Review Article

Microglial heterogeneity in chronic pain

George Sideris-Lampretsas^{*}, Marzia Malcangio

Wolfson Centre for Age-Related Diseases, Institute of Psychiatry, Psychology and Neuroscience, King's College London, Guys' Campus, London Bridge, London SE1 1UL, UK

ARTICLE INFO

Keywords:

Chronic pain
Neuropathic pain
Microglia
Neuroinflammation

ABSTRACT

In this review, we report existing preclinical evidence on how the CNS compartment as well as sex affect microglia functions in health. We highlight that recent advances in transcriptomics analyses have led to thorough characterization of disease-associated microglial states in mice and humans. We then consider the specific scenario of peripheral nerve or tissue injury which induce expression of a specific subset of genes in microglia in the dorsal horn of the spinal cord. We suggest the intriguing possibility that future studies may disclose the existence of a unique microglia transcriptional profile that is associated with chronic pain conditions. We also collect evidence that microglial activation in pain-related areas of the brain can be observed in models of neuropathic pain in agreement with recent neuroimaging studies in chronic pain patients. Based on the evidence discussed here, we predict that future studies on the neuroimmune interactions in chronic pain should complement our current understanding of microglia functions, but also adventure in using novel approaches such as scRNA-seq, spatial transcriptomics, CYTOF and transmission electron microscopy to provide a more complete characterization of the function, transcriptome and structure of microglia in chronic pain.

1. Introduction

In both brain and spinal cord, microglia are ubiquitous resident immune cells that constantly surveil the parenchyma via highly dynamic processes and interactions with neurons, vasculature and other glial cells (Bernier et al., 2019; Cserép et al., 2019). Unlike other tissue-resident and non-parenchymal brain macrophages, microglia originate from erythromyeloid progenitors in the yolk sac that arise earlier than other mononuclear phagocytes (Ginhoux et al., 2010; Utz et al., 2020). However, similar to tissue-resident macrophages, microglia markedly rely on environmental cues that shape their transcriptional profile and functional properties (Gosselin et al., 2014). Through multiple interactions with surrounding cells, microglia assume diverse biological roles that aim to maintenance of homeostasis. Such roles range from securing functional development of neuronal circuits to responding and regulating neuronal excitability and clearing the CNS parenchyma from potential pathogens or dead cells (Sierra et al., 2010; Stevens et al., 2007; Umpierre and Wu, 2020). Upon CNS injury or inflammatory insult, microglia shift from a homeostatic role to a reactive state that guarantees neuroprotection but can also lead to pronounced neuroinflammation (Stratoulas et al., 2019). This divergence from homeostasis is clearly represented at cellular and subcellular levels by the

occurrence of morphological changes -rapid regulation of both motility and direction of branches- and prominent transcriptional changes (Davalos et al., 2005; Nimmerjahn et al., 2005). The advent of genome-wide association studies (GWAS) has marked an important paradigm shift in our understanding of microglia in disease states as they implicate genetic variants, that are either solely expressed by microglia or regulate microglia responses, as risk factors for neurodegenerative diseases (Jansen et al., 2019) and neuropsychiatric disorders (Sekar et al., 2016). Therefore, microglia are involved in disease aetiology and actively support and modulate neuronal function.

Although most neuronal and glial cells are known to be diverse across the CNS (Bayraktar et al., 2020; Marques et al., 2016; Saunders et al., 2018), microglial heterogeneity has only recently become the centre of attention. At first, with the intention to classify microglia functional modalities, microglial heterogeneity was based on the expression of membrane markers and morphological configurations, which resulted in the generation of fixed phenotypic categories. For instance, the M1/M2 framework has been adopted from the macrophage field to describe pro-inflammatory (M1) and anti-inflammatory (M2) functions of microglia following CNS insult. However, the use of this oversimplified nomenclature is widely criticized as misleading (Ransohoff, 2016) whereas there is more consensus towards using single-cell

^{*} Corresponding author.E-mail addresses: george.sideris@kcl.ac.uk (G. Sideris-Lampretsas), marzia.malcangio@kcl.ac.uk (M. Malcangio).<https://doi.org/10.1016/j.bbi.2021.06.005>

Received 11 March 2021; Received in revised form 24 May 2021; Accepted 8 June 2021

Available online 15 June 2021

0889-1591/© 2021 Elsevier Inc. All rights reserved.

RNA sequencing (RNA-seq) analysis to characterize microglial heterogeneity. In the healthy murine brain, RNA-seq approach has demonstrated that microglia share a conserved expression of core genes that regulate homeostatic functions (Bennett et al., 2016; Butovsky et al., 2014; Li et al., 2019). This homeostatic gene signature is associated with various roles played by microglia in a region- and time-dependent fashion (Butovsky et al., 2014; De Biase et al., 2017; Masuda et al., 2019). Furthermore, microglia are endowed with the ability to sense the environment and readily respond to external stimuli across the CNS by changing the expression of the transcriptional homeostatic signature. For instance, in mouse models of Alzheimer's disease (AD), multiple sclerosis and neuroinflammation, microglial heterogeneity manifests through the establishment of cell subsets that alter their transcriptional profile through down-regulation of some homeostatic genes (Cx3cr1, Tmem119, P2ry12, Sall1, Hexb, Fcrls) and up-regulation of disease-specific genes (Apoe, Trem2, Ctss, Axl) (Jordão et al., 2019; Keren-Shaul et al., 2017; Krasemann et al., 2017). In support of the concept that diverse microglial profiles can be observed in response to pathology, changes in gene expression are accompanied by alteration at the molecular level (Mrdjen et al., 2018), and for instance, microglia adjacent to amyloid plaques, the pathological hallmark of AD, express specific subsets of protein markers (Kamphuis et al., 2016; Parhizkar et al., 2019).

Several comprehensive reviews discuss microglial heterogeneity in the context of regional diversity, sexual dimorphism, healthy development, ageing and nervous system diseases (Masuda et al., 2020; Stratosoulas et al., 2019; Tan et al., 2019). We discuss microglial heterogeneity in the context of chronic pain. In the last two decades, it has become apparent that under neuropathic pain conditions following peripheral damage, dorsal horn microglia in the spinal cord play a causal role in the establishment and maintenance of nociceptive mechanisms via well-defined pathways for communication with neurons (Malcangio, 2019). Typical examples of neuroimmune interactions include 1) neuron-derived ATP activation of microglial P₂X₄/BDNF pathway which results in a TrkB-mediated reduction of GABAergic inhibition of dorsal horn neurons and an overall increase in excitability (Coull et al., 2005; Masuda et al., 2016) 2) microglial P₂X₇ receptor activation by neuronal ATP that promotes release of the cysteine protease Cathepsin S which liberates neuronal fractalkine (CX₃CL₁) chemokine domain that further stimulates microglia to secrete pro-nociceptive cytokines via activation of CX₃CR₁ receptor (Clark et al., 2007a; Malcangio, 2019) and 3) *de novo* expression of colony-stimulating factor 1 (CSF1) in injured nociceptors which drives the expression of neuropathic pain-associated genes in dorsal horn microglia through binding to CSF1 receptor/DAP12 (Guan et al., 2016). Despite significant progress in our understanding of microglial pathways that facilitate nociceptive transmission, so far very few studies investigated microglial heterogeneous transcriptional changes in chronic pain states. Hence, this review provides an overview on the contemporary framework under which microglial heterogeneity is being studied in the CNS in the context of disease, sex, and intraregional differences. Then against this background, we discuss microglial heterogeneity in chronic pain. Our prediction is that the advent of novel technologies will facilitate a better characterization of functions, transcriptome and structure of microglia and their dynamic role in the establishment and maintenance of chronic pain. This type of approach may lead to the identification of innovative microglial targets for chronic pain treatments.

2. Microglial heterogeneity in the adult CNS

2.1. Membrane markers and morphology

With the aim to understand microglial functions in healthy murine CNS, early studies examined microglial distribution, morphology and membrane marker expression and suggested that CNS regional density accounts for microglia's ability to adapt and respond to local cues from

neuronal environments. Specifically, immunostaining of the membrane marker F4/80 indicates that microglia density in thalamus (101 cells/mm²), hippocampus (120 cells/mm²) and substantia nigra (134 cells/mm²) is higher than in cortex (65 cells/mm²) and cerebellum (73 cells/mm²) (Lawson et al., 1990). Furthermore, the high degree of microglia spatial organization prompted investigations into possible region-specific expression of immunoregulatory membrane markers (CD11b, CD40, CD45, CD80, CD86, F4/80, TREM2b and CCR9) (de Haas et al., 2008). Indeed, regional differences do exist as *ex vivo* flow cytometry shows that CD11b expression is high in spinal microglia, whereas CD40 is lower in cortical microglia than in cerebellum. Moreover, hippocampal microglia express the lowest levels of CD45, CD80, CXCR3, and CCR9 and the highest expression for F4/80 (de Haas et al., 2008). Independently from the extent of expression, all markers can be detected in microglia derived from all brain regions (de Haas et al., 2008), indicating that in the healthy CNS, microglia share a common homeostatic profile of membrane markers, which are finely tuned by local microenvironments. Furthermore, microglia morphology can be affected by CNS cytoarchitecture and there are several pieces of evidence to support this conclusion. For example, measurements of areas and perimeters of F4/80⁺ cells show that grey matter microglia possess longer primary processes and more complex arborization compared to white matter microglia (Lawson et al., 1990). Likewise, three-dimensional reconstruction of individual cells indicates that microglia morphology varies significantly between the basal ganglia nuclei since cells exhibit complex process structure in the substantia nigra and display fewer branches and less coverage of microenvironment in the ventral tegmental area (VTA) (De Biase et al., 2017). Then, automated confocal tissue imaging coupled with morphological modelling reveals that cerebellar microglia have smaller cell bodies but significantly larger cytoplasm compared to microglia in frontal cortex, hippocampus and striatum (Verdonk et al., 2016). Moreover, the mean complexity of primary ramifications is higher in striatum and cortex, whilst microglia in cerebellum shows lower complexity (Verdonk et al., 2016).

2.2. Transcriptomic heterogeneity across the CNS

Under control conditions, microglia homeostatic profiles are associated with the constant expression of a cluster of transcripts as revealed by bulk RNAs sequencing analysis of whole brain microglia. These genes, namely the microglia sensome, encode proteins that recognize ligands and microbes and include P2ry12, Tmem119, Cx3cr1, Csf1r, Dap12, Trem2, and Cd33 (Hickman et al., 2013). Some of these genes are known risk factors for late-onset sporadic AD, suggesting that uncontrolled survey of the environment can have detrimental effects on neuronal function. Transcripts such as P2ry12, P2ry13, Tmem119, Siglech, Trem2 and Cx3cr1 are mostly enriched in microglia, as for instance when compared to peripheral macrophages by cluster analysis, suggesting a significant influence of neuronal microenvironment (Gosselin et al., 2014; Hickman et al., 2013). Moreover, since P2ry12, Fcrls, Tmem119, Olfrml3, Hexb and Tgfbf1 are only enriched in microglia compared to the other CNS cells, these genes are considered as key microglia-enriched homeostatic genes (Bennett et al., 2016; Butovsky et al., 2014) (Fig. 1). Similar conclusions can be drawn about the human microglial homeostatic transcriptome outlined by RNA sequencing of microglia isolated from post-mortem (Galatro et al., 2017) or surgically-removed (Gosselin et al., 2017) tissue. Specifically, microglia express a conserved core gene program of orthologous genes from rodents to humans, namely P2RY12, CX3CR1, C3, CSFR1, C1QA-C. Nonetheless, notable differences also emerge as human microglia express several human-restricted transcripts, such as CD58, GNLY, CLECL1, CD89, and APOBEC3C, involved in immune pathways, which may reflect functional dissimilarities between species (Galatro et al., 2017; Geirsdottir et al., 2019).

These observations collectively suggest that there is a unique microglia transcriptomic signature, termed homeostatic, which enables

Fig. 1. Microglial heterogeneity in the Alzheimer's brain and systemic inflammation - Characteristic genes of homeostatic and disease associated microglia (DAM) transcriptional profiles. A) Under healthy conditions microglia express a cluster of transcripts encoding proteins for sensing endogenous ligands and neuronal activity. B) Microglia in the AD brain respond to amyloid pathology by shifting to a distinct transcriptional profile, termed disease-associated microglia (DAM), which are characterized by the downregulation of several homeostatic genes while a subset of core genes related to environmental interactions is significantly dysregulated. Please note that the genes included in this figure have been identified in AD mouse models (Keren-Shaul et al., 2017; Krasemann et al., 2017). C) Systemic inflammation induces a distinct population of microglia in the brain, characterized by the downregulation of several homeostatic genes and the upregulation of classical pro-inflammatory genes (Sousa et al., 2018).

microglial surveillance of the healthy CNS. However, they also raise the intriguing question of how a set of gene transcripts can account for a multitude of functions and compartment-specific heterogeneity of microglia. The answer is that interregional differences do exist and they do not represent separate microglial populations but rather by a transcriptional continuum of the homeostatic gene signature. For instance, coupling transcriptomics analysis with deep microscopy of microglia in the basal ganglia, has revealed that microglia express key homeostatic genes and the highest expression in substantia nigra (De Biase et al., 2017), similar to early observations (Lawson et al., 1990). Regional differences can be observed in lysosome content and transcripts involved in oxidative phosphorylation and cell metabolism; for example, in the ventral tegmental area (VTA), microglia has low lysosome content and low expression of relevant genes, suggesting that metabolic requirements may be associated with interregional variability (De Biase et al., 2017).

This evidence is substantiated in depletion paradigms which allow microglia to repopulate and re-establish region-specific properties and demonstrate that the microenvironment is sufficient to induce interregional variability at both transcriptional and functional level (De Biase et al., 2017). In addition, power component analysis has demonstrated, that despite sharing the expression of several key homeostatic genes, microglia in cerebellum and hippocampus are more immune vigilant (and express *Clec7a*, *Stat1*, *Stat4*, *Ifit2*, *H2-D1* and *H2-Aa*) than in striatum and cortex where energy metabolism constitutes the most enriched signalling pathway (e.g. *Cd47* and *Cd300a*) (Grabert et al., 2016). Moreover, microglia in cerebellum acquire a phagocytic phenotype (with expression of *Apoe*, *Axl*, *Cd74*, *Lilrb4*, and *Ms4a7*) that controls clearance of dying cells (Ayata et al., 2018), while striatal microglia engage with surveillance of neuronal activation, which is inextricably linked to suppression of neuronal activity (Badimon et al., 2020). Recent single-cell transcriptomics studies have revealed limited heterogeneity in murine microglia isolated from the cortex, cerebellum, hippocampus, and striatum (Li et al., 2019; Masuda et al., 2019), highlighting that further analysis is required to fully resolve this discrepancy. As an example, high-throughput sequencing and spatial

transcriptomics with mechanistic analyses, would help establish how regional cues shape microglia homeostatic identity in the healthy CNS.

2.3. Microglial heterogeneity in sexes

Heterogeneity in microglial gene expression profiles can be observed between males and females in health and disease. It is becoming increasingly appreciated that a combination of intrinsic (sex chromosome) and extrinsic (hormone exposure) factors modulate microglial function (Villa et al., 2019). Microglia differentiate in a sex-dependent fashion in the adult mouse hippocampus, where transcriptional sex differences correlate with ultrastructural differences of microglia. For instance, male microglia display higher volume and number of branches, as well as total area covered (Hanamsagar et al., 2017). Nevertheless, basal phagocytosis of fluorescence beads in cortical and hippocampal slices is comparable between males and females (Guneykaya et al., 2018). Moreover, male microglia in cortex and hippocampus show higher soma size and antigen-presenting compared to female microglia (Guneykaya et al., 2018). These differences are also reflected at transcriptional level as next-generation sequencing shows increased expression of *P2rx7*, *P2rx4*, and *P2ry12* in male and *Irf3* in female microglia (Guneykaya et al., 2018). In line with these results, male, but not female, microglia are enriched in genes involved in inflammatory processes such as *Irf7*, *Il1rm*, *C3*, *C4a* and *C3ar1* (Villa et al., 2018). Remarkably, sex differences in microglia appear not to be dependent on circulating sex hormones, as female microglia transplanted to male mouse brain retained their initial properties and mediate neuroprotection (Villa et al., 2018), perhaps suggesting a permanent distinction established during embryo development. If sex differences were to be found in human microglial responses, then an intriguing question would be to figure out whether sex-dependent propensity to neuroinflammation contributes to different susceptibility to neurological disorders between the sexes. Indeed, the concept that microglia constitute an active player in the onset and progression of neurological and neurodegenerative diseases is a breakthrough in the field.

2.4. Microglial heterogeneity in neurological and neurodegenerative diseases

In mouse models of AD, brain microglia respond to amyloid pathology by acquiring a distinct profile which is termed disease-associated microglia (DAM). This profile is characterized by down-regulation of homeostatic genes such as *Cx3cr1*, *Tmem119* and *P2ry12* and up-regulation of other genes such as *Trem2*, *ApoE* and *Ctsb* (Keren-Shaul et al., 2017; Krasemann et al., 2017; Sala Frigerio et al., 2019) (Fig. 1). Consistent with microglia's susceptibility to respond to microenvironmental changes, DAM signature is inextricably linked to microglia in the vicinity of amyloid plaques (Hammond et al., 2019; Keren-Shaul et al., 2017; Sala Frigerio et al., 2019). Thus, DAMs constitute a fraction of the microglia population in diseased brains as most microglia express homeostatic genes (Keren-Shaul et al., 2017). The unique transcriptional profile of DAMs adjacent to amyloid pathology is reflected at molecular level (Mrdjen et al., 2018) as these microglia express specific markers such as *ApoE*, *Trem2* and *CD11c* (Kamphuis et al., 2016; Parhizkar et al., 2019). Sub-clusters of brain microglia that show expression profiles similar to DAMs are also found in mouse models of ALS (Krasemann et al., 2017), MS (Hammond et al., 2019; Masuda et al., 2019) and in ageing brain (Hammond et al., 2019; Sala Frigerio et al., 2019), and these observations suggest that microglia is endowed with an innate program in responses in neurodegeneration. Supporting this notion, genes typical of DAM signature are upregulated after exposure to phosphatidylserine, a marker of apoptotic neurons (Krasemann et al., 2017). However, despite the agreement of preclinical data, recent studies in humans have yielded mixed results. Microglia in the prefrontal cortex of AD patients assume a profile reminiscent of DAM in mice, although several AD-associated genes, such as *C1QB* and *CD14*, are not found in the mouse models of AD (Mathys et al., 2019). Adding to complexity, human microglia isolated from AD dorsolateral prefrontal cortex shares minimal similarities with mouse DAM (Zhou et al., 2020), and display upregulation of homeostatic genes such as *Cx3cr1*, *Tmem119* and *P2ry12* (Zhou et al., 2020). We find it rather intriguing that the AD brain microglia transcriptional profile is reminiscent of microglia profile in the dorsal horn of spinal cord in neuropathic pain models (Masuda et al., 2012). Specifically, following peripheral nerve injury, in the dorsal horn, the increased activity of the first pain synapse is associated with a microglial response that includes up-regulation of transcription factor *Irf-8* which regulates the expression of *Tlr2*, *P2ry12*, *Cx3cr1*, *Il1b*, *Ctss*, and *P2rx4* (Masuda et al., 2012). These observations suggest that distant from the site of injury, microglia acquire a well-defined transcriptional profile in response to neuronal hyperexcitability. It is tempting to suggest that, in a similar fashion, in the AD brain microglia shift to such an *Irf-8* driven transcriptional signature in response to dysregulation of neuronal activity. Despite the general enthusiasm towards novel technologies, some concerns are expressed on the limitations of single nucleus (sn)RNA-seq to describe fully human microglial transcriptional profile, as several transcripts such as *APOE*, *CST3*, *SPP1*, and *CD74*, which are typical of disease heterogeneity, are underrepresented in the nuclei compared to whole cells (Thrupp et al., 2020). Furthermore, there are still some limitations for scRNA-seq technologies as low-expression genes are hardly detected, while owing to the low amount of starting material, scRNA-seq studies produce noisier and more variable data compared to bulk sequencing (Gerrits et al., 2020). More knowledge needs to be acquired to define beneficial versus detrimental roles of microglia, and establish whether disease stage, examined area and concomitant diseases might account for the disparate data generated in human studies.

2.5. Unique microglial signatures in response to systemic inflammation

Systemic inflammation can influence microglia phenotype in the CNS through intricate crosstalk mechanisms, including infiltration of peripheral immune cells and cytokines through the blood–brain barrier

(BBB). For example, in a model of acute inflammation, 24 h after a single systemic injection of lipopolysaccharide (LPS) (Gram-negative bacterial endotoxin) (4 mg/kg intraperitoneal (i.p.)), brain microglia shift from a homeostatic state to a heterogeneous reactive signature. Microglia upregulate *Il1b*, *Tnf* and *Ccl2* and downregulate *Fcrls*, *Tmem119*, *Siglech*, *Gpr34*, *P2ry12*, *Tyrbp* and *Trem2* (Sousa et al., 2018) (Fig. 1), and this phenotype bears little similarities to the DAM signature observed in models of neurodegenerative disorders, suggesting that separate gene modalities regulate microglia responses to peripheral inflammation and neurodegeneration (Sousa et al., 2018). Microglial transcriptional heterogeneity in response to systemic LPS may be the result of spatial heterogeneity as well as diverse proliferation properties of microglia in specific CNS compartments (Sousa et al., 2018). For instance, after LPS administration (100 ug/kg i.p.), microglia changes can be observed in the circumventricular organs (organum of vasculosum of lamina terminalis, subfornical organ and area postrema), all characterized by high vascular permeability, but not in hippocampus and cortex (Furube et al., 2018). Similarly, in the TNF transgenic (TNFtg) model of Rheumatoid Arthritis (RA), systemic inflammation is associated with i) down-regulation of homeostatic genes, ii) a distinct microglia transcriptional signature (enrichment in genes involved in inflammatory signalling: *Cxcl13*, *Ccl2*, *Ccl8*, *C1s1*, *C3*, *C4b*) and iii) expression of activation markers (*CD45⁺* and *CD169⁺* in *IBA-1⁺* cells) in thalamic, striatal and cortical, but not hippocampal and cerebellar microglia (Süß et al., 2020). We speculate that these observations may prove informative on a possible role of microglia in arthritis pain, as the TNFtg model is characterized by hind paw mechanical hypersensitivity, and increased neuronal metabolic activity in pain-related areas such as thalamus and somatosensory cortex following the application of thermal noxious stimuli, as assessed by functional MRI (Hess et al., 2011). It is possible that a combination of inflammatory factors from the periphery and neuronal activation in the pain pathways affect transcriptional and molecular changes of microglia in pain-related areas. Future studies will improve our understanding of the mechanisms which control the interplay between peripheral signals and microglia, and orchestrate disruption of microglial homeostatic transcriptional profile in response to peripheral inflammation.

3. Chronic pain and microglial heterogeneity

3.1. Chronic pain

In contrast to acute pain which is an adaptive process and a defensive mechanism from harmful stimuli, chronic pain is maladaptive, outlasts noxious stimuli and poorly controlled by current analgesics. Harmful stimuli are detected in the periphery of the body by specialized sensory neurons, namely the nociceptors, which transmit electrical impulses to the dorsal horn of the spinal cord. From this first relay station, nociceptive signalling continues to the higher centres in the brain where pain is perceived and subcortical and cortical structures are engaged to modulate pain (Clark et al., 2007a, 2007b; Nieto et al., 2016). Lesions to the peripheral nervous system, as caused by mechanical nerve trauma, and damage to tissues, as in diseases such as RA, can result in long-lasting neuropathic and inflammatory pain, respectively. Under chronic pain conditions, the increased nociceptive input from the periphery triggers the physiological plasticity and long-lasting transcriptional and post-translational changes in CNS neurons defined as central sensitization. Such sensitized pain system leads to exaggerated responses to noxious stimuli (hyperalgesia) and responses to non-noxious stimuli (allodynia). Furthermore, microglia in the vicinity of sensitized neurons respond to the neuronal activity and contribute to central sensitization mechanisms as well as to the development and maintenance of allodynia and hyperalgesia (Malcangio, 2019). Specifically, preclinical data demonstrate a correlation between allodynia and microgliosis in the dorsal horn of the spinal cord, and microgliosis can also be observed in pain-related regions of the brain such as the ventral tegmental area and

the infralimbic cortex (Chung et al., 2017; Taylor et al., 2017) (Table 1). However, there are instances in which neuropathic allodynia is not associated with microgliosis and microglia undergo morphological changes as well as transcriptional changes that can regulate nociceptive mechanisms (Colburn et al., 1997; Ji et al., 2013; Tsuda et al., 2003; Ulmann et al., 2008). Human studies are in agreement with the mounting evidence of microglial involvement in chronic pain mechanisms (Albrecht et al., 2019).

3.2. Transcriptomics data indicative of microglial functions in the spinal cord

Whilst there is a large body of research that provides evidence for microglia pronociceptive properties and identifies microglial targets for pain relief, a definition of microglial heterogeneity in either preclinical or clinical settings has just started to be addressed. The comparison between genes involved in DAM signature and transcriptomics data from microglia under neuropathic pain conditions has revealed minimal or no correlation (Fig. 1 and Fig. 2), as dorsal horn microglia are enriched with Ctss, P2ry12, Fcrls, Cx3Cr1, Csfr1, Tlr4, Tnf, Il1b and C5ar1 (Denk et al., 2016; Jeong et al., 2016; Krasemann et al., 2017). This has also been observed at the protein level, as both P2Y12 and CX3CR1 levels are increased in spinal microglia after nerve injury, further highlighting that the DAM signature is not encountered in models of chronic pain (Kobayashi et al., 2008; Tozaki-Saitoh et al., 2008). This evidence also suggests that homeostatic transcript dysregulation in disease can undergo up-regulation as well as down-regulation. At 7 days after spinal nerve ligation, when allodynia is highly significant, dorsal horn microglia transcriptional profiling shows expression of C4b, Cd244, Cst1, Stat1, Cst7 and Ifi30, and unchanged expression of homeostatic genes such as Cx3Cr1, Cst3, P2ry12, Fcrls and Csfr1 (Denk et al., 2016). Gene ontology (GO) enrichment analysis of the upregulated genes has identified involvement of alternative complement pathways suggesting that complement-mediated regulation of synaptic activity in the dorsal horn of the spinal cord might be a typical feature of neuropathic pain (Denk et al., 2016). Moreover, alterations of interferon-gamma (IFN γ) pathways suggest a specific influence of

immunomodulatory factors on the microglial response in neuropathic pain (Denk et al., 2016). Consistently, microarray gene profiling of dorsal horn microglia at day 1 and day 7 after nerve injury, has disclosed differential expression of 559 genes and these include Tlr4, Tlr3, Tlr7, Il6, C5ar1 and Gria1 (Jeong et al., 2016). Indeed, microglia display transcriptomic heterogeneity after peripheral nerve injury, with early-phase (day 1) dysregulated genes which are linked to complement and chemotaxis pathways, and late-phase (day 7) genes involved in the interferons and phospholipid metabolism pathways (Jeong et al., 2016). Following sciatic nerve injury, microglia proliferate in the mediolateral dorsal horn which is the termination area for the central axons of injured primary afferent fibres, and *de novo* release of CSF-1 by injured fibres plays a significant role in such proliferation (Gu et al., 2016; Guan et al., 2016) (Fig. 2). This scenario raises the prospect that microglia in the vicinity of injured fibre terminals would be more likely to adapt to neuronal changes, while microglia in neighbouring neurons would maintain a homeostatic profile. In this context, there is evidence that ipsilateral dorsal horn microglia undergo a CSF1R-DAP12-dependent induction of Itgam, Cx3cr1, Bdnf and Ctss (Clark et al., 2007a; Clark and Malcangio, 2012) (Fig. 2). Considering that these genes are implicated in neuropathic pain mechanisms (Guan et al., 2016; Staniland et al., 2010), their expression is indicative of a specific neuropathic pain signature in microglia. Much less progress has been made in the inflammatory pain settings in which no dorsal horn microglial transcriptomic changes could be detected in the late phase of the collagen antibody-induced arthritis model of RA (Fernandez-Zafra et al., 2019). Yet, it is noteworthy that the extent of dorsal horn microglia proliferation and activation following joint/paw inflammation is less significant than after nerve injury (Staniland et al., 2010) (Fig. 2). Therefore, to identify a specific signature, microglia will need to be isolated exclusively from the superficial laminae of the dorsal horn where the central terminals of the joint/paw nociceptors engage with microglia.

Although we possess preclinical evidence that microglia alter their transcriptional signatures in response to increased neuronal activity which is associated with allodynia and hyperalgesia, more studies are required to be able to mechanistically link transcriptional signatures to functional outputs in the context of chronic pain. Indeed, microglia are

Table 1

An overview of animal models of neuropathic and inflammatory pain that have been used by studies cited in this review.

Model of chronic pain	Injury	Description	Microglia/Behaviour	Reference
Spared Nerve Injury (SNI)	Axotomy of tibial and common peroneal nerves, the sural nerve is left intact	The mechanical allodynia and thermal hyperalgesia are established 3 days after injury and are maintained for several weeks.	Spared nerve injury induces persistent spinal cord microgliosis up at least up to day 14. Microgliosis in several brain regions has also been reported.	Chung et al., 2017; Taylor et al., 2017; Guan et al., 2016; Sorge et al., 2011, 2015; Li et al., 2016; Liu et al., 2017; Gui et al., 2016; Xu et al., 2017; Mai et al., 2021; Clark et al., 2015
Sciatic nerve ligation (SNL)	Tight ligation of one-third to half of sciatic nerve	The mechanical allodynia and thermal hyperalgesia are established 3 days after injury and are maintained at least for a month.	Sciatic nerve ligation is associated with increased dorsal horn microglia at all time points (Day 7,14,21).	Denk et al., 2016; Staniland et al., 2010
Spinal nerve transection (SNT)	Transection of lumbar spinal nerves	Alterations in behaviour such as mechanical allodynia and thermal hyperalgesia have been noted to develop within the first day and persist for several weeks post-injury	Microgliosis is present at Day 3 surgery, and Day 7 post surgery, in parallel to the development of mechanical hypersensitivity	Jeong et al., 2016; Gu et al., 2016
Chronic constriction injury (CCI)	Four loose ligatures around the sciatic nerve	The behavioral changes have been noted to develop within a week, later peak during the second week and persist for at least 7 weeks after the surgery	Microgliosis is prevalent in the spinal cord following the increased allodynia at least until week 8. Microgliosis in the amygdala and mPFC can be observed 4- and 8-weeks post-surgery.	Barcelon et al., 2019 but also see Colburn et al., 1997
Collagen antibody-induced arthritis (CAIA)	Intravenous injection with an anti-collagen type-II cocktail containing 5 different monoclonal autoantibodies	CAIA induces transient joint inflammation and persistent mechanical hypersensitivity in male and female mice up to Day 54	Microgliosis was associated with hypersensitivity both at Day 15 (acute phase) and at Day 54 (chronic phase).	Fernandez-Zafra et al., 2019
Collagen-induced arthritis (CIA)	Intradermal injection at the base of the tail with collagen/CFA emulsion	CIA induces mechanical hypersensitivity seven days after the injection and persists at least up to day 14.	Mechanical hypersensitivity and microgliosis were prevalent after day 7 after collagen immunization in the absence of noticeable paw swelling.	Nieto et al., 2016

Fig. 2. Microglial responses in various regions of the CNS in chronic pain states. A) In health, spinal microglia express a cluster of transcripts such as Cx3cr1, P2ry12 and Tmem119, which are characteristic transcripts of the homeostatic microglial profile. B) After nerve injury, microglia proliferate in the mediolateral dorsal horn and upregulate well-characterised transcripts such as Ctss, Cx3cr1, Itgam, and Bdnf, hinting the existence of neuropathic pain associated microglia. C) Although extensive microgliosis is present at the dorsal horn in mouse models of inflammatory pain, little is known about the transcriptomic underpinnings that regulate microglia proliferation and activation. C) Preclinical and clinical data indicates that compartment-specific activation of microglia in pain-related areas of the CNS might influence nociceptive mechanisms.

well placed to detect and modulate neuronal hyperexcitability in the dorsal horn of the spinal cord, similar to the brain (Badimon et al., 2020; Cserép et al., 2019). Therefore, the interplay of dorsal horn microglia with different neuronal subtypes, and microglial metabolic demands in response to nociceptor activation, would be intriguing.

3.3. Microglial sexual dimorphism in chronic pain

Sex differences have been described to prevail under multiple chronic pain conditions, with women more commonly reporting increased sensitivity to pain (Mogil, 2020). Several recent reviews have discussed immune-mediated sex differences in chronic pain both in preclinical and clinical settings (Mogil, 2020; Sorge and Strath, 2018). Hence, here we will only briefly introduce paradigm-shifting work that has been insightful as to how microglial and immune heterogeneity endows sex-dependent pain sensitivity.

In a seminal study, Sorge et al. reported that the intrathecal administration of glial inhibitors in the lumbar spinal cord reverses neuropathic mechanical hypersensitivity in male, but not female mice (Sorge et al., 2015). Specifically, even though microgliosis is observed in both sexes (Lopes et al., 2017; Mapplebeck et al., 2018; Nieto et al., 2016), microglia are integral to the development of neuropathic pain in male mice, since female mice engage the adaptive immune system (Sorge et al., 2015). However, the relevance of T cell-mediated mechanisms in female microglia remains to be consolidated as the extent of T cells infiltration in the dorsal horn under neuropathic pain conditions is under debate (Denk et al., 2016; Gattlen et al., 2016; Malcangio, 2017). Further microglial sexual dimorphic response has been seen following

intrathecal administration of LPS which is associated with allodynia (Sorge et al., 2011). Again, only male mice spinal cord microglia manifest TLR4-sensitive allodynia, although TLR-4 expression does not differ between male and female spinal microglia, supporting the possibility that microglial responses can be sex-dependent (Sorge et al., 2011).

Overall, preclinical evidence suggests that microglia play mechanistic roles in the development of allodynia in male and less so in female. Future studies will shed light on whether distinct microglial pathways and immune cells are engaged under chronic pain conditions in males and females.

3.4. Differences between spinal and cerebral microglia in chronic pain

Accumulating evidence suggests that spinal cord microglia response to an insult can differ from brain microglia (Xuan et al., 2019). Upon virus infection, spinal cord microglia show higher expression of membrane markers (such as CD45 and CD11b) than brain microglia (Olson, 2010), in agreement with evidence for regional diversity of immunoregulatory protein expression (de Haas et al., 2008), and further supporting regional differences in microglial functions discussed above. Notwithstanding that bulk RNA-seq analysis of brain and spinal cord microglia reveals that a subset of key homeostatic genes is similarly expressed under normal conditions, the expression levels of genes involved in microglial activation (Clec7a, Spp1, Lpl, Axl, Ms4a4c, and Ms4a6c) and interferon signalling (Ifi204, Ifi207, Ifi209, and Oasl2) is higher in spinal cord than brain microglia (Wang et al., 2020). Of note, Tgfb1 higher expression in spinal microglia is in line with Tgf-β pathway effects on microglia survival and neuroprotective roles in

neurodegeneration (Butovsky et al., 2014; Wang et al., 2020), and P2ry12 higher expression is indicative of microglia-mediated suppression of hyperexcitability and maintenance of homeostasis (Cserép et al., 2019; Wang et al., 2020). On a similar vein, one of the first studies to observe that microglia respond differently to neuropathic allodynia in different areas of the CNS in rats demonstrated upregulation of CD11b/c and MHCII, and MHCII⁺/CD172a⁺ ratio in microglia in the spinal dorsal horn, in contrast to the prefrontal cortex where these markers were downregulated (Li et al., 2016). After peripheral nerve injury, there is evidence for microglia-derived TNF- α mediating an increase in excitatory synaptic connectivity in the dorsal horn of the spinal cord and a decrease in hippocampal connectivity (Liu et al., 2017). Most importantly, microglia are responsible for both structural and electrophysiological changes of synaptic plasticity in response to peripheral injury in the CNS as both neuropathic allodynia and changes in neuronal connectivity can be prevented by microglia depletion (Liu et al., 2017).

Altogether, this evidence suggests that microglia in the spinal cord behave differently from brain microglia under healthy and chronic pain conditions and suggest that further elucidation of regional differences may lead to the identification of innovative and region-specific therapeutic targets.

4. Regional microglia activation in chronic pain states

4.1. Sensory discriminative

In addition to microglia heterogeneous immune responses across the CNS, there is clinical and preclinical evidence that alteration in microglia properties occurs in pain-related areas that are closely linked with both the sensory-discriminative and the affective-motivational component of chronic pain (Austin and Fiore, 2019). Preclinical data in neuropathic pain models indicates that microglia influence nociceptive mechanisms in a variety of brain regions (Fig. 3), as both Iba-1 gene expression and microglia cell-soma size are increased in areas such as contralateral primary sensory cortex, thalamus, ventral tegmental area (VTA), nucleus accumbens (NAc) and amygdala, but not the motor

cortex (Taylor et al., 2017). Furthermore, in a model of central post-stroke pain (CPSP), which is characterized by hyperalgesia and allodynia, supraspinal microglia undergoes microgliosis in thalamus and primary somatosensory cortex (Hiraga et al., 2020). Since microglia depletion prevents the development of pain-like behaviour, the assumption is that microglia assume a pronociceptive phenotype in the thalamus which is integral to the development of allodynia (Hiraga et al., 2020). Notably, human studies have also outlined that microglial responsiveness is critical in chronic pain, since PET-imaging studies using the TSPO-¹¹C-PBR28 tracer in people with low back pain showed glial activation in both thalamus and paracentral lobule of the somatosensory cortex (Loggia et al., 2015). These results are corroborated in people with fibromyalgia, where upregulation of TSPO-¹¹C-PBR28 tracer binding can be observed in the cortex and positively correlates to fatigue (Albrecht et al., 2019) (Fig. 3). Nonetheless, another study using the same tracer to detect brain neuroinflammation in people with RA, did not detect any significant changes compared to healthy individuals, yet it described a correlation between lower cerebral TSPO binding and higher disease activity, although it should be noted that the majority of the RA patients were on therapeutic treatment with common disease-modifying anti-rheumatic drugs, like methotrexate (Forsberg et al., 2019). Here, it is critical to mention that TSPO-¹¹C-PBR28 tracer results are not directly indicative of microglia functional roles as TSPO is expressed by microglia, astrocytes and endothelial cells (Turkheimer et al., 2015).

Altogether, these findings suggest that microglial activation takes place in the sensory-discriminative pain pathway in chronic pain states. It remains to be addressed whether the interaction between microglia and neuronal activity in the sensory-discriminative areas provide a mechanistic understanding of how supraspinal microglia are affected and regulate the neuronal outputs that generate hypersensitivity or antinociception.

4.2. Affective emotional comorbidities

Chronic pain is frequently co-morbid with affective disturbances,

Fig. 3. Microglial responses in brain areas associated with the sensory-discriminative and affective-motivational components of chronic pain. A simplified depiction of the growing evidence stemming from preclinical and clinical studies showing the activation of microglia in brain regions involved in processing the sensory and the emotional components of pain. Microglia responses include increased expression of CD68, p-p38, Iba1, as well as enlargement of the cell soma and microgliosis. VTA- ventral tegmental area, PAG- periaqueductal gray, NAc- nucleus accumbens, PFC- prefrontal cortex.

such as depression, anhedonia and cognitive decline (Bushnell et al., 2013). There are multiple reports on preclinical neuropathic pain as being accompanied by depression-like behaviour, memory deficits, with recent studies linking aberrant microglial functions with the development of such symptoms (Austin and Fiore, 2019; Barcelon et al., 2019; Fiore and Austin, 2019). For instance, several weeks after the development of neuropathic allodynia, the detection of depression-like behaviour is associated with microgliosis, increased p-p38 MAPK/IBA-1 co-staining and soma enlargement in hippocampus, amygdala and medial prefrontal cortex (mPFC) (Barcelon et al., 2019; Fiore and Austin, 2019) (Fig. 3). Such regional heterogeneity in microglial activation may contribute to the development of depressive-like behaviour, as it only manifested with concomitant behavioural disturbances (Fiore and Austin, 2019). Under these circumstances, in PFC, NAc and hippocampus, microglia become more phagocytic, overexpress CD68 (marker of activation) and release pro-inflammatory cytokines which can give rise to memory deficits, and depression-like behaviour following peripheral nerve injury (Gui et al., 2016; Xu et al., 2017). There is also evidence that peripheral cytokines can activate microglia and affect neuronal properties in a region-specific manner (Clark et al., 2015; Liu et al., 2017). The suggestion is that following peripheral nerve injury, locally-produced IL-1 β can act as a mediator of immune-to-brain signalling that leads to increased microglia density in brain regions associated with memory deficits and depression, which are not causally related to neuropathic allodynia (Gui et al., 2016). Specifically, an anti-IL-1 β antibody and genetic deletion of IL-1 β receptor (IL-1R1) can prevent IL-1 β up-regulation and microgliosis in the PFC, hippocampus, amygdala and nucleus accumbens, as well as reverse depression-like behaviours in neuropathic conditions (Gui et al., 2016) (Fig. 3). Memory impairment associated with neuropathic pain has also been recently linked to circulating leukocytes and plasma C-X-C motif chemokine 12 (CXCL12) which can affect microgliosis in some regions of the brain (Mai et al., 2021). Evidence indicates an increase of CD45⁺CD11b⁺Ly6C^{high} circulating monocytes up to 21 days after the nerve injury and increased microgliosis and perivascular macrophage number in hippocampus of mice of both sexes (Mai et al., 2021). Microgliosis is observed in thalamus, somatosensory cortex S1, amygdala and cingulate cortex nine days after the injury (Mai et al., 2021) (Fig. 3). Critically, clodronate liposome-mediated depletion of monocytes within 3 days after nerve injury, can attenuate microgliosis in the hippocampus and prevent memory deficits, suggesting that peripheral immune cells constantly communicate and regulate regional microglial function in health and disease (Mai et al., 2021). These results underline that aberrant microglia function can be linked to the emotional comorbidities in chronic pain and also support evidence that peripheral inflammation can induce functional changes in microglia.

Collectively, these findings provide evidence that microglia may be involved in the development of some chronic pain co-morbidities and offer the opportunity for future research to define microglial transcriptome and mechanistic insights into neuron-to-microglia interactions.

5. Conclusions

Microglia can assume diverse states to integrate inward and outward signals within microenvironments and under homeostasis, microglia exert multiple functions. Upon stimulation by a pathogen or in disease states, some microglia diverge from homeostatic profiles and acquire distinct modes of activation: cells alter morphology and membrane marker expression, as well as core transcriptional profiles (Mathys et al., 2019; Sala Frigerio et al., 2019). Thus, the association of a microglia subpopulation with a single disease or specific function becomes an oversimplification as activation states can coexist within the same CNS compartments (Ransohoff, 2016). Categorization of microglial profiles is of little value if not accompanied by the specific context (age, sex, region, disease, species) in which these cells are studied. In all cases,

microglia constitute an active player in the onset and progression of neurological and neurodegenerative diseases and they are pivotal to maintain communication with neurons under chronic pain conditions. We can foresee a dramatic expansion of the chronic pain research interest in the role of these cells, which will undoubtedly lead to the identification of innovative targets for analgesia.

Acknowledgements

The authors acknowledge funds from the European Union's Horizon 2020 research and innovation programme "TOBeATPAIN" under the Marie Skłodowska-Curie grant agreement No 764860.

We specially thank Ana Rita Alves da Silva for providing the image showing microgliosis in the dorsal horn of the spinal cord in animals with nerve injury (Fig. 2B).

References

- Albrecht, D.S., Forsberg, A., Sandström, A., Bergan, C., Kadetoff, D., Protsenko, E., Lampa, J., Lee, Y.C., Höglund, C.O., Catana, C., Cervenka, S., Akeju, O., Lekander, M., Cohen, G., Halldin, C., Taylor, N., Kim, M., Hooker, J.M., Edwards, R., Napadow, V., Kosek, E., Loggia, M.L., 2019. Brain glial activation in fibromyalgia – a multi-site positron emission tomography investigation. *Brain. Behav. Immun.* 75, 72–83. <https://doi.org/10.1016/j.bbi.2018.09.018>.
- Austin, P.J., Fiore, N.T., 2019. Supraspinal neuroimmune crosstalk in chronic pain states. *Curr. Opin. Physiol.* 11, 7–15. <https://doi.org/10.1016/j.cophys.2019.03.008>.
- Ayata, P., Badimon, A., Strasburger, H.J., Duff, M.K., Montgomery, S.E., Loh, Y.H.E., Ebert, A., Pimenova, A.A., Ramirez, B.R., Chan, A.T., Sullivan, J.M., Purushothaman, I., Scarpa, J.R., Goate, A.M., Busslinger, M., Shen, L., Losic, B., Schaefer, A., 2018. Epigenetic regulation of brain region-specific microglia clearance activity. *Nat. Neurosci.* 21, 1049–1060. <https://doi.org/10.1038/s41593-018-0192-3>.
- Badimon, A., Ayata, P., Strasburger, H.J., Chen, X., Ikegami, A., Graves, S.M., Ishikawa, M., Chan, A.T., Wang, Y.-C., Loh, Y.E., Wheeler, M.A., Jiang, J.X., Robson, S.C., Sebra, R., Kenny, P.J., Surmeier, D.J., Colonna, M., Quintana, F.J., Wake, H., Gradinaru, V., Schaefer, A., 2020. Negative feedback control of neuronal activity by microglia. *Nature*. <https://doi.org/10.1038/s41586-020-2777-8>.
- Barcelon, E.E., Cho, W.-H., Jun, S.B., Lee, S.J., 2019. Brain microglial activation in chronic pain-associated affective disorder. *Front. Neurosci.* 13, 1–10. <https://doi.org/10.3389/fnins.2019.00213>.
- Bayraktar, O.A., Bartels, T., Holmqvist, S., Kleshchevnikov, V., Martirosyan, A., Poliodakakis, D., Ben Haim, L., Young, A.M.H., Batiuk, M.Y., Prakash, K., Brown, A., Roberts, K., Paredes, M.F., Kawaguchi, R., Stockley, J.H., Sabour, K., Chang, S.M., Huang, E., Hutchinson, P., Ullian, E.M., Hemberg, M., Coppola, G., Holt, M.G., Geschwind, D.H., Rowitch, D.H., 2020. Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map. *Nat. Neurosci.* 10.1038/s41593-020-0602-1.
- Bennett, M.L., Bennett, F.C., Liddel, S.A., Ajami, B., Zamanian, J.L., Fernhoff, N.B., Mulinyawe, S.B., Bohlen, C.J., Adil, A., Tucker, A., Weissman, I.L., Chang, E.F., Li, G., Grant, G.A., Hayden Gephart, M.G., Barres, B.A., 2016. New tools for studying microglia in the mouse and human CNS. *Proc. Natl. Acad. Sci. U. S. A.* 113, E1738–E1746. <https://doi.org/10.1073/pnas.1525528113>.
- Bernier, L.-P., Bohlen, C.J., York, E.M., Choi, H.B., Kamyabi, A., Dissing-Olesen, L., Hefendehl, J.K., Collins, H.Y., Stevens, B., Barres, B.A., MacVicar, B.A., 2019. Nanoscale surveillance of the brain by microglia via cAMP-regulated filopodia. *Cell Rep.* 27, 2895–2908.e4. <https://doi.org/10.1016/j.celrep.2019.05.010>.
- Bushnell, M.C., Ceko, M., Low, L.A., 2013. Cognitive and emotional control of pain and its disruption in chronic pain. *Nat. Rev. Neurosci.* 14, 502–511. <https://doi.org/10.1038/nrn3516>.
- Butovsky, O., Jedrychowski, M.P., Moore, C.S., Cialic, R., Lanser, A.J., Gabriely, G., Koeglperger, T., Dake, B., Wu, P.M., Doykan, C.E., Fanek, Z., Liu, L., Chen, Z., Rothstein, J.D., Ransohoff, R.M., Gygi, S.P., Antel, J.P., Weiner, H.L., 2014. Identification of a unique TGF- β -dependent molecular and functional signature in microglia. *Nat. Neurosci.* 17, 131–143. <https://doi.org/10.1038/nn.3599>.
- Chu Sin Chung, P., Panigada, T., Cardis, R., Decosterd, I., Gosselin, R.D., 2017. Peripheral nerve injury induces a transitory microglial reaction in the rat infralimbic cortex. *Neurosci. Lett.* 655, 14–20. <https://doi.org/10.1016/j.neulet.2017.06.037>.
- Clark, Anna K., Gentry, C., Bradbury, E.J., McMahon, S.B., Malcangio, M., 2007a. Role of spinal microglia in rat models of peripheral nerve injury and inflammation. *Eur. J. Pain* 11, 223–230. <https://doi.org/10.1016/j.ejpain.2006.02.003>.
- Clark, A.K., Gruber-Schoffnegger, D., Drdla-Schutting, R., Gerhold, K.J., Malcangio, M., Sandkühler, J., 2015. Selective activation of microglia facilitates synaptic strength. *J. Neurosci.* 35, 4552–4570. <https://doi.org/10.1523/JNEUROSCI.2061-14.2015>.
- Clark, A.K., Malcangio, M., 2012. Microglial signalling mechanisms: cathepsin S and Fractalkine. *Exp. Neurol.* 234, 283–292. <https://doi.org/10.1016/j.expneurol.2011.09.012>.
- Clark, A.K., Yip, P.K., Grist, J., Gentry, C., Staniland, A.A., Marchand, F., Dehvari, M., Wotherspoon, G., Winter, J., Ullah, J., Bevan, S., Malcangio, M., 2007b. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proc. Natl. Acad. Sci.* 104, 10655–10660. <https://doi.org/10.1073/pnas.0610811104>.

- Colburn, R.W., Deleo, J.A., Rickman, A.J., Yeager, M.P., Kwon, P., Hickey, W.F., 1997. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. *J. Neuroimmunol.* 79, 163–175. [https://doi.org/10.1016/S0165-5728\(97\)00119-7](https://doi.org/10.1016/S0165-5728(97)00119-7).
- Coull, J.A.M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M.W., De Koninck, Y., 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021. <https://doi.org/10.1038/nature04223>.
- Cserép, C., Pósfai, B., Lénárt, N., Fekete, R., László, Z.I., Lele, Z., Orsolits, B., Molnár, G., Heindl, S., Schwarcz, A.D., Ujvári, K., Környei, Z., Tóth, K., Szabadits, E., Sperlág, B., Baranyi, M., Csiba, L., Hortobágyi, T., Maglóczy, Z., Martincz, B., Szabó, G., Erdélyi, F., Szipőcs, R., Tamkun, M.M., Gesierich, B., Duering, M., Katona, I., Liesz, A., Tamás, G., Dénes, Á., 2019. Microglia monitor and protect neuronal function via specialized somatic purinergic junctions. *Science* (80-). 6752, eaax6752. <https://doi.org/10.1126/science.aax6752>.
- Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., Gan, W.B., 2005. ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.* 8, 752–758. <https://doi.org/10.1038/nn1472>.
- De Biase, L.M., Schuebel, K.E., Usvfeld, Z.H., Jair, K., Hawes, I.A., Cimbro, R., Zhang, H. Y., Liu, Q.R., Shen, H., Xi, Z.X., Goldman, D., Bonci, A., 2017. Local cues establish and maintain region-specific phenotypes of basal ganglia microglia. *Neuron* 95, 341–356.e6. <https://doi.org/10.1016/j.neuron.2017.06.020>.
- de Haas, A.H., Boddeke, H.W.G.M., Biber, K., 2008. Region-specific expression of immunoregulatory proteins on microglia in the healthy CNS. *Glia* 56, 888–894. <https://doi.org/10.1002/glia.20663>.
- Denk, F., Crow, M., Didangelos, A., Lopes, D.M., McMahon, S.B., 2016. Persistent alterations in microglial enhancers in a model of chronic pain. *Cell Rep.* 15, 1771–1781. <https://doi.org/10.1016/j.celrep.2016.04.063>.
- Fernandez-Zafra, T., Gao, T., Jurczak, A., Sandor, K., Hore, Z., Agalave, N.M., Su, J., Estelius, J., Lampa, J., Hokfelt, T., Wiesenfeld-Hallin, Z., Xu, X., Denk, F., Svensson, C.I., 2019. Exploring the transcriptome of resident spinal microglia after collagen antibody-induced arthritis. *Pain* 160, 224–236. <https://doi.org/10.1097/j.pain.0000000000001394>.
- Fiore, N.T., Austin, P.J., 2019. Peripheral nerve injury triggers neuroinflammation in the medial prefrontal cortex and ventral hippocampus in a subgroup of rats with coincident affective behavioural changes. *Neuroscience* 416, 147–167. <https://doi.org/10.1016/j.neuroscience.2019.08.005>.
- Forsberg, A., Lampa, J., Estelius, J., Cervenká, S., Farde, L., Halldin, C., Lekander, M., Olgart Höglund, C., Kosek, E., 2019. Disease activity in rheumatoid arthritis is inversely related to cerebral TSPO binding assessed by [11C]PBR28 positron emission tomography. *J. Neuroimmunol.* 334 <https://doi.org/10.1016/j.jneuroim.2019.577000>.
- Furue, E., Kawai, S., Inagaki, H., Takagi, S., Miyata, S., 2018. Brain region-dependent heterogeneity and dose-dependent difference in transient microglia population increase during lipopolysaccharide-induced inflammation. *Sci. Rep.* 8, 1–15. <https://doi.org/10.1038/s41598-018-20643-3>.
- Galat, T.F., Holtman, I.R., Lerario, A.M., Vainchtein, I.D., Brouwer, N., Sola, P.R., Veras, M.M., Pereira, T.F., Leite, R.E.P., Möller, T., Wes, P.D., Sogayar, M.C., Laman, J.D., Den Dunnen, W., Pasqualucci, C.A., Oba-Shinjo, S.M., Boddeke, E.W.G. M., Marie, S.K.N., Eggen, B.J.L., 2017. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat. Neurosci.* 20, 1162–1171. <https://doi.org/10.1038/nn.4597>.
- Gattlen, C., Clarke, C.B., Pillar, N., Kirschmann, G., Pertin, M., Decosterd, I., Gosselin, R. D., Suter, M.R., 2016. Spinal cord t-cell infiltration in the rat spared nerve injury model: A time course study. *Int. J. Mol. Sci.* 17, 1–12. <https://doi.org/10.3390/ijms17030352>.
- Geirsdottir, L., David, E., Keren-Shaul, H., Weiner, A., Bohlen, S.C., Neuber, J., Balic, A., Giladi, A., Sheban, F., Dutertre, C.A., Pfeifle, C., Peri, F., Raffo-Romero, A., Vizoli, J., Matiassek, K., Scheiwe, C., Meckel, S., Mätz-Rensing, K., van der Meer, F., Thormodsson, F.R., Stadelmann, C., Zilkha, N., Kimchi, T., Ginhoux, F., Ulitsky, I., Erny, D., Amit, I., Prinz, M., 2019. Cross-species single-cell analysis reveals divergence of the primate microglia program. *Cell* 179, 1609–1622.e16. <https://doi.org/10.1016/j.cell.2019.11.010>.
- Gerrits, E., Heng, Y., Boddeke, E.W.G.M., Eggen, B.J.L., 2020. Transcriptional profiling of microglia; current state of the art and future perspectives. *Glia* 68, 740–755. <https://doi.org/10.1002/glia.23767>.
- Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M.F., Conway, S.J., Ng, L.G., Stanley, E.R., Samokhvalov, I.M., Merad, M., 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* (80-) 701, 841–845. <https://doi.org/10.1126/science.1194637>.
- Gosselin, D., Link, V.M., Romanoski, C.E., Fonseca, G.J., Eichenfield, D.Z., Spann, N.J., Stender, J.D., Chun, H.B., Garner, H., Geissmann, F., Glass, C.K., 2014. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* 159, 1327–1340. <https://doi.org/10.1016/j.cell.2014.11.023>.
- Gosselin, D., Skola, D., Coufal, N.G., Holtman, I.R., Schlachetzki, J.C.M., Sajti, E., Jaeger, B.N., O'Connor, C., Fitzpatrick, C., Pasillas, M.P., Pena, M., Adair, A., Gonda, D.D., Levy, M.L., Ransohoff, R.M., Gage, F.H., Glass, C.K., 2017. An environment-dependent transcriptional network specifies human microglia identity. *Science* (80-). 356, 1248–1259. <https://doi.org/10.1126/science.aal3222>.
- Grabert, K., Michael, T., Karavolos, M.H., Clohisey, S., Baillie, J.K., Stevens, M.P., Freeman, T.C., Summers, K.M., McColl, B.W., 2016. Microglial brain region-dependent diversity and selective regional sensitivities to aging. *Nat. Neurosci.* 19, 504–516. <https://doi.org/10.1038/nn.4222>.
- Gu, N., Peng, J., Murugan, M., Wang, X., Eyo, U.B., Sun, D., Ren, Y., DiCicco-Bloom, E., Young, W., Dong, H., Wu, L.J., 2016. Spinal microgliosis due to resident microglial proliferation is required for pain hypersensitivity after peripheral nerve injury. *Cell Rep.* 16, 605–614. <https://doi.org/10.1016/j.celrep.2016.06.018>.
- Guan, Z., Kuhn, J.A., Wang, X., Colquitt, B., Solorzano, C., Vaman, S., Guan, A.K., Evans-Reinisch, Z., Braz, J., Devor, M., Abboud-Werner, S.L., Lanier, L.L., Lomvardas, S., Basbaum, A.I., 2016. Injured sensory neuron-derived CSF1 induces microglial proliferation and DAPI2-dependent pain. *Nat. Neurosci.* 19, 94–101. <https://doi.org/10.1038/nn.4189>.
- Gui, W.S., Wei, X., Mai, C.L., Murugan, M., Wu, L.J., Xin, W.J., Zhou, L.J., Liu, X.G., 2016. Interleukin-1 β overproduction is a common cause for neuropathic pain, memory deficit, and depression following peripheral nerve injury in rodents. *Mol. Pain* 12, 1–15. <https://doi.org/10.1177/1744806916646784>.
- Guneaykaya, D., Ivanov, A., Hernandez, D.P., Haage, V., Wojtas, B., Meyer, N., Maricos, M., Jordan, P., Buonfiglioli, A., Glielnievski, B., Ochocka, N., Cömert, C., Friedrich, C., Artiles, L.S., Kaminska, B., Mertins, P., Beule, D., Kettenmann, H., Wolf, S.A., 2018. Transcriptional and translational differences of microglia from male and female brains. *Cell Rep.* 24, 2773–2783.e6. <https://doi.org/10.1016/j.celrep.2018.08.001>.
- Hammond, T.R., Dufort, C., Dissing-Olesen, L., Giera, S., Young, A., Wysoker, A., Walker, A.J., Gergits, F., Segel, M., Nemesh, J., Marsh, S.E., Saunders, A., Macosko, E., Ginhoux, F., Chen, J., Franklin, R.J.M., Piao, X., McCarroll, S.A., Stevens, B., 2019. Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. *Immunity* 1–19. <https://doi.org/10.1016/j.immuni.2018.11.004>.
- Hanamsagar, R., Alter, M.D., Block, C.S., Sullivan, H., Bolton, J.L., Bilbo, S.D., 2017. Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia* 65, 1504–1520. <https://doi.org/10.1002/glia.23176>.
- Hess, A., Axmann, R., Rech, J., Finzel, S., Heindl, C., Kreitz, S., Sergeeva, M., Saake, M., Garcia, M., Kollias, G., Straub, R.H., Sporns, O., Doerfler, A., Brune, K., Schett, G., 2011. Blockade of TNF- α rapidly inhibits pain responses in the central nervous system. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3731–3736. <https://doi.org/10.1073/pnas.1011774108>.
- Hickman, S.E., Kingery, N.D., Ohsumi, T.K., Borowsky, M.L., Wang, L.C., Means, T.K., El Khoury, J., 2013. The microglial sensome revealed by direct RNA sequencing. *Nat. Neurosci.* 16, 1896–1905. <https://doi.org/10.1038/nn.3554>.
- Hiraga, S., Itokazu, T., Hoshiko, M., Takaya, H., Nishibe, M., Yamashita, T., 2020. Microglial depletion under thalamic hemorrhage ameliorates mechanical allodynia and suppresses aberrant axonal sprouting. *JCI Insight* 5. <https://doi.org/10.1172/jci.insight.131801>.
- Jansen, I.E., Savage, J.E., Watanabe, K., Bryois, J., Williams, D.M., Steinberg, S., Sealock, J., Karlsson, I.K., Hägg, S., Athanasou, L., Voyle, N., Proitsi, P., Wittecler, A., Stringer, S., Aarsland, D., Almdahl, I.S., Andersen, F., Bergh, S., Bettella, F., Bjornsson, S., Brækhus, A., Bräthen, G., de Leeuw, C., Desikan, R.S., Djurovic, S., Dumitrescu, L., Fladby, T., Hohman, T.J., Jonsson, P.V., Kiddle, S.J., Rongve, A., Saltvedt, I., Sando, S.B., Selbæk, G., Shoaib, M., Skene, N.G., Snaedal, J., Stordal, E., Ulstein, I.D., Wang, Y., White, L.R., Hardy, J., Hjerling-Lefler, J., Sullivan, P.F., van der Flier, W.M., Dobson, R., Davis, L.K., Stefansson, H., Stefansson, K., Pedersen, N. L., Ripke, S., Andreassen, O.A., Posthuma, D., 2019. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat. Genet.* 51, 404–413. <https://doi.org/10.1038/s41588-018-0311-9>.
- Jeong, H., Na, Y.J., Lee, K., Kim, Y.H., Lee, Y., Kang, M., Jiang, B.C., Il Yeom, Y., Wu, L.J., Gao, Y.J., Kim, J., Oh, S.B., 2016. High-resolution transcriptome analysis reveals neuropathic pain gene-expression signatures in spinal microglia after nerve injury. *Pain* 157, 964–976. <https://doi.org/10.1097/j.pain.0000000000000470>.
- Ji, R.R., Berta, T., Nedergaard, M., 2013. Glia and pain: is chronic pain a gliopathy? *Pain* 154, S10–S28. <https://doi.org/10.1016/j.pain.2013.06.022>.
- Jordão, M.J.C., Sankowski, R., Brendecke, S.M., Sagar, Locatelli, G., Tai, Y.-H., Tay, T.L., Schramm, E., Armbruster, S., Hagemeyer, N., Groß, O., Mai, D., Çiçek, Ö., Falk, T., Kerschensperger, M., Grün, D., Prinz, M., 2019. Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. *Science* (80-). 363, eaat7554. <https://doi.org/10.1126/SCIENCE.AAT7554>.
- Kamphuis, W., Kooijman, L., Schetter, S., Orre, M., Hol, E.M., 2016. Transcriptional profiling of CD11c-positive microglia accumulating around amyloid plaques in a mouse model for Alzheimer's disease. *Biochim. Biophys. Acta – Mol. Basis Dis.* 1862, 1847–1860. <https://doi.org/10.1016/j.bbadis.2016.07.007>.
- Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T.K., David, E., Baruch, K., Lara-Astaiso, D., Toth, B., Itzkovitz, S., Colonna, M., Schwartz, M., Amit, I., 2017. A unique microglia type associated with restricting development of Alzheimer's Disease. *Cell* 169, 1276–1290.e17. <https://doi.org/10.1016/j.cell.2017.05.018>.
- Kobayashi, K., Yamanaka, H., Fukuoka, T., Dai, Y., Obata, K., Noguchi, K., 2008. P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. *J. Neurosci.* 28, 2892–2902. <https://doi.org/10.1523/JNEUROSCI.5589-07.2008>.
- Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., Beckers, L., O'Loughlin, E., Xu, Y., Fanek, Z., Greco, D.J., Smith, S.T., Tweet, G., Humulock, Z., Zrzavy, T., Conde-Sanroman, P., Gacias, M., Weng, Z., Chen, H., Tjón, E., Mazaheri, F., Hartmann, K., Madi, A., Ulrich, J.D., Glatzel, M., Worthmann, A., Heeren, J., Budnik, B., Lemere, C., Ikezu, T., Heppner, F.L., Litvak, V., Holtzman, D. M., Lassmann, H., Weiner, H.L., Ochando, J., Haass, C., Butovsky, O., 2017. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* 47, 566–581.e9. <https://doi.org/10.1016/j.immuni.2017.08.008>.
- Lawson, L.J., Perry, V.H., Dri, P., Gordon, S., 1990. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 39, 151–170. [https://doi.org/10.1016/0306-4522\(90\)90229-W](https://doi.org/10.1016/0306-4522(90)90229-W).

- Li, Q., Cheng, Z., Zhou, L., Darmanis, S., Neff, N.F., Okamoto, J., Gulati, G., Bennett, M. L., Sun, L.O., Clarke, L.E., Marschallinger, J., Yu, G., Quake, S.R., Wyss-Coray, T., Barres, B.A., 2019. Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. *Neuron* 101, 207–223.e10. <https://doi.org/10.1016/j.neuron.2018.12.006>.
- Li, Z., Wei, H., Piirainen, S., Chen, Z., Kalso, E., Pertovaara, A., Tian, L., 2016. Spinal versus brain microglial and macrophage activation traits determine the differential neuroinflammatory responses and analgesic effect of minocycline in chronic neuropathic pain. *Brain. Behav. Immun.* 58, 107–117. <https://doi.org/10.1016/j.bbi.2016.05.021>.
- Liu, Y., Zhou, L.-J., Wang, J., Li, D., Ren, W.-J., Peng, J., Wei, X., Xu, T., Xin, W.-J., Pang, R.-P., Li, Y.-Y., Qin, Z.-H., Murugan, M., Mattson, M.P., Wu, L.-J., Liu, X.-G., 2017. TNF- α differentially regulates synaptic plasticity in the hippocampus and spinal cord by microglia-dependent mechanisms after peripheral nerve injury. *J. Neurosci.* 37, 871–881. <https://doi.org/10.1523/JNEUROSCI.2235-16.2016>.
- Loggia, M.L., Chonde, D.B., Akeju, O., Arabasz, G., Catana, C., Edwards, R.R., Hill, E., Hsu, S., Izquierdo-Garcia, D., Ji, R.R., Riley, M., Wasan, A.D., Zürcher, N.R., Albrecht, D.S., Vangel, M.G., Rosen, B.R., Napadow, V., Hooker, J.M., 2015. Evidence for brain glial activation in chronic pain patients. *Brain* 138, 604–615. <https://doi.org/10.1093/brain/awu377>.
- Lopes, D.M., Malek, N., Edye, M., Jager, S.B., McMurray, S., McMahon, S.B., Denk, F., 2017. Sex differences in peripheral not central immune responses to pain-inducing injury. *Sci. Rep.* 7, 1–8. <https://doi.org/10.1038/s41598-017-16664-z>.
- Mai, C.L., Tan, Z., Xu, Y.N., Zhou, J.J., Huang, Z.H., Wang, D., Zhang, H., Gui, W.S., Zhang, J., Lin, Z.J., Meng, Y.T., Wei, X., Jie, Y.T., Grace, P.M., Wu, L.J., Zhou, L.J., Liu, X.G., 2021. CXCL12-mediated monocyte transmigration into brain perivascular space leads to neuroinflammation and memory deficit in neuropathic pain. *Theranostics* 11, 1059–1078. <https://doi.org/10.7150/thno.44364>.
- Malcangio, M., 2019. Role of the immune system in neuropathic pain. *Scand. J. Pain* 1–5. <https://doi.org/10.1515/sjpain-2019-0138>.
- Malcangio, M., 2017. Spinal mechanisms of neuropathic pain: Is there a P2X4-BDNF controversy? *Neurobiol. Pain* 1, 1–5. <https://doi.org/10.1016/j.jynpai.2017.04.001>.
- Mapplebeck, J.C.S., Dalgarno, R., Tu, Y., Moriarty, O., Beggs, S., Kwok, C.H.T., Halievski, K., Assi, S., Mogil, J.S., Trang, T., Salter, M.W., 2018. Microglial P2X4R-evoked pain hypersensitivity is sexually dimorphic in rats. *Pain* 159, 1. <https://doi.org/10.1097/j.pain.0000000000001265>.
- Marques, S., Zeisel, A., Codeluppi, S., Van Bruggen, D., Falcão, A.M., Xiao, L., Li, H., Häring, M., Hochgerner, H., Romanov, R.A., Gyllborg, D., Muñoz-Manchado, A.B., La Manno, G., Lönnerberg, P., Floridia, E.M., Rezayee, F., Ernfors, P., Arenas, E., Hjerling-Leffler, J., Harkany, T., Richardson, W.D., Linnarsson, S., Castelo-Branco, G., 2016. Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science* (80-). 352, 1326–1329. <https://doi.org/10.1126/science.aaf6463>.
- Masuda, T., Ozono, Y., Mikuriya, S., Kohro, Y., Tozaki-Saitoh, H., Iwatsuki, K., Uneyama, H., Ichikawa, R., Salter, M.W., Tsuda, M., Inoue, K., 2016. Dorsal horn neurons release extracellular ATP in a VOLT-dependent manner that underlies neuropathic pain. *Nat. Commun.* 7, 1–11. <https://doi.org/10.1038/ncomms12529>.
- Masuda, T., Sankowski, R., Staszewski, O., Böttcher, C., Amann, L., Sagar, Scheiwe, C., Nessler, S., Kunz, P., van Loo, G., Coenen, V.A., Reinacher, P.C., Michel, A., Sure, U., Gold, R., Grün, D., Priller, J., Stadelmann, C., Prinz, M., 2019. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* 566, 388–392. <https://doi.org/10.1038/s41586-019-0924-x>.
- Masuda, T., Sankowski, R., Staszewski, O., Prinz, M., 2020. Microglia heterogeneity in the single-cell era. *Cell Rep.* 30, 1271–1281. <https://doi.org/10.1016/j.celrep.2020.01.010>.
- Masuda, T., Tsuda, M., Yoshinaga, R., Tozaki-Saitoh, H., Ozato, K., Tamura, T., Inoue, K., 2012. IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. *Cell Rep.* 1, 334–340. <https://doi.org/10.1016/j.celrep.2012.02.014>.
- Mathys, H., Davila-Velderrain, J., Peng, Z., Gao, F., Mohammadi, S., Young, J.Z., Menon, M., He, L., Abdurrob, F., Jiang, X., Martorell, A.J., Ransohoff, R.M., Hafler, B.P., Bennett, D.A., Kellis, M., Tsai, L.-H., 2019. Single-cell transcriptomic analysis of Alzheimer's disease. *Nature*. <https://doi.org/10.1038/s41586-019-1195-2>.
- Mogil, J.S., 2020. Qualitative sex differences in pain processing: emerging evidence of a biased literature. *Nat. Rev. Neurosci.* 1–13. <https://doi.org/10.1038/s41583-020-0310-6>.
- Mrdjen, D., Pavlovic, A., Hartmann, F.J., Schreiner, B., Utz, S.G., Leung, B.P., Lelios, I., Heppner, F.L., Kipnis, J., Merkler, D., Greter, M., Becher, B., 2018. High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease. *Immunity* 48, 380–395.e6. <https://doi.org/10.1016/j.immuni.2018.01.011>.
- Nieto, F.R., Clark, A.K., Grist, J., Hathway, G.J., Chapman, V., Malcangio, M., 2016. Neuron-immune mechanisms contribute to pain in early stages of arthritis. *J. Neuroinflammation* 13, 96. <https://doi.org/10.1186/s12974-016-0556-0>.
- Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* (80-) 308, 1314–1319. <https://doi.org/10.1126/science.1110647>.
- Olson, J.K., 2010. Immune response by microglia in the spinal cord. *Ann. N. Y. Acad. Sci.* 1198, 271–278. <https://doi.org/10.1111/j.1749-6632.2010.05536.x>.
- Parhizkar, S., Arzberger, T., Brendel, M., Kleinberger, G., Deussing, M., Focke, C., Nuscher, B., Xiong, M., Ghasemigharag, A., Katzmarski, N., Krasemann, S., Lichtenthaler, S.F., Müller, S.A., Colombo, A., Monasor, L.S., Tahirovic, S., Herms, J., Willem, M., Pettkus, N., Butovsky, O., Bartenstein, P., Edbauer, D., Rominger, A., Ertürk, A., Grathwohl, S.A., Neher, J.J., Holtzman, D.M., Meyer-Luehmann, M., Haass, C., 2019. Loss of TREM2 function increases amyloid seeding but reduces plaque-associated ApoE. *Suppl. Nat. Neurosci.* 10.1038/s41593-018-0296-9.
- Ransohoff, R.M., 2016. A polarizing question: Do M1 and M2 microglia exist. *Nat. Neurosci.* 19, 987–991. <https://doi.org/10.1038/nn.4338>.
- Sala Frigerio, C., Wolfs, L., Fattorelli, N., Thrupp, N., Voytyuk, I., Schmidt, I., Mancuso, R., Chen, W.-T., Woodbury, M.E., Srivastava, G., Moeller, T., Hudry, E., Das, S., Saido, T.C., Karran, E., Hyman, B.T., Perry, V.H., Fiers, M., De Strooper, B., 2019. The major risk factors for Alzheimer's disease: age, sex and genes, modulate the microglia response to A β plaques. *Cell Rep.* 1293–1306. <https://doi.org/10.1016/j.celrep.2019.03.099>.
- Saunders, A., Macosko, E.Z., Wysoker, A., Goldman, M., Krienen, F.M., de Rivera, H., Bien, E., Baum, M., Bortolin, L., Wang, S., Goeva, A., Nemesh, J., Kamitaki, N., Brumbaugh, S., Kulp, D., McCarroll, S.A., 2018. Molecular diversity and specializations among the cells of the adult mouse brain. *Cell* 174, 1015–1030.e16. <https://doi.org/10.1016/j.cell.2018.07.028>.
- Sekar, A., Bialas, A.R., De Rivera, H., Davis, A., Hammond, T.R., Kamitaki, N., Tooley, K., Presumey, J., Baum, M., Van Doren, V., Genovese, G., Rose, S.A., Handsaker, R.E., Daly, M.J., Carroll, M.C., Stevens, B., McCarroll, S.A., 2016. Schizophrenia risk from complex variation of complement component 4. *Nature* 530, 177–183. <https://doi.org/10.1038/nature16549>.
- Sierra, A., Encinas, J.M., Deudero, J.J.P., Chancey, J.H., Enikolopov, G., Overstreet-Wadiche, L.S., Tsirka, S.E., Maletic-Savatic, M., 2010. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495. <https://doi.org/10.1016/j.stem.2010.08.014>.
- Sorge, R.E., LaCroix-Fralish, M.L., Tuttle, A.H., Sotocinal, S.G., Austin, J.S., Ritchie, J., Chanda, M.L., Graham, A.C., Topham, L., Beggs, S., Salter, M.W., Mogil, J.S., 2011. Spinal cord toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. *J. Neurosci.* 31, 15450–15454. <https://doi.org/10.1523/JNEUROSCI.3859-11.2011>.
- Sorge, R.E., Mapplebeck, J.C.S., Rosen, S., Beggs, S., Taves, S., Alexander, J.K., Martin, L. J., Austin, J.S., Sotocinal, S.G., Chen, D., Yang, M., Shi, X.Q., Huang, H., Pillon, N.J., Bilan, P.J., Tu, Y., Klip, A., Ji, R.R., Zhang, J., Salter, M.W., Mogil, J.S., 2015. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat. Neurosci.* 18, 1081–1083. <https://doi.org/10.1038/nn.4053>.
- Sorge, R.E., Strath, L.J., 2018. Sex differences in pain responses. *Curr. Opin. Physiol.* 6, 75–81. <https://doi.org/10.1016/j.cophys.2018.05.006>.
- Sousa, C., Golebiewska, A., Poovathingal, S.K., Kaoma, T., Pires-Afonso, Y., Martina, S., Coowar, D., Azuaje, F., Skupin, A., Balling, R., Biber, K., Niclou, S.P., Michelucci, A., 2018. Single-cell transcriptomics reveals distinct inflammation-induced microglia signatures. *EMBO Rep.* 19, 1–17. <https://doi.org/10.15252/embr.201846171>.
- Staniland, A.A., Clark, A.K., Wodarski, R., Sasso, O., Maione, F., D'Acquisto, F., Malcangio, M., 2010. Reduced inflammatory and neuropathic pain and decreased spinal microglial response in fractalkine receptor (CX3CR1) knockout mice. *J. Neurochem.* 114, 1143–1157. <https://doi.org/10.1111/j.1471-4159.2010.06837.x>.
- Stevens, B., Allen, N.J., Vazquez, L.E., Howell, G.R., Christopherson, K.S., Nouri, N., Micheva, K.D., Mehallow, A.K., Huberman, A.D., Stafford, B., Sher, A., Litke, A.M.M., Lambris, J.D., Smith, S.J., John, S.W.M., Barres, B.A., 2007. The classical complement cascade mediates CNS synapse elimination. *Cell* 131, 1164–1178. <https://doi.org/10.1016/j.cell.2007.10.036>.
- Stratoulas, V., Venero, J.L., Tremblay, M., Joseph, B., 2019. Microglial subtypes: diversity within the microglial community. *EMBO J.* 1–18. <https://doi.org/10.15252/emboj.2019101997>.
- Süß, P., Hoffmann, A., Rothe, T., Ouyang, Z., Baum, W., Staszewski, O., Schett, G., Prinz, M., Krönke, G., Glass, C.K., Winkler, J., Schlachetzki, J.C.M., 2020. Chronic peripheral inflammation causes a region-specific myeloid response in the central nervous system. *Cell Rep.* 30, 4082–4095.e6. <https://doi.org/10.1016/j.celrep.2020.02.109>.
- Tan, Y.L., Yuan, Y., Tian, L., 2019. Microglial regional heterogeneity and its role in the brain. *Mol. Psychiatry*. 10.1038/s41380-019-0609-8.
- Taylor, A.M.W., Mehrabani, S., Liu, S., Taylor, A.J., Cahill, C.M., 2017. Topography of microglial activation in sensory- and affect-related brain regions in chronic pain. *J. Neurosci. Res.* 95, 1330–1335. <https://doi.org/10.1002/jnr.23883>.
- Thrupp, N., Sala Frigerio, C., Wolfs, L., Skene, N.G., Fattorelli, N., Poovathingal, S., Fournie, Y., Matthews, P.M., Theys, T., Mancuso, R., de Strooper, B., Fiers, M., 2020. Single-nucleus RNA-Seq is not suitable for detection of microglial activation genes in humans. *Cell Rep.* 32, 108189. <https://doi.org/10.1016/j.celrep.2020.108189>.
- Tozaki-Saitoh, H., Tsuda, M., Miyata, H., Ueda, K., Kohsaka, S., Inoue, K., 2008. P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. *J. Neurosci.* 28, 4949–4956. <https://doi.org/10.1523/JNEUROSCI.0323-08.2008>.
- Tsuda, Makoto, Shigemoto, Mogami, Yukari, Koizumi, Schuichi, Mizokoshi, Akito, 2003. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424, 1–6. <https://doi.org/10.1038/nature01859.1>.
- Turkheimer, F.E., Rizzo, G., Bloomfield, P.S., Howes, O., Zanotti-Fregonara, P., Bertoldo, A., Veronesi, M., 2015. The methodology of TSPO imaging with positron emission tomography. *Biochem. Soc. Trans.* <https://doi.org/10.1042/BST20150058>.
- Ulmann, L., Hatcher, J.P., Hughes, J.P., Chaumont, S., Green, P.J., Conquet, F., Buell, G. N., Reeve, A.J., Chessell, I.P., Rassendren, F., 2008. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J. Neurosci.* 28, 11263–11268. <https://doi.org/10.1523/JNEUROSCI.2308-08.2008>.
- Umpierre, A.D., Wu, L.J., 2020. How microglia sense and regulate neuronal activity. *Glia* 1–17. <https://doi.org/10.1002/glia.23961>.
- Utz, S.G., See, P., Mildenberger, W., Thion, M.S., Silvina, A., Lutz, M., Ingelfinger, F., Rayan, N.A., Lelios, I., Buttgeriet, A., Asano, K., Prabhakar, S., Garel, S., Becher, B., Ginhoux, F., Greter, M., 2020. Early fate defines microglia and non-parenchymal

- brain macrophage development. *Cell* 1–17. <https://doi.org/10.1016/j.cell.2020.03.021>.
- Verdonk, F., Roux, P., Flamant, P., Fiette, L., Bozza, F.A., Simard, S., Lemaire, M., Plaud, B., Shorte, S.L., Sharshar, T., Chrétien, F., Danckaert, A., 2016. Phenotypic clustering: a novel method for microglial morphology analysis. *J. Neuroinflammation* 13. <https://doi.org/10.1186/s12974-016-0614-7>.
- Villa, A., Della Torre, S., Maggi, A., 2019. Sexual differentiation of microglia. *Front. Neuroendocrinol.* 52, 156–164. <https://doi.org/10.1016/j.yfrne.2018.11.003>.
- Villa, A., Gelosa, P., Castiglioni, L., Cimino, M., Rizzi, N., Pepe, G., Lolli, F., Marcello, E., Sironi, L., Vegeto, E., Maggi, A., 2018. Sex-specific features of microglia from adult mice. *Cell Rep.* 23, 3501–3511. <https://doi.org/10.1016/j.celrep.2018.05.048>.
- Wang, P.L., Yim, A.K.Y., Kim, K.W., Avey, D., Czepliewski, R.S., Colonna, M., Milbrandt, J., Randolph, G.J., 2020. Peripheral nerve resident macrophages share tissue-specific programming and features of activated microglia. *Nat. Commun.* 11, 2552. <https://doi.org/10.1038/s41467-020-16355-w>.
- Xu, N., Tang, X.H., Pan, W., Xie, Z.M., Zhang, G.F., Ji, M.H., Yang, J.J., Zhou, M.T., Zhou, Z.Q., 2017. Spared nerve injury increases the expression of microglia M1 markers in the prefrontal cortex of rats and provokes depression-like behaviors. *Front. Neurosci.* 11, 1–12. <https://doi.org/10.3389/fnins.2017.00209>.
- Xuan, F.-L., Chithanathan, K., Lilleväli, K., Yuan, X., Tian, L., 2019. Differences of microglia in the brain and the spinal cord. *Front. Cell. Neurosci.* 13, 1–5. <https://doi.org/10.3389/fncel.2019.00504>.
- Zhou, Y., Song, W.M., Andhey, P.S., Swain, A., Levy, T., Miller, K.R., Poliani, P.L., Cominelli, M., Grover, S., Gilfillan, S., Cella, M., Ulland, T.K., Zaitsev, K., Miyashita, A., Ikeuchi, T., Sainouchi, M., Kakita, A., Bennett, D.A., Schneider, J.A., Nichols, M.R., Beausoleil, S.A., Ulrich, J.D., Holtzman, D.M., Artyomov, M.N., Colonna, M., 2020. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat. Med.* 26, 131–142. <https://doi.org/10.1038/s41591-019-0695-9>.