hippocampus, and this modulation appears to differ depending on the activation phenotype of microglia and the specific factors that they release. It is now clear that the range of impact of microglia on adult-born neurons is wider than previously thought, as demonstrated by the anti-neurogenic and pro-neurogenic effects of opposite pro-inflammatory and anti-inflammatory polarized microglia. Previous strategies aimed to maintain functional neurogenesis have mainly focused on decreasing microglia activation. While recent data highlight the potential neuroprotective role of microglia following brain injury, it appears that transforming their phenotype toward alternative activation states could optimize the production, migration, and integration of neurons. Future studies are needed to: (i) characterize the phenotype of microglia activation and the microglia-released factors following brain injury, taking into account the nature of the injury and the timing following the injury; (ii) understand how specific microglia activation states and microglia-released factors impact functional neurogenesis, including migration and functional integration; (iii) identify ways to induce activation of microglia that would support functional neurogenesis in the injured brain. These steps are of critical importance to develop immune-mediated strategies to promote efficient adult-born neurons integration for the maintenance or improvement of hippocampus-dependent cognitive function.

ACKNOWLEDGMENTS

We would like to thank Professor John R. Fike for the editorial help with this manuscript. This work was supported by the NIH R01 CA133216 (Susanna Rosi) and the Alzheimer's Association IIRG-11-202064 (Susanna Rosi).

REFERENCES

Altman, J., and Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* 124, 319–335. doi: 10.1002/cne.901240303

Andersson, P. B., Perry, V. H., and Gordon, S. (1991). The kinetics and morphological characteristics of the macrophage-microglial response to kainic acid-induced neuronal degeneration. *Neuroscience* 42, 201–214. doi: 10.1016/0306-4522(91)90159-L

Annenkov, A. (2009). The insulin-like growth factor (IGF) receptor type 1 (IGF1R) as an essential component of the signalling network regulating neurogenesis. *Mol. Neurobiol.* 40, 195–215. doi: 10.1007/s12035-009-8081-0

Battista, D., Ferrari, C. C., Gage, F. H., and Pitossi, F. J. (2006). Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. *Eur. J. Neurosci.* 23, 83–93. doi: 10.1111/j.1460-9568.2005.04539.x

Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., et al. (1997). A new class of membrane-bound chemokine with a CX3C motif. *Nature* 385, 640–644. doi: 10.1038/385640a0

Belarbi, K., Arellano, C., Ferguson, R., Jopson, T., and Rosi, S. (2012a). Chronic neuroinflammation impacts the recruitment of adult-born neurons into behaviorally relevant hippocampal networks. *Brain Behav. Immun.* 26, 18–23. doi: 10.1016/j.bbi.2011.07.225

Belarbi, K., Jopson, T., Tweedie, D., Arellano, C., Luo, W., Greig, N. H., et al. (2012b). TNF-alpha protein synthesis inhibitor restores neuronal function and reverses cognitive deficits induced by chronic neuroinflammation. *J. Neuroinflammation* 9, 23. doi: 10.1186/1742-2094-9-23

Belarbi, K., Burnouf, S., Fernandez-Gomez, F. J., Laurent, C., Lestavel, S., Figeac, M., et al. (2011). Beneficial effects of exercise in a transgenic mouse model of Alzheimer's disease-like Tau pathology. *Neurobiol. Dis.* 43, 486–494. doi: 10.1016/j.nbd.2011.04.022

Belarbi, K., Jopson, T., Arellano, C., Fike, J. R., and Rosi, S. (2013). CCR2 deficiency prevents neuronal dysfunction and cognitive impairments induced by cranial irradiation. *Cancer Res.* 73, 1201–1210. doi: 10.1158/0008-5472.CAN-12-2989

Bellucci, A., Westwood, A. J., Ingram, E., Casamenti, F., Goedert, M., and Spillantini, M. G. (2004). Induction of inflammatory mediators and microglial activation in mice transgenic for mutant human P301S tau protein. *Am. J. Pathol.* 165, 1643–1652. doi: 10.1016/S0002-9440(10)63421-9

Belmadani, A., Tran, P. B., Ren, D., and Miller, R. J. (2006). Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. *J. Neurosci.* 26, 3182–3191. doi: 10.1523/JNEUROSCI.0156-06.2006

Benzing, W. C., Wujek, J. R., Ward, E. K., Shaffer, D., Ashe, K. H., Younkin, S. G., et al. (1999). Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. *Neurobiol. Aging* 20, 581–589. doi: 10.1016/S0197-4580(99)00065-2

Biebl, M., Cooper, C. M., Winkler, J., and Kuhn, H. G. (2000). Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci. Lett.* 291, 17–20. doi: 10.1016/S0304-3940(00)01368-9

Block, M. L., Zecca, L., and Hong, J. S. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat. Rev. Neurosci.* 8, 57–69. doi: 10.1038/nrn2038

Borges, K., Gearing, M., Mcdermott, D. L., Smith, A. B., Almonte, A. G., Wainer, B. H., et al. (2003). Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model. *Exp. Neurol.* 182, 21–34. doi: 10.1016/S0014-4886(03)00086-4

Bornemann, K. D., Wiederhold, K. H., Pauli, C., Ermini, F., Stalder, M., Schnell, L., et al. (2001). Abeta-induced inflammatory processes in microglia cells of APP23 transgenic mice. *Am. J. Pathol.* 158, 63–73. doi: 10.1016/S0002-9440(10)63945-4

Bruel-Jungerman, E., Davis, S., Rampon, C., and Laroche, S. (2006). Long-term potentiation enhances neurogenesis in the adult dentate gyrus. *J. Neurosci.* 26, 5888–5893. doi: 10.1523/JNEUROSCI.0782-06.2006

Butovsky, O., Talpalar, A. E., Ben-Yaakov, K., and Schwartz, M. (2005). Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. *Mol. Cell. Neurosci.* 29, 381–393. doi: 10.1016/j.mcn.2005.03.005

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A. E., Pluchino, S., et al. (2006). Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31, 149–160. doi: 10.1016/j.mcn.2005.10.006

Cacci, E., Claasen, J. H., and Kokaia, Z. (2005). Microglia-derived tumor necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells in vitro. *J.*

Neurosci. Res. 80, 789–797. doi: 10.1002/jnr.20531

Chirumamilla, S., Sun, D., Bullock, M. R., and Colello, R. J. (2002). Traumatic brain injury induced cell proliferation in the adult mammalian central nervous system. *J. Neurotrauma* 19, 693–703. doi: 10.1089/089771502601 39084

Cho, K. O., and Kim, S. Y. (2010). Effects of brain insults and pharmacological manipulations on the adult hippocampal neurogenesis. *Arch. Pharm. Res.* 33, 1475–1488. doi: 10.1007/s12272-010-1002-y

Choi, Y. S., Cho, H. Y., Hoyt, K. R., Naegele, J. R., and Obrietan, K. (2008). IGF-1 receptor-mediated ERK/MAPK signaling couples status epilepticus to progenitor cell proliferation in the subgranular layer of the dentate gyrus. *Glia* 56, 791–800. doi: 10.1002/glia.20653

Choi, Y. S., Cho, K. O., and Kim, S. Y. (2007). Asymmetry in enhanced neurogenesis in the rostral dentate gyrus following kainic acid-induced status epilepticus in adult rats. *Arch. Pharm. Res.* 30, 646–652. doi: 10.1007/BF02977661

Choi, Y. S., Lee, M. Y., Sung, K. W., Jeong, S. W., Choi, J. S., Park, H. J., et al. (2003). Regional differences in enhanced neurogenesis in the dentate gyrus of adult rats after transient forebrain ischemia. *Mol. Cells* 16, 232–238.

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D. Jr., Fragniere, A., Tyers, P., et al. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* 325, 210–213. doi: 10.1126/science.1173215

Colton, C. A. (2009). Heterogeneity of microglial activation in the innate immune response in the brain. *J. Neuroimmune Pharmacol.* 4, 399–418. doi: 10.1007/s11481-009-9164-4

Dash, P. K., Mach, S. A., and Moore, A. N. (2001). Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. *J. Neurosci. Res.* 63, 313–319. doi: 10.1002/1097-4547(20010215)63:4<313::AID-JNR1025>3.0.CO;2-4

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758. doi: 10.1038/nn1472

Ekdahl, C. T., Claasen, J. H., Bonde, S., Kokaia, Z., and Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13632–13637. doi: 10.1073/pnas.2234031100

Emery, D. L., Fulp, C. T., Saatman, K. E., Schutz, C., Neugebauer, E., and Mcintosh, T. K. (2005). Newly born granule cells in the dentate gyrus rapidly extend axons into the hippocampal CA3 region following experimental brain injury. *J. Neurotrauma* 22, 978–988. doi: 10.1089/neu.2005.22.978

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., et al. (1998). Neurogenesis in the adult human hippocampus. *Nat. Med.* 4, 1313–1317. doi: 10.1038/3305

Esposito, M. S., Piatti, V. C., Laplagne, D. A., Morgenstern, N. A., Ferrari, C. C., Pitossi, F. J., et al. (2005). Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J. Neurosci.* 25, 10074–10086. doi: 10.1523/JNEUROSCI.3114-05.2005

Frautschy, S. A., Yang, F., Irrizarry, M., Hyman, B., Saido, T. C., Hsiao, K., et al. (1998). Microglial response to amyloid plaques in APPsw transgenic mice. *Am. J. Pathol.* 152, 307–317.

Fujioka, H., and Akema, T. (2010). Lipopolysaccharide acutely inhibits proliferation of neural precursor cells in the dentate gyrus in adult rats. *Brain Res.* 1352, 35–42. doi: 10.1016/j.brainres.2010.07.032

Garcia, J. A., Pino, P. A., Mizutani, M., Cardona, S. M., Charo, I. F., Ransohoff, R. M., et al. (2013). Regulation of adaptive immunity by the fractalkine receptor during autoimmune inflammation. *J. Immunol.* 19, 1063–1072. doi: 10.4049/jimmunol.1300040

Ge, S., Yang, C. H., Hsu, K. S., Ming, G. L., and Song, H. (2007). A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* 54, 559–566. doi: 10.1016/j.neuron.2007.05.002

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845. doi: 10.1126/science.1194637

Goodman, T., Trouche, S., Massou, I., Verret, L., Zerwas, M., Roullet, P., et al. (2010). Young hippocampal neurons are critical for recent and remote spatial memory in adult mice. *Neuroscience* 171, 769–778. doi: 10.1016/j.neuroscience.2010.09.047

Gordon, M. N., Holcomb, L. A., Jantzen, P. T., Dicarlo, G., Wilcock, D., Boyett, K. W., et al. (2002). Time course of the development of Alzheimer-like pathology in the doubly transgenic PS1 + APP mouse. *Exp. Neurol.* 173, 183–195. doi: 10.1006/exnr.2001.7754

Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., et al. (2008). Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol. Psychiatry* 13, 717–728. doi: 10.1038/sj.mp.4002055

Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., Mcgaugh, J. L., Worley, P. F., et al. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J. Neurosci.* 20, 3993–4001.

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

Heldmann, U., Thored, P., Claasen, J. H., Arvidsson, A., Kokaia, Z., and Lindvall, O. (2005). TNF-alpha antibody infusion impairs survival of stroke-generated neuroblasts in adult rat brain. *Exp. Neurol.* 196, 204–208. doi: 10.1016/j.expneurol.2005.07.024

Hoehn, B. D., Palmer, T. D., and Steinberg, G. K. (2005). Neurogenesis in rats after focal cerebral ischemia is enhanced by indomethacin. *Stroke* 36, 2718–2724. doi: 10.1161/01.STR.0000190020.30282.cc

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012). Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J. Neurosci.* 32, 15106–15111. doi: 10.1523/JNEUROSCI.1167-12.2012

Hung, Y. W., Lai, M. T., Tseng, Y. J., Chou, C. C., and Lin, Y. Y. (2013). Monocyte chemoattractant protein-1 affects migration of hippocampal neural progenitors following status epilepticus in rats. *J. Neuroinflammation* 10, 11. doi: 10.1186/1742-2094-10-11

Iosif, R. E., Ekdahl, C. T., Ahlenius, H., Pronk, C. J., Bonde, S., Kokaia, Z., et al. (2006). Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. *J. Neurosci.* 26, 9703–9712. doi: 10.1523/JNEUROSCI.2723-06.2006

Jakubs, K., Bonde, S., Iosif, R. E., Ekdahl, C. T., Kokaia, Z., Kokaia, M., et al. (2008). Inflammation regulates functional integration of neurons born in adult brain. *J. Neurosci.* 28, 12477–12488. doi: 10.1523/JNEUROSCI.3240-08.2008

Jessberger, S., and Kempermann, G. (2003). Adult-born hippocampal neurons mature into activity-dependent responsiveness. *Eur. J. Neurosci.* 18, 2707–2712. doi: 10.1111/j.1460-9568.2003.02986.x

Jungenitz, T., Radic, T., Jedlicka, P., and Schwarzacher, S. W. (2013). High-frequency stimulation induces gradual immediate early gene expression in maturing adult-generated hippocampal granule cells. *Cereb. Cortex.* doi: 10.1093/cercor/bht035 [Epub ahead of print].

Jurgens, H. A., and Johnson, R. W. (2012). Dysregulated neuronal-microglial cross-talk during aging, stress and inflammation. *Exp. Neurol.* 233, 40–48. doi: 10.1016/j.expneurol.2010.11.014

Kee, N., Teixeira, C. M., Wang, A. H., and Frankland, P. W. (2007). Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat. Neurosci.* 10, 355–362. doi: 10.1038/nn1847

Kee, N. J., Preston, E., and Wojtowicz, J. M. (2001). Enhanced neurogenesis after transient global ischemia in the dentate gyrus of the rat. *Exp. Brain Res.* 136, 313–320. doi: 10.1007/s002210000591

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130, 391–399. doi: 10.1242/dev.00203

Kempermann, G., Kuhn, H. G., and Gage, F. H. (1997). Genetic influence on neurogenesis in the dentate gyrus of adult mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 10409–10414. doi: 10.1073/pnas.94.19.10409

Kernie, S. G., Erwin, T. M., and Parada, L. F. (2001). Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. *J. Neurosci. Res.* 66, 317–326. doi: 10.1002/jnr.10013

Kernie, S. G., and Parent, J. M. (2010). Forebrain neurogenesis after focal Ischemic and traumatic brain injury. *Neurobiol. Dis.* 37, 267–274. doi: 10.1016/j.nbd.2009.11.002

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553. doi: 10.1152/physrev.00011.2010

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., et al. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and *Irf*8-dependent pathways. *Nat. Neurosci.* 16, 273–280. doi: 10.1038/nn.3318

Kiyota, T., Ingraham, K. L., Swan, R. J., Jacobsen, M. T., Andrews, S. J., and Ikezu, T. (2012). AAV serotype 2/1-mediated gene delivery of anti-inflammatory interleukin-10 enhances neurogenesis and cognitive function in APP + PS1 mice. *Gene Ther.* 19, 724–733. doi: 10.1038/gt.2011.126

Kiyota, T., Okuyama, S., Swan, R. J., Jacobsen, M. T., Gendelman, H. E., and Ikezu, T. (2010). CNS expression of anti-inflammatory cytokine interleukin-4 attenuates Alzheimer's disease-like pathogenesis in APP + PS1 bigenic mice. *FASEB J.* 24, 3093–3102. doi: 10.1096/fj.10-155317

Kohman, R. A., and Rhodes, J. S. (2013). Neurogenesis, inflammation and behavior. *Brain Behav. Immun.* 27, 22–32. doi: 10.1016/j.bbi.2012.09.003

Koo, J. W., and Duman, R. S. (2008). IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc. Natl. Acad. Sci. U.S.A.* 105, 751–756. doi: 10.1073/pnas.0708092105

Kuzumaki, N., Ikegami, D., Imai, S., Narita, M., Tamura, R., Yajima, M., et al. (2010). Enhanced IL-1beta production in response to the activation of hippocampal glial cells impairs neurogenesis in aged mice. *Synapse* 64, 721–728. doi: 10.1002/syn.20800

Lalancette-Hebert, M., Gowing, G., Simard, A., Weng, Y. C., and Kriz, J. (2007). Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. *J. Neurosci.* 27, 2596–2605.

Laplagne, D. A., Kamienkowski, J. E., Esposito, M. S., Piatti, V. C., Zhao, C., Gage, F. H., et al. (2007). Similar GABAergic inputs in dentate granule cells born during embryonic and adult neurogenesis. *Eur. J. Neurosci.* 25, 2973–2981. doi: 10.1111/j.1460-9568.2007.05549.x

Lawson, L. J., Perry, V. H., Dri, P., and Gordon, S. (1990). 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

Lehnardt, S., Massillon, L., Follett, P., Jensen, F. E., Ratan, R., Rosenberg, P. A., et al. (2003). Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8514–8519. doi: 10.1073/pnas.1432609100

Liu, J., Solway, K., Messing, R. O., and Sharp, F. R. (1998). Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. *J. Neurosci.* 18, 7768–7778.

Liu, Z., Fan, Y., Won, S. J., Neumann, M., Hu, D., Zhou, L., et al. (2007). Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. *Stroke* 38, 146–152. doi: 10.1161/01.STR.0000251791.64910.cd

London, A., Cohen, M., and Schwartz, M. (2013). Microglia and monocyte-derived macrophages: functionally distinct populations that act in concert in CNS plasticity and repair. *Front. Cell. Neurosci.* 7:34. doi: 10.3389/fncel.2013.00034

Markakis, E. A., and Gage, F. H. (1999). Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *J. Comp. Neurol.* 406, 449–460. doi: 10.1002/(SICI)1096-9861(19990419)406:4<449::AID-CNE3>3.0.CO;2-I

Mathieu, P., Piantanida, A. P., and Pitossi, F. (2010). Chronic expression of transforming growth factor-beta enhances adult neurogenesis. *Neuroimmunomodulation* 17, 200–201. doi: 10.1159/000258723

Matsuoka, Y., Picciano, M., Malester, B., Lafrancois, J., Zehr, C., Daeschner, J. M., et al. (2001). Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease. *Am. J. Pathol.* 158, 1345–1354. doi: 10.1016/S0002-9440(10)64085-0

McNaughton, B. L., Barnes, C. A., Gerrard, J. L., Gothard, K., Jung, M. W., Knierim, J. J., et al. (1996). Deciphering the hippocampal polyglot: the hippocampus as a path integration system. *J. Exp. Biol.* 199, 173–185.

Meda, L., Bernasconi, S., Bonaiuto, C., Sozzani, S., Zhou, D., Otvos, L. Jr., et al. (1996). Beta-amyloid (25–35) peptide and IFN-gamma synergistically induce the production of the chemotactic cytokine MCP-1/JE in monocytes and microglial cells. *J. Immunol.* 157, 1213–1218.

Michelucci, A., Heurtaux, T., Grandbarbe, L., Morga, E., and Heuschling, P. (2009). 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

Miyashita, T., Kubik, S., Lewandowski, G., and Guzowski, J. F. (2008). Networks of neurons, networks of genes: an integrated view of memory consolidation. *Neurobiol. Learn. Mem.* 89, 269–284. doi: 10.1016/j.nlm.2007.08.012

Mizoue, L. S., Bazan, J. F., Johnson, E. C., and Handel, T. M. (1999). Solution structure and dynamics of the CX3C chemokine domain of fractalkine and its interaction with an N-terminal fragment of CX3CR1. *Biochemistry* 38, 1402–1414. doi: 10.1021/bi9820614

Mizumatsu, S., Monje, M. L., Morhardt, D. R., Rola, R., Palmer, T. D., and Fike, J. R. (2003). Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. *Cancer Res.* 63, 4021–4027.

Monje, M. L., Mizumatsu, S., Fike, J. R., and Palmer, T. D. (2002). Irradiation induces neural precursor-cell dysfunction. *Nat. Med.* 8, 955–962. doi: 10.1038/nm749

Monje, M. L., Toda, H., and Palmer, T. D. (2003). Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 302, 1760–1765. doi: 10.1126/science.1088417

Mu, Y., and Gage, F. H. (2011). Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol. Neurodegener.* 6, 85. doi: 10.1186/1750-1326-6-85

Nakashiba, T., Cushman, J. D., Pelkey, K. A., Renaudineau, S., Buhl, D. L., Mchugh, T. J., et al. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell* 149, 188–201. doi: 10.1016/j.cell.2012.01.046

Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., et al. (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* 110, 429–441. doi: 10.1016/S0092-8674(02)00862-0

Neumann, J., Gunzer, M., Gutzeit, H. O., Ullrich, O., Reymann, K. G., and Dinkel, K. (2006). Microglia provide neuroprotection after ischemia. *FASEB J.* 20, 714–716. doi: 10.1096/fj.05-4882fje

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

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458. doi: 10.1126/science.1202529

Parent, J. M., Elliott, R. C., Pleasure, S. J., Barbaro, N. M., and Lowenstein, D. H. (2006). Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. *Ann. Neurol.* 59, 81–91. doi: 10.1002/ana.20699

Parent, J. M., Yu, T. W., Leibowitz, R. T., Geschwind, D. H., Sloviter, R. S., and Lowenstein, D. H. (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* 17, 3727–3738.

Piao, C. S., Stoica, B. A., Wu, J., Sabirzhanov, B., Zhao, Z., Cabatbat, R., et al. (2013). Late exercise reduces neuroinflammation and cognitive dysfunction after traumatic brain injury. *Neurobiol. Dis.* 54, 252–263. doi: 10.1016/j.nbd.2012.12.017

Porta, C., Rimoldi, M., Raes, G., Brys, L., Ghezzi, P., Di Liberto, D., et al. (2009). Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14978–14983. doi: 10.1073/pnas.0809784106

Prinz, M., and Priller, J. (2010). Tickets to the brain: role of CCR2 and CX3CR1 in myeloid cell entry in the CNS. *J. Neuroimmunol.* 224, 80–84. doi: 10.1016/j.jneuroim.2010.05.015

Qin, L., Liu, Y., Hong, J. S., and Crews, F. T. (2013). NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic neurodegeneration following systemic LPS administration. *Glia* 61, 855–868. doi: 10.1002/glia.22479

Raber, J., Fan, Y., Matsumori, Y., Liu, Z., Weinstein, P. R., Fike, J. R., et al. (2004a). Irradiation attenuates neurogenesis and exacerbates ischemia-induced deficits. *Ann. Neurol.* 55, 381–389. doi: 10.1002/ana.10853

Raber, J., Rola, R., Lefevour, A., Morhardt, D., Curley, J., Mizumatsu,

S., et al. (2004b). Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat. Res.* 162, 39–47. doi: 10.1667/RR3206

Ramirez-Amaya, V., Marrone, D. F., Gage, F. H., Worley, P. F., and Barnes, C. A. (2006). Integration of new neurons into functional neural networks. *J. Neurosci.* 26, 12237–12241. doi: 10.1523/JNEUROSCI.2195-06.2006

Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., et al. (2011). CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. *J. Neurosci.* 31, 16241–16250. doi: 10.1523/JNEUROSCI.3667-11.2011

Rola, R., Fishman, K., Baure, J., Rosi, S., Lamborn, K. R., Obenaus, A., et al. (2008). Hippocampal neurogenesis and neuroinflammation after cranial irradiation with (56)Fe particles. *Radiat. Res.* 169, 626–632. doi: 10.1667/RR1263.1

Rosi, S. (2011). Neuroinflammation and the plasticity-related immediate-early gene Arc. *Brain Behav. Immun.* 25(Suppl. 1), S39–S49. doi: 10.1016/j.bbi.2011.02.003

Rosi, S., Andres-Mach, M., Fishman, K. M., Levy, W., Ferguson, R. A., and Fike, J. R. (2008). Cranial irradiation alters the behaviorally induced immediate-early gene arc (activity-regulated cytoskeleton-associated protein). *Cancer Res.* 68, 9763–9770. doi: 10.1158/0008-5472.CAN-08-1861

Rosi, S., Belarbi, K., Ferguson, R. A., Fishman, K., Obenaus, A., Raber, J., et al. (2012). Trauma-induced alterations in cognition and Arc expression are reduced by previous exposure to 56Fe irradiation. *Hippocampus* 22, 544–554. doi: 10.1002/hipo.20920

Rosi, S., Ramirez-Amaya, V., Vazdarjanova, A., Worley, P. F., Barnes, C. A., and Wenk, G. L. (2005). Neuroinflammation alters the hippocampal pattern of behaviorally induced Arc expression. *J. Neurosci.* 25, 723–731. doi: 10.1523/JNEUROSCI.4469-04.2005

Sandoval, C. J., Martinez-Claros, M., Bello-Medina, P. C., Perez, O., and Ramirez-Amaya, V. (2011). When are new hippocampal neurons, born in the adult brain, integrated into the network that processes spatial information? *PLoS ONE* 6:e17689. doi: 10.1371/journal.pone.0017689

Sasaki, A., Kawarabayashi, T., Murakami, T., Matsubara, E., Ikeda, M., Hagiwara, H., et al. (2008). Microglial activation in brain lesions with tau deposits: comparison of human tauopathies and tau transgenic mice TgTauP301L. *Brain Res.* 1214, 159–168. doi: 10.1016/j.brainres.2008.02.084

Sasaki, A., Shoji, M., Harigaya, Y., Kawarabayashi, T., Ikeda, M., Naito, M., et al. (2002). Amyloid cored plaques in Tg2576 transgenic mice are characterized by giant plaques, slightly activated microglia, and the lack of paired helical filament-typed, dystrophic neurites. *Virchows Arch.* 441, 358–367. doi: 10.1007/s00428-002-0643-8

Schilling, M., Besselmann, M., Muller, M., Strecker, J. K., Ringelstein, E. B., and Kiefer, R. (2005). Predominant phagocytic activity of resident microglia over hematogenous macrophages following transient focal cerebral ischemia: an investigation using green fluorescent protein transgenic bone marrow chimeric mice. *Exp. Neurol.* 196, 290–297. doi: 10.1016/j.expneurol.2005.08.004

Schmidt-Hieber, C., Jonas, P., and Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* 429, 184–187. doi: 10.1038/nature02553

Schwartz, M., and Shechter, R. (2010). Systemic inflammatory cells fight off neurodegenerative disease. *Nat. Rev. Neurol.* 6, 405–410. doi: 10.1038/nrneurol.2010.71

Seki, T., Namba, T., Mochizuki, H., and Onodera, M. (2007). Clustering, migration, and neurite formation of neural precursor cells in the adult rat hippocampus. *J. Comp. Neurol.* 502, 275–290. doi: 10.1002/cne.21301

Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., et al. (2013). Dynamics of hippocampal neurogenesis in adult humans. *Cell* 153, 1219–1227. doi: 10.1016/j.cell.2013.05.002

Stalder, M., Phinney, A., Probst, A., Sommer, B., Staufenbiel, M., and Jucker, M. (1999). Association of microglia with amyloid plaques in brains of APP23 transgenic mice. *Am. J. Pathol.* 154, 1673–1684. doi: 10.1016/S0002-9440(10)65423-5

Sun, D., Mcginn, M. J., Zhou, Z., Harvey, H. B., Bullock, M. R., and Colello, R. J. (2007). Anatomical integration of newly generated dentate granule neurons following traumatic brain injury in adult rats and its association to cognitive recovery. *Exp. Neurol.* 204, 264–272. doi: 10.1016/j.expneurol.2006.11.005

Tada, E., Parent, J. M., Lowenstein, D. H., and Fike, J. R. (2000). X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. *Neuroscience* 99, 33–41. doi: 10.1016/S0306-4522(00)00151-2

Thored, P., Heldmann, U., Gomes-Leal, W., Gisler, R., Darsalia, V., Taneera, J., et al. (2009). Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* 57, 835–849. doi: 10.1002/glia.20810

Toni, N., Laplagne, D. A., Zhao, C., Lombardi, G., Ribak, C. E., Gage, F. H., et al. (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nat. Neurosci.* 11, 901–907. doi: 10.1038/nn.2156

Tran, P. B., Banisadr, G., Ren, D., Chenn, A., and Miller, R. J. (2007). Chemokine receptor expression by neural progenitor cells in neurogenic regions of mouse brain. *J. Comp. Neurol.* 500, 1007–1033. doi: 10.1002/cne.21229

Tremblay, M. E., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Trouche, S., Bontempi, B., Roullet, P., and Rampon, C. (2009). Recruitment of adult-generated neurons into functional hippocampal networks contributes to updating and strengthening of spatial memory. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5919–5924. doi: 10.1073/pnas.0811054106

Turrin, N. P., and Rivest, S. (2004). Innate immune reaction in response to seizures: implications for the neuropathology associated with epilepsy. *Neurobiol. Dis.* 16, 321–334. doi: 10.1016/j.nbd.2004.03.010

Vallieres, L., Campbell, I. L., Gage, F. H., and Sawchenko, P. E. (2002). Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J. Neurosci.* 22, 486–492.

Vezzani, A., Ravizza, T., Balosso, S., and Aronica, E. (2008). Glia as a source of cytokines: implications for neuronal excitability and survival. *Epilepsia* 49(Suppl. 2), 24–32. doi: 10.1111/j.1528-1167.2008.01490.x

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). 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

Wu, M. D., Hein, A. M., Moravan, M. J., Shaftel, S. S., Olschowka, J. A., and O'Banion, M. K. (2012). Adult murine hippocampal neurogenesis is inhibited by sustained IL-1beta and not rescued by voluntary running. *Brain Behav. Immun.* 26, 292–300. doi: 10.1016/j.bbi.2011.09.012

Yagita, Y., Kitagawa, K., Ohtsuki, T., Takasawa, K., Miyata, T., Okano, H., et al. (2001). Neurogenesis by progenitor cells in the ischemic adult rat hippocampus. *Stroke* 32, 1890–1896. doi: 10.1161/01.STR.32.8.1890

Yi, M. H., Zhang, E., Kang, J. W., Shin, Y. N., Byun, J. Y., Oh, S. H., et al. (2012). Expression of CD200 in alternative activation of microglia following an excitotoxic lesion in the mouse hippocampus. *Brain Res.* 1481, 90–96. doi: 10.1016/j.brainres.2012.08.053

Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S. M., Iwata, N., Saido, T. C., et al. (2007). Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* 53, 337–351. doi: 10.1016/j.neuron.2007.01.010

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 April 2013; accepted: 21 August 2013; published online: 06 September 2013.

*Citation: Belarbi K and Rosi S (2013) Modulation of adult-born neurons in the inflamed hippocampus. Front. Cell. Neurosci. **7**:145. doi: 10.3389/fncel.2013.00145*

This article was submitted to the journal Frontiers in Cellular Neuroscience.

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 13 March 2013
doi: 10.3389/fncel.2013.00022

Microglial aging in the healthy CNS: phenotypes, drivers, and rejuvenation

Wai T. Wong*

Unit on Neuron-Glia Interactions in Retinal Disease, National Eye Institute, National Institutes of Health, Bethesda, MD, USA

Edited by:
Hiroaki Wake, National Institute for Basic Biology, Japan

Reviewed by:
Rafael Linden, Federal University of Rio de Janeiro, Brazil
Uwe-Karsten Hanisch, Univesrity of Göttingen, Germany
Gaylia J. Harry, National Institute of Environmental Health Sciences, USA

***Correspondence:**
Wai T. Wong, Unit on Neuron-Glia Interactions in Retinal Disease, National Eye Institute, National Institutes of Health, 6 Center Drive, 6/217, Bethesda, MD 20892, USA.
e-mail: wongw@nei.nih.gov

Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and age-related macular degeneration (AMD), share two characteristics in common: (1) a disease prevalence that increases markedly with advancing age, and (2) neuroinflammatory changes in which microglia, the primary resident immune cell of the CNS, feature prominently. These characteristics have led to the hypothesis that pathogenic mechanisms underlying age-related neurodegenerative disease involve aging changes in microglia. If correct, targeting features of microglial senescence may constitute a feasible therapeutic strategy. This review explores this hypothesis and its implications by considering the current knowledge on how microglia undergo change during aging and how the emergence of these aging phenotypes relate to significant alterations in microglial function. Evidence and theories on cellular mechanisms implicated in driving senescence in microglia are reviewed, as are "rejuvenative" measures and strategies that aim to reverse or ameliorate the aging microglial phenotype. Understanding and controlling microglial aging may represent an opportunity for elucidating disease mechanisms and for formulating novel therapies.

Keywords: microglia, aging, priming, neurodegeneration, rejuvenation, inflammation, activation, senescence

INTRODUCTION

As the predominant resident immune cell in the central nervous system (CNS), microglia has been causally linked to the development of neurodegenerative diseases in which neuroinflammation plays a prominent role. As the prevalence of such diseases, which include Alzheimer's disease, Parkinson's disease, and age-related macular degeneration (AMD), demonstrates a strong association with increasing age, aging changes in microglia have been hypothesized to play a prominent role in disease pathogenesis. Over the last few years, new and exciting discoveries with respect to microglial physiology and function have provided key insights that provide a conceptual framework for thinking about the connection between microglial aging and neurodegenerative disease. First, we have learned from studies employing parabiosis to create chimerism in circulating bone-marrow derived precursors that microglia are long-lived cells in the healthy CNS, with residence times that extend across much of an animal's life-span, and are therefore susceptible to aging factors *in situ* (Ajami et al., 2007; Mildner et al., 2007). Second, a critical mass of studies focused on characterizing microglia in the aged CNS have elucidated a combination of anatomical, morphological, physiological, and molecular changes exhibited by aging microglia that now provides a comprehensive depiction of the senescent microglial phenotype. Third, a combination of detailed ultrastructure studies and live-cell imaging techniques has provided novel insights into the constitutive cellular interactions conducted by microglia through which the everyday functional roles of microglia can be discerned. These data allow us to conceptually connect the features of the aging microglial phenotype with significant and consequential alterations in microglial function in a way that is helpful for thinking about mechanisms of disease pathogenesis and for formulating strategies of disease prevention and treatment.

This review aims to summarize what is currently known about: (1) the nature of the aging microglial phenotype, (2) how microglial aging can impact the function of microglia in their everyday roles, as well as in their response to perturbations, (3) what mechanisms underlie the generation of the aging microglial phenotype, and (4) whether aging changes in microglia may be successfully reversed (i.e., if aged microglia may be therapeutically rejuvenated).

THE AGING PHENOTYPE OF MICROGLIA

AGING CHANGES IN MICROGLIAL DENSITY, DISTRIBUTION, AND RAMIFIED MORPHOLOGY

How do microglia in the uninjured and healthy CNS change with aging? Microglia in the healthy young CNS have a typical ramified morphology and are distributed throughout the neural parenchyma in a "space-filling" manner, providing efficient spatial coverage of the entire CNS milieu. This orderly arrangement of ramified microglia reflects the presence of organizing factors whose identity, mechanism, and purpose are still rather mysterious. Interestingly, as the CNS ages, the fidelity of these regulatory factors appears to diminish as the numbers, distribution, and morphological features of microglia progressively changes with time, reflecting aging-sensitive alterations in microglia function.

The total number and density of microglia have been noted to increase significantly with age in various CNS compartments, including the hippocampus (Mouton et al., 2002), visual and auditory cortices (Tremblay et al., 2012), and the retina (Damani et al., 2011). These increases may be contributed by a low rate

of basal microglia proliferation (Lawson et al., 1992; Ajami et al., 2007) or otherwise a slow incremental recruitment of monocytes or macrophages from the periphery. The significance of increasing microglial numbers is unclear—one hypothesis suggests that it may be a compensatory mechanism to maintain overall function as each individual aged microglial cell declines in function with aging (Streit and Xue, 2010). The increase may also be driven by a cumulative history of environmental influences (e.g., infections, injuries, inflammatory insults) that have triggered episodes of microglial proliferation that did not fully revert back to the basal level (Ajami et al., 2007). Currently, functional significance of increased microglial numbers remains unknown.

With aging, the order and regularity of the mosaic distribution of microglia in the CNS also appears to deteriorate. Microglia in the aged mouse cortex are distributed less evenly than in the young cortex; neighboring cells occasionally have somata that are closely juxtaposed, instead of being evenly spaced and clearly separated (Tremblay et al., 2012). In the healthy young retina, microglia have an unique distribution as horizontally tiled arrays that are limited only to the laminated inner layers of retina, with no microglia found in the outer retinal layers (Santos et al., 2008). With aging, this distribution pattern breaks down as microglia translocate into the formerly "microglial-free" zone of the outer retina and accumulate in the subretinal space (Xu et al., 2008; Damani et al., 2011). The consequences of aging-related changes in microglial distribution are not generally obvious; however in the retina, this redistribution brings microglia into novel contact with photoreceptors and retinal pigment epithelial (RPE) cells, initiating immune changes in those cells that recapitulate pathologic phenotypes in AMD (Ma et al., 2009, 2012). Finding out what drives age-related microglia redistribution may be therapeutically relevant to AMD treatment and prevention (Karlstetter et al., 2010; Buschini et al., 2011). These studies comparing the distribution of microglia in young and aged animals rely on the ability of microglial markers such as Iba1 to consistently reveal microglia across different ages. Although it is theoretically possible that unknown subsets of microglia may change their expression of these markers with aging to elude detection and confound the results described, the stability of Iba1 expression across aging (unpublished data) and different activation states, makes this unlikely.

In addition, microglia undergo changes in their ramified morphology with aging. In the mouse retina, aged microglia demonstrate smaller dendritic arbors relative to young microglia which are less branched and are have smaller total process lengths (Damani et al., 2011). In the aging mouse cortex and hippocampus, microglial dendritic arbors are similarly smaller, more variable in size, less circularly symmetric, and more elongated in shape (Sierra et al., 2007; Tremblay et al., 2012). In aged human (but not in rodent) brains, sporadic microglial cells have been observed to exhibit dystrophic morphologies in which dendritic arbors appear deramified, with residual processes showing increased tortuosity, cytoplasmic beading, and fragmentation, reflective of ongoing cytorrhexis (Streit et al., 2004). As microglial processes are constitutively dynamic structures whose contact with surrounding cells mediate a host of functions including immune surveillance (Nimmerjahn et al., 2005), synaptic regulation (Wake et al., 2009; Tremblay et al., 2010; Schafer et al., 2012), regulation of neuronal activity and neurogenesis (Nakanishi and Wu, 2009; Nakanishi et al., 2011), these age-related reductions in the structure of ramified processes are likely to be functionally influential and indeed detrimental to the efficiency of these functions. A summary of changes in microglial distribution, morphology, and behavior associated with aging is provided in **Table 1**.

AGING CHANGES IN BASAL STATES OF MICROGLIAL ACTIVATION

In addition to changes in anatomy, microglia demonstrates aging changes in their immune physiology. Multiple lines of evidence have indicated that microglia in the aged CNS show increased basal states of activation. In histopathology studies of the aging human brain, microglial morphologies exhibit a perinuclear cytoplasm hypertrophy and retracted processes, reminiscent of activated microglia (Sheng et al., 1998; Miller and Streit, 2007). Aged microglial immunophenotypes also resemble those of activated microglia, with increased expression of major histocompatibility complex II (MHCII) and CD11b (Rogers et al., 1988; Perry et al., 1993; Frank et al., 2006; Ziv et al., 2006). Molecular markers

Table 1 | Summary of changes in microglial distribution, morphology, and behavior associated with aging.

Phenotypes
CHANGES IN MICROGLIAL DISTRIBUTION
Increase in microglial numbers/density in neural parenchyma
Decrease in regularity in distribution
Translocation into areas not previously occupied by microglia (e.g., to the outer layers of the retina)
CHANGES IN MICROGLIAL MORPHOLOGY
Decrease in individual microglial ramification (dendritic arbor area, branching, and total process length)
Appearance of morphological changes suggestive of increased activation state (e.g., perinuclear cytoplasmic hypertrophy, retraction of processes)
Sporadic appearance of dystrophic microglia in aged human brains
CHANGES IN MICROGLIAL DYNAMIC BEHAVIOR
Decrease in rate of process movement
Decrease in rate of migration to focal tissue injury

typically found to be up-regulated in activated microglia activation (e.g., Iba1, OX6) are also increased in aged microglia in the absence of injury or disease (Ogura et al., 1994; Frank et al., 2006). In healthy aged humans, *in vivo* positron emission tomography (PET) using R-[^{11}C]PK11195 revealed increased ligand binding in several cortical and subcortical areas, indicating increased levels of basal microglial activation (Schuitemaker et al., 2010).

Consistent with these markers of increased activation, aged microglia are found to express increased levels of effector molecules associated with activated microglia. Increased expression of inflammatory cytokines (such as IL1β, TNF-α, IL6) are detected in aged microglia studied *in situ* (Sheng et al., 1998), isolated *ex vivo* (Sierra et al., 2007; Njie et al., 2012), or when cultured *in vitro* (Ye and Johnson, 1999). Interestingly, studies examining the levels of anti-inflammatory cytokines such as IL10 are less uniform, with differing associations with aging described in different studies (Ye and Johnson, 2001; Sierra et al., 2007). On the whole, there is consensus on the increased activated basal state of microglia in healthy aging, indicating that aged microglia may contribute to the graded chronic states of "para-inflammation"(Medzhitov, 2008) that is associated with the increased susceptibility of the aged CNS to neurodegenerative diseases in which chronic neuroinflammation feature.

INFLUENCE OF AGING ON EVERYDAY FUNCTIONS OF MICROGLIA

AGING MICROGLIA AND NEUROTROPHIC FUNCTIONS

Given the above descriptions for how microglia change in their structural and basal activation state with aging, how can we connect these aging phenotypes to potential changes in "everyday" functions of microglia? Concepts concerning the constitutive functions of microglia in a healthy CNS have been changing and developing in an exciting way over the last few years as key discoveries reveal new functional roles. A number of metaphors have arisen in the literature concerning these roles: microglia, for their role in dynamic immune surveillance, have been likened to "cops on the beat" (Raivich, 2005). For their role in constitutive pruning and maintenance of synapses, they have been named as "constant gardeners" (Hughes, 2012). Microglia also appear to maintain a conducive environment in the CNS for the healthy function and survival of neurons, prompting the description of "industrious housekeepers" (Streit and Xue, 2009). With regards to the latter function, the presence of ramified microglia have been associated with the provision of trophic support for neurons; neuronal cell death in injury models increases with maneuvers that result in the depletion of microglia (Montero et al., 2009; Vinet et al., 2012). Conversely, repletion of microglia or introduction of exogenous microglia result in neuronal rescue in injury models (Kitamura et al., 2004; Imai et al., 2007), driven possibly by microglial production of trophic influences such as growth factors (Imai et al., 2007; Boscia et al., 2009), adenosine (Lauro et al., 2010), TNF-α (Lambertsen et al., 2009), or via the clearance of deleterious byproducts of neurotransmission (e.g., glutamate) (Persson et al., 2005; Persson and Ronnback, 2012) and metabolism (e.g., iron and excess oligodendroglial membrane) (Fitzner et al., 2011; Ward et al., 2011). As such, the senescent debilitation of microglia, suggested by the emergence of dystrophic, fragmented microglia in aged human brains, have been associated with a decline of neuroprotective function, and with it, an increased vulnerability of the CNS. Indeed, the so-called "microglial dysfunction hypothesis" (Streit and Xue, 2009) articulates that the age-related susceptibility to neurodegenerative disease in human is causally connected to the age-related microglial deficiency in neuroprotective function. In support of this view, dystrophic microglia have been co-localized with areas of neurodegeneration and tau pathology in Alzheimer's brains (Streit et al., 2009), indicating that neurodegeneration in these examples follow from the failure of local support from senescent microglia.

AGING MICROGLIA AND MAINTENANCE OF NEURONAL ACTIVITY AND SYNAPSES

As the "constant gardener" of synapses, microglia in recent studies have been found to constitutively regulate synaptic structure and neuronal activity. The constant dynamic behavior of microglial processes (Davalos et al., 2005; Nimmerjahn et al., 2005) appears not to be a random, cell-autonomous feature but are instead have their overall rate, extent, and direction regulated and guided by neuronal activity (Fontainhas et al., 2011; Li et al., 2012). These dynamic processes are observed to make repeated contact with synaptic structures and influence synaptic stability and pruning in an activity-dependent manner (Wake et al., 2009; Tremblay et al., 2010; Paolicelli et al., 2011; Schafer et al., 2012), potentially altering overall activity patterns in neuronal networks (Pascual et al., 2012). Microglia are capable of modulating the activity of neurons by directly contacting neuronal somata with their processes, down-regulating the activity of contacted neurons (Li et al., 2012). Taken together, these forms of regulation seem to constitute a homeostatic mechanism regulating overall levels of activity in the CNS; neurons with greater levels of activity release the attractant ATP in an activity-dependent manner via probenecid-sensitive pannexin-1 channels (Fontainhas et al., 2011; Li et al., 2012), inducing microglia to migrate and polarize their processes toward them, and which are then reduced in their activity following microglial contact.

Is this "gardening" function of microglia influenced by aging? In our work, we have observed that aged microglia interestingly decline in their dynamic motility of their processes and in their migration rates through neural parenchyma (Damani et al., 2011). These decrements may translate into less frequent and extensive synaptic and neuronal contacts and prolonged microglia response times. These may potentially be related to (1) age-related changes in the structure and electrotonic properties of dendrites and dendritic spines (Duan et al., 2003; Kabaso et al., 2009; Morrison and Baxter, 2012) and (2) a decreased homeostatic regulation of neuronal activity. Also, we had found that microglia migration to sites of neural injury is slower to initiate in aged animals—but once accomplished, microglial aggregation tends to be more sustained and less reversible. Dysregulated fluxes of microglia can therefore result in more sustained imbalances in activity homeostasis, and possibly contribute to differential responses to excitotoxic damage observed in young versus aged brains (Campuzano et al., 2008).

AGING MICROGLIA AND ADULT NEUROGENESIS

An additional constitutive role that microglia play involves the regulation of adult neurogenesis. It is known that adult neurogenesis diminishes with aging, driven perhaps by a declining pool of the neural stem cells (Medrano et al., 2009; Encinas and Sierra, 2012), or a cell-autonomous loss of proliferative capacity in precursor cells (Kuhn et al., 1996). However, as microglia are capable of influencing neurogenesis *in vitro* studies (Butovsky et al., 2006; Walton et al., 2006; Cacci et al., 2008) and are associated with altered neurogenesis *in vivo* studies (Ekdahl et al., 2003; Monje et al., 2003), the contribution of aging microglia to this decrease has been examined. In one study in which neurosphere formation from dissociated hippocampus tissue was used as a measure of neuronal precursor cell (NPC) activity, lower levels of neurogenesis was found in 9-month-old mouse hippocampal tissue relative to 2-month-old tissue (Vukovic et al., 2012). While depletion of microglia from hippocampal tissue did not alter neurogenesis at 2 months, it resulted in increased neurogenesis at 9 months, indicating that microglia in the aged hippocampus, but not in the young hippocampus, contribute to an age-related suppression of NPC activity.

What controls the ability of microglia to influence neurogenesis? The ability of aged microglial to limit neurogenesis has been related to the increasing pro-inflammatory basal state of aging microglia, in which pro-inflammatory mediators produced by aged microglia can inhibit neurogenesis (Ekdahl et al., 2003; McPherson et al., 2011). The connection between microglia and neurogenesis is, however, complicated by findings that different subtypes of microglia in the CNS may affect neurogenesis differentially. Depletion of all microglia from dissociated hippocampi of young, exercising mice reduces neurosphere formation *in vitro*, suggesting that the entire population of microglia post-exercise exerts a net positive contribution to neurogenesis (Vukovic et al., 2012). However, depletion of only the $Csf1r^{+}MHCII^{+}$ subpopulation of microglia conversely increased neurosphere formation, indicating that this subset may instead hold back neurogenesis. These results present the possibility that subpopulations of pro- and anti-neurogenesis microglia change in their representation as a function of aging and may differentially regulate neurogenesis in different brain areas. Also, microglial effects on neurogenesis may also be modulated by signals in the aging brain environment. CX3CL1-CX3CR1 signaling in particular appears influential in conferring on microglia their pro-neurogenesis effects (Bachstetter et al., 2011). CX3CL1 levels in the aged hippocampus were found to decline in concert with decreasing rates of neurogenesis. Revealingly, exogenous CX3CL1 application was found to increase neurogenesis, and is effective only in the presence of microglia (Bachstetter et al., 2011; Vukovic et al., 2012).

The types of communication occurring between microglia and neural precursor cells (NPC) are not fully understood. Microglia can influence NPCs via secreted factors that direct their migration and differentiation (Aarum et al., 2003; Walton et al., 2006). These may occur either by direct signaling or by modifying the microenvironment of the neurogenic niche (Battista et al., 2006). Alternatively, microglia can physically interact with neural precursors by direct contact, influencing neurogenesis by phagocytosis of apoptotic newborn neurons (Sierra et al., 2010). However, this form of microglial phagocytosis appears unaffected by aging or by microglial activation, further reflecting a nuanced relationship between microglial function and neurogenesis.

INFLUENCE OF AGING ON MICROGLIA RESPONSES TO PERTURBATIONS

In the above sections, we have largely considered how the aging phenotype in microglia influences their functions in a healthy uninjured CNS. The question that is considered in this section concerns how aging in microglia influences their ability to respond appropriately to perturbations such as injury, infections, or disease. The classic notion concerning microglial response to perturbation is the acquisition of an activated state. Microglia demonstrates a particular activation status that is a function of the balance of extrinsic signals in their milieu (Hanisch and Kettenmann, 2007). These signals are composed of either "On" signals that induce greater activation, or "Off" signals that induces the maintenance of, or a return to, a resting basal state (Van Rossum and Hanisch, 2004; Biber et al., 2007). Also relevant to this picture are intrinsic, cell-autonomous factors and mechanisms within microglia that influence and limit the magnitude and duration of activation responses. While these mechanisms have not been fully characterized, they include nuclear receptor signaling (Saijo et al., 2011, 2013) and microRNA-regulated gene expression (Ponomarev et al., 2013). Optimal microglial responses to perturbation comprise of a rapid, pro-inflammatory activation, necessary to produce efficient responses to injury and infections, as well as a well-timed and expeditious resolution of activation, required to avoid sustained damage to the CNS.

There is accumulating evidence that microglial responses to CNS perturbations become more dysregulated with aging. In the aged brain, in addition to the mild increases in basal microglial activation, microglia demonstrates increased priming or sensitization, and as a result generates responses to inflammatory stimuli that are exaggerated and prolonged. In numerous studies involving animal models of infectious and inflammatory challenge (Sparkman et al., 2005; Sierra et al., 2007; Abraham et al., 2008; Barrientos et al., 2009a,b; Norden and Godbout, 2012), hemorrhagic stroke (Wasserman and Schlichter, 2008; Wasserman et al., 2008), cognitive and physiological stressors (Buchanan et al., 2008; Rosczyk et al., 2008), and neurological injury induced by axotomy (Conde and Streit, 2006), neurotoxins, (Sugama et al., 2003) or trauma (Sandhir et al., 2008), responses generated in aged animals tend in general to be larger and more sustained, and culminate in more severe anatomical degeneration and functional debilitation relative to those produced in young animals. These larger inflammatory responses appear to be causally related to increased neurological deficits; in studies involving *E. coli*- and lipopolysaccharide (LPS)-induced systemic infection in aged mice, inhibition of IL-1β signaling blocked both the neuroinflammatory response as well as the associated cognitive deficits (Frank et al., 2010; Barrientos et al., 2012). Augmented neuroinflammatory responses are thought to suppress growth factor signaling, particularly that involving BDNF (Barrientos et al., 2004; Chapman et al., 2012), which is directly associated to neurological dysfunction.

What mechanisms result in age-related priming of microglia? An altered balance of "On" vs. "Off" extrinsic signals in the aging brain milieu, as well as the altered reception of these signals by microglia, constitute potential mechanisms. Microglial expression of pattern recognition receptors (PRRs) such as TLR1, TLR2, TLR4, TLR5, TLR7, and CD14 are up-regulated with increasing age (Letiembre et al., 2007). "Off" signaling involving CX3CL1-CX3CR1 and CD200-CD200R signaling also undergoes aging changes as the expression levels of "Off" ligands, CX3CL1 (Bachstetter et al., 2011) and CD200 (Frank et al., 2006; Lyons et al., 2007), as well as "Off" receptor CX3CR1 (Wynne et al., 2010), all decrease with advancing age. These reductions in "Off" signaling in aged microglia may be the basis for a more chronic activation and prolonged pro-inflammatory responses, a phenotype that is milder but mechanistically similar to the more pronounced pro-inflammatory effects observed in CX3CR1- and CD200-knockout mouse models (Hoek et al., 2000; Corona et al., 2010). The ability of exogenous CX3CL1 to reverse phenotypes associated with microglial aging (decreased neurogenesis, activated immunophenotype) also underscore its involvement as a driver of age-related priming of microglia (Bachstetter et al., 2011).

ATP signaling via purinergic receptors on microglia surfaces may also contribute to aging-related responses. It has been found that microglial responses to local tissue injury in the forms of increased process dynamism and polarization and directed migration are mediated by the local release of ATP as an injury signal (Davalos et al., 2005; Farber and Kettenmann, 2006; Haynes et al., 2006). In previous work, we found that dynamic microglial responses to the changes in ATP concentrations in the environment are age-related (Damani et al., 2011). As different P2 purinergic receptors have been described to vary with age (Crain et al., 2009; Damani et al., 2011), changes in the reception of ATP-related signals by microglia may contribute to changing dynamic responses in aging microglia.

WHAT ARE SOME DRIVERS OF MICROGLIAL SENESCENCE?

What mechanisms result in the development of senescent phenotypes in microglia? A number of theories have been proposed (summarized in **Table 2**). One theory invokes age-related replicative senescence, which refers to the finite capability of cells to undergo repeated cycles of replication. Microglia may undergo low levels of replication under steady state conditions (Lawson et al., 1992) and can proliferative quickly in response to perturbations (Ajami et al., 2007). In the course of a lifetime, these cycles can culminate in the shortening of telomeres and the attainment of replicative senescence (Olovnikov, 1996). Microglia induced to replicate *in vitro* do in fact demonstrate progressive telomere attrition (Flanary and Streit, 2004), and telomere lengths in microglia are shorter in aged compared to young brains (Flanary et al., 2007). Levels of telomerase, an enzyme that can extend the length of telomeres, also decrease in microglia with age, indicating that aging microglia cannot as successfully re-lengthen telomeres following injury-activated replication (Flanary and Streit, 2005). While replicative senescence in microglia may constrain their ability to go on proliferating, how it can drive the emergence of other senescent microglial phenotypes is unclear.

Alternately, microglia aging may result from the accumulation of biological changes that build up within microglia as a consequence of physiological activities sustained over time. Microglia in the aged brain accrue prominent amounts of autofluorescent lipofuscin (Sierra et al., 2007; Tremblay et al., 2012), that appears on ultrastructure studies as lysosomal inclusions (Peinado et al., 1998; Tremblay et al., 2012). Aged retinal microglia located in the subretinal space also demonstrates autofluorescent lipofuscin granules which increase in number with age (Xu et al., 2008). This intracellular accumulation is thought to result from microglia phagocytizing lipofuscin or its precursor molecules from nearby cells; autofluorescent granules found in subretinal microglia have similar spectral characteristics as those found in adjacent RPE cells, suggesting their common origin. Age-related lipofuscin buildup in microglia may indeed be influential on microglial physiology. In recent work, we found that cultured retinal microglia accumulating A2E, a prominent component of ocular lipofuscin, (1) developed a less ramified and more activated morphology, (2) demonstrated changes in gene expression that favored a more pro-inflammatory profile with an increased M1/M2 polarization, and (3) exhibited changes in the expression of complement regulatory proteins that promoted increased complement activation(Ma et al., 2013), all of which correspond to the basal state of increased activation typical in senescence.

Another candidate mechanism invokes the concept of oxidative stress. Microglia are prominent producers of oxidative products including reactive oxygen species (ROS) in the CNS. Mitochondria-derived ROS are produced in close proximity to mitochondria DNA (mtDNA) which encode components of the mitochondria electron transfer complexes. In aged microglia, mtDNA damage accumulates and leads to dysfunction in the respiratory chain and an over-production of ROS (Corral-Debrinski et al., 1992; Lin et al., 2002). Concurrently, decreased microglial autophagy due to age-related accumulation of lipofuscin slows down the turnover of senescent mitochondria, adding to the increase in oxidative stress within microglia (Kurz et al., 2008). As the activation of NF-κB mediated transcription in microglia,

Table 2 | Summary of proposed drivers of microglial senescence.

Drivers	Effects
Shortening of telomeres	Induction of replicative senescence and decreased ability to proliferate
Accumulation of intracellular lipofuscin	Decreased autophagy, leading to decreased organelle (e.g., mitochondria) turnover, increased ROS production, increased microglial activation
Accumulation of mtDNA mutations	Dysfunction in respiratory chain and over-production of ROS, leading to increased microglial activation
Increased iron load	Increased intracellular iron leads to increased ROS production and microglial activation

which regulates the expression of multiple pro-inflammatory cytokines, is positively modulated by ROS (Toledano and Leonard, 1991), this increased redox state may serve to prime microglia responses to perturbations, resulting in augmented microglial activation states observed in aged microglia (Nakanishi et al., 2011).

The age-related increase of oxidative stress as a driving force for microglia senescence has also been related to iron management in aging microglia. Levels of iron, and its storage protein, ferritin, increase with age in brain tissues (Benkovic and Connor, 1993; Bartzokis et al., 1997; Kwan et al., 2012). Microglia, which express ferritin, are thought to play a role in iron homeostasis (Kaneko et al., 1989; Cheepsunthorn et al., 1998; Widmer and Grune, 2005). As the iron load managed by microglia increases in the aging brain, the risk of oxidative damage to microglia increases as the labile nature of intracellular Fe^{2+} can readily lead to the formation of ROS. Microglial activation with LPS has been shown to increase ferritin expression in microglia and total iron content in brain areas (Hunter et al., 2008). The related natures of increased iron load, ROS production, and increased activation can potentially drive a feed-forward process that accelerates the formation of senescent microglial phenotypes. Indeed, dystrophic microglia in aged brains, as well as those with Alzheimer's and Huntington's disease, have been associated with ferritin immunopositivity and accumulation (Simmons et al., 2007; Lopes et al., 2008), suggesting a link between increased iron load and the development of dystrophy in individual microglial cells.

CAN MICROGLIA BE REJUVENATED?

A discussion of the roles of microglial senescence in brain aging and susceptibility to age-related neurodegeneration leads naturally to the question of whether it is possible to prevent or reverse the aging phenotypes of microglia. Proposed mechanisms for the etiology of microglial senescence and the descriptions of senescent microglial phenotypes present opportunities to formulate therapeutic strategies for achieving this, as well as outcome measures for which these therapies can be assessed. A number of these therapeutic approaches has been discussed or investigated in the literature and are listed thematically in the sections below (**Table 3**). Although there is currently a dearth of preventative therapies for age-related neurological diseases, research aimed at microglial "rejuvenation" may constitute a fruitful strategy in generating these in the future.

ANTI-INFLAMMATORY AND ANTI-OXIDANT APPROACHES

The pro-inflammatory and primed characteristics of the aging microglia phenotype prompt the use of an anti-inflammatory approach to alleviate the consequences of microglial aging. Minocycline, a highly bioavailable tetracycline-derived antibiotic with anti-inflammatory properties (Yrjanheikki et al., 1998, 1999; Tikka et al., 2001), has been investigated as an inhibitor of microglial activation in numerous animal models of neuroinflammation and CNS injury (Kim and Suh, 2009). Specific to its effect on aging microglia, minocycline has been shown to ameliorate pro-inflammatory cytokine production and sickness behavior

Table 3 | Proposed therapeutic approaches for the rejuvenation of senescent microglia.

Approaches	Effects
ANTI-INFLAMMATORY AND ANTIOXIDANT APPROACHES	
Minocycline	Inhibits microglial activation, decreases microglial proinflammatory cytokine production
IL1RA	Inhibits IL1β-mediated proinflammatory signaling
Dietary supplementation with antioxidants: flavonoids (e.g., luteolin, quercetin, genistein, hesperetin), retinoids/carotenoids (e.g., astaxanthin, crocin, crocetin, retinoic acid, lutein, zeaxanthin), vitamins (E and D3)	Exerts antioxidant and anti-inflammatory effects, decreases markers of neuroinflammation
PREVENTING OR REVERSING MICROGLIAL "RUN-DOWN" IN AGING	
Decreasing lipofuscin accumulation with visual cycle modulators (ACU-4429, fenretinide)	Partially inhibits the visual cycle to decrease ocular lipofuscin formation in retinal microglia
Stimulation of microglial autophagy (e.g., anti-lipolytic drugs, rapamycin)	Increases autophagy to promote mitochondria turnover in microglia
Stimulation of TFAM expression or activity (e.g., resveratrol, brimonidine)	Inhibits accumulation of mtDNA mutations in microglial mitochondria, decreasing ROS production
MODULATION OF THE AGING MICROGLIAL MILIEU	
Stimulation of CX3CL1-CX3CR1 signaling	Decreases microglial activation states
Stimulation of CD200-CD200R signaling [stimulation of IL4 signaling, fibroblast growth loop (FGL)]	Decreases microglial activation states
Exercise	Decreases microglial activation states, up-regulates proliferation of neural precursor cells
REPLACEMENT OF AGED MICROGLIA	
Depletion, followed by autologous or exogenous repletion by bone marrow derived cells	Enables the replacement of endogenous aged microglia with "replacement" immune cells that can carry out microglial functions
Cell-based therapies involving stem cells	Enables the replacement of endogenous aged microglia with "replacement" immune cells that can carry out microglial functions

following LPS-administration in aged mice (Henry et al., 2008) and to reduce aging-exacerbated deficits in long-term potentiation (LTP) in the aged rat hippocampus (Griffin et al., 2006; Liu et al., 2012). Clinical studies involving the use of minocycline for neurodegenerative diseases are ongoing; results of completed studies have produced both positive and negative results in separate disease contexts, revealing the need for further investigations (Plane et al., 2010). In contrast to minocycline, which has broad and somewhat varied anti-inflammatory actions, specific and targeted therapeutic approaches have also been considered. The central role of IL1β in mediating age-related pro-inflammatory effects of microglia has led to investigations of the IL1 receptor antagonist, IL1RA, as a potential therapy. Administration of IL1RA was found to ameliorate deficits in long term memory (Frank et al., 2006), impairments in theta-burst late-phase LTP (Chapman et al., 2010), and sickness behavior (Abraham and Johnson, 2009a) following immune challenges by *E. coli* or LPS administration. These findings may prompt future clinical investigations involving approved inhibitors of IL-1 signaling (anakinra, rilonacept, canakinumab) for the treatment of neurodegenerative disease (Dinarello et al., 2012).

As the generation of ROS and increased oxidative stress has been associated with the activation of pro-inflammatory programs in primed microglia (Verri et al., 2012), anti-oxidant approaches involving the use of dietary compounds have been examined in multiple studies, with the goal of reducing microglial activation in the course of reducing oxidative stress (McGahon et al., 1999; Gemma et al., 2002; Abraham and Johnson, 2009b). Flavonoids, a class of plant phenolics found widely in a variety of fruits, vegetables, and grains, have been investigated in the forms of luteolin, quercetin, genistein, hesperetin, among others, and have been found to decrease markers of neuroinflammation (Jang and Johnson, 2010; Izzi et al., 2012). Dietary administration of luteolin, one of the more potent agents in this regard (Comalada et al., 2006), decreased inflammatory markers and improved spatial working memory in aged mice (Jang et al., 2008, 2010). Dietary components of the retinoid/carotenoid class (which include astaxanthin, crocin, crocetin, retinoic acid, lutein, zeaxanthin, and their synthetic derivatives) have also been found to decrease microglia and macrophage activation in numerous *in vitro* studies (Dheen et al., 2005; Xu and Drew, 2006; Kim et al., 2008, 2010; Nam et al., 2010; Bian et al., 2012). Anti-oxidant supplements such as alpha-tocopherol (Vitamin E) (Murray and Lynch, 1998; Berg et al., 2005) and vitamin D3 (Moore et al., 2005) have been found to exert anti-inflammatory benefits. While the combination of *in vitro*, animal model, and epidemiological studies indicates the promise of dietary supplementation, verification of benefit in large scale controlled clinical studies is a necessary next step. Currently, there are few therapies of this nature that have been proven and approved for age-related neurodegenerative disease. One exception is AMD, a disease in which activated microglia in the aged retina feature (Gupta et al., 2003; Ma et al., 2013), for which anti-oxidant supplementation with vitamin A, C, E, and zinc, constitutes the current standard of care in reducing risk for disease progression (Age-Related Eye Disease Study Research, 2001). Results for a large scale trial involving supplementation with the carotenoids, lutein and zeaxanthin, and omega-3 fatty acids, for preventing AMD progression are expected in mid-2013 (AREDS2 Research Group et al., 2012).

PREVENTING OR REVERSING MICROGLIAL "RUN-DOWN" IN AGING

The concept that aging microglia accumulate progressive physiological changes (build-up of lipofuscin, increasing number of mtDNA mutations) that drive the emergence of senescent phenotypes suggest the feasibility of strategies that aim to ameliorate or reverse these changes. Measures targeted at lipofuscin accumulation in microglia may help alleviate lysosomal dysfunction, increase autophagy, decrease ROS production, and in general, ameliorate the detrimental phenotypes of aging microglia (Nakanishi and Wu, 2009). In the retina, lipofuscin accumulates in microglia of aged and AMD retinas (Ma et al., 2013) and is compositionally akin to that accumulating in nearby RPE cells (Xu et al., 2008). Current clinical studies of visual cycle modulators (ACU-4429, fenretinide) (Kubota et al., 2012; Mata et al., 2012) that aim to decrease lipofuscin accumulation in RPE cells may also be helpful in decreasing accumulation in retinal microglia. Alternatively, measures that stimulate autophagy, a process slowed by lipofuscin accumulation, may also be helpful. Anti-lipolytic drugs and rapamycin, which are agents found increase autophagy (Donati, 2006; Cai et al., 2012), have also been associated with reductions in age-related oxidative damage (Donati et al., 2006; Yang and Ming, 2012) and microglial activation (Dello Russo et al., 2012), and may therefore be useful in the therapy of neurodegenerative disease (Chong et al., 2012). Early stage clinical trials have been conducted with rapamycin for AMD and multiple sclerosis (NCT00095329 and 00766649) but results are not currently available.

With respect to the age-dependent accumulation of mtDNA mutations in aged microglia, a nucleus-encoded protein called transcription factor A, mitochondrial (TFAM) has been found to promote mtDNA transcription and maintain its architectural structure (Kanki et al., 2004). TFAM overexpression can reduce ROS production and NF-κB activation *in vitro* and decrease age-related mtDNA damage and IL1β expression in hippocampal microglia *in vivo* (Hayashi et al., 2008). As a result, compounds that can stimulate TFAM expression or activity, such as resveratrol (Lagouge et al., 2006; Vetterli et al., 2011) and brimonidine (Lee et al., 2012a) may be useful in this strategy.

MODULATION OF THE AGING MICROGLIAL MILIEU

The increased basal inflammatory state and priming in aged microglia that result from an age-dependent shift in balance between "On" and "Off" signals may be another target for microglial "rejuvenation." During aging, the expression levels of "Off" ligands CX3CL1 and CD200 decrease concurrently with increases in microglial activation status; the causal influence of these changes are demonstrated by the ability of exogenous CX3CL1 or a CD200 fusion protein to ameliorate phenotypes associated with microglial aging (Lyons et al., 2007, 2009a,b; Cox et al., 2012; Vukovic et al., 2012). These results present these signaling pathways as promising molecular targets for intervention. For the CX3CL1-CX3CR1 signaling axis, despite increasing

attention in different disease areas, suitable modulatory agents have yet to be investigated in clinical studies (D'Haese et al., 2012). For the CD200-CD200R axis, measures that increase IL4 (phospholipid microparticles-incorporating phosphatidylserine, atorvastatin), which up-regulates CD200 expression, can improve age-related decreases in LTP attributed to microglial aging (Nolan et al., 2005; Lyons et al., 2007, 2009b, 2011; Clarke et al., 2008). Also, a neural cell adhesion molecule (NCAM)-derived peptide, FGL, which can increase CD200 expression *in vitro* and *in vivo*, has been found to abrogate age-related increases in glial activation and synaptic changes (Downer et al., 2009, 2010; Ojo et al., 2012). Discovery of other "Off" signaling pathways can augment this strategy of modulating the milieu of aging microglia in ways that promote microglial quiescence.

EXERCISE

Physical exercise has been associated with a number of salubrious effects on CNS in animal studies, including increasing adult neurogenesis, improving performance on cognitive tasks, and ameliorating various declining neurological parameters observed with aging (Van Praag et al., 1999, 2005; Kim et al., 2004; Blackmore et al., 2009). Also, exercise has been implicated in improving aspects of systemic immune function (Woods, 2005). Recent studies have connected these two classes of effects in showing that exercise may exert its effects on the CNS, at least in part, through the modulation of microglia activity. The exaggerated neuroinflammatory response following a peripheral immune challenge in aged animals, which is associated with increased cytokine production, reduced BDNF expression, and increased cognitive deficits, was found to be completely reversed by voluntary exercise (Barrientos et al., 2011). This effect of exercise appears to be mediated by the "de-priming" of aged microglia as microglia from isolated the brains from exercising mice similarly demonstrated a more modest pro-inflammatory response to LPS administered *in vitro* relative to those from non-exercising mice. In a separate study, exercise was found to promote adult neurogenesis by stimulating greater NPC proliferation (Vukovic et al., 2012). These effects again appear to be mediated via microglia as prior depletion of microglia completely eliminates them. In this study, exercise seems to influence microglia through increased expression of CX3CL1, which confers on microglia the ability to up-regulate NPC proliferation. In other studies, exercise in animals was found to decrease age-related basal microglial proliferation (Kohman et al., 2012), and to lower microglial activation in models of hypoxia (Lin et al., 2012), high-fat diet (Yi et al., 2012), tau overexpression (Leem et al., 2011), and closed-head injury (Chen et al., 2012), further supporting the notion that exercise can play a role in deactivating and de-priming microglia. The molecular mechanisms underlying how exercise mediates these effects will be a fascinating area for future study, and may discover agents that can elicit exercise-induced effects without the need for actual exercise.

REPLACEMENT OF MICROGLIA AS THERAPY?

Although microglia are long-lived residents of the CNS and are not readily turned-over in the uninjured CNS, it remains plausible that aged microglia, instead of being modulated, may actually be wholly replaced or reinforced by exogenously introduced cells capable of carrying out the functions of young, non-senescent microglia. Studies in organotypic hippocampal slice cultures have shown that brain slices deprived of microglia can be again replenished by exogenously introduced cultured microglia which are able to colonize the brain parenchyma, acquire a ramified morphology, and confer neuroprotection to excitotoxic injury (Vinet et al., 2012). Animal studies have demonstrated that lethal irradiation followed by bone marrow transplantation allows the elimination of endogenous microglia and their replacement by bone marrow-derived cells which acquire features of microglia within the CNS parenchyma (Simard and Rivest, 2004). This technique of "microglial replacement" has enabled microglia expressing a mutant version of a particular gene in a transgenic mouse to be replaced by wild type microglia expressing a functional version of the same gene. These microglial "replacements" have been successful in alleviating the neurological and systemic symptoms in *MECP2* mutant mice, a model for Rett syndrome (Derecki et al., 2012), in correcting abnormal grooming behavior in *Hoxb8* mutant mice, a possible model for obsessive-compulsive disorder (Chen et al., 2010), and in increasing survival in *SOD1G93A* transgenic mice, a model for amyotrophic lateral sclerosis (ALS) (Lee et al., 2012b). Conversely, replacement of wild type microglia with transgenic microglia deficient in MyD88 signaling was found to increase disease susceptibility in a mouse ALS model (Kang and Rivest, 2007). Even in the absence of bone-marrow transplantation, the depletion of brain microglia has been found to induce an influx of peripheral monocytes into the CNS that repopulates the microglia-depleted areas (Varvel et al., 2012). These "replacement" cells intriguingly resemble endogenous microglia in distribution, morphology, and physiology, suggesting the notion that worn-out, aged brain microglia can perhaps be replaced by fresher and more able recruits from the periphery. The possibility that we can harness our intrinsic capability to "self-rejuvenate" our CNS microglia using our peripheral immune system is a radical but tantalizing concept.

The ability of microglia to be replenished in the CNS by peripherally derived cells that can take on features and functions of microglia present the possibility that these approaches may be in the future be translated into cell-based therapies to rejuvenating the population of senescent microglia in aged brains. Further studies still remain to be done regarding (1) possible sources of replacement cells (including stem cells sources) (Selvaraj et al., 2012), (2) the physiological functioning of newly integrated cells in an aged brain milieu, and (3) the use of brain conditioning regimens to optimize the spatial and temporal extents of microglial replacement (Capotondo et al., 2012) in order to increase and to realize the feasibility of such strategies.

ACKNOWLEDGMENTS

Research in our laboratory is supported by the National Eye Institute Intramural Research Program. Acknowledgment is made for grant support to the donors of Macular Degeneration Research (MDR), a program of the American Health Assistance Foundation (AHAF, now the BrightFocus Foundation).

REFERENCES

Aarum, J., Sandberg, K., Haeberlein, S. L., and Persson, M. A. (2003). Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15983–15988.

Abraham, J., Jang, S., Godbout, J. P., Chen, J., Kelley, K. W., Dantzer, R., et al. (2008). Aging sensitizes mice to behavioral deficits induced by central HIV-1 gp120. *Neurobiol. Aging* 29, 614–621.

Abraham, J., and Johnson, R. W. (2009a). Central inhibition of interleukin-1beta ameliorates sickness behavior in aged mice. *Brain Behav. Immun.* 23, 396–401.

Abraham, J., and Johnson, R. W. (2009b). Consuming a diet supplemented with resveratrol reduced infection-related neuroinflammation and deficits in working memory in aged mice. *Rejuvenation Res.* 12, 445–453.

Age-Related Eye Disease Study Research, G. (2001). Risk factors associated with age-related nuclear and cortical cataract: a case-control study in the Age-Related Eye Disease Study, AREDS Report No. 5. *Ophthalmology* 108, 1400–1408.

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

Areds2 Research Group, G., Chew, E. Y., Clemons, T., Sangiovanni, J. P., Danis, R., Domalpally, A., et al. (2012). The Age-related Eye Disease Study 2 (AREDS2): study design and baseline characteristics (AREDS2 Report Number 1). *Ophthalmology* 119, 2282–2289.

Bachstetter, A. D., Morganti, J. M., Jernberg, J., Schlunk, A., Mitchell, S. H., Brewster, K. W., et al. (2011). Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol. Aging* 32, 2030–2044.

Barrientos, R. M., Frank, M. G., Crysdale, N. Y., Chapman, T. R., Ahrendsen, J. T., Day, H. E., et al. (2011). Little exercise, big effects: reversing aging and infection-induced memory deficits, and underlying processes. *J. Neurosci.* 31, 11578–11586.

Barrientos, R. M., Frank, M. G., Hein, A. M., Higgins, E. A., Watkins, L. R., Rudy, J. W., et al. (2009a). Time course of hippocampal IL-1 beta and memory consolidation impairments in aging rats following peripheral infection. *Brain Behav. Immun.* 23, 46–54.

Barrientos, R. M., Watkins, L. R., Rudy, J. W., and Maier, S. F. (2009b). Characterization of the sickness response in young and aging rats following E. *coli* infection. *Brain Behav. Immun.* 23, 450–454.

Barrientos, R. M., Hein, A. M., Frank, M. G., Watkins, L. R., and Maier, S. F. (2012). Intracisternal interleukin-1 receptor antagonist prevents postoperative cognitive decline and neuroinflammatory response in aged rats. *J. Neurosci.* 32, 14641–14648.

Barrientos, R. M., Sprunger, D. B., Campeau, S., Watkins, L. R., Rudy, J. W., and Maier, S. F. (2004). BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. *J. Neuroimmunol.* 155, 119–126.

Bartzokis, G., Beckson, M., Hance, D. B., Marx, P., Foster, J. A., and Marder, S. R. (1997). MR evaluation of age-related increase of brain iron in young adult and older normal males. *Magn. Reson. Imaging* 15, 29–35.

Battista, D., Ferrari, C. C., Gage, F. H., and Pitossi, F. J. (2006). Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. *Eur. J. Neurosci.* 23, 83–93.

Benkovic, S. A., and Connor, J. R. (1993). Ferritin, transferrin, and iron in selected regions of the adult and aged rat brain. *J. Comp. Neurol.* 338, 97–113.

Berg, B. M., Godbout, J. P., Chen, J., Kelley, K. W., and Johnson, R. W. (2005). alpha-Tocopherol and selenium facilitate recovery from lipopolysaccharide-induced sickness in aged mice. *J. Nutr.* 135, 1157–1163.

Bian, Q., Qin, T., Ren, Z., Wu, D., and Shang, F. (2012). Lutein or zeaxanthin supplementation suppresses inflammatory responses in retinal pigment epithelial cells and macrophages. *Adv. Exp. Med. Biol.* 723, 43–50.

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci.* 30, 596–602.

Blackmore, D. G., Golmohammadi, M. G., Large, B., Waters, M. J., and Rietze, R. L. (2009). Exercise increases neural stem cell number in a growth hormone-dependent manner, augmenting the regenerative response in aged mice. *Stem Cells* 27, 2044–2052.

Boscia, F., Esposito, C. L., Di Crisci, A., De Franciscis, V., Annunziato, L., and Cerchia, L. (2009). GDNF selectively induces microglial activation and neuronal survival in CA1/CA3 hippocampal regions exposed to NMDA insult through Ret/ERK signalling. *PLoS ONE* 4:e6486. doi: 10.1371/journal.pone.0006486

Buchanan, J. B., Sparkman, N. L., Chen, J., and Johnson, R. W. (2008). Cognitive and neuroinflammatory consequences of mild repeated stress are exacerbated in aged mice. *Psychoneuroendocrinology* 33, 755–765.

Buschini, E., Piras, A., Nuzzi, R., and Vercelli, A. (2011). Age related macular degeneration and drusen: neuroinflammation in the retina. *Prog. Neurobiol.* 95, 14–25.

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A. E., Pluchino, S., et al. (2006). Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31, 149–160.

Cacci, E., Ajmone-Cat, M. A., Anelli, T., Biagioni, S., and Minghetti, L. (2008). *In vitro* neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* 56, 412–425.

Cai, Z., Zhao, B., Li, K., Zhang, L., Li, C., Quazi, S. H., et al. (2012). Mammalian target of rapamycin: a valid therapeutic target through the autophagy pathway for Alzheimer's disease? *J. Neurosci. Res.* 90, 1105–1118.

Campuzano, O., Castillo-Ruiz, M. M., Acarin, L., Castellano, B., and Gonzalez, B. (2008). Distinct pattern of microglial response, cyclooxygenase-2, and inducible nitric oxide synthase expression in the aged rat brain after excitotoxic damage. *J. Neurosci. Res.* 86, 3170–3183.

Capotondo, A., Milazzo, R., Politi, L. S., Quattrini, A., Palini, A., Plati, T., et al. (2012). Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation. *Proc. Natl. Acad. Sci. U.S.A.* 109, 15018–15023.

Chapman, T. R., Barrientos, R. M., Ahrendsen, J. T., Hoover, J. M., Maier, S. F., and Patterson, S. L. (2012). Aging and infection reduce expression of specific brain-derived neurotrophic factor mRNAs in hippocampus. *Neurobiol. Aging* 33, 832.e1–832.e14.

Chapman, T. R., Barrientos, R. M., Ahrendsen, J. T., Maier, S. F., and Patterson, S. L. (2010). Synaptic correlates of increased cognitive vulnerability with aging: peripheral immune challenge and aging interact to disrupt theta-burst late-phase long-term potentiation in hippocampal area CA1. *J. Neurosci.* 30, 7598–7603.

Cheepsunthorn, P., Palmer, C., and Connor, J. R. (1998). Cellular distribution of ferritin subunits in postnatal rat brain. *J. Comp. Neurol.* 400, 73–86.

Chen, M. F., Huang, T. Y., Kuo, Y. M., Yu, L., Chen, H. I., and Jen, C. J. (2012). Early post-injury exercise reverses memory deficits and retards the closed-head injury in mice. *J. Physiol.* 591, 985–1000.

Chen, S. K., Tvrdik, P., Peden, E., Cho, S., Wu, S., Spangrude, G., et al. (2010). Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* 141, 775–785.

Chong, Z. Z., Shang, Y. C., Wang, S., and Maiese, K. (2012). Shedding new light on neurodegenerative diseases through the mammalian target of rapamycin. *Prog. Neurobiol.* 99, 128–148.

Clarke, R. M., Lyons, A., O'Connell, F., Deighan, B. F., Barry, C. E., Anyakoha, N. G., et al. (2008). A pivotal role for interleukin-4 in atorvastatin-associated neuroprotection in rat brain. *J. Biol. Chem.* 283, 1808–1817.

Comalada, M., Ballester, I., Bailon, E., Sierra, S., Xaus, J., Galvez, J., et al. (2006). Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure-activity relationship. *Biochem. Pharmacol.* 72, 1010–1021.

Conde, J. R., and Streit, W. J. (2006). Effect of aging on the microglial response to peripheral nerve injury. *Neurobiol. Aging* 27, 1451–1461.

Corona, A. W., Huang, Y., O'Connor, J. C., Dantzer, R., Kelley, K. W., Popovich, P. G., et al. (2010). Fractalkine receptor (CX3CR1) deficiency sensitizes mice to the behavioral changes induced by lipopolysaccharide. *J. Neuroinflammation* 7:93. doi: 10.1186/1742-2094-7-93

Corral-Debrinski, M., Horton, T., Lott, M. T., Shoffner, J. M., Beal, M. F., and Wallace, D. C. (1992). Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nat. Genet.* 2, 324–329.

Cox, F. F., Carney, D., Miller, A. M., and Lynch, M. A. (2012). CD200 fusion protein decreases microglial

activation in the hippocampus of aged rats. *Brain Behav. Immun.* 26, 789–796.

Crain, J. M., Nikodemova, M., and Watters, J. J. (2009). Expression of P2 nucleotide receptors varies with age and sex in murine brain microglia. *J. Neuroinflammation* 6:24. doi: 10.1186/1742-2094-6-24

Damani, M. R., Zhao, L., Fontainhas, A. M., Amaral, J., Fariss, R. N., and Wong, W. T. (2011). Age-related alterations in the dynamic behavior of microglia. *Aging Cell* 10, 263–276.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., et al. (2005). ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat. Neurosci.* 8, 752–758.

Dello Russo, C., Lisi, L., Feinstein, D. L., and Navarra, P. (2012). mTOR kinase, a key player in the regulation of glial functions: relevance for the therapy of multiple sclerosis. *Glia* 61, 301–311.

Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., et al. (2012). Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105–109.

D'Haese, J. G., Friess, H., and Ceyhan, G. O. (2012). Therapeutic potential of the chemokine-receptor duo fractalkine/CX3CR1: an update. *Expert Opin. Ther. Targets* 16, 613–618.

Dheen, S. T., Jun, Y., Yan, Z., Tay, S. S., and Ling, E. A. (2005). Retinoic acid inhibits expression of TNF-alpha and iNOS in activated rat microglia. *Glia* 50, 21–31.

Dinarello, C. A., Simon, A., and Van Der Meer, J. W. (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat. Rev. Drug Discov.* 11, 633–652.

Donati, A. (2006). The involvement of macroautophagy in aging and anti-aging interventions. *Mol. Aspects Med.* 27, 455–470.

Donati, A., Taddei, M., Cavallini, G., and Bergamini, E. (2006). Stimulation of macroautophagy can rescue older cells from 8-OHdG mtDNA accumulation: a safe and easy way to meet goals in the SENS agenda. *Rejuvenation Res.* 9, 408–412.

Downer, E. J., Cowley, T. R., Cox, F., Maher, F. O., Berezin, V., Bock, E., et al. (2009). A synthetic NCAM-derived mimetic peptide, FGL, exerts anti-inflammatory properties via IGF-1 and interferon-gamma modulation. *J. Neurochem.* 109, 1516–1525.

Downer, E. J., Cowley, T. R., Lyons, A., Mills, K. H., Berezin, V., Bock, E., et al. (2010). A novel anti-inflammatory role of NCAM-derived mimetic peptide, FGL. *Neurobiol. Aging* 31, 118–128.

Duan, H., Wearne, S. L., Rocher, A. B., Macedo, A., Morrison, J. H., and Hof, P. R. (2003). Age-related dendritic and spine changes in corticocortically projecting neurons in macaque monkeys. *Cereb. Cortex* 13, 950–961.

Ekdahl, C. T., Claasen, J. H., Bonde, S., Kokaia, Z., and Lindvall, O. (2003). Inflammation is detrimental for neurogenesis in adult brain. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13632–13637.

Encinas, J. M., and Sierra, A. (2012). Neural stem cell deforestation as the main force driving the age-related decline in adult hippocampal neurogenesis. *Behav. Brain Res.* 227, 433–439.

Farber, K., and Kettenmann, H. (2006). Purinergic signaling and microglia. *Pflugers Arch.* 452, 615–621.

Fitzner, D., Schnaars, M., Van Rossum, D., Krishnamoorthy, G., Dibaj, P., Bakhti, M., et al. (2011). Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J. Cell. Sci.* 124, 447–458.

Flanary, B. E., Sammons, N. W., Nguyen, C., Walker, D., and Streit, W. J. (2007). Evidence that aging and amyloid promote microglial cell senescence. *Rejuvenation Res.* 10, 61–74.

Flanary, B. E., and Streit, W. J. (2004). Progressive telomere shortening occurs in cultured rat microglia, but not astrocytes. *Glia* 45, 75–88.

Flanary, B. E., and Streit, W. J. (2005). Effects of axotomy on telomere length, telomerase activity, and protein in activated microglia. *J. Neurosci. Res.* 82, 160–171.

Fontainhas, A. M., Wang, M., Liang, K. J., Chen, S., Mettu, P., Damani, M., et al. (2011). Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and GABAergic neurotransmission. *PLoS ONE* 6:e15973. doi: 10.1371/journal.pone.0015973

Frank, M. G., Barrientos, R. M., Biedenkapp, J. C., Rudy, J. W., Watkins, L. R., and Maier, S. F. (2006). mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. *Neurobiol. Aging* 27, 717–722.

Frank, M. G., Barrientos, R. M., Hein, A. M., Biedenkapp, J. C., Watkins, L. R., and Maier, S. F. (2010). IL-1RA blocks E. *coli*-induced suppression of Arc and long-term memory in aged F344xBN F1 rats. *Brain Behav. Immun.* 24, 254–262.

Gemma, C., Mesches, M. H., Sepesi, B., Choo, K., Holmes, D. B., and Bickford, P. C. (2002). Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. *J. Neurosci.* 22, 6114–6120.

Griffin, R., Nally, R., Nolan, Y., McCartney, Y., Linden, J., and Lynch, M. A. (2006). The age-related attenuation in long-term potentiation is associated with microglial activation. *J. Neurochem.* 99, 1263–1272.

Gupta, N., Brown, K. E., and Milam, A. H. (2003). Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. *Exp. Eye Res.* 76, 463–471.

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394.

Hayashi, Y., Yoshida, M., Yamato, M., Ide, T., Wu, Z., Ochi-Shindou, M., et al. (2008). Reverse of age-dependent memory impairment and mitochondrial DNA damage in microglia by an overexpression of human mitochondrial transcription factor a in mice. *J. Neurosci.* 28, 8624–8634.

Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W. B., et al. (2006). The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* 9, 1512–1519.

Henry, C. J., Huang, Y., Wynne, A., Hanke, M., Himler, J., Bailey, M. T., et al. (2008). Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. *J. Neuroinflammation* 5:15. doi: 10.1186/1742-2094-5-15

Hoek, R. M., Ruuls, S. R., Murphy, C. A., Wright, G. J., Goddard, R., Zurawski, S. M., et al. (2000). Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 290, 1768–1771.

Hughes, V. (2012). Microglia: the constant gardeners. *Nature* 485, 570–572.

Hunter, R. L., Liu, M., Choi, D. Y., Cass, W. A., and Bing, G. (2008). Inflammation and age-related iron accumulation in F344 rats. *Curr. Aging Sci.* 1, 112–121.

Imai, F., Suzuki, H., Oda, J., Ninomiya, T., Ono, K., Sano, H., et al. (2007). Neuroprotective effect of exogenous microglia in global brain ischemia. *J. Cereb. Blood Flow Metab.* 27, 488–500.

Izzi, V., Masuelli, L., Tresoldi, I., Sacchetti, P., Modesti, A., Galvano, F., et al. (2012). The effects of dietary flavonoids on the regulation of redox inflammatory networks. *Front. Biosci.* 17, 2396–2418.

Jang, S., Dilger, R. N., and Johnson, R. W. (2010). Luteolin inhibits microglia and alters hippocampal-dependent spatial working memory in aged mice. *J. Nutr.* 140, 1892–1898.

Jang, S., and Johnson, R. W. (2010). Can consuming flavonoids restore old microglia to their youthful state? *Nutr. Rev.* 68, 719–728.

Jang, S., Kelley, K. W., and Johnson, R. W. (2008). Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. *Proc. Natl. Acad. Sci. U.S.A.* 105, 7534–7539.

Kabaso, D., Coskren, P. J., Henry, B. I., Hof, P. R., and Wearne, S. L. (2009). The electrotonic structure of pyramidal neurons contributing to prefrontal cortical circuits in macaque monkeys is significantly altered in aging. *Cereb. Cortex* 19, 2248–2268.

Kaneko, Y., Kitamoto, T., Tateishi, J., and Yamaguchi, K. (1989). Ferritin immunohistochemistry as a marker for microglia. *Acta Neuropathol.* 79, 129–136.

Kang, J., and Rivest, S. (2007). MyD88-deficient bone marrow cells accelerate onset and reduce survival in a mouse model of amyotrophic lateral sclerosis. *J. Cell Biol.* 179, 1219–1230.

Kanki, T., Nakayama, H., Sasaki, N., Takio, K., Alam, T. I., Hamasaki, N., et al. (2004). Mitochondrial nucleoid and transcription factor A. *Ann. N.Y. Acad. Sci.* 1011, 61–68.

Karlstetter, M., Ebert, S., and Langmann, T. (2010). Microglia in the healthy and degenerating retina: insights from novel mouse models. *Immunobiology* 215, 685–691.

Kim, H. S., and Suh, Y. H. (2009). Minocycline and neurodegenerative diseases. *Behav. Brain Res.* 196, 168–179.

Kim, J. H., Na, H. J., Kim, C. K., Kim, J. Y., Ha, K. S., Lee, H., et al. (2008). The non-provitamin A carotenoid, lutein, inhibits NF-kappaB-dependent gene expression

through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-kappaB-inducing kinase pathways: role of H(2)O(2) in NF-kappaB activation. *Free Radic. Biol. Med.* 45, 885–896.

Kim, Y. H., Koh, H. K., and Kim, D. S. (2010). Down-regulation of IL-6 production by astaxanthin via ERK-, MSK-, and NF-kappaB-mediated signals in activated microglia. *Int. Immunopharmacol.* 10, 1560–1572.

Kim, Y. P., Kim, H., Shin, M. S., Chang, H. K., Jang, M. H., Shin, M. C., et al. (2004). Age-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats. *Neurosci. Lett.* 355, 152–154.

Kitamura, Y., Takata, K., Inden, M., Tsuchiya, D., Yanagisawa, D., Nakata, J., et al. (2004). Intracerebroventricular injection of microglia protects against focal brain ischemia. *J. Pharmacol. Sci.* 94, 203–206.

Kohman, R. A., Deyoung, E. K., Bhattacharya, T. K., Peterson, L. N., and Rhodes, J. S. (2012). Wheel running attenuates microglia proliferation and increases expression of a proneurogenic phenotype in the hippocampus of aged mice. *Brain Behav. Immun.* 26, 803–810.

Kubota, R., Boman, N. L., David, R., Mallikaarjun, S., Patil, S., and Birch, D. (2012). Safety and effect on rod function of ACU-4429, a novel small-molecule visual cycle modulator. *Retina* 32, 183–188.

Kuhn, H. G., Dickinson-Anson, H., and Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* 16, 2027–2033.

Kurz, T., Terman, A., Gustafsson, B., and Brunk, U. T. (2008). Lysosomes and oxidative stress in aging and apoptosis. *Biochim. Biophys. Acta* 1780, 1291–1303.

Kwan, J. Y., Jeong, S. Y., Van Gelderen, P., Deng, H. X., Quezado, M. M., Danielian, L. E., et al. (2012). Iron accumulation in deep cortical layers accounts for MRI signal abnormalities in ALS: correlating 7 Tesla MRI and pathology. *PLoS ONE* 7:e35241. doi: 10.1371/journal.pone.0035241

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., et al. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. *Cell* 127, 1109–1122.

Lambertsen, K. L., Clausen, B. H., Babcock, A. A., Gregersen, R., Fenger, C., Nielsen, H. H., et al. (2009). Microglia protect neurons against ischemia by synthesis of tumor necrosis factor. *J. Neurosci.* 29, 1319–1330.

Lauro, C., Cipriani, R., Catalano, M., Trettel, F., Chece, G., Brusadin, V., et al. (2010). Adenosine A1 receptors and microglial cells mediate CX3CL1-induced protection of hippocampal neurons against Glu-induced death. *Neuropsychopharmacology* 35, 1550–1559.

Lawson, L. J., Perry, V. H., and Gordon, S. (1992). Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* 48, 405–415.

Lee, D., Kim, K. Y., Noh, Y. H., Chai, S., Lindsey, J. D., Ellisman, M. H., et al. (2012a). Brimonidine blocks glutamate excitotoxicity-induced oxidative stress and preserves mitochondrial transcription factor a in ischemic retinal injury. *PLoS ONE* 7:e47098. doi: 10.1371/journal.pone.0047098

Lee, J. C., Seong, J., Kim, S. H., Lee, S. J., Cho, Y. J., An, J., et al. (2012b). Replacement of microglial cells using Clodronate liposome and bone marrow transplantation in the central nervous system of SOD1(G93A) transgenic mice as an *in vivo* model of amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.* 418, 359–365.

Leem, Y. H., Lee, Y. I., Son, H. J., and Lee, S. H. (2011). Chronic exercise ameliorates the neuroinflammation in mice carrying NSE/htau23. *Biochem. Biophys. Res. Commun.* 406, 359–365.

Letiembre, M., Hao, W., Liu, Y., Walter, S., Mihaljevic, I., Rivest, S., et al. (2007). Innate immune receptor expression in normal brain aging. *Neuroscience* 146, 248–254.

Li, Y., Du, X. F., Liu, C. S., Wen, Z. L., and Du, J. L. (2012). Reciprocal regulation between resting microglial dynamics and neuronal activity *in vivo*. *Dev. Cell* 23, 1189–1202.

Lin, C., Wu, C. J., Wei, I. H., Tsai, M. H., Chang, N. W., Yang, T. T., et al. (2012). Chronic treadmill running protects hippocampal neurons from hypobaric hypoxia-induced apoptosis in rats. *Neuroscience* 231, 216–224.

Lin, M. T., Simon, D. K., Ahn, C. H., Kim, L. M., and Beal, M. F. (2002). High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. *Hum. Mol. Genet.* 11, 133–145.

Liu, X., Wu, Z., Hayashi, Y., and Nakanishi, H. (2012). Age-dependent neuroinflammatory responses and deficits in long-term potentiation in the hippocampus during systemic inflammation. *Neuroscience* 216, 133–142.

Lopes, K. O., Sparks, D. L., and Streit, W. J. (2008). Microglial dystrophy in the aged and Alzheimer's disease brain is associated with ferritin immunoreactivity. *Glia* 56, 1048–1060.

Lyons, A., Downer, E. J., Crotty, S., Nolan, Y. M., Mills, K. H., and Lynch, M. A. (2007). CD200 ligand receptor interaction modulates microglial activation *in vivo* and *in vitro*: a role for IL-4. *J. Neurosci.* 27, 8309–8313.

Lyons, A., Lynch, A. M., Downer, E. J., Hanley, R., O'Sullivan, J. B., Smith, A., et al. (2009a). Fractalkine-induced activation of the phosphatidylinositol-3 kinase pathway attentuates microglial activation *in vivo* and *in vitro*. *J. Neurochem.* 110, 1547–1556.

Lyons, A., McQuillan, K., Deighan, B. F., O'reilly, J. A., Downer, E. J., Murphy, A. C., et al. (2009b). Decreased neuronal CD200 expression in IL-4-deficient mice results in increased neuroinflammation in response to lipopolysaccharide. *Brain Behav. Immun.* 23, 1020–1027.

Lyons, A., Murphy, K. J., Clarke, R., and Lynch, M. A. (2011). Atorvastatin prevents age-related and amyloid-beta-induced microglial activation by blocking interferon-gamma release from natural killer cells in the brain. *J. Neuroinflammation* 8:27. doi: 10.1186/1742-2094-8-27

Ma, W., Coon, S., Zhao, L., Fariss, R. N., and Wong, W. T. (2013). A2E accumulation influences retinal microglial activation and complement regulation. *Neurobiol. Aging* 34, 943–960.

Ma, W., Zhao, L., Fontainhas, A. M., Fariss, R. N., and Wong, W. T. (2009). Microglia in the mouse retina alter the structure and function of retinal pigmented epithelial cells: a potential cellular interaction relevant to AMD. *PLoS ONE* 4:e7945. doi: 10.1371/journal.pone.0007945

Ma, W., Zhao, L., and Wong, W. T. (2012). Microglia in the outer retina and their relevance to pathogenesis of age-related macular degeneration. *Adv. Exp. Med. Biol.* 723, 37–42.

Mata, N. L., Lichter, J. B., Vogel, R., Han, Y., Bui, T. V., and Singerman, L. J. (2012). Investigation of oral fenretinide for treatment of geographic atrophy in age-related macular degeneration. *Retina* 33, 498–507.

McGahon, B. M., Murray, C. A., Horrobin, D. F., and Lynch, (1999). Age-related changes in oxidative mechanisms and LTP are reversed by dietary manipulation. *Neurobiol. Aging* 20, 643–653.

McPherson, C. A., Aoyama, M., and Harry, G. J. (2011). Interleukin (IL)-1 and IL-6 regulation of neural progenitor cell proliferation with hippocampal injury: differential regulatory pathways in the subgranular zone (SGZ) of the adolescent and mature mouse brain. *Brain Behav. Immun.* 25, 850–862.

Medrano, S., Burns-Cusato, M., Atienza, M. B., Rahimi, D., and Scrable, H. (2009). Regenerative capacity of neural precursors in the adult mammalian brain is under the control of p53. *Neurobiol. Aging* 30, 483–497.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature* 454, 428–435.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., et al. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat. Neurosci.* 10, 1544–1553.

Miller, K. R., and Streit, W. J. (2007). The effects of aging, injury and disease on microglial function: a case for cellular senescence. *Neuron Glia Biol.* 3, 245–253.

Monje, M. L., Toda, H., and Palmer, T. D. (2003). Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 302, 1760–1765.

Montero, M., Gonzalez, B., and Zimmer, J. (2009). Immunotoxic depletion of microglia in mouse hippocampal slice cultures enhances ischemia-like neurodegeneration. *Brain Res.* 1291, 140–152.

Moore, M. E., Piazza, A., McCartney, Y., and Lynch, M. A. (2005). Evidence that vitamin D3 reverses age-related inflammatory changes in the rat hippocampus. *Biochem. Soc. Trans.* 33, 573–577.

Morrison, J. H., and Baxter, M. G. (2012). The ageing cortical synapse: hallmarks and implications for cognitive decline. *Nat. Rev. Neurosci.* 13, 240–250.

Mouton, P. R., Long, J. M., Lei, D. L., Howard, V., Jucker, M., Calhoun, M. E., et al. (2002). Age and gender effects on microglia and astrocyte numbers in brains of mice. *Brain Res.* 956, 30–35.

Murray, C. A., and Lynch, M. A. (1998). Dietary supplementation with vitamin E reverses the age-related deficit in long term potentiation in dentate gyrus. *J. Biol. Chem.* 273, 12161–12168.

Nakanishi, H., Hayashi, Y., and Wu, Z. (2011). The role of microglial mtDNA damage in age-dependent prolonged LPS-induced sickness behavior. *Neuron Glia Biol.* 7, 17–23.

Nakanishi, H., and Wu, Z. (2009). Microglia-aging: roles of microglial lysosome- and mitochondria-derived reactive oxygen species in brain aging. *Behav. Brain Res.* 201, 1–7.

Nam, K. N., Park, Y. M., Jung, H. J., Lee, J. Y., Min, B. D., Park, S. U., et al. (2010). Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. *Eur. J. Pharmacol.* 648, 110–116.

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318.

Njie, E. G., Boelen, E., Stassen, F. R., Steinbusch, H. W., Borchelt, D. R., and Streit, W. J. (2012). *Ex vivo* cultures of microglia from young and aged rodent brain reveal age-related changes in microglial function. *Neurobiol. Aging* 33, 195.e1–195.e12.

Nolan, Y., Maher, F. O., Martin, D. S., Clarke, R. M., Brady, M. T., Bolton, A. E., et al. (2005). Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. *J. Biol. Chem.* 280, 9354–9362.

Norden, D. M., and Godbout, J. P. (2012). Microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol. Appl. Neurobiol.* 39, 19–34.

Ogura, K., Ogawa, M., and Yoshida, M. (1994). Effects of ageing on microglia in the normal rat brain: immunohistochemical observations. *Neuroreport* 5, 1224–1226.

Ojo, B., Rezaie, P., Gabbott, P. L., Davies, H., Colyer, F., Cowley, T. R., et al. (2012). Age-related changes in the hippocampus (loss of synaptophysin and glial-synaptic interaction) are modified by systemic treatment with an NCAM-derived peptide, FGL. *Brain Behav. Immun.* 26, 778–788.

Olovnikov, A. M. (1996). Telomeres, telomerase, and aging: origin of the theory. *Exp. Gerontol.* 31, 443–448.

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458.

Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2012). Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U.S.A.* 109, E197–E205.

Peinado, M. A., Quesada, A., Pedrosa, J. A., Torres, M. I., Martinez, M., Esteban, F. J., et al. (1998). Quantitative and ultrastructural changes in glia and pericytes in the parietal cortex of the aging rat. *Microsc. Res. Tech.* 43, 34–42.

Perry, V. H., Matyszak, M. K., and Fearn, S. (1993). Altered antigen expression of microglia in the aged rodent CNS. *Glia* 7, 60–67.

Persson, M., Brantefjord, M., Hansson, E., and Ronnback, L. (2005). Lipopolysaccharide increases microglial GLT-1 expression and glutamate uptake capacity *in vitro* by a mechanism dependent on TNF-alpha. *Glia* 51, 111–120.

Persson, M., and Ronnback, L. (2012). Microglial self-defence mediated through GLT-1 and glutathione. *Amino Acids* 42, 207–219.

Plane, J. M., Shen, Y., Pleasure, D. E., and Deng, W. (2010). Prospects for minocycline neuroprotection. *Arch. Neurol.* 67, 1442–1448.

Ponomarev, E. D., Veremeyko, T., and Weiner, H. L. (2013). MicroRNAs are universal regulators of differentiation, activation, and polarization of microglia and macrophages in normal and diseased CNS. *Glia* 61, 91–103.

Raivich, G. (2005). Like cops on the beat: the active role of resting microglia. *Trends Neurosci.* 28, 571–573.

Rogers, J., Luber-Narod, J., Styren, S. D., and Civin, W. H. (1988). Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol. Aging* 9, 339–349.

Rosczyk, H. A., Sparkman, N. L., and Johnson, R. W. (2008). Neuroinflammation and cognitive function in aged mice following minor surgery. *Exp. Gerontol.* 43, 840–846.

Saijo, K., Collier, J. G., Li, A. C., Katzenellenbogen, J. A., and Glass, C. K. (2011). An ADIOL-ERbeta-CtBP transrepression pathway negatively regulates microglia-mediated inflammation. *Cell* 145, 584–595.

Saijo, K., Crotti, A., and Glass, C. K. (2013). Regulation of microglia activation and deactivation by nuclear receptors. *Glia* 61, 104–111.

Sandhir, R., Onyszchuk, G., and Berman, N. E. (2008). Exacerbated glial response in the aged mouse hippocampus following controlled cortical impact injury. *Exp. Neurol.* 213, 372–380.

Santos, A. M., Calvente, R., Tassi, M., Carrasco, M. C., Martin-Oliva, D., Marin-Teva, J. L., et al. (2008). Embryonic and postnatal development of microglial cells in the mouse retina. *J. Comp. Neurol.* 506, 224–239.

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705.

Schuitemaker, A., Van Der Doef, T. F., Boellaard, R., Van Der Flier, W. M., Yaqub, M., Windhorst, A. D., et al. (2010). Microglial activation in healthy aging. *Neurobiol. Aging* 33, 1067–1072.

Selvaraj, V., Jiang, P., Chechneva, O., Lo, U. G., and Deng, W. (2012). Differentiating human stem cells into neurons and glial cells for neural repair. *Front. Biosci.* 17, 65–89.

Sheng, J. G., Mrak, R. E., and Griffin, W. S. (1998). Enlarged and phagocytic, but not primed, interleukin-1 alpha-immunoreactive microglia increase with age in normal human brain. *Acta Neuropathol.* 95, 229–234.

Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495.

Sierra, A., Gottfried-Blackmore, A. C., McEwen, B. S., and Bulloch, K. (2007). Microglia derived from aging mice exhibit an altered inflammatory profile. *Glia* 55, 412–424.

Simard, A. R., and Rivest, S. (2004). Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J.* 18, 998–1000.

Simmons, D. A., Casale, M., Alcon, B., Pham, N., Narayan, N., and Lynch, G. (2007). Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. *Glia* 55, 1074–1084.

Sparkman, N. L., Martin, L. A., Calvert, W. S., and Boehm, G. W. (2005). Effects of intraperitoneal lipopolysaccharide on Morris maze performance in year-old and 2-month-old female C57BL/6J mice. *Behav. Brain Res.* 159, 145–151.

Streit, W. J., Braak, H., Xue, Q. S., and Bechmann, I. (2009). Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. *Acta Neuropathol* 118, 475–485.

Streit, W. J., Sammons, N. W., Kuhns, A. J., and Sparks, D. L. (2004). Dystrophic microglia in the aging human brain. *Glia* 45, 208–212.

Streit, W. J., and Xue, Q. S. (2009). Life and death of microglia. *J. Neuroimmun. Pharmacol.* 4, 371–379.

Streit, W. J., and Xue, Q. S. (2010). The brain's aging immune system. *Aging Dis.* 1, 254–261.

Sugama, S., Yang, L., Cho, B. P., Degiorgio, L. A., Lorenzl, S., Albers, D. S., et al. (2003). Age-related microglial activation in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. *Brain Res.* 964, 288–294.

Tikka, T., Fiebich, B. L., Goldsteins, G., Keinanen, R., and Koistinaho, J. (2001). Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J. Neurosci.* 21, 2580–2588.

Toledano, M. B., and Leonard, W. J. (1991). Modulation of transcription factor NF-kappa B binding activity by oxidation-reduction *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* 88, 4328–4332.

Tremblay, M. E., Lowery, R. L., and Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 8:e1000527. doi: 10.1371/journal.pbio.1000527

Tremblay, M. E., Zettel, M. L., Ison, J. R., Allen, P. D., and Majewska, A. K. (2012). Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. *Glia* 60, 541–558.

Van Praag, H., Kempermann, G., and Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse

dentate gyrus. *Nat. Neurosci.* 2, 266–270.

Van Praag, H., Shubert, T., Zhao, C., and Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *J. Neurosci.* 25, 8680–8685.

Van Rossum, D., and Hanisch, U. K. (2004). Microglia. *Metab. Brain Dis.* 19, 393–411.

Varvel, N. H., Grathwohl, S. A., Baumann, F., Liebig, C., Bosch, A., Brawek, B., et al. (2012). Microglial repopulation model reveals a robust homeostatic process for replacing CNS myeloid cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 18150–18155.

Verri, M., Pastoris, O., Dossena, M., Aquilani, R., Guerriero, F., Cuzzoni, G., et al. (2012). Mitochondrial alterations, oxidative stress and neuroinflammation in Alzheimer's disease. *Int. J. Immunopathol. Pharmacol.* 25, 345–353.

Vetterli, L., Brun, T., Giovannoni, L., Bosco, D., and Maechler, P. (2011). Resveratrol potentiates glucose-stimulated insulin secretion in INS-1E beta-cells and human islets through a SIRT1-dependent mechanism. *J. Biol. Chem.* 286, 6049–6060.

Vinet, J., Weering, H. R., Heinrich, A., Kalin, R. E., Wegner, A., Brouwer, N., et al. (2012). Neuroprotective function for ramified microglia in hippocampal excitotoxicity. *J. Neuroinflammation* 9:27. doi: 10.1186/1742-2094-9-27

Vukovic, J., Colditz, M. J., Blackmore, D. G., Ruitenberg, M. J., and Bartlett, P. F. (2012). Microglia modulate hippocampal neural precursor activity in response to exercise and aging. *J. Neurosci.* 32, 6435–6443.

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., and Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses *in vivo* and determine the fate of ischemic terminals. *J. Neurosci.* 29, 3974–3980.

Walton, N. M., Sutter, B. M., Laywell, E. D., Levkoff, L. H., Kearns, S. M., Marshall, G. P., et al. (2006). Microglia instruct subventricular zone neurogenesis. *Glia* 54, 815–825.

Ward, R. J., Crichton, R. R., Taylor, D. L., Della Corte, L., Srai, S. K., and Dexter, D. T. (2011). Iron and the immune system. *J. Neural Transm.* 118, 315–328.

Wasserman, J. K., and Schlichter, L. C. (2008). White matter injury in young and aged rats after intracerebral hemorrhage. *Exp. Neurol.* 214, 266–275.

Wasserman, J. K., Yang, H., and Schlichter, L. C. (2008). Glial responses, neuron death and lesion resolution after intracerebral hemorrhage in young vs. aged rats. *Eur. J. Neurosci.* 28, 1316–1328.

Widmer, R., and Grune, T. (2005). Iron uptake of the normoxic, anoxic and postanoxic microglial cell line RAW 264.7. *Biofactors* 24, 247–254.

Woods, J. A. (2005). Physical activity, exercise, and immune function. *Brain Behav. Immun.* 19, 369–370.

Wynne, A. M., Henry, C. J., Huang, Y., Cleland, A., and Godbout, J. P. (2010). Protracted downregulation of CX3CR1 on microglia of aged mice after lipopolysaccharide challenge. *Brain Behav. Immun.* 24, 1190–1201.

Xu, H., Chen, M., Manivannan, A., Lois, N., and Forrester, J. V. (2008). Age-dependent accumulation of lipofuscin in perivascular and subretinal microglia in experimental mice. *Aging Cell* 7, 58–68.

Xu, J., and Drew, P. D. (2006). 9-Cis-retinoic acid suppresses inflammatory responses of microglia and astrocytes. *J. Neuroimmunol.* 171, 135–144.

Yang, Z., and Ming, X. F. (2012). mTOR signalling: the molecular interface connecting metabolic stress, aging and cardiovascular diseases. *Obes. Rev.* 13(Suppl. 2), 58–68.

Ye, S. M., and Johnson, R. W. (1999). Increased interleukin-6 expression by microglia from brain of aged mice. *J. Neuroimmunol.* 93, 139–148.

Ye, S. M., and Johnson, R. W. (2001). An age-related decline in interleukin-10 may contribute to the increased expression of interleukin-6 in brain of aged mice. *Neuroimmunomodulation* 9, 183–192.

Yi, C. X., Al-Massadi, O., Donelan, E., Lehti, M., Weber, J., Ress, C., et al. (2012). Exercise protects against high-fat diet-induced hypothalamic inflammation. *Physiol. Behav.* 106, 485–490.

Yrjanheikki, J., Keinanen, R., Pellikka, M., Hokfelt, T., and Koistinaho, J. (1998). Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc. Natl. Acad. Sci. U.S.A.* 95, 15769–15774.

Yrjanheikki, J., Tikka, T., Keinanen, R., Goldsteins, G., Chan, P. H., and Koistinaho, J. (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13496–13500.

Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., et al. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat. Neurosci.* 9, 268–275.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 December 2012; paper pending published: 22 January 2013; accepted: 21 February 2013; published online: 13 March 2013.

Citation: Wong WT (2013) Microglial aging in the healthy CNS: phenotypes, drivers, and rejuvenation. Front. Cell. Neurosci. 7:22. doi: 10.3389/fncel.2013.00022