

This is an Accepted Manuscript of an article published by Elsevier in
Experimental Neurology

Final publication is available at

<http://www.sciencedirect.com/science/article/pii/S0014488612000088>

© 2014. This manuscript version is made available under the CC-BY-NC-ND 4.0
license <http://creativecommons.org/licenses/by-nc-nd/4.0/>

Multiple Mechanisms of Microglia: A Gatekeeper's Contribution to Pain States

Manuel B. Graeber¹ and MacDonald J. Christie²

¹Brain Tumor Research Laboratories,²Neuropharmacology Laboratory, The Brain and Mind Research Institute, University of Sydney, Sydney, Australia

Running title: Microglial control of pain

Address for correspondence:

Professor Manuel B. Graeber MD PhD FRCPATH
Neuropathologist
Barnet-Cropper Chair of Brain Tumour Research
The Brain and Mind Research Institute
University of Sydney
94 Mallett St, Camperdown
Sydney NSW 2050
Australia
Tel: ☐ 61 2 91144008
Fax: ☐ 61 2 93510731
Email: manuel@graeber.net

Abstract

Microglia are gatekeepers in the CNS for a wide range of pathological stimuli and they blow the whistle when things go wrong. Collectively, microglia form a CNS tissue alarm system (Kreutzberg's "sensor of pathology"), and their involvement in physiological pain is in line with this function. However, pathological neuropathic pain is characterized by microglial activation that is unwanted and considered to contribute to or even cause tactile allodynia, hyperalgesia and spontaneous pain. Such abnormal microglial behavior seems likely due to an as yet ill-understood disturbance of microglial functions unrelated to inflammation. The idea that microglia have roles in the CNS that differ from those of peripheral macrophages has gained momentum with the discovery of their separate, pre-haematopoietic lineage during embryonic development and their direct interactions with synapses.

Key words

CNS autonomous and specific mechanisms of microglial activation – diseased microglia – inflammation – normal pain – pathological pain

Introduction

Central pain can be defined as pain arising as a consequence of dysfunction of the central somatosensory system (Finnerup, 2008; Treede et al., 2008). This condition is highly unpleasant and does not serve a useful role, i.e. it is unwanted and pathological. In recent years, microglia have been increasingly implicated in the mechanisms underlying abnormal pain states, notably post-traumatic neuropathic pain. However, central pain can also arise from other acquired conditions such as spinal cord and brain injury, multiple sclerosis, stroke, Parkinson's disease, tumors, and epilepsy (Finnerup, 2008). In particular, spinal cord injury frequently results in intractable chronic central pain syndromes (Knerlich-Lukoschus et al., 2008). Activation of microglia is a feature of all these pathologies so a closer look at the microglial cell and its exact role in central pain seems justified. This article discusses how multiple microglial mechanisms including the release of signaling and effector molecules such as cytokines, chemokines and prostaglandins, microglia-synapse interactions and the endocannabinoid (eCB) pathway may contribute to central pain states. The paper further provides links to individual microglial mechanisms that are discussed in greater detail in the respective articles of this Special issue.

A rapidly growing body of evidence suggests that microglia have a causal role in the pathogenesis of persistent neuropathic pain (Inoue and Tsuda, 2009; Narita et al., 2006). For instance, microglial NADPH oxidase 2-generated reactive oxygen species appear to be involved in the initiation of neuropathic pain in mice (Kim et al., 2010). In line with this view, many compounds, which modify microglial functions successfully, alleviate neuropathic pain in experimental models (Toda et al., 2011). Microglia also seem to be involved in the ethanol-induced neuropathic pain-like state in the rat (Narita et al., 2007) and could play a role in the development of pain following chronic exposure to opioids (Horvath et al., 2010; Zhou et al., 2010). Black and Waxman discuss sodium channels and microglial function in their article of this Special issue and demonstrate that blocking sodium channels significantly attenuates several effector functions of

microglia activated by exposure to ATP and LPS, supporting a role for sodium channel activity in the molecular pathways governing microglial activation. Consequently, improving our understanding of the multiple mechanisms that underlie the microglial involvement in central pain may aid the development of new and more effective pain therapeutics.

Microglia as a tissue alarm system

Activation of microglia in response to pathological stimuli is a very common phenomenon because microglial cells have a very low threshold of activation and respond to essentially all known types of CNS disease states. As a result, microglia have been termed *sensors of pathology* (“a sensor for pathological events in the CNS”) in what has become the most highly cited publication (Web of Science) of the microglia literature (Kreutzberg, 1996). Microglia are normal residents of brain and spinal cord parenchyma. They need to be distinguished from stromal macrophages in a perivascular location both in terms of cellular origin and function as well as from invading blood-borne cells, which can enter the CNS under certain conditions. In contrast, microglia are true parenchymal constituents and directly neighbour nerve cells as well as other glia. Their cell processes, like that of astrocytes, are in close contact with synapses and have been proposed to form the “tetrapartite synapse” (De Leo et al., 2006). We have suggested that microglial cells have an important role in the maintenance of synaptic integrity in normal CNS (Graeber, 2010) and they may have the potential to monitor microelectromagnetic fields (Graeber et al., 2011), which are generated by axons and dendrites. Microglia have been demonstrated to constantly sample their microenvironment (Nimmerjahn et al., 2005), and the microenvironment of synapses in particular (Wake et al., 2009). These functional characteristics put microglia in an ideal position to act as a tissue alarm system.

Microglial activation versus inflammation

The concept of microglial activation was introduced and the term “activated microglial cells” was coined (Graeber et al., 1988) when it became obvious that microglia are capable of up-regulating certain molecules *de novo* that are not normally expressed in the CNS (Graeber et al., 2011). This behavior attests to the

pronounced plasticity of microglial cells, which concerns their morphology as well as their functional capabilities. M1 and M2 polarised states have also been described (Michelucci et al., 2009). Importantly, microglial activation is finely graded as well as dynamic (Graeber et al., 2011). Thus, activated microglia may adopt different phenotypes in response to various stimuli and can become secondarily altered by for example systemic inflammation. Furthermore, their activation appears to be brain region-specific (Choi et al., 2011). These findings indicate that each case of microglial activation has to be assessed in context and that CNS autonomous and specific mechanisms of microglial activation have to be distinguished from external influences such as cytokines that may penetrate the blood-brain-barrier under conditions of systemic infection (Perry, 2010).

Once activated microglial cells can up-regulate a number of molecules that are known to be involved in inflammatory reactions in peripheral organs. This has led to the suggestion that activated microglial cells form part of an inflammatory process in most if not all CNS pathologies. However, the overly generous application of the term “neuroinflammation” to a wide range of CNS conditions is not justified and misleading (Graeber et al., 2011). Accordingly, we do not consider central pain an inflammatory condition. The terminological confusion surrounding microglial activation and inflammation is especially problematic in the field of pain research because there is true inflammatory pain (pain related to inflammation affecting peripheral tissues that are supplied with nociceptive fibers) while spinal microglia activation is not closely correlated with such peripheral inflammation (Lin et al., 2007). Yet, there may be a contributing role of spinal cord-infiltrating CD4 (+) T lymphocytes following nerve injury-induced neuropathic pain (Cao and DeLeo, 2008). Thus, clarity of the terms used to describe microglial activation is of great importance, particularly in association with pain. The neuropathological designation microgliosis refers to population changes of microglia, which occur in various disease settings but not in isolation. Microgliosis is invariably accompanied by astrogliosis but may be dominant (hence the name!). Therefore, the term microgliosis is merely descriptive and not tied to a specific pathology or molecular mechanism. Calvo and Bennett in this Special issue write on it and mechanisms of microglial changes after peripheral nerve injury.

Multiple mechanisms of microglial activation

There is a plethora of ways to get microglia excited. The relevance of this important fact for the field of pain research becomes clear from studies demonstrating that post-injury intrathecal minocycline, a general microglia activation inhibitor, represents an effective therapeutic intervention for treating spinal nerve ligation-induced neuropathic pain as well (Mei et al., 2011). However, the precise molecular mechanisms underlying the microglial involvement in central pain are still being elucidated. Five pathways have attracted special attention in which the following molecules play central roles: **fractalkine** (chemokine (C-X3-C motif) ligand 1; CX3CL1), **interferon-gamma**, **monocyte chemoattractant protein-1** (chemokine (C-C motif) ligand 2; CCL2), **TLR4** (toll-like receptor 4), and **P2X4** (purinergic receptor P2X, ligand-gated ion channel, 4) (Smith, 2010).

P2X4 is an ATP receptor (Inoue, 2006) (also see the article by Trang et al. in this Special issue), and ATP is a known pain mediator (Ulmann et al., 2008). Up-regulation of P2X4 receptors in spinal cord microglia is crucial for tactile allodynia, which is an untreatable pathological pain reaction that occurs after peripheral nerve injury (Biber et al., 2011). The articles in this special issue by Inoue and Tsuda and by Crown focus on the purinergic system and intracellular signaling mechanisms, respectively. Expression of P2X4 receptor by lesional activated microglia during formalin-induced peripheral inflammatory pain has been reported (Guo et al., 2005), and a unifying mechanism has been proposed for pain hypersensitivity after peripheral nerve injury through P2X4R-evoked increase in Ca²⁺ and activation of p38-MAPK leading to the synthesis and exocytotic release of BDNF from microglia (Trang et al., 2009). BDNF is a crucially important pain-signalling molecule that is of relevance for the communication between microglia and neurons and which causes the shift in the neuronal anion gradient that underlies neuropathic pain (Coull et al., 2005). Accordingly, BDNF has been suggested to serve as the final common path in the generation of neuropathic pain (Biggs et al., 2010). Indeed, elevated BDNF expression in the dorsal horn appears to be required for the development

and maintenance of neuropathic pain, and minocycline can only prevent mechanical allodynia in the early stages, likely by inhibiting BDNF release from microglia (Zhang et al., 2011). Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release as well as neuropathic pain (Ulmann et al., 2008). P2X4 receptors in spinal microglia are also up-regulated after chronic morphine treatment and inhibition of this up-regulation blunts development of morphine tolerance (Horvath et al., 2010).

The **TLR** family currently includes a total of 13 receptors that are responsible for the recognition of highly conserved structural motifs that are used by the innate immune system to defend the host against pathogens (He et al., 2011). The article by Nicotra et al. in this Special issue discusses toll-like receptors in chronic pain in detail. It may suffice to mention here that TLR2 plays a critical role secondary to activation of the μ -opioid receptor in morphine-induced microglia activation and dependence (Zhang et al., 2010) and that there is spinal glial TLR4-mediated nociception and production of prostaglandin E(2) and TNF (Saito et al., 2010). It has been discovered recently that opioids prime microglia to undergo apoptosis through TLR9 and μ OR (He et al., 2011).

Chemokines play a key role in mediating neuronal-microglial communication, which leads to increased nociception (Clark et al., 2011). Monocyte-chemoattractant protein-1 (CCL2) is an important mediator of microglia activation in neuropathic pain (Thacker et al., 2009). It is released in an activity dependent manner from the central terminals of primary sensory neurons (Thacker et al., 2009). After spinal cord injury, CCL21 release from spinothalamic tract neurons has been reported to induce microglial activation in the ventral posterolateral thalamus to cause neuropathic pain (Zhao et al., 2007). Since expression of CCR2, the MCP-1/CCL2 receptor, in either resident microglia or bone marrow-derived microglia is sufficient for the development of mechanical allodynia, to effectively relieve neuropathic pain, both CNS resident microglia and blood-borne macrophages need to be targeted (Zhang et al., 2007). It has been proposed

that TNF α and IL-1 β released from activated microglia induce CCL2 expression in astrocytes, which, in turn, regulates the excitability of pain transmission neurons in the dorsal horn (Kim et al., 2010).

The receptor for *interferon-gamma* (IFN- γ R) is regarded as another key element in the molecular machinery through which resting spinal microglia transform into an activated state that drives neuropathic pain (Tsuda et al., 2009). Rosiglitazone, a peroxisome proliferator-activated receptor- γ agonist, has been found to down-regulate interferon- γ -induced gene expression of cyclooxygenase-2 and inducible nitric oxide synthase and to reduce the chemotactic response to CCL2 (Takahashi et al., 2011). Thus, the macrophage-mediated effects of rosiglitazone attenuate tactile allodynia in the early phase of neuropathic pain development (Takahashi et al., 2011).

Fractalkine is also an important pain-associated biological molecule. For instance, spinal nerve injury, but not peripheral inflammation, induces the expression of CX3CL1 (fractalkine) in astrocytes and upregulates CX3CR1 in microglia in the spinal cord (Lindia, et al., 2005). For more information on fractalkine and microglial signaling mechanisms see the article by Clark and Malcangio in this special issue.

In addition to the microglial activation mechanisms detailed above, there are alternative pathways of microglial activation such as via P2X7R and P2Y(12). It has been demonstrated that P2X7R participates in the onset and persistence of neuropathic pain and that spinal P2X7R mediate microglia activation (He et al., 2011). Others have reported that blocking the P2X7 receptor prevents morphine-induced microglial activation and inhibits development but not maintenance of morphine tolerance (Zhou et al., 2010). Furthermore, it has been suggested that production of cytokines and other mediators that could result in neuropathic pain happens via P2Y(12), a metabotropic ATP receptor (Kobayashi et al., 2008). Activation of this receptor by released ATP or its hydrolyzed products activates the p38 mitogen-activated protein kinase (MAPK) pathway (Kobayashi et al., 2008). Therefore, blocking microglial P2Y(12)R has been proposed as a viable strategy for treating neuropathic pain (Tozaki-Saitoh et al., 2008).

Importance of the endocannabinoid (eCB) system

eCBs are key regulators of synaptic transmission throughout the CNS (Heifets and Castillo, 2009). Within neurons, retrograde eCB signaling from postsynaptic cells to presynaptic nerve terminals has been implicated in short-term and long-term synaptic depression (LTD) at both excitatory, and inhibitory synapses throughout the CNS (Heifets and Castillo, 2009), although LTD at inhibitory synapses is most prominent. Disruption of inhibitory synaptic transmission in the dorsal horn of the spinal cord is a crucial contributor to neuropathic pain (Sandkuhler, 2009). Activated microglia contribute to failure of inhibitory synaptic transmission in spinal cord (Coull et al., 2005; Coull, et al., 2003). Microglia also express eCBs and cannabinoid receptors but the contribution of this microglia-cannabinoid signaling system to synaptic plasticity in spinal cord and neuropathic pain is still not well understood.

The cannabinoid type 1 receptor (CBR1) is expressed widely by neurons and is one of the most abundant G protein-coupled receptors (GPCR) found in the CNS (Mackie, 2005). It is also expressed by microglia, albeit at low levels (Ashton and Glass, 2007; Stella, 2009). The CBR2 is the other major target for eCBs, particularly 2-arachidonyl glycerol. Stimulation of CBR2 with selective CBR2 agonists inhibits neuropathic pain in various experimental models (Anand et al., 2009; Sagar et al., 2010). Microglia might also express other, “abnormal” cannabinoid receptors (Stella, 2009). There is very little expression of CBR2 in healthy CNS tissue but profound upregulation in activated microglia (Ashton and Glass, 2007; Stella, 2009). Microglia are a very rich source of 2-arachidonyl glycerol (Muccioli et al., 2007) and production of this eCB is controlled by activation of P2X7 receptors (Witting et al., 2004). Stimulation of microglial CBR2 by eCBs is associated with increased proliferation and chemotaxis in vitro and reduces release of pro-nociceptive mediators including TNF α and free radicals (Ashton and Glass, 2007; Romero-Sandoval et al., 2009). CBR2 also exist on resting spinal glia and perivascular cells, which has been interpreted as suggesting an

immunoregulatory role of these receptors in the CNS (Romero-Sandoval et al., 2008). Accordingly, eCB signaling in microglial cells has become a focus of attention in neuroprotection and neuropathic pain (Ashton and Glass, 2007; Stella, 2009).

ECBs produced by microglia may directly modulate neuropathic pain as well as contribute to functional re-organization of spinal nociceptive processing in neuropathic pain models. ECB concentrations are elevated in spinal cord in neuropathic pain models and microglia appear to contribute to this (Guasti et al., 2009; Sagar et al., 2010). Activation of CBR1 by eCBs in spinal cord can produce both anti- and pro-nociceptive actions (Christie and Mallet, 2009; Pacher, 2006). Although CBR1 agonists can inhibit both acute and neuropathic pain, eCBs produced by intense stimulation of thermal nociceptive nerves can also produce mechanical hyperalgesia via a CBR1 mediated suppression of inhibitory synapses (Pernia-Andrade et al., 2009). The latter effect wanes during development of neuropathic pain, possibly due to the pain promoting adaptations to inhibitory synapses caused by activated microglia (Christie and Mallet, 2009). BDNF released by activated microglia collapses the chloride gradient of some pain transmission neurons in the dorsal horn of the spinal cord, causing a shift to excitation at inhibitory synapses (Coull et al., 2005; Coull et al., 2003). ECBs acting on CBR1 at inhibitory synapses could suppress this aberrant excitatory activity. ECBs acting at CBR2 on microglia should also have a pain relieving action in neuropathic pain by blunting release of pro-nociceptive mediators including BDNF, TNF α and free radicals. Elevated concentrations of eCBs from microglia could also contribute to silencing of inhibitory synapses. Although this has not been directly shown in neuropathic spinal cord, it has been demonstrated that altered 2-arachidonoylglycerol (2-AG) signalling could contribute to synapse silencing in Alzheimer's disease (Mulder et al., 2011).

ECB actions on microglia are not limited to direct modulation of synaptic transmission in the spinal cord. CBR2 stimulation may also modulate polarization and migration of microglia in spinal cord to focus activated microglia at sites of nerve injury. Interestingly, CB2R protect against alcoholic liver disease by

regulating Kupffer cell polarization in mice (Louvet et al., 2011) suggesting that microglial cell polarization may be influenced by CB2R activation as well. In cultured microglial cells, CB2R are expressed at the leading edge of lamellipodia, which is consistent with the migratory behavior of microglial cells (Walter et al., 2003). The involvement of CB2R is not limited to microglia in neuropathic pain. Deletion of CB2R selectively in hematopoietic cells has been reported to exacerbate the development of neuropathic pain (Racz et al., 2008). This manipulation resulted in an extended range of microglial activation beyond the spinal cord ipsilateral to injured nerves into contralateral dorsal horn. ECB-microglia interactions in central pain associated with spinal cord injury are more widespread than the spinal cord involving changes in CB1R expression in brain areas harbouring circuitry of chronic pain conditions (Knerlich-Lukoschus et al., 2011).

Microglia are not alone

It is important to note that microglia are not the only glial cell type that has been shown to be involved in the pathogenesis of neuropathic pain. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathic pain (Raghavendra et al., 2003). Specifically, it has been suggested that astrocytes play a role in the initiation of acute pain and in the maintenance of chronic pain while microglial activation closely correlates with the early phase of neuropathic pain (Romero-Sandoval et al., 2008). Activated astrocytes can release cytokines (e.g., IL-1 β) and chemokines (e.g., CCL2) and thus enhance and prolong persistent pain states in the spinal cord (Gao and Ji, 2010). Unsurprisingly, both activated astrocytes and microglia of the locus coeruleus react to acute cardiac injury (Zhang et al., 2009). In mice there is astrocyte activation in the cingulate cortex in chronic pain (Kuzumaki et al., 2007).

Extracellular signal-regulated kinase (ERK), a mitogen activated-protein kinase (MAPK), is sequentially activated in microglia and astrocytes by spinal nerve ligation (Zhuang et al., 2005) which supports the idea

of distinct roles for these two glial cell types in the temporal evolution of neuropathic pain (Zhuang et al., 2005). It has been confirmed in different neuropathic pain models that activation of spinal microglia appears before that of astrocytes (Miraucourt et al., 2011). Accordingly, proliferation of dorsal horn astrocytes after peripheral nerve injury takes place later than that of microglia (Tsuda et al., 2011).

A prostaglandin E2 (PGE2)-dependent, ERK1/2-regulated microglia-neuron signaling pathway that mediates the microglial component of pain maintenance after injury to the spinal cord has been proposed (Zhao et al., 2007). Furthermore, ERK5 activated in spinal microglia and DRG neurons is thought to contribute to the development of neuropathic pain (Obata et al., 2007). Nerve injury also induces a striking increase in IL-18 and IL-18R expression in the dorsal horn with IL-18 found to be upregulated in activated microglia and IL-18R increased in astrocytes, respectively (Miyoshi et al., 2008). These IL-18-mediated microglia-astrocyte interactions in the spinal cord are considered to have a substantial role in the generation of tactile allodynia (Miyoshi et al., 2008). Furthermore, it has been proposed that glycine disinhibition makes tactile stimuli able to activate astrocytes, which may produce d-serine and enable NMDA receptor activation and thus allodynia (Miraucourt et al., 2011). The glutamate transporter-1 (GLT-1), which is expressed on astrocytes and probably correlates with astrocyte activation, has also been implicated in the induction and/or maintenance of neuropathic pain (Wang et al., 2008).

The JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to the development of signs of neuropathic pain in rat (Dominguez et al., 2008). Importantly, inhibitors of JAK-STAT3 signalling suppress astrocyte proliferation, which leads to a recovery from tactile allodynia (Tsuda et al., 2011). Recent studies have identified astrocyte-specific pain-associated molecules (Inoue and Tsuda, 2009). Thus, astrocyte signaling may also represent a target for the treatment of chronic pain (Gao and Ji, 2010). It is worth noting that local anesthetics such as ropivacaine may provide a new approach to glial cell inhibition and, therefore, therapeutic strategies for neuropathic pain (Toda et al., 2011). Ropivacaine provides prolonged analgesia in the dorsal spinal cord possibly by suppressing astrocyte activation in an

NGF-independent and microglial activation in an NGF-dependent manner (Toda et al., 2011). For more information on spatial and temporal activation of spinal glial cells see the article by Gwak et al. in this Special issue.

In addition to central nervous system (CNS) microglia, it has been suggested to target invading macrophage-derived PGE₂, IL-6 and calcitonin gene-related peptide in injured nerve to treat neuropathic pain (Ma and Quirion, 2006). Leptin which is produced by adipocytes in injured peripheral nerves and facilitates the development of neuropathic pain via macrophage stimulation (Maeda et al., 2009) may be another promising target. Additional evidence in support of the role played by myeloid cells in the causation of central pain is provided by work suggesting that macrophage migration inhibitory factor (MIF) and macrophage inflammatory protein-1alpha(MIP-1alpha) contribute to neuropathic pain-like hypersensitivity (Kiguchi et al., 2010; Wang et al., 2011). However, the role of MIF appears to be broader because this factor has been proposed to have a role in endometriosis-associated pain (Akoum et al., 2006) and in the sex differences observed in chronic pain conditions (Aloisi et al., 2005). Furthermore, a statistically significant inverse correlation between histologically determined macrophage infiltration and post-operative pain after lumbar disc surgery was observed as long as 10 years ago (Rothoerl et al., 2002). Diabetes is yet another important disease condition where macrophages seem to play a painful role. Depleting macrophages by means of liposome-encapsulated clodronate reduces mechanical allodynia in diabetic rats without affecting thermal hyperalgesia suggesting that depletion of macrophages in diabetes can postpone the development of diabetic neuropathic pain (Mert et al., 2009). For more information on microglia and their involvement in diabetic pain neuropathy see the article by Talbot and Couture in this Special issue.

Central pain: the microglial gatekeeper turned ugly

CNS pathologies associated with neuropathic pain are characterized by microglial activation that is unwanted because it contributes to or even causes central pain. However, a pathological situation does not mean that microglia automatically behave in a pathology-amplifying way. After all they are proven guardians of the brain and spinal cord and this rule applies to the vast majority of disease conditions where microglia not only clear debris or fight infections but also participate in repair processes. Yet, there are disease conditions that can affect microglia directly or indirectly (Graeber, 2010). Similarly, while microglia behave normally in physiological pain, they obviously do not do so when activated in central pain (Hulsebosch, 2008). This is evidenced by the fact that exacerbated microglial activation is associated with the severity of neuropathic pain symptoms (Berger et al., 2011). However, the exact nature and extent of microglial dysfunction in pathological pain remain largely unknown at present, the apparent absence of a specific pathomorphology being one characteristic and upregulation of microglial purinergic receptors another (see article by Inoue and Tsuda, this issue). Furthermore, a theory of glia-driven mood and cognitive dysfunction in chronic pain has been proposed (Panigada and Gosselin, 2011).

In order to understand abnormal microglial behavior better it may be helpful to look at other dysfunctional states where microglia seem involved and which also lack overt microglial pathology but to look in greater detail including at their ultrastructure and relationship to synapses (Tremblay et al., 2010). In addition, although our understanding of microglial activation is incomplete and only a few regulatory mechanisms are known at present, it seems advisable to assess each case of microglial activation in context. Therefore, CNS autonomous specific microglial activation needs to be distinguished from potentially confounding processes, which extend beyond the nervous system such as systemic infection and which can influence the activation state of microglial cells within the CNS. For in vitro evidence supporting the existence of differential responses in human and rodent microglia and macrophages see the article from the DeLeo

laboratory in this Special issue. Psychological stress represents another potentially confounding factor. Notably, microglia have been proposed to mediate the effects of psychological stress on prefrontal neuronal function and prefrontal cortex-regulated behavior (Hinwood et al., 2011). Chronic stress also reversibly gates eCB synaptic plasticity at inhibitory synapses in the amygdala, and in vivo augmentation of 2-AG levels prevents both synaptic and behavioral adaptations to chronic stress (Sumislawski et al., 2011). Chronic psychological stress is associated with visceral hyperalgesia and increased expression of spinal NK1 receptors and microglia appear to have a key role in the process (Bradesi et al., 2008). Microglial activation further takes place in response to repeated social defeat-induced anxiety which is dependent on the activation of β -adrenergic and IL-1 receptors (Wohleb et al., 2011). Minocycline, the well-known antibiotic with inhibitory effects on microglial activation, has even been suggested to influence the ways humans make decisions (Watabe et al., 2011). Thus, microglial-neuronal interactions exhibit an unexpectedly high level of sophistication.

The idea that microglia exert CNS specific functions in normal CNS that are unrelated to inflammation and differ from those of peripheral macrophages - which do not live in an electrical organ - has gained additional momentum recently with the discovery of a separate (pre-haematopoietic) myeloid lineage of the microglia during embryogenesis (Ginhoux et al., 2010). Accordingly, induction of complement in spinal cord microglia that results in anaphylatoxin C5a-mediated pain hypersensitivity (Griffin et al., 2007) could be related to synaptic alterations. Such a non-immune function of complement has been suggested (Stevens et al., 2007). For the relationship between microglial activation and complement receptor expression see Graeber (2010).

Conclusions and outlook

Resistance to morphine (Mika, 2008) characterizes neuropathic pain. Therefore, elucidation of the mechanisms underlying opioid receptor control by microglia seems important. Moreover, there is an

obvious need for ultrastructural studies of microglia-synapse interactions in pain states. This seems important because it has been suggested that the mechanisms that produce neuropathic pain after exposure to chemotherapeutics may be fundamentally different from those operating after nerve trauma (Zheng et al., 2010). Thus, different pain states should be characterized not only at the molecular but also at the cellular (including ultrastructure) and systemic (including tissue) level. Knowing precisely what differences to normal microglial activation exist may be important for the assessment of novel therapeutics for the treatment of chronic pain (O'Callaghan and Miller, 2010). Finally, we argue in favor of more research on psychosocial factors in central pain focusing on the connection between psychological stress and microglial activation.

References

1. Akoum, A., Metz, C. N., Al-Akoum, M., and Kats, R., 2006. Macrophage migration inhibitory factor expression in the intrauterine endometrium of women with endometriosis varies with disease stage, infertility status, and pelvic pain. *Fertil. Steril.* 85, 1379-1385.
2. Aloisi, A. M., Pari, G., Ceccarelli, I., Vecchi, I., Ietta, F., Lodi, L., and Paulesu, L., 2005. Gender-related effects of chronic non-malignant pain and opioid therapy on plasma levels of macrophage migration inhibitory factor (MIF). *Pain* 115, 142-151.
3. Anand, P., Whiteside, G., Fowler, C. J., and Hohmann, A. G., 2009. Targeting CB2 receptors and the endocannabinoid system for the treatment of pain. *Brain Res. Rev.* 60, 255-266.
4. Ashton, J. C., and Glass, M., 2007. The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. *Curr. Neuropharmacol.* 5, 73-80.
5. Berger, J. V., Deumens, R., Goursaud, S., Schafer, S., Lavand'homme, P., Joosten, E. A., and Hermans, E., 2011. "Enhanced neuroinflammation and pain hypersensitivity after peripheral nerve injury in rats expressing mutated superoxide dismutase 1". *J. Neuroinflammation* 8, 33.
6. Biber, K., Tsuda, M., Tozaki-Saitoh, H., Tsukamoto, K., Toyomitsu, E., Masuda, T., Boddeke, H., and Inoue, K., 2011. Neuronal CCL21 up-regulates microglia P2X4 expression and initiates neuropathic pain development. *EMBO J.* 30, 1864–1873.
7. Biggs, J. E., Lu, V. B., Stebbing, M. J., Balasubramanyan, S., and Smith, P. A., 2010. Is BDNF sufficient for information transfer between microglia and dorsal horn neurons during the onset of central sensitization? *Mol. Pain* 6, 44.
8. Bradesi, S., Svensson, C., Steinauer, J., Pothoulakis, C., Yaksh, T., and Mayer, E., 2008. Role of spinal microglia in visceral hyperalgesia and NK1R up-regulation in a rat model of chronic stress. *Gastroenterology* 136, 1339–1348.

9. Cao, L., and DeLeo, J. A., 2008. CNS-infiltrating CD4+ T lymphocytes contribute to murine spinal nerve transection-induced neuropathic pain. *Eur. J. Immunol.* 38, 448-458.
10. Choi, H. K., Ryu, H. J., Kim, J.-E., Jo, S.-M., Choi, H.-C., Song, H.-K., and Kang, T.-C., 2011. The roles of P2X7 receptor in regional-specific microglial responses in the rat brain following status epilepticus. *Neurol. Sci.*, doi:10.1007/s10072-011-0740-z.
11. Christie, M. J., and Mallet, C., 2009. Endocannabinoids can open the pain gate. *Science Signaling* 2, pe57.
12. Clark, A. K., Staniland, A. A., and Malcangio, M., 2011. Fractalkine/CX3CR1 signalling in chronic pain and inflammation. *Curr. Pharmaceutical Biotechnology*, in press.
13. Coull, J. A. M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M. W., and De Koninck, Y., 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017-1021.
14. Coull, J. A. M., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., S  k, A., De Koninck, P., and De Koninck, Y., 2003. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* 424, 938-942.
15. De Leo, J. A., Tawfik, V. L., and Lacroix-Fralish, M. L., 2006. The tetrapartite synapse: path to CNS sensitization and chronic pain. *Pain* 122, 17-21.
16. Dominguez, E., Rivat, C., Pommier, B., Mauborgne, A., and Pohl, M., 2008. JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat. *J. Neurochem.* 107, 50-60.
17. Finnerup, N. B., 2008. A review of central neuropathic pain states. *Curr. Opin. Anaesthesiol.* 21, 586-589.
18. Gao, Y.-J., and Ji, R.-R., 2010. Targeting astrocyte signaling for chronic pain. *Neurotherapeutics* 7, 482-493.

19. 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., and Merad, M., 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841-845.
20. Graeber, M. B., 2010. Changing face of microglia. *Science* 330, 783-788.
21. Graeber, M. B., Streit, W.J., and Kreutzberg, G.W., 1988. Axotomy of the rat facial nerve leads to increased CR3 complement receptor expression by activated microglial cells. *J. Neurosci. Res.* 21, 18-24.
22. Graeber, M. B., Wei, L., and Rodriguez, M., 2011. Role of microglia in CNS inflammation *FEBS Letters*, in press.
23. Griffin, R. S., Costigan, M., Brenner, G. J., Ma, C. H. E., Scholz, J., Moss, A., Allchorne, A. J., Stahl, G. L., and Woolf, C. J., 2007. Complement induction in spinal cord microglia results in anaphylatoxin C5a-mediated pain hypersensitivity. *J. Neurosci.* 27, 8699-8708.
24. Guasti, L., Richardson, D., Jhaveri, M., Eldeeb, K., Barrett, D., Elphick, M. R., Alexander, S. P., Kendall, D., Michael, G. J., and Chapman, V., 2009. Minocycline treatment inhibits microglial activation and alters spinal levels of endocannabinoids in a rat model of neuropathic pain. *Mol. Pain* 5, 35.
25. Guo, L.-H., Trautmann, K., and Schluesener, H. J., 2005. Expression of P2X4 receptor by lesional activated microglia during formalin-induced inflammatory pain. *J. Neuroimmunol.* 163, 120-127.
26. He, L., Li, H., Chen, L., Miao, J., Jiang, Y., Zhang, Y., Xiao, Z., Hanley, G., Li, Y., Zhang, X., Lesage, G., Peng, Y., and Yin, D., 2011. Toll-like receptor 9 is required for opioid-induced microglia apoptosis. *PLoS ONE* 6, e18190.
27. He, W.-J., Cui, J., Du, L., Zhao, Y.-D., Burnstock, G., Zhou, H.-D., and Ruan, H.-Z., 2011. Spinal P2X7 receptor mediates microglia activation-induced neuropathic pain in the sciatic nerve injury rat model. *Behav. Brain Res.*, doi:10.1016/j.bbr.2011.09.015.

28. Heifets, B. D., and Castillo, P. E., 2009. Endocannabinoid Signaling and Long-Term Synaptic Plasticity. *Annu. Rev. Physiol.* 71, 283-306.
29. Hinwood, M., Morandini, J., Day, T. A., and Walker, F. R., 2011. Evidence that microglia mediate the neurobiological effects of chronic psychological stress on the medial prefrontal cortex. *Cerebral Cortex*, doi:10.1093/cercor/bhr229.
30. Horvath, R. J., Romero-Sandoval, E. A., and DeLeo, J.A., Inhibition of microglial P2X4 receptors attenuates morphine tolerance, Iba1, GFAP and μ opioid receptor protein expression while enhancing perivascular microglial ED2. *Pain* 150, 401-413.
31. Hulsebosch, C.E., 2008. Gliopathy ensures persistent inflammation and chronic pain after spinal cord injury. *Exp. Neurol.* 214, 6–9.
32. Inoue, K., and Tsuda, M., 2009. Microglia and neuropathic pain. *Glia* 57, 1469–1479.
33. Inoue, K., 2006. ATP receptors of microglia involved in pain. *Novartis Found Symp* 276, 263-272; discussion 273-281.
34. Kiguchi, N., Maeda, T., Kobayashi, Y., Fukazawa, Y., and Kishioka, S., 2010. Macrophage inflammatory protein-1 α mediates the development of neuropathic pain following peripheral nerve injury through interleukin-1 β up-regulation. *Pain* 149, 305-315.
35. Kim, D., You, B., Jo, E.-K., Han, S.-K., Simon, M. I., and Lee, S. J., 2010. NADPH oxidase 2-derived reactive oxygen species in spinal cord microglia contribute to peripheral nerve injury-induced neuropathic pain. *Proc. Natl. Acad. Sci. USA* 107, 14851-14856.
36. Knerlich-Lukoschus, F., Juraschek, M., Blömer, U., Lucius, R., Mehdorn, H. M., and Held-Feindt, J., 2008. Force-dependent development of neuropathic central pain and time-related CCL2/CCR2 expression after graded spinal cord contusion injuries of the rat. *J. Neurotrauma* 25, 427-448.
37. Knerlich-Lukoschus, F., Noack, M., von der Ropp-Brenner, B., Lucius, R., Mehdorn, H. M., and Held-Feindt, J., 2011. Spinal cord injuries induce changes in CB1 cannabinoid receptor and C-C

- chemokine expression in brain areas underlying circuitry of chronic pain conditions. *J. Neurotrauma* 28, 619-634.
38. 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.
 39. Kreutzberg, G. W., 1996. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.* 19, 312-318.
 40. Kuzumaki, N., Narita, M., Hareyama, N., Niikura, K., Nagumo, Y., Nozaki, H., Amano, T., and Suzuki, T., 2007. Chronic pain-induced astrocyte activation in the cingulate cortex with no change in neural or glial differentiation from neural stem cells in mice. *Neurosci. Lett.* 415, 22-27.
 41. Lin, T., Li, K., Zhang, F.-Y., Zhang, Z.-K., Light, A. R., and Fu, K.-Y., 2007. Dissociation of spinal microglia morphological activation and peripheral inflammation in inflammatory pain models. *J. Neuroimmunol.* 192, 40-48.
 42. 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.
 43. Louvet, A., Teixeira-Clerc, F., Chobert, M.-N., Deveaux, V., Pavoine, C., Zimmer, A., Pecker, F., Mallat, A., and Lotersztajn, S., 2011. Cannabinoid CB2 receptors protect against alcoholic liver disease by regulating Kupffer cell polarization in mice. *Hepatology*, doi:10.1002/hep.24524.
 44. Ma, W., and Quirion, R., 2006. Targeting invading macrophage-derived PGE2, IL-6 and calcitonin gene-related peptide in injured nerve to treat neuropathic pain. *Expert Opin. Ther. Targets* 10, 533-546.
 45. Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb. Exp. Pharmacol.* 168, 299-325.

46. Maeda, T., Kiguchi, N., Kobayashi, Y., Ikuta, T., Ozaki, M., and Kishioka, S., 2009. Leptin derived from adipocytes in injured peripheral nerves facilitates development of neuropathic pain via macrophage stimulation. *Proc. Natl. Acad. Sci. USA* 106, 13076-13081.
47. Mei, X.-P., Xu, H., Xie, C., Ren, J., Zhou, Y., Zhang, H., and Xu, L.-X., 2011. Post-injury administration of minocycline: an effective treatment for nerve-injury induced neuropathic pain. *Neurosci. Res.* 70, 305-312.
48. Mert, T., Gunay, I., Ocal, I., Guzel, A. I., Inal, T. C., Sencar, L., and Polat, S., 2009. Macrophage depletion delays progression of neuropathic pain in diabetic animals. *Naunyn Schmiedebergs Arch. Pharmacol.* 379, 445-452.
49. 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.
50. Mika, J., 2008. Modulation of microglia can attenuate neuropathic pain symptoms and enhance morphine effectiveness. *Pharmacol. Reports* 60, 297-307.
51. Miraucourt, L. S., Peirs, C., Dallel, R., and Voisin, D. L., 2011. Glycine inhibitory dysfunction turns touch into pain through astrocyte-derived d-serine. *Pain* 152, 1340-1348.
52. Miyoshi, K., Obata, K., Kondo, T., Okamura, H., and Noguchi, K., 2008. Interleukin-18-mediated microglia/astrocyte interaction in the spinal cord enhances neuropathic pain processing after nerve injury. *J. Neurosci.* 28, 12775-12787.
53. Muccioli, G. G., Xu, C., Odah, E., Cudaback, E., Cisneros, J. A., Lambert, D. M., Lopez Rodriguez, M. L., Bajjalieh, S., and Stella, N., 2007. Identification of a novel endocannabinoid-hydrolyzing enzyme expressed by microglial cells. *J. Neurosci.* 27, 2883-2889.
54. Mulder, J., Zilberter, M., Pasquaré, S. J., Alpár, A., Schulte, G., Ferreira, S. G., Köfalvi, A., Martín-Moreno, A. M., Keimpema, E., Tanila, H., Watanabe, M., Mackie, K., Hortobágyi, T., de Ceballos, M.

- L., and Harkany, T., 2011. Molecular reorganization of endocannabinoid signalling in Alzheimer's disease. *Brain* 134, 1041-1060.
55. Narita, M., Miyoshi, K., Narita, M., and Suzuki, T., 2007. Involvement of microglia in the ethanol-induced neuropathic pain-like state in the rat. *Neurosci. Lett.* 414, 21-25.
56. Narita, M., Yoshida, T., Nakajima, M., Narita, M., Miyatake, M., Takagi, T., Yajima, Y., and Suzuki, T., 2006. Direct evidence for spinal cord microglia in the development of a neuropathic pain-like state in mice. *J. Neurochem.* 97, 1337-1348.
57. Nimmerjahn, A., Kirchhoff, F., and Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314-1318.
58. O'Callaghan, J. P., and Miller, D. B., 2010. Spinal glia and chronic pain. *Metab. Clin. Exp.* 59 Suppl. 1, S21-26.
59. Obata, K., Katsura, H., Mizushima, T., Sakurai, J., Kobayashi, K., Yamanaka, H., Dai, Y., Fukuoka, T., and Noguchi, K., 2007. Roles of extracellular signal-regulated protein kinases 5 in spinal microglia and primary sensory neurons for neuropathic pain. *J. Neurochem.* 102, 1569-1584.
60. Pacher, P., 2006. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* 58, 389-462.
61. Panigada, T., and Gosselin, R.-D., 2011. Behavioural alteration in chronic pain: Are brain glia involved? *Med Hypotheses*, doi:10.1016/j.mehy.2011.06.036.
62. Pernia-Andrade, A. J., Kato, A., Witschi, R., Nyilas, R., Katona, I., Freund, T. F., Watanabe, M., Filitz, J., Koppert, W., Schuttler, J., Ji, G., Neugebauer, V., Marsicano, G., Lutz, B., Vanegas, H., and Zeilhofer, H. U., 2009. Spinal endocannabinoids and CB1 receptors mediate C-fiber-induced heterosynaptic pain sensitization. *Science* 325, 760-764.
63. Perry, V. H., 2010. Contribution of systemic inflammation to chronic neurodegeneration. *Acta Neuropathol.* 120, 277-286.

64. Racz, I., Nadal, X., Alferink, J., Baños, J. E., Rehnelt, J., Martín, M., Pintado, B., Gutierrez-Adan, A., Sanguino, E., Manzanares, J., Zimmer, A., and Maldonado, R., 2008. Crucial role of CB(2) cannabinoid receptor in the regulation of central immune responses during neuropathic pain. *J. Neurosci.* 28, 12125-12135.
65. 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.
66. Romero-Sandoval, A., Chai, N., Nutile-McMenemy, N., and DeLeo, J. A., 2008. A comparison of spinal Iba1 and GFAP expression in rodent models of acute and chronic pain. *Brain Res.* 1219, 116-126.
67. Romero-Sandoval, A., Nutile-McMenemy, N., and DeLeo, J. A., 2008. Spinal microglial and perivascular cell cannabinoid receptor type 2 activation reduces behavioral hypersensitivity without tolerance after peripheral nerve injury. *Anesthesiology* 108, 722-734.
68. Romero-Sandoval, E., Horvath, R., Landry, R., and Deleo, J., 2009. Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation. *Mol. Pain* 5, 25.
69. Rothoerl, R. D., Woertgen, C., and Brawanski, A., 2002. Pain resolution after lumbar disc surgery is influenced by macrophage tissue infiltration. A prospective consecutive study on 177 patients. *J. Clin. Neurosci.* 9, 633-636.
70. Sagar, D. R., Jhaveri, M. D., Richardson, D., Gray, R. A., De Lago, E., Fernández-Ruiz, J., Barrett, D. A., Kendall, D. A., and Chapman, V., 2010. Endocannabinoid regulation of spinal nociceptive processing in a model of neuropathic pain. *Eur. J. Neurosci.* 31, 1414-1422.
71. Saito, O., Svensson, C. I., Buczynski, M. W., Wegner, K., Hua, X.-Y., Codeluppi, S., Schaloske, R. H., Deems, R. A., Dennis, E. A., and Yaksh, T. L., 2010. Spinal glial TLR4-mediated nociception and production of prostaglandin E(2) and TNF. *Br. J. Pharmacol.* 160, 1754-1764.

72. Sandkuhler, J., 2009. Models and mechanisms of hyperalgesia and allodynia. *Physiol. Reviews* 89, 707-758.
73. Smith, H. S., 2010. Activated microglia in nociception. *Pain Physician* 13, 295-304.
74. Stella, N., 2009. Endocannabinoid signaling in microglial cells. *Neuropharmacology* 56, 244–253.
75. Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., Micheva, K. D., Mehalow, A. K., Huberman, A. D., Stafford, B., Sher, A., Litke, A. M., Lambris, J. D., Smith, S. J., John, S. W. M., and Barres, B. A., 2007. The classical complement cascade mediates CNS synapse elimination. *Cell* 131, 1164-1178.
76. Sumislawski, J. J., Ramikie, T. S., and Patel, S., 2011. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. *Neuropsychopharmacology*, doi:10.1038/npp.2011.166.
77. Takahashi, Y., Hasegawa-Moriyama, M., Sakurai, T., and Inada, E., 2011. The macrophage-mediated effects of the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone attenuate tactile allodynia in the early phase of neuropathic pain development. *Anesthesia and Analgesia*, doi:10.1213/ANE.0b013e31821b220c.
78. Thacker, M. A., Clark, A. K., Bishop, T., Grist, J., Yip, P. K., Moon, L. D. F., Thompson, S. W. N., Marchand, F., and McMahon, S. B., 2009. CCL2 is a key mediator of microglia activation in neuropathic pain states. *Eur.J. Pain* 13, 263-272.
79. Toda, S., Sakai, A., Ikeda, Y., Sakamoto, A., and Suzuki, H., 2011. A local anesthetic, ropivacaine, suppresses activated microglia via a nerve growth factor-dependent mechanism and astrocytes via a nerve growth factor-independent mechanism in neuropathic pain. *Mol. Pain* 7, 2.
80. 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.

81. Trang, T., Beggs, S., Wan, X., and Salter, M. W., 2009. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J. Neurosci.* 29, 3518-3528.
82. Treede, R.-D., Jensen, T. S., Campbell, J. N., Cruccu, G., Dostrovsky, J. O., Griffin, J. W., Hansson, P., Hughes, R., Nurmikko, T., and Serra, J., 2008. Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* 70, 1630-1635.
83. Tremblay, M.È., Lowery, R.L., and Majewska, A.K. 2010. Microglial interactions with synapses are modulated by visual experience. *PLoS Biology* 8 (11) e1000527
84. Tsuda, M., Kohro, Y., Yano, T., Tsujikawa, T., Kitano, J., Tozaki-Saitoh, H., Koyanagi, S., Ohdo, S., Ji, R.-R., Salter, M. W., and Inoue, K., 2011. JAK-STAT3 pathway regulates spinal astrocyte proliferation and neuropathic pain maintenance in rats. *Brain* 134, 1127-1139.
85. Tsuda, M., Masuda, T., Kitano, J., Shimoyama, H., Tozaki-Saitoh, H., and Inoue, 2009. IFN- γ receptor signaling mediates spinal microglia activation driving neuropathic pain. *Proc. Natl. Acad. Sci. USA* 106, 8032–8037.
86. Ulmann, L., Hatcher, J. P., Hughes, J. P., Chaumont, S., Green, P. J., Conquet, F., Buell, G. N., Reeve, A. J., Chessell, I. P., and 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.
87. 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.
88. Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., and Stella, N., 2003. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.* 23, 1398-1405.

89. Wang, F., Xu, S., Shen, X., Guo, X., Peng, Y., and Yang, J., 2011. Spinal macrophage migration inhibitory factor is a major contributor to rodent neuropathic pain-like hypersensitivity. *Anesthesiology* 114, 643-659.
90. Wang, W., Wang, W., Wang, Y., Huang, J., Wu, S., and Li, Y.-Q., 2008. Temporal changes of astrocyte activation and glutamate transporter-1 expression in the spinal cord after spinal nerve ligation-induced neuropathic pain. *Anat. Rec.* 291, 513-518.
91. Watabe, M., Kato, T. A., Monji, A., Horikawa, H., and Kanba, S., 2011. Does minocycline, an antibiotic with inhibitory effects on microglial activation, sharpen a sense of trust in social interaction? *Psychopharmacology*, doi:10.1007/s00213-011-2509-8.
92. 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. USA* 101, 3214-3219.
93. Wohleb, E. S., Hanke, M. L., Corona, A. W., Powell, N. D., Stiner, L. t. M., Bailey, M. T., Nelson, R. J., Godbout, J. P., and Sheridan, J. F., 2011. β -Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. *J. Neurosci.* 31, 6277-6288.
94. Zhang, J., Cheng, H., Chen, Yi, F., Li, W., Luan, R., Guo, W., Lv, A., Rao, Z., and Wang, H., 2009. Involvement of activated astrocyte and microglia of locus coeruleus in cardiac pain processing after acute cardiac injury. *Neurol. Res.* 31, 432-438.
95. 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.
96. Zhang, X., Xu, Y., Wang, J., Zhou, Q., Pu, S., Jiang, W., and Du, D., 2011. The effect of intrathecal administration of glial activation inhibitors on dorsal horn BDNF overexpression and hind paw mechanical allodynia in spinal nerve ligated rats. *J. Neural Transm.*, doi:10.1007/s00702-011-0713-7.

97. Zhang, Y., Li, H., Li, Y., Sun, X., Zhu, M., Hanley, G., Lesage, G., and Yin, D., 2010. Essential role of Toll-like receptor 2 in morphine-induced microglia activation in mice. *Neurosci. Lett.*, doi:10.1016/j.neulet.2010.11.063.
98. Zhao, P., Waxman, S. G., and Hains, B. C., 2007. Extracellular signal-regulated kinase-regulated microglia-neuron signaling by prostaglandin E2 contributes to pain after spinal cord injury. *J. Neurosci.* 27, 2357-2368.
99. Zhao, P., Waxman, S. G., and Hains, B. C., 2007. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. *J. Neurosci.* 27, 8893-8902.
100. Zheng, F. Y., Xiao, W.-H., and Bennett, G. J., 2011. The response of spinal microglia to chemotherapy-evoked painful peripheral neuropathies is distinct from that evoked by traumatic nerve injuries. *Neuroscience* 176, 447–454.
101. Zhou, D., Chen, M.-L., Zhang, Y.-Q., and Zhao, Z.-Q., 2010. Involvement of spinal microglial P2X7 receptor in generation of tolerance to morphine analgesia in rats. *J. Neurosci.* 30, 8042-8047.
102. Zhuang, Z.-Y., Gerner, P., Woolf, C. J., and Ji, R.-R., 2005. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. *Pain* 114, 149-159.