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Review

Neuroinflammation in osteoarthritis: From pain to mood disorders

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ABSTRACT

Osteoarthritis (OA) is the most common form of musculoskeletal disease, and its prevalence is increasing due to the aging of the population. Chronic pain is the most burdensome symptom of OA that significantly lowers patients' quality of life, also due to its frequent association with emotional comorbidities, such as anxiety and depression. In recent years, both chronic pain and mood alterations have been linked to the development of neuroinflammation in the peripheral nervous system, spinal cord and supraspinal brain areas. Thus, mechanisms at the basis of the development of the neuroinflammatory process may indicate promising targets for novel treatment for pain and affective comorbidities that accompany OA. In order to assess the key role of neuroinflammation in the maintenance of chronic pain and its potential involvement in development of psychiatric components, the monoiodoacetate (MIA) model of OA in rodents has been used and validated. In the present commentary article, we aim to summarize up-to-date results achieved in this experimental model of OA, focusing on glia activation and cytokine production in the sciatic nerve, dorsal root ganglia (DRGs), spinal cord and brain areas. The association of a neuroinflammatory state with the development of pain and anxiety- and depression-like behaviors are discussed. Results suggest that cells and molecules involved in neuroinflammation may represent novel targets for innovative pharmacological treatments of OA pain and mood comorbidities.

1. Introduction

Osteoarthritis (OA) is the most frequent form of musculoskeletal disease and affects millions of people in the world, especially the elderly. Thus, considering the increase in life expectancy, the number of subjects suffering from this pathology is growing every year [1,2].

It is well known that OA is a complex pathology that involves all the structures, tissues, and cells that compose the joint, in which both mechanical and inflammatory factors cause progressive joint degeneration [3–5]. As a direct consequence, functional impairment represents an important negative trait of the pathology, but the main symptom that deeply and negatively impacts patients is pain [6]. Signs of progressive joint damage, including cartilage erosion, subchondral bone sclerosis, synovitis, bone remodeling with osteophyte formation, and meniscal damage may theoretically all contribute to pain onset and maintenance [3,7]. However, in OA patients, no direct correlation has been found between structural joint harm and the intensity or persistence of pain [8]. Indeed, although OA patients are known to complain of both joint and referred pain (i.e., pain in areas adjacent to the affected joint) [9,10], it has been reported that a subset of patients continues to feel pain even after a technically successful joint replacement [11]. These clinical observations describe the complexity of the genesis and maintenance of OA pain, suggesting the existence of a neuropathic component, given that either pain arises also in areas outside of the injury site or it manifests after the peripheral nociceptive input has been removed [12,13].

In OA-damaged joints, different cell populations such as synoviocytes, inflammatory cells, and chondrocytes produce chemokines, cytokines, and proteases that can sensitize primary sensory neuronal afferents [8,12]. The continuous increase in nociceptive inputs from the periphery further results in central sensitization in the dorsal horn of the spinal cord. Indeed, pathological changes in the joint cause hyperexcitability of second-order spinal neurons [8,12], by reducing their firing thresholds and enhancing their responses to knee stimulation.

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Moreover, sensitized dorsal horn neurons expand their receptive fields, a mechanism that underlies the spread of hypersensitivity from the affected joint (either knee, hip or ankle joints which could all be affected by OA) to adjacent areas. The expansion of receptive fields and reduction of mechanical thresholds around the joint area are consistently observed in OA patients [14].

Nowadays, there is no cure for OA, with currently available treatments only focusing on temporary symptomatic pain relief and on the reduction of inflammation, often leaving patients with considerable pain and functional disability. Paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), steroids and in some rare cases opioids are the most prescribed pain-relieving therapies for OA [15].

Interestingly, it is now recognized that OA pain is frequently accompanied by co-morbid affective manifestations, such as anxiety and depression [16,17], which could additionally impact the patient's quality of life and aggravate pain perception, contributing to the transition to chronic pain. It has been hypothesized that the underlying mechanisms involved in chronic pain processes are also implicated in the development of memory deficits and psychiatric disorders due to a neurobiological overlap between pain processing and stressful and emotional signals [18,19]. In particular, a pivotal role for neuroinflammation, an inflammatory response in the central (CNS) and peripheral nervous system (PNS), in the pathogenesis of both chronic pain and depressive/anxious diseases is now emerging [20–24]. Hallmarks of neuroinflammation are the activation of glial cells (such as microglia, astrocytes and satellite glial cells, SGCs), and macrophage infiltration. During a neuroinflammatory condition, all these cell populations release chemokines and other mediators, and through neuroimmune interactions modify pain signaling [23] at key stations in pain transmission (including dorsal root ganglia, DRGs, and the dorsal horn of the spinal cord). Similarly, the presence of activated non-neuronal cells in supraspinal areas is currently proposed as a predisposing factor in the development of emotional and psychiatric disorders [18].

Despite the above-mentioned evidence, the role of neuroinflammation as a common substrate for pain chronicization and the development of anxious-depressive disorders in OA has begun to be considered and studied only recently. Due to the complexity of investigating these underlying mechanisms in patients, preclinical models of OA in rodents have been developed and utilized to try to decipher the existence of links among pain, emotional disturbances and neuroinflammation in PNS and CNS.

The aim of this review is therefore to discuss preclinical evidence on the key role of neuroinflammation in the maintenance of chronic OA pain and its potential involvement in the development of OA-related anxious-depressive components, and to propose a translation of currently available results to a clinical setting.

2. Neuroinflammation

Inflammation is a protective response against external pathogens or damaged cells, which promotes their elimination and, consequently, the resolution of infections or wound healing. Several studies have shown that, following injury or infections, also the nervous system exhibits the typical features of inflammation, named neuroinflammation [25], a localized form of inflammation that occurs in PNS (nerves and ganglia) and CNS (spinal cord and brain) [20]. Physiologically, it represents a defense mechanism that initially protects the brain by eliminating pathogens, promoting tissue repair, and removing cellular debris. A prolonged inflammatory response, however, becomes detrimental and consequently inhibits tissue regeneration [26]. Neuroinflammation can indeed be classified as neuroprotective or neurodegenerative, depending on whether its effects last for a short amount of time or become chronic and endure for a prolonged time, resulting in nervous system damage [25]. There is growing evidence that numerous factors, such as trauma or the normal aging process, contribute to neuroinflammation. It is also a major cause and the driver of the progression of several

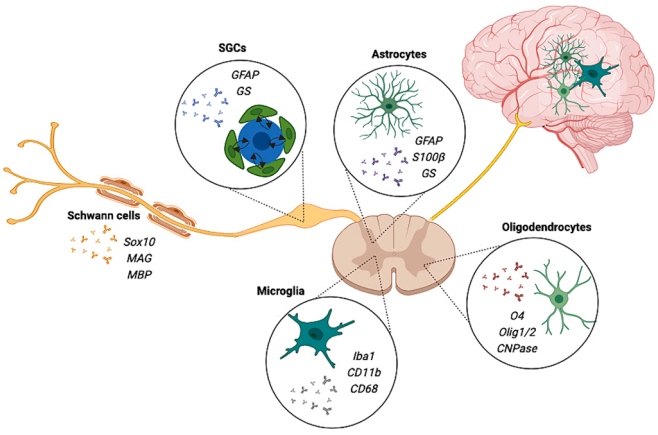

Fig. 1. Schematic representation of the glial cell populations involved in neuroinflammation and of their typical markers. After PNS injury or peripheral inflammatory stimuli, Schwann cells and SGCs become activated, express specific markers, and release pro-inflammatory cytokines, thus contributing to the generation and maintenance of an inflammatory environment. Microglia cells and astrocytes are the main actors involved in CNS inflammation. After inflammatory stimuli, both cell types can exert pro-inflammatory functions, which are neurotoxic and detrimental, or anti-inflammatory functions, which are instead beneficial. Depending on their state of activation, and on whether they are exerting neuroprotective or neurotoxic functions, these cells express different markers. The CNS is also populated by oligodendrocytes, which, upon injury, express a wide range of inflammatory mediators and several receptors, which enable them to sense and react to inflammation. Created with BioRender.com.

neurodegenerative and neuropsychiatric diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and depression [27,28]. In addition, neuroinflammation is associated with different types of pain involving the CNS, such as central neuropathic pain, but also quite unexpectedly with other pain conditions, such as OA pain. Indeed, it is now clear that some painful conditions for a long time considered as being exquisitely peripheral (e.g., OA pain or chronic constriction injury of the sciatic nerve) also manifest typical features of centrally driven pain, including signs of neuroinflammation in the PNS and CNS [20,25].

Neuroinflammation is characterized by vascular changes resulting in increased blood–brain barrier (BBB) permeability, which leads to increased invasion of leukocytes, activation of glial cells, and eventually production of inflammatory mediators, including cytokines and chemokines [20].

In the PNS, the main types of glial cells are Schwann cells (SCs), which provide myelin sheaths to peripheral nerves and maintain homeostasis of the neuronal microenvironment, and satellite glial cells (SGCs), which envelop the cell bodies of primary sensory neurons within the DRGs and trigeminal ganglia (TGs) and are coupled together through gap junctions. In response to inflammatory stimuli and nerve injury, these cells are activated before the central glia and release inflammatory mediators that sensitize nociceptors at the level of axons and cell bodies [29]. After peripheral nerve injury, activated SCs release proinflammatory cytokines, such as tumor necrosis factor (TNF-α) and interleukin (IL)-1β [30]. SGCs, on the other hand, actively participate in neuroinflammation by upregulating glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS), which represent markers of their activation under pathophysiological conditions, as described in Fig. 1 and in [31]. During neuroinflammation, an increase in SGC-SGC and neuron-SGC coupling mediated by gap junctions and an increased release of proinflammatory mediators, such as TNF-α, IL-1β, IL-6 and CX3CL1, also occur. SGCs are also believed to regulate the environment around neuronal bodies under pathological conditions by releasing additional factors that modulate neuronal activity, such as adenosine 5′-

triphosphate (ATP), nitric oxide and prostaglandins [30]. Besides, infiltrating macrophages and immune cells collaborate with SGCs to sensitize sensory neurons through the release of multiple pro-inflammatory mediators [32,33].

The main non-neuronal key actors in CNS inflammation are microglia cells and astrocytes. Both glial cell types can have pro-inflammatory or anti-inflammatory functions, leading to detrimental or beneficial effects during neuroinflammation, depending on the timing, underlying signaling mechanisms and complex intercellular interactions [34].

Microglia cells are ubiquitously distributed in the brain and spinal cord and represent the principal innate immune cells and the first cell population to react following pathological lesions. Their main functions are to detect changes in the surrounding environment through their sensomes, to migrate to injured sites, to remodel synapses, and to control myelin homeostasis [25]. Finally, following inflammatory stimuli, microglia cells become activated, change their morphology from

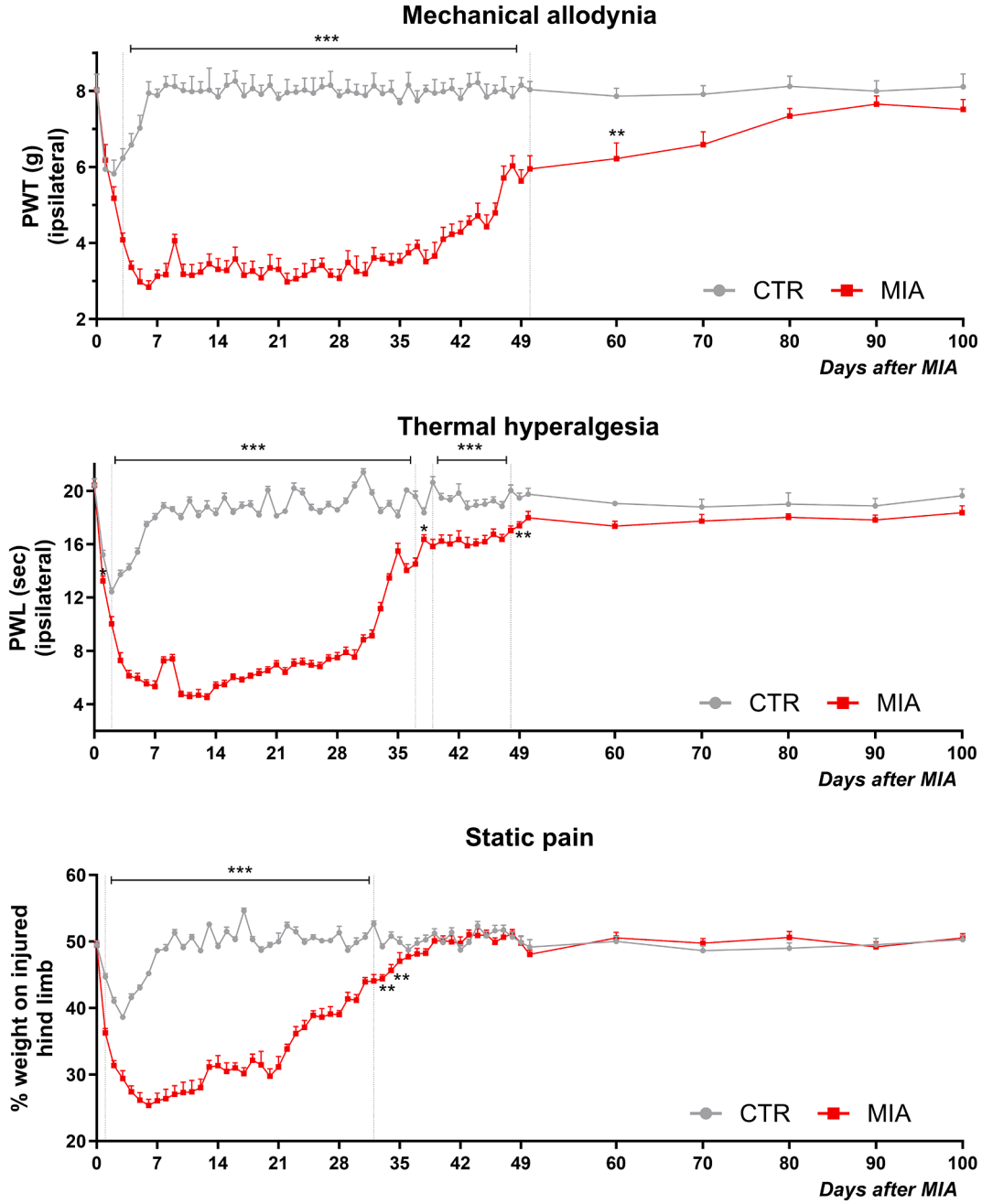

Fig. 2. Time course of the onset of pain-like behavior after induction of OA in mice. Mechanical allodynia (A), thermal hyperalgesia (B) and weight-bearing responses (C) were assessed in 11-week old male C57BL/6J mice, before (day 0) and after a single intra-articular administration of MIA (1 mg in 10 μl saline in the right knee; Merck Life Science, Milan, Italy). Control (CTR) mice were intra-articularly treated in the right knee with 10 μl saline on day 0. Tests to evaluate pain-like behavior were conducted as previously described [53]. Briefly, mechanical allodynia was evaluated on OA paw (mid-plantar surface) using dynamic plantar aesthesiometer (von Frey filament Ø 0.5 mm, setting: 10 g in 10 s; Ugo Basile, Italy). Thermal hyperalgesia was assessed on OA paw (mid-plantar surface) by plantar test with a constant intensity radiant heat source (beam diameter 0.5 cm and intensity 20 I.R.; Ugo Basile, Italy). Weight-bearing asymmetry changes in the weight bearing on hind limbs were tested using incapacitance tester (interval: 3sec; Linton Instruments, Norfolk, UK). Data represent the mean ± SEM of 8 mice/group. Statistical analysis was performed by two-way ANOVA followed by Bonferroni's post-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to CTR. PWT, paw withdrawal threshold; PWL, paw withdraw latency.

ramified to ameboid [29], and release pro-inflammatory cytokines, such as TNF-α, IL-1β and chemokines, and recruit infiltrating immune cells to eliminate the inflammatory triggers and to contribute to damage repair [26]. Depending on their state of activation, on the pathological condition, and on whether they are exerting a pro- or anti-inflammatory function, microglia cells express different markers that are summarized in [35] (see Fig. 1). Although the common definition of M1 pro-inflammatory and M2 anti-inflammatory microglia subpopulations has currently been overcome by a more dynamic and integrated view of microglia functions [35], the expression of specific markers and the morphology of microglia are still considered very useful tools to describe the stage of tissue inflammation or its resolution upon pharmacological interventions.

Astrocytes are the largest glial cell population in the brain and spinal cord and play an active and critical role in maintaining brain homeostasis. Currently, GFAP, GS and S100β are the main markers used to assess astrocyte activation state (as shown in Fig. 1 and summarized in [34]). Astrocytes are activated under various pathological conditions and convert to a reactive state, called astrogliosis, characterized by morphological changes, significantly increased GFAP expression and cell proliferation, which is believed to be associated with a loss of astrocyte homeostatic functions [30,36]. Similar to microglia, astrocyte response to danger signals during neuroinflammation can be beneficial or detrimental depending on the stimuli from the inflamed environment. During acute neuroinflammation, reactive astrocytes perform neuroprotective functions through the secretion of neurotrophins to support damaged neurons and the formation of a glial scar to enclose the damaged area, thereby limiting the spread of a cytotoxic milieu and promoting axon regeneration [34]. However, during chronic neuroinflammation, astrocytes are exposed to a variety of danger signals, resulting in an increase in their reactivity that contributes to demyelination and neurodegeneration. Pro-inflammatory reactive astrocytes upregulate several genes (e.g., complement cascade genes) and induce pro-inflammatory factors (e.g., IL-1β and TNF-α), with damaging functions [25,26,36].

In addition to microglia and astrocytes, the CNS is also populated by oligodendrocytes (OLs), highly specialized cells whose main role is to constitute myelin [37]. Based on several studies, it is now clear that OLs have also immunomodulatory properties, through which they actively contribute to the immune-inflammatory response in neurodegenerative diseases. This is relevant not only to neuroinflammatory conditions but also to other neurological disorders in which inflammation strongly contributes to neurodegeneration. Indeed, selective loss of myelin and OLs contributes to their pathogenesis [38]. Several mechanisms are known to generate OL stress, but one of the most important factors is inflammation. Studies suggest that pro-inflammatory cytokines have the potential to impair OLs already during their development [25].

Overall, published literature demonstrates that neuroinflammation represents a complex response to CNS and PNS injuries, involves a wide number of cellular and molecular actors, and could represent a valid target for many central and peripheral pathological conditions.

3. The MIA rodent model of OA

Several OA models have been developed in the last years [24,39,40]. Among them, models of spontaneous OA in rodents are unreliable and time-consuming, therefore the most successful rodent models of the disease are based on either surgically- or chemically-induced OA. Surgical models include induction of damage to the anterior cruciate ligament and partial or complete meniscectomy, but they have not been frequently utilized to study OA-related pain due to the technical difficulties of the interventions and to the often unpredictable time course of the development of painful symptoms [41].

For these reasons, chemically-induced OA models are generally preferred. They usually consist of a single intra-articular injection of substances (e.g., monoiodoacetate - MIA - papain or collagenase) which target different components of the joint [40,42]. Injection is usually performed in the knee, but other joints such as the hip and the ankle can be exposed to the drug as well [43–45]. Based on its wide use as a reliable OA rodent model, we focused the present review on data obtained in both mice and rats injected with MIA into the knee joint.

Although artificial induction of OA cannot fully recapitulate the natural onset of the human disease, it is nevertheless useful to originate robust and reproducible pain phenotypes, making these models particularly adequate for studying the molecular pain pathways and for testing the efficacy of new pharmacological agents to treat OA pain [46].

MIA-induced OA is the most widely used rodent model to study the underlying mechanisms and to develop new analgesics for OA pain [47–49]. MIA is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, which causes dose-dependent cell death by disrupting cellular glycolysis [48]. Thus, the knee intra-articular injection of MIA results in histopathological alterations and functional impairment like some of the features observed in the early phases of human OA. Since the site of injection is restricted to the joint space, intra-articular injection of MIA causes chondrocyte cell death only, leading to cartilage degeneration and subsequent subchondral bone alterations [50,51]. As previously mentioned, the MIA model is of simple induction and leads to the development of a robust disease model that mimics human OA pain; however, the injection into the joint capsule must be extremely precise and carried out by expert personnel. Indeed, the release of MIA outside the joint space could cause the death of the animal.

Although some variables, such as the dose of injected MIA, may change the time course of the development of joint damage and of pain-related behaviors as well as the extent of pain intensity, the presence of sensory hypersensitivity is generally observed starting from 3 days after MIA injection and remains overtly present up to 28 days [48,52–54]. Hypersensitivity may last longer, but most studies are interrupted 4–5 weeks after MIA injection for ethical reasons [55].

3.1. Pain-related behavior in the MIA model of OA

Pain assessment in animals is challenging. In the MIA model, Von Frey filament or the dynamic plantar aesthesiometer tests are employed to measure alterations of nociceptive mechanical thresholds (allodynia) in the hind paw rather than in the injected knee joint, since assessing joint pain threshold is technically difficult in rodents. Measurements performed on the paw reflect refers OA knee joint pain. Indeed, as mentioned above, during experimental OA, joint afferents typically expand their receptive fields to areas adjacent to the injected joint [56], as it spontaneously happens in human OA [14]. In addition, another commonly used method for pain evaluation is the incapacitance test, which measures the weight distribution between both hindlimbs, thus providing a measurement of static pain [57]. Although mechanical thresholds and weight distribution are more commonly used, thermal hyperalgesia has been reported in the MIA model as well (Fig. 2) [53].

4. Inflammation in MIA-induced rodent models of OA

4.1. Inflammatory response in the joints

After MIA injection, a strong inflammatory response develops in the knee joint, as described in [23,24,48]. Proinflammatory cytokines such as IL-1β, IL-6 and TNF-α are significantly increased around 3 days after OA induction and their levels remain elevated for several weeks [8,23,54].

Several chemokines, such as chemokine ligand (CCL) 2 and its receptor CCR2 are also found increased in the MIA-treated joint [58]. Recently, among chemokines, our research group identified the overexpression of the prokineticin system (PKS) in the knee joint [54], which includes a ligand, prokineticin (PK)2, and two GPCRs (PKR1 and PKR2), and exerts pro-inflammatory and pronociceptive effects [59–62]. Synoviocytes, infiltrating inflammatory cells, chondrocytes, as well as

Table 1
Summary of published data on the development of neuroinflammation after induction of OA by injection of MIA in rodents. The table summarizes the main changes in the expression of markers/mediators of neuroinflammation following its progression in key tissues in rodent models of MIA-induced OA. See text for details.

Animal model	Sciatic nerve	DRGs	Spinal Cord	HPC	PFC	Ref.
Sprague Dawley rats, adult males MIA: 3 mg in 50 μl saline, right knee joint	n.a.	n.a.	**CD11b and GFAP**: ↑T21	n.a.	n.a.	[85]
Sprague Dawley rats, females, 6-week-old MIA: 2 mg in 25 μl saline, right knee joint	n.a.	**ATF3**: = T7; ↑T14, T21 and T28	**Iba1**: = T7; ↑T14, T21 and T28	n.a.	n.a.	[8]
Sprague Dawley rats, adult males MIA: 1 mg in 50 μl saline, left knee joint	n.a.	n.a.	**Iba1**: ↑T7, T14 and T28 **GFAP**: = T7 and T14; ↑ T28	n.a.	n.a.	[80]
Wistar rats, adult males MIA: 0.3, 1, 2 mg in 25 μl saline, left knee joint	n.a.	**ATF3**: ↑T3, T7, T14, T21 and T31	n.a.	n.a.	n.a.	[71]
Sprague Dawley rats, adult males MIA: 1 or 2 mg in 25 μl saline, left knee joint	n.a.	**ATF3**: ↑T3, T7 and T14	**Iba1 and ATF3**: = T3 and T14; ↑T7	n.a.	n.a.	[68]
Sprague Dawley rats, adult males MIA: 3 mg in 25 μl saline, left knee joint	n.a.	n.a.	**IL-6, GFAP and CD11b**: ↑T21	n.a.	n.a.	[87]
Sprague Dawley and Wistar Kyoto rats, adult males MIA: 1 mg in 50 μl saline, left knee joint	n.a.	n.a.	**Iba1 and GFAP**:↑T21	n.a.	n.a.	[81]
Sprague Dawley rats, adult males MIA: 2 mg in 25 μl saline, left knee joint	n.a.	**ATF3**: = T4 and T14	**Iba1, GFAP, IL-6 and TNFα:** = T4 and T14 **IL-1β:** = T4; ↑T14	n.a.	n.a.	[84]
Sprague Dawley rats, adult males MIA: 0.8, 1.6 or 2.4 in 40 μl saline, tiobiotalar joint	n.a.	**ATF3**: ↑T35 **Iba1 and GFAP**: = T7 and T14; ↑T35 and T42	n.a.	n.a.	n.a.	[45]
Sprague Dawley rats, adult males MIA: 4.8 mg in 60 μl saline, right knee joint	n.a.	n.a.	**IL-1β, IL-6 and TNFα**:↑ T14 and T28	n.a.	n.a.	[88]
Sprague Dawley rats, adult males MIA: 1 mg in 50 μl saline, knee joint	n.a.		**IL-1β, IL-6 and TNFα:** ↑T3, T7, T14 and T21 **Iba1**: = T3 and T7; ↑T14 and T21 **GFAP**: = T3, T7 and T14; ↑T21	n.a.	n.a.	[82]
Sprague Dawley rats, adult males MIA: 1 mg in 50 μl saline, knee joint	n.a.	**IL-1β, IL-6 and TNFα:** = T3; ↑T7, T14 and T21	**IL-1β:** ↑T3, T7, T14 and T21 **IL-6:** = T3 and T7; ↑T14 and T21 **TNFα:** = T3; ↑T7, T14 and T21 **Iba1:** ↑T18 **GFAP**: = T18	n.a.	n.a.	[73]
C57BL/6J mice, 8–10-week-old males MIA: 0.5, 0.75 and 1 mg in 10 μl saline, left knee joint	n.a.	**ATF3**: ↑T7	**Iba1**: = T7; ↑T28 **GFAP**: = T7 and T28	n.a.	n.a.	[72]
Swiss Albino mice, 9–12-week-old males MIA: 1 mg in 10 μl saline, knee joint	n.a.	**Iba1**: = T15 (trend ↑)	**TNFα**: ↑T1 and T15 **IL-1β**: = T1; ↑T15 **Iba1**: ↑T15	n.a.	n.a.	[83]
Female mice, 6–8-week-old MIA: 0.15 mg in 10 μl saline, right knee joint	n.a.	n.a.	n.a.	**CD11b**: ↑T29 **GFAP**: = T29	n.a.	[102]
C57BL/6J mice, 10-week-old males MIA: 1 mg in 10 μl saline, right knee joint	**IL-1β, IL-6, CD68, GFAP and ATF3**:↑T21	**IL-1β, CD68 and ATF3**: ↑T21 **IL-6 and GFAP**: = T21	**IL-1β, IL-6, GFAP, CD68 and ATF3**:↑T21	n.a.	n.a.	[66]
C57BL/6J mice, 10-week-old males MIA: 1 mg in 10 μl saline, right knee joint	**IL-1β, IL-6, TNFα, CD68, GFAP**: ↑T14 **ATF3**: = T14	**IL-1β, CD68, GFAP and ATF3**: ↑T14 **IL-6 and TNFα**: = T14	**IL-6, CD11b, GFAP and ATF3**: ↑T14 **IL-1β and TNFα**: = T14	n.a.	n.a.	[53]

(*continued on next page*)

Table 1 (*continued*)

Animal model	Sciatic nerve	DRGs	Spinal Cord	HPC	PFC	Ref.
C57BL/6J mice, 10-week-old males MIA: 1 mg in 10 μl saline, right knee joint	n.a.	n.a.	n.a.	**TNFα, Iba1 and GFAP**: ↑T14 **IL-6**: = T14	**IL-6, TNFα, Iba1 and GFAP**: ↑T14	[52]
C57BL/6J mice, 10-week-old males MIA: 1 mg in 10 μl saline, right knee joint	**PK2, PKR1, IL-1β, IL-6, CD11b, GFAP and ATF3**: ↑T28	n.a.	**PK2, PKR1, IL-1β, IL-6 and GFAP**: ↑T28 **CD11b and ATF3**:= T28	**PK2, IL-1β, TNFα and GFAP**: ↑T28 **IL-6 and Iba1**: = T28	**IL-1β, TNFα and Iba1**: ↑T28 **PK2, IL-6 and GFAP**:= T28	[54]

HPC: hippocampus; PFC: prefrontal cortex; =: not modified in MIA-treated vs CTR animals; ↑: increased in MIA-treated vs CTR animals; n.a.: not available; T: time (days) after MIA injection.

infrapatellar fat may express PKRs and produce PK2 [63,64], whose upregulation could sustain an inflammatory loop in turn promoting the recruitment of inflammatory cells, the release of pro-inflammatory/algogenic mediators and a direct sensitization of nociceptors [61,62]. A close link between the PKS and known inflammatory pathways is demonstrated by the fact that administration of diclofenac to MIA mice, besides relieving pain, also exerts a significant reduction of PK2 and its receptors in OA knee joint [54]. Interestingly, a recent paper identified the presence of high levels of PK2 also in the knee synovial fluid of OA patients, drawing the attention to this chemokine as a possible new marker and/or target for OA treatment [65].

4.2. OA-induced neuroinflammation in the PNS

Since most pain measurements are performed at the paw level (i.e., referred pain) and the sciatic nerve and its distal afferents (e.g., the tibial, common peroneal and sural nerves) innervate the paw, this station represents the first tissue where a neuroimmune interaction may be possible after OA induction. Therefore, in a series of studies we analyzed the activation of non-neuronal components in the sciatic nerve of MIA mice at different time points [53,54,66]. Briefly, from 14 to 28 days after MIA injection elevated levels of the proinflammatory cytokines IL-1β and IL-6 were constantly present, and GFAP expression, which in the nerve can identify activated SCs, steadily increased. Instead, the overexpression of markers related to macrophage activation, such as CD11b and CD68, peaked precociously at 14 days, but then slowly decreased, although it remained significantly elevated up to 28 days after MIA injection (unpublished data; [53,54,66]).

Activated or infiltrating macrophages could be responsible for the increase of inflammatory markers in the sciatic nerve and may be a consequence of demyelination [67]. Indeed, Thakur and colleagues [13,68] and Muley and coworkers [67] observed significant demyelination of the sciatic nerve in the MIA rat and mouse models. Demyelination and neuroinflammation are largely interrelated, and sciatic nerve damage and subsequent neuroinflammation are also confirmed by the expression of the transcription factor 3 (ATF3), a marker of neuronal damage/stress, which gradually increases over time [53,54,66]. We found a stable upregulation of PK2 and its receptors in the sciatic nerve as well (unpublished data, [54]). Although no direct evidence of the cellular source of PK2 are available in MIA models, results from our previous studies in different neuropathic pain models [69] suggest that both infiltrating macrophages and activated SCs may be the primary source of PK2.

Since the cell bodies of sensory neurons that innervate the joints are located in the DRGs, neuroinflammation has been evaluated in this tissue as well. As shown in the sciatic nerve, a time-dependent increase of the neuronal stress marker ATF3 was reported at 3, 7 and 14 days after MIA injection [53,68,70,71], with a more limited increase 7 days after MIA injection demonstrated in [72]. Furthermore, our group [66] observed that at later observation times (i.e., 21 days after MIA injection) ATF3 expression is still upregulated and Bourassa and colleagues [45] demonstrated an increase of this stress signal at 5 weeks after MIA. Overall, these data confirm how intra-joint degeneration processes impact PNS tissues. IL-1β, IL-6 and TNF-α levels were also reported to be increased in rat DRGs as early as 7 days after MIA injection [73]. Our group confirmed the presence of high IL-1β levels in mouse DRGs both at 14 and 21 days after OA induction. Moreover, we also observed the presence of activated SGCs in DRGs, as demonstrated by elevated GFAP expression at 14, 21 and 28 days after induction of OA (unpublished data; [53,66]).

In addition to the activation of resident glia, recent research described a role for DRG-infiltrating immune cells, in particular macrophages, as key contributors in the development of OA-associated pain [74]. Authors further observed that these infiltrating macrophages express typical markers of the M1 pro-inflammatory phenotype and how their switch to an anti-inflammatory M2 phenotype may be beneficial for pain relief [75,76]. To support these data, we also observed the overexpression of macrophage markers in mouse DRGs, which was particularly evident between 14 and 21 days after MIA injection [53,66]. Interestingly, depleting macrophages before induction of OA in mice resolved pain-like behaviors by day 7 without affecting the initial development of pain. Authors demonstrated that inhibition of a member of the transforming growth factor (TGF)-β signaling pathway (i.e. myostatin) during established OA fully reverted pain and macrophage DRG infiltration [75].

As summarized in Table 1, unfortunately, results from the few research papers on the neuroimmune interaction in DRGs in the MIA model are not always coherent, also because different methodologies have been used; thus, further work is needed to assess its role in the development of OA-related pain. Although we are aware that it is difficult and likely incorrect to make comparisons among different animal models, based on available data our opinion is that the involvement of neuroinflammation in DRGs in the MIA model appears less relevant than in other preclinical models of chronic neuropathic pain, such as chemotherapy-induced or partial nerve ligation-induced neuropathic pain [59,60,72,77]. Our impression is that infiltrating macrophages, rather than activated resident SGCs, may be relevant for the maintenance of OA pain.

4.3. Neuroinflammation in the spinal cord of MIA-induced OA

Primary afferent fibers innervating the knee joint project to several spinal cord segments and terminate in both the superficial and deeper laminae, where they make synapse with second-order dorsal horn neurons [78], which become hyperexcitable following pathological changes in the joint, with a reduction of their firing threshold and an enhancement of their responses to knee stimulation (see also section 3.1). Furthermore, sensitized dorsal horn neurons expand their receptive fields, a mechanism that underlies the spread of hypersensitivity from the knee joint to adjacent areas [56]. Thus, neuroinflammation in the spinal cord is a distinctive sign of persistent pain [79], and it has been increasingly reported also in the MIA model both in mice and rats, as summarized below.

There is a general consensus about the activation of spinal cord microglia in MIA-induced OA (Table 1). Most studies on rodent models (both mice and rats) investigated the development of microgliosis at

different times after MIA injection, by analyzing the expression levels of the microglia marker Iba1, either as mRNA or protein by Western blotting and/or immunohistochemistry. Studies with other markers, such as CD11b and CD68, provided similar results.

Sagar and colleagues [80] demonstrated microglia activation in rat spinal cord as early as 7 days after OA induction, which remained elevated at 14 and 28 days. Other studies in rats confirmed that microgliosis is observed at 14 and 21 days after MIA administration [73,81,82], while Bourassa and colleagues [45] reported elevated Iba1 expression at later time points, i.e. 6 weeks after OA induction, thus suggesting persistence of microglia involvement over time. Additionally, also in mice OA models, activation of microglia can be detected at 15 [83] and at 28 days after MIA injection [72]. Furthermore, a series of studies from our group demonstrated increased CD11b and CD68 expression at 14 and 21 days after OA induction in mice [53,66]. However, in contrast with other reports, we observed that microglia marker overexpression disappeared at 28 days after MIA injection [54]. Finally, a study in rats did not find any significant alteration of microglia markers either at a very early phase (4 days) or at later time points (14 days post-MIA injection) [84].

In contrast, astrocyte activation, as measured by GFAP expression, is less clearly evident, with some studies reporting no astrocyte response [72,85], while others demonstrate increased GFAP immunoreactivity [86,87]. In particular, elevated GFAP expression was found only at a late phase of the disease in rats, meaning starting from 21 days after MIA injection [80–82]. On the contrary, we found a significant increase in GFAP expression at 14, 21 and 28 days after MIA injection in mice [53,54,66].

Overall, from the above-mentioned studies, it can be summed up that continuous nociceptive inputs from the joint significantly affect the activation of non-neuronal cells in the spinal cord. Moreover, published results lead to hypothesize that microglia may represent the driving force in the development of pathological neuroimmune interaction which in turn leads to a stable but less consistent astrocyte involvement.

Furthermore, in addition to the demonstration of glial cell activation, the identification of the signaling molecules that are produced and released by glia and immune cells in the spinal cord, and that either directly sensitize neurons or further fuel the activation of glial cells, is of utmost importance. Indeed, a few papers evaluated the levels of pro-inflammatory cytokines in spinal cord of rat and mice after MIA injection. In a rat model of OA, Sun and coworkers [73,82] reported a time-dependent increase of IL-1β, TNF-α and IL-6 expression, as early as 3 days after MIA injection and gradually increasing up to 21 days. Elevated levels of pro-inflammatory cytokines were also reported by Li and colleagues [88] and Lokwood and coworkers [84] at 14 and 28 days.

The pattern of cytokine upregulation is slightly different in the mouse model of OA. We found an upregulation of IL-6 starting from 14 days after OA induction which remains elevated at later time points (i.e., 21 and 28 days after MIA injection), while IL-1β was increased starting from 21 days after OA induction [52,54,66]. We also found over-expression of the chemokine PK2 (unpublished data; [54]).

It is well documented that the expression of cytokines, such as IL-1β, TNF-α and IL-6, and of neuropeptides can be regulated by NF-κB [89], a key transcription factor that plays a fundamental role in inflammatory diseases, including autoimmune disorders, cancer and neuro-degeneration. Activation of NF-κB induces TNF-α, IL-1β and cyclo-oxygenase (COX)-2 upregulation in the spinal cord, in turn leading to pain hypersensitivity following peripheral inflammation [90]. Consistently, NF-κB activation has been reported in OA rats as early as 7 days after MIA injection and remained constant up to 28 days. These results suggest that the activation of NF-κB/p65 in the spinal cord may induce upregulation of inflammatory mediators, such as TNF-α, IL-1β, and IL-6, also in OA, thus contributing to the development of neuroinflammation [88].

To the best of our knowledge, no data are currently available on the involvement of oligodendrocytes in OA-induced pro-inflammatory alterations at the spinal cord level. Since their role in the development of neuroinflammatory responses is now clearly emerging (see section 2 and Fig. 1), it will be worth evaluating their contribution to spinal neuronal sensitization in the MIA rodent models as well.

In conclusion, although currently available data are still limited, a significant involvement of neuroinflammatory components in the spinal cord is really evident in MIA-induced OA, in line with other chronic pain conditions such as neuropathic pain. Spinal neuroinflammation likely contributes to the chronicization of pain as an underlying mechanism of advanced knee OA.

5. Mood alterations and supraspinal neuroinflammation in MIA models of OA

It has now become evident that chronic pain affects various aspects of patient's quality of life, including mood, sleep and cognitive processes [91,92]. Indeed, mood disorders, such as major depressive disorders and anxiety, are frequently observed in chronic pain patients [16,17,93]. In turn, these co-morbidities can aggravate pain perception, leading to the generation of a vicious circle which further contributes to sustaining chronic pain in OA as well [94]. It is therefore extremely important to understand the underlying mechanisms to develop innovative treatments that can simultaneously control the nociceptive, affective and cognitive manifestations of OA. Therefore, preclinical studies have recently started to address the anxio-depressive-like consequences of chronic pain in several pain models, including MIA-induced OA [95].

As a general issue, we are perfectly aware of the drawbacks and intrinsic limitations of the behavioral tests that are generally employed to detect mood alterations in rodents. Nevertheless, they have been validated for their reliability and have proved to be useful in preclinical settings and to extrapolate data to be applied to the clinics. A detailed description of their advantages and limitations goes beyond the scope of this paper, and we therefore address the reader to a recently published extensive review on this topic [96]. In brief, the elevated plus maze (EPM), the open field (OF) and the light/dark box (LDB) are the most frequently used tests to assess the presence of anxiety-like conditions [97]. Tail suspension (TST) and forced swimming (FST) tests are instead generally accepted and validated methods to evaluate the presence of a depressive-like behavior [98], while the novel object recognition test (NOR) is the most frequently utilized to detect memory impairment, that is usually also associated with depression [99].

Most of the studies that reported psychiatric manifestations in the MIA rodent models also attempted to evaluate the underlying mechanisms, by measuring the expression of mediators and neuro-inflammatory markers in various brain areas.

As reported above, the studies on OA-related pain measurements were performed both in rats and in mice. Conversely, the few papers dealing with depression and anxiety-like behaviors have been mostly conducted in mice.

LaPorta and coworkers [100] demonstrated that a weak anxiety state, evaluated with the EPM test, was already present at 7 days and became more severe at 21 days after MIA injection. Cognitive impairment, assessed with the NOR test, was also present in OA mice with a similar time course. These authors did not look for neuroinflammation in the brain, since their study was only focused on the role of the endocannabinoid system. Subsequently, Carcolè and colleagues [101] also observed a time-dependent increase in the anxiety-like condition (EPM test) in OA mice. Indeed, a mild anxiety status was observed 11 days after MIA injection, while it was clearly evident at 21 days. Besides, in this paper authors also found a clear depressive-like condition, as evaluated with the FST, 25 days from OA induction. Additionally, in parallel with behavioral alterations, authors detected significant microgliosis, as demonstrated by an increase of the total number and the soma perimeter of microglial cells in the prelimbic and infralimbic areas. Moreover, by employing several tests (i.e., EPM and OF for anxiety; FST and TST for depression) Batallè and colleagues [102,103] demonstrated

anxiety- and depression-like behavior in mice 29 days after MIA injection. Moreover, animals also displayed cognitive impairment [103]. In parallel with these behavioral dysfunctions, authors also found supraspinal biochemical alterations: the microglia marker CD11b was upregulated in the hippocampus, while astrocytic makers were not modified. Moreover, an increase in nitric oxide (NO) synthase was observed both in the hippocampus and the amygdala. Studies from our group confirmed the development of altered mood behaviors and cognitive function in mice at 14 [53] and 28 days [54] after MIA injection by applying the same behavioral tests (i.e., LDT, EPM and OF for anxiety; TST and FST for depression; NOR for cognitive impairment) to evaluate anxiety- and depressive-like behaviors. Additionally, we detected signs of neuroinflammation both in the hippocampus and in the prefrontal cortex, although with slight differences depending on the time point and brain area. Indeed, the hippocampus was characterized by astrogliosis both at 14 and 28 days after MIA injection, but only at the latter time point upregulation of pro-inflammatory cytokines was also detected. Similarly, in the prefrontal cortex, IL-1β and TNF-α were overexpressed at 28 days after MIA administration; however, significant glial activation was observed 14 days post-induction of OA, while at 28 days GFAP (astrocyte marker) returned to physiological levels [52,54].

Finally, an interesting paper by Burston and coworkers [81] tested two rat strains, characterized by differences in basal anxiety levels. Following induction of OA, the basal high-anxiety Wistar Kyoto rats developed more severe pain symptoms in comparison to low-anxiety Sprague Dawley rats, further confirming that anxiety may impact on pain perception. Authors also found significant activation of astrocytes both in the periaqueductal gray and in the anterior cingulate cortex, which well correlated with the levels of anxiety and pain in the two strains.

In conclusion, the very few studies analyzing the relationship between OA pain and mood disorders in MIA rodent models consistently agree on the significant presence of anxiety- and depression-like behaviors. It must be also underlined that the development of the psychoactive comorbidities starts when painful symptoms persist for at least two weeks, indicating the importance of pain chronicization in the negative modulation of mood. From a clinical point of view, this observation is extremely relevant, since it suggests that a prompt and efficacious approach to pain can prevent the subsequent and long-lasting development of highly invalidating mood comorbidities.

6. Therapeutic approaches targeting neuroinflammation in MIA models

Since its implementation, the MIA model in rodents has been extensively utilized not only to study the mechanisms underlying OA-induced pain, but also to check and validate the efficacy of different drug treatments, nutraceuticals and non-pharmacological approaches to counteract pain, disability, histological modifications and joint damage. More recently, thanks to emerging evidence on the involvement of neuroinflammation in pain chronicization, also the impact of different treatments on neuroinflammatory pathways has started to be evaluated in preclinical models of OA.

Indeed, research in this field has moved in two directions: on one hand, to propose drugs that specifically target non-neuronal cells and have been shown to switch off or dampen neuroinflammatory pathways; on the other, to re-evaluate whether classical OA therapeutic regimens may exert their favorable effects also by targeting neuroinflammation.

Chronic treatment with the microglia inhibitors minocycline and fluorocitrate was found effective in reducing allodynia and neuroinflammation as well, suggesting a pivotal role for glia cells in maintaining OA-related hypersensitivity in the MIA model [45,80]. Besides, we recently demonstrated how the pharmacological blockade of PK2, a chemokine overexpressed in the CNS and PNS in OA pain (see above), also ameliorates painful symptoms and neuroinflammation [54]. Li and colleagues [88] showed how the inhibition of NF-κB activation and translocation in the spinal cord is effective in controlling both pain hypersensitivity and neuroinflammation.

Among standard analgesic drugs currently used to treat pain in OA patients, NSAIDs are prevalent, followed by opioids in selected patients. In MIA rodent models of OA, NSAIDs such as diclofenac and nimesulide relieved hypersensitivity, significantly reduced microglia and astrocyte activation, as well as decreased the levels of pro-inflammatory cytokines in both DRGs and spinal cord [54,80]. Moreover, in parallel with the reduction of painful symptoms, a precocious treatment with diclofenac was able to prevent the development of anxious-depressive-like behaviors as well, also by decreasing neuroinflammatory activation in brain areas [54].

In recent years, the ability of NSAIDs to modulate and blunt neuroinflammation in the brain has been actively studied as novel therapeutic approach in neurodegenerative diseases, such as AD and PD [104]. Also based on available data in these different pathological settings, at the moment we cannot state whether the positive effect of this class of drugs on neuroinflammation in the MIA models is due to their direct effect on the glia component or to the reduction of pain that may represent a primary neuroinflammatory trigger.

The use of opioids in non-cancer pain remains a debated question, due to the well-known adverse effects related to their chronic use. In the MIA model opioid administration exerts a significant analgesic effect [52,53,55,105], but the interplay among morphine, opioid receptors and neuroinflammation is complex and multifaceted, as recently extensively studied [106]. Chronic morphine administration has been associated with microglia activation and cytokine production in the spinal cord and this activation was suggested as one of the mechanisms underlying tolerance and opioid-induced hyperalgesia [107,108]. The impact exerted by morphine on brain neuroinflammation is debated, with often contradictory literature data, showing both increased [109,110] and decreased [111,112] central neuroinflammation. Work from our group clearly indicates that one week of morphine treatment in MIA model, at a dose able to relieve pain, blunted the activation of microglia and astrocytes and the overexpression of proinflammatory cytokines both in the PNS and CNS, and prevented the development of anxio-depressive behavior [52,53]. Once more it can be hypothesized that in OA models the final effect of opioids on neuroinflammation is likely due to the combination of their effect in relieving pain in turn blunting pain-induced neuroinflammation, balanced by a potential direct effect on the glia components. It is also possible that the doses and the duration of opioid treatment may be relevant to understand their interactions with neuroinflammation. However novel pharmacological approaches specifically targeted on neuroinflammation must be actively sought. In this direction, Carcolè and coworkers [83] also demonstrated how the pharmacological blockade of Sigma-1 receptor (s1R), that is expressed by several cell types in the CNS including microglia, inhibits mechanical hypersensitivity, cognitive deficits and depressive-like states associated with OA pain in mice. A series of studies by Batallè and coworkers [102,103] showed how hydrogen sulfide slow-release donors counteracted pain and mood alterations, reducing neuroinflammatory markers.

Considering the presence of a neuropathic component in MIA-induced pain, a few studies evaluated the effect of treatment with antidepressants in MIA rats, since these drugs represent the first line treatment for neuropathic pain. The serotonin-noradrenaline reuptake inhibitor (SNRI) duloxetine was effective in relieving MIA-related hypersensitivity [105,113–115] especially when administered in the late phase of the disease. Yoneda and colleagues [115] also demonstrated that the effect of this SNRI was due to the modulation of endogenous descending inhibitor control. Interestingly, Nastić and coworkers [114] showed that the novel antidepressant vortioxetine counteracted pain and was also able to restore the cognitive impairment (as measured with the NOR test) in MIA rats. However, none of these studies performed any biochemical analysis in order to evaluate the effect of antidepressant treatments on neuroinflammation. Thus, this represents an issue of great

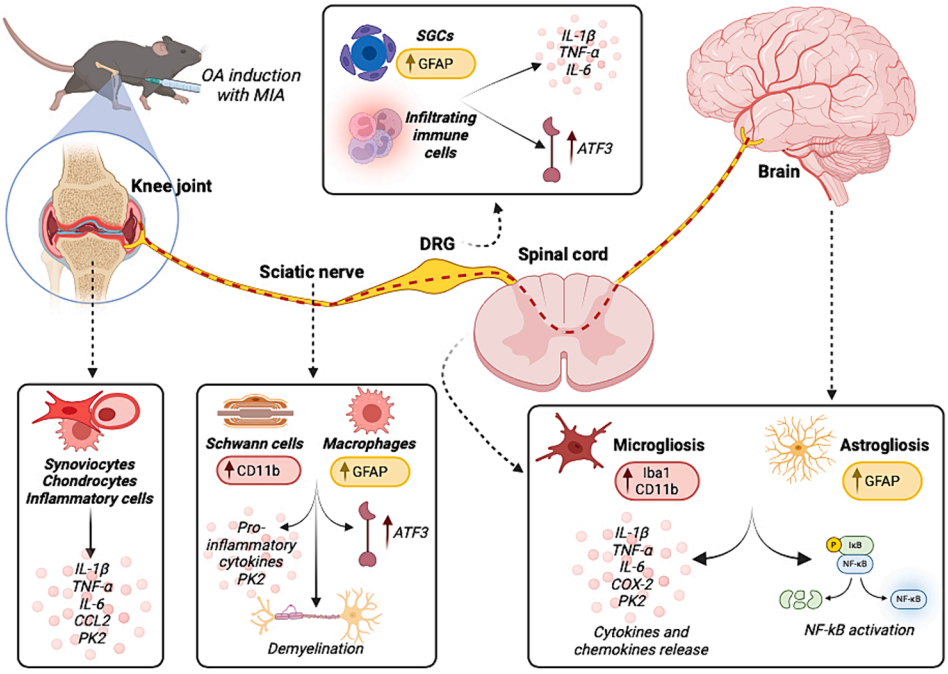

Fig. 3. Schematic representation of the known neuroinflammatory mechanisms underlying the development and progression of OA pain. The cartoon summarizes the main cell populations involved in neuroinflammatory pathways underlying OA pain in the knee joint, in the sciatic nerve, in DRGs and in the spinal cord, and the main mediators released by these cells. See text for details. Created with BioRender.com.

interest for future research.

Also, more advanced approaches, such as the epigenetic regulation of pro-inflammatory cytokines production [73] or the treatment with mesenchymal stem cells and their secretome, were able not only to counteract allodynia and hyperalgesia but also significantly blunted the neuroinflammatory status in the PNS and CNS [66,82].

We can therefore hypothesize that an efficacious control of the neuroinflammatory components during OA may represent a promising strategy to counteract both pain and related comorbidities.

7. Limitations and conclusion

In summary, recent research on the MIA model has generated crucial and novel insights into the mechanisms involved in OA pain, strongly highlighting the involvement of neuroinflammation in the PNS and CNS. Moreover, these studies have suggested that glia activation may be at the basis of the development of mood and cognitive comorbidities that frequently accompany OA as well (summarized in Fig. 3).

We are aware that the number of studies on this topic is still limited and that some bias need to be addressed. For example, aging is one of the major risk factors for the development of OA and research is increasingly unveiling how age-related systemic and local inflammation can contribute to the progression of OA joint damage [116]. Indeed, only 3 published original papers reported the use of aged mice in MIA-induced OA, with contrasting results. From two of them [52,53], the basal presence of an inflammatory and neuroinflammatory condition in aged mice clearly emerged, with further aggravation following MIA injection. Conversely, Ogbonna and coworkers [117] reported reduced spinal cord microglia activation in old mice after exposure to MIA.

Another variable that has not been adequately taken into consideration is sex, since it represents a major determinant in the inflammatory process and differences between male and female OA patients in both disease progression and pain burden have been widely reported [118]. In preclinical studies on MIA models, there is no agreement on this issue, since some studies were conducted in female while others in male mice, but no direct comparisons have been performed [119]. Interestingly, it has been suggested that the development and maintenance of neuroinflammation may be dimorphic, with a preferential activation of different cell types, such as microglia/astrocytes or infiltrating immune cells in males and females, respectively [120,121]. Whether this is the case in OA-induced neuroinflammation still needs to be elucidated.

These aspects deserve an in-depth evaluation in order to translate basic research results into innovative, and if possible personalized therapies for OA patients. However, we are confident that the precise understanding and elucidation of the neuroimmune interactions in OA pain will soon indicate new targets to develop novel therapeutic approaches that could ameliorate pain, associated mood disorders and the overall quality of life of OA patients.

CRediT authorship contribution statement

Giada Amodeo: Writing – review & editing, Investigation, Data curation. **Giulia Magni:** Writing – review & editing, Investigation. **Giulia Galimberti:** Writing – review & editing, Investigation. **Benedetta Riboldi:** Writing – review & editing, Writing – original draft. **Silvia Franchi:** Writing – review & editing, Investigation, Conceptualization. **Paola Sacerdote:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Stefania Ceruti:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

PS and GA conceptualized and drafted the manuscript; GG and SF read and corrected the manuscript. SC integrated contributions from all authors; GA drafted Fig. 2 and Table 1; GM and BR drafted the section on Neuroinflammation and Figs. 1 and 3; all authors read and approved the final version of the manuscript.

References

[1] M. Cross, E. Smith, D. Hoy, S. Nolte, I. Ackerman, M. Fransen, L. Bridgett, S. Williams, F. Guillemin, C.L. Hill, L.L. Laslett, G. Jones, F. Cicuttini, R. Osborne, T. Vos, R. Buchbinder, A. Woolf, L. March, The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study, Ann. Rheum. Dis. 73 (2014) 1323–1330, https://doi.org/10.1136/ANNRHEUMDIS-2013-204763.

[2] V.L. Johnson, D.J. Hunter, The epidemiology of osteoarthritis, Best Pract. Res. Clin. Rheumatol. 28 (2014) 5–15, https://doi.org/10.1016/J.BERH.2014.01.004.

[3] A.J. Barr, T.M. Campbell, D. Hopkinson, S.R. Kingsbury, M.A. Bowes, P. G. Conaghan, A systematic review of the relationship between subchondral bone features, pain and structural pathology in peripheral joint osteoarthritis, Arthritis Res. Ther. 17 (2015) 228, https://doi.org/10.1186/s13075-015-0735-x.

[4] D.J. Hunter, S. Bierma-Zeinstra, Osteoarthritis, Lancet 393 (2019) 1745–1759, https://doi.org/10.1016/S0140-6736(19)30417-9.

[5] D.T. Felson, Osteoarthritis as a disease of mechanics, Osteoarthritis Cartilage 21 (2013) 10–15, https://doi.org/10.1016/J.JOCA.2012.09.012.

[6] S. Perrot, T. Anne-Priscille, Pain in osteoarthritis from a symptom to a disease, Best Pract. Res. Clin. Rheumatol. 37 (2023) 101825, https://doi.org/10.1016/j.berh.2023.101825.

[7] R. Rainbow, W. Ren, L. Zeng, Inflammation and joint tissue interactions in OA: implications for potential therapeutic approaches, Arthritis 2012 (2012) 1–8, https://doi.org/10.1155/2012/741582.

[8] S. Orita, T. Koshi, T. Mitsuka, M. Miyagi, G. Inoue, G. Arai, T. Ishikawa, E. Hanaoka, K. Yamashita, M. Yamashita, Y. Eguchi, T. Toyone, K. Takahashi, S. Ohtori, Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee, BMC Musculoskelet. Disord. 12 (2011) 144, https://doi.org/10.1186/1471-2474-12-144.

[9] P. Bajaj, P. Bajaj, T. Graven-Nielsen, L. Arendt-Nielsen, Osteoarthritis and its association with muscle hyperalgesia: an experimental controlled study, Pain 93 (2001) 107–114, https://doi.org/10.1016/S0304-3959(01)00300-1.

[10] A.M. Khan, E. McLoughlin, K. Giannakas, C. Hutchinson, J.G. Andrew, Hip osteoarthritis: where is the pain? Ann. R. Coll. Surg. Engl. 86 (2004) 119–121, https://doi.org/10.1308/003588404322827518.

[11] H. Lundblad, A. Kreicbergs, K.Å. Jansson, Prediction of persistent pain after total knee replacement for osteoarthritis, J. Bone Joint Surg. Br. 90 (2008) 166–171, https://doi.org/10.1302/0301-620X.90B2.19640.

[12] H.G. Schaible, Mechanisms of chronic pain in osteoarthritis, Curr. Rheumatol. Rep. 14 (2012) 549–556, https://doi.org/10.1007/S11926-012-0279-X.

[13] M. Thakur, A.H. Dickenson, R. Baron, Osteoarthritis pain: nociceptive or neuropathic? Nat. Rev. Rheumatol. 10 (2014) 374–380, https://doi.org/10.1038/NRRHEUM.2014.47.

[14] L. Arendt-Nielsen, H. Nie, M.B. Laursen, B.S. Laursen, P. Madeleine, O. H. Simonsen, T. Graven-Nielsen, Sensitization in patients with painful knee osteoarthritis, Pain 149 (2010) 573–581, https://doi.org/10.1016/J.PAIN.2010.04.003.

[15] S.N. El-Tallawy, R. Nalamasu, G.I. Salem, J.A.K. LeQuang, J.V. Pergolizzi, P. J. Christo, Management of musculoskeletal pain: an update with emphasis on chronic musculoskeletal pain, Pain Ther. 10 (2021) 181–209, https://doi.org/10.1007/S40122-021-00235-2.

[16] A. Sharma, P. Kudesia, Q. Shi, R. Gandhi, Anxiety and depression in patients with osteoarthritis: impact and management challenges, Open Access Rheumatol. 8 (2016) 103–113, https://doi.org/10.2147/OARRR.S93516.

[17] J. Axford, A. Butt, C. Heron, J. Hammond, J. Morgan, A. Alavi, J. Bolton, M. Bland, Prevalence of anxiety and depression in osteoarthritis: use of the Hospital Anxiety and Depression Scale as a screening tool, Clin. Rheumatol. 29 (2010) 1277–1283, https://doi.org/10.1007/S10067-010-1547-7.

[18] M. Zhuo, Neural mechanisms underlying anxiety-chronic pain interactions, Trends Neurosci 39 (2016) 136–145, https://doi.org/10.1016/J.TINS.2016.01.006.

[19] D.L. Goldenberg, The interface of pain and mood disturbances in the rheumatic diseases, Semin. Arthritis Rheum. 40 (2010) 15–31, https://doi.org/10.1016/J.SEMARTHRIT.2008.11.005.

[20] R.R. Ji, A. Nackley, Y. Huh, N. Terrando, W. Maixner, Neuroinflammation and central sensitization in chronic and widespread pain, Anesthesiology 129 (2018) 343–366, https://doi.org/10.1097/ALN.0000000000002130.

[21] M. Matsuda, Y. Huh, R.R. Ji, Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain, J. Anesth. 33 (2019) 131–139, https://doi.org/10.1007/S00540-018-2579-4.

[22] T. Geraghty, D.R. Winter, R.J. Miller, R.E. Miller, A.M. Malfait, Neuroimmune interactions and osteoarthritis pain: focus on macrophages, Pain Rep 6 (2021) E892, https://doi.org/10.1097/PR9.0000000000000892.

[23] M.J. Wood, R.E. Miller, A.M. Malfait, The genesis of pain in osteoarthritis: inflammation as a mediator of osteoarthritis pain, Clin. Geriatr. Med. 38 (2022) 221–238, https://doi.org/10.1016/J.CGER.2021.11.013.

[24] R.X. Zhang, K. Ren, R. Dubner, Osteoarthritis pain mechanisms: basic studies in animal models, Osteoarthritis Cartilage 21 (2013) 1308–1315, https://doi.org/10.1016/J.JOCA.2013.06.013.

[25] A. Rauf, H. Badoni, T. Abu-Izneid, A. Olatunde, M.M. Rahman, S. Painuli, P. Semwal, P. Wilairatana, M.S. Mubarak, Neuroinflammatory markers: key indicators in the pathology of neurodegenerative diseases, Molecules 27 (2022) 3194, https://doi.org/10.3390/MOLECULES27103194.

[26] H.S. Kwon, S.H. Koh, Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes, Transl. Neurodegener. 9 (2020) 42, https://doi.org/10.1186/S40035-020-00221-2.

[27] R. Troubat, P. Barone, S. Leman, T. Desmidt, A. Cressant, B. Atanasova, B. Brizard, W. El Hage, A. Surget, C. Belzung, V. Camus, Neuroinflammation and depression: A review, Eur. J. Neurosci. 53 (2021) 151–171, https://doi.org/10.1111/EJN.14720.

[28] D.M. Teleanu, A.G. Niculescu, I.I. Lungu, C.I. Radu, O. Vladâcenco, E. Roza, B. Costăchescu, A.M. Grumezescu, R.I. Teleanu, An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases, Int. J. Mol. Sci. 23 (2022) 5938, https://doi.org/10.3390/IJMS23115938.

[29] G. Magni, B. Riboldi, S. Ceruti, Modulation of glial cell functions by the gut-brain axis: A role in neurodegenerative disorders and pain transmission, Cells 12 (2023) 1612, https://doi.org/10.3390/CELLS12121612.

[30] X.X. Fang, M.N. Zhai, M. Zhu, C. He, H. Wang, J. Wang, Z.J. Zhang, Inflammation in pathogenesis of chronic pain: Foe and friend, Mol. Pain 19 (2023), https://doi.org/10.1177/17448069231178176, 17448069231178176.

[31] J. Lu, D. Wang, J. Xu, H. Zhang, W. Yu, New Insights on the Role of Satellite Glial Cells, Stem Cell Rev. Rep. 19 (2023) 358–367, https://doi.org/10.1007/S12015-022-10460-7.

[32] R. Simeoli, K. Montague, H.R. Jones, L. Castaldi, D. Chambers, J.H. Kelleher, V. Vacca, T. Pitcher, J. Grist, H. Al-Ahdal, L.F. Wong, M. Perretti, J. Lai, P. Mouritzen, P. Heppenstall, M. Malcangio, Exosomal cargo including microRNA regulates sensory neuron to macrophage communication after nerve trauma, Nat. Commun. 8 (2017) 1778, https://doi.org/10.1038/S41467-017-01841-5.

[33] R. Yang, J. Du, L. Li, X. Xu, S. Liang, Central role of purinergic receptors with inflammation in neuropathic pain-related macrophage-SGC-neuron triad, Neuropharmacology 228 (2023) 109445, https://doi.org/10.1016/J.NEUROPHARM.2023.109445.

[34] I. Bozic, D. Savic, I. Lavrnja, Astrocyte phenotypes: Emphasis on potential markers in neuroinflammation, Histol. Histopathol. 36 (2021) 267–290. https://doi.org/10.14670/HH-18-284.

[35] R.C. Paolicelli, A. Sierra, B. Stevens, M.E. Tremblay, A. Aguzzi, B. Ajami, I. Amit, E. Audinat, I. Bechmann, M. Bennett, F. Bennett, A. Bessis, K. Biber, S. Bilbo, M. Blurton-Jones, E. Boddeke, D. Brites, B. Brône, G.C. Brown, O. Butovsky, M. J. Carson, B. Castellano, M. Colonna, S.A. Cowley, C. Cunningham, D. Davalos, P. L. De Jager, B. de Strooper, A. Denes, B.J.L. Eggen, U. Eyo, E. Galea, S. Garel, F. Ginhoux, C.K. Glass, O. Gokce, D. Gomez-Nicola, B. González, S. Gordon, M. B. Graeber, A.D. Greenhalgh, P. Gressens, M. Greter, D.H. Gutmann, C. Haass, M. T. Heneka, F.L. Heppner, S. Hong, D.A. Hume, S. Jung, H. Kettenmann, J. Kipnis, R. Koyama, G. Lemke, M. Lynch, A. Majewska, M. Malcangio, T. Malm, R. Mancuso, T. Masuda, M. Matteoli, B.W. McColl, V.E. Miron, A.V. Molofsky, M. Monje, E. Mracsko, A. Nadjar, J.J. Neher, U. Neniskyte, H. Neumann, M. Noda, B. Peng, F. Peri, V.H. Perry, P.G. Popovich, C. Pridans, J. Priller, M. Prinz, D. Ragozzino, R.M. Ransohoff, M.W. Salter, A. Schaefer, D.P. Schafer, M. Schwartz, M. Simons, C.J. Smith, W.J. Streit, T.L. Tay, L.H. Tsai, A. Verkhratsky, R. von Bernhardi, H. Wake, V. Wittamer, S.A. Wolf, L.J. Wu, T. Wyss-Coray, Microglia states and nomenclature: A field at its crossroads, Neuron 110 (2022) 3458–3483, https://doi.org/10.1016/J.NEURON.2022.10.020.

[36] A. Buffo, C. Rolando, S. Ceruti, Astrocytes in the damaged brain: molecular and cellular insights into their reactive response and healing potential, Biochem. Pharmacol. 79 (2010) 77–89, https://doi.org/10.1016/J.BCP.2009.09.014.

[37] S. Pandey, K. Shen, S.H. Lee, Y.A.A. Shen, Y. Wang, M. Otero-García, N. Kotova, S. T. Vito, B.I. Laufer, D.F. Newton, M.G. Rezzonico, J.E. Hanson, J.S. Kaminker, C. J. Bohlen, T.J. Yuen, B.A. Friedman, Disease-associated oligodendrocyte responses across neurodegenerative diseases, Cell Rep. 40 (2022) 111189, https://doi.org/10.1016/J.CELREP.2022.111189.

[38] M. Boccazzi, S. Raffaele, M. Fumagalli, Not only myelination: the immune-inflammatory functions of oligodendrocytes, Neural Regen. Res. 17 (2022) 2661–2663, https://doi.org/10.4103/1673-5374.342678.

[39] E.L. Kuyinu, G. Narayanan, L.S. Nair, C.T. Laurencin, Animal models of osteoarthritis: classification, update, and measurement of outcomes, J. Orthop. Surg. Res. 11 (2016) 19, https://doi.org/10.1186/S13018-016-0346-5.

[40] R.E. Miller, A.M. Malfait, Osteoarthritis pain: What are we learning from animal models? Best Pract. Res. Clin. Rheumatol. 31 (2017) 676–687, https://doi.org/10.1016/J.BERH.2018.03.003.

[41] M. Alves-Simões, Rodent models of knee osteoarthritis for pain research, Osteoarthritis Cartilage 30 (2022) 802–814, https://doi.org/10.1016/J.JOCA.2022.01.010.

[42] K. Lampropoulou-Adamidou, P. Lelovas, E.V. Karadimas, C. Liakou, I. K. Triantafillopoulos, I. Dontas, N.A. Papaioannou, Useful animal models for the research of osteoarthritis, Eur. J. Orthop. Surg. Traumatol. 24 (2014) 263–271, https://doi.org/10.1007/S00590-013-1205-2.

[43] S. Yoh, Y. Kawarai, S. Hagiwara, S. Ortiz, J. Nakamura, S. Miyamoto, T. Suzuki, T. Akazawa, Y. Shiko, Y. Kawasaki, S. Ohtori, Intra-articular injection of

monoiodoacetate induces diverse hip osteoarthritis in rats, depending on its dose, BMC Musculoskelet. Disord. 23 (2022) 494, https://doi.org/10.1186/S12891-022-05454-Y.
[44] S. Jimbo, Y. Terashima, A. Teramoto, T. Takebayashi, I. Ogon, K. Watanabe, T. Sato, N. Ichise, N. Tohse, T. Yamashita, Antinociceptive effects of hyaluronic acid on monoiodoacetate-induced ankle osteoarthritis in rats, J. Pain Res. 12 (2019) 191–200, https://doi.org/10.2147/JPR.S186413.
[45] V. Bourassa, H. Deamond, N. Yousefpour, M.A. Fitzcharles, A. Ribeiro-Da-Silva, Pain-related behavior is associated with increased joint innervation, ipsilateral dorsal horn gliosis, and dorsal root ganglia activating transcription factor 3 expression in a rat ankle joint model of osteoarthritis, Pain Rep 5 (2020) E846, https://doi.org/10.1097/PR9.0000000000000846.
[46] A. Eitner, G.O. Hofmann, H.G. Schaible, Mechanisms of osteoarthritic pain. Studies in humans and experimental models, Front Mol Neurosci 10 (2017) 349, https://doi.org/10.3389/FNMOL.2017.00349.
[47] H. Jin, Y. Yang, G. Lei, C. Zeng, K. He, Y. Wang, C. Deng, J. Wei, X. Li, H. Li, Pain intensity and trajectory following intra-articular injection of mono-iodoacetate in experimental osteoarthritis: A meta-analysis of in vivo studies, Cartilage 14 (2023) 86–93, https://doi.org/10.1177/19476035221144748.
[48] J. De Sousa Valente, The pharmacology of pain associated with the monoiodoacetate model of osteoarthritis, Front. Pharmacol. 10 (2019) 974, https://doi.org/10.3389/FPHAR.2019.00974.
[49] T. Pitcher, J. Sousa-Valente, M. Malcangio, The monoiodoacetate model of osteoarthritis pain in the mouse, J. Vis. Exp. 2016 (2016) 53746, https://doi.org/10.3791/53746.
[50] C. Guingamp, P. Gegout-Pottie, L. Philippe, B. Terlain, P. Netter, P. Gillet, Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility, morphology, and biochemistry, Arthritis Rheum. 40 (1997) 1670–1679, https://doi.org/10.1002/ART.1780400917.
[51] R.E. Guzman, M.G. Evans, S. Bove, B. Morenko, K. Kilgore, Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis, Toxicol. Pathol. 31 (2003) 619–624, https://doi.org/10.1080/01926230390241800.
[52] G. Amodeo, S. Franchi, S. D'Agnelli, G. Galimberti, M. Baciarello, E.G. Bignami, P. Sacerdote, Supraspinal neuroinflammation and anxio-depressive-like behaviors in young- and older- adult mice with osteoarthritis pain: the effect of morphine, Psychopharmacology (Berl) 240 (2023) 2131–2146, https://doi.org/10.1007/S00213-023-06436-1.
[53] G. Amodeo, S. Franchi, G. Galimberti, L. Comi, S. D'Agnelli, M. Baciarello, E. G. Bignami, P. Sacerdote, Osteoarthritis pain in old mice aggravates neuroinflammation and frailty: the positive effect of morphine treatment, Biomedicines 10 (2022) 2847, https://doi.org/10.3390/BIOMEDICINES10112847.
[54] G. Galimberti, G. Amodeo, G. Magni, B. Riboldi, G. Balboni, V. Onnis, S. Ceruti, P. Sacerdote, S. Franchi, Prokineticin system is a pharmacological target to counteract pain and its comorbid mood alterations in an osteoarthritis murine model, Cells 12 (2023) 2255, https://doi.org/10.3390/CELLS12182255.
[55] F.Y. Han, D.A. Brockman, J.R. Nicholson, L. Corradini, M.T. Smith, Pharmacological characterization of the chronic phase of the monoiodoacetate-induced rat model of osteoarthritis pain in the knee joint, Clin. Exp. Pharmacol. Physiol. 48 (2021) 1515–1522, https://doi.org/10.1111/1440-1681.13551.
[56] A.R. Haywood, G.J. Hathway, V. Chapman, Differential contributions of peripheral and central mechanisms to pain in a rodent model of osteoarthritis, Sci. Rep. 8 (2018) 7122, https://doi.org/10.1038/S41598-018-25581-8.
[57] S.E. Bove, S.L. Calcaterra, R.M. Brooker, C.M. Huber, R.E. Guzman, P.L. Juneau, D.J. Schrier, K.S. Kilgore, Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis, Osteoarthritis Cartilage 11 (2003) 821–830, https://doi.org/10.1016/S1063-4584(03)00163-8.
[58] E.G. Yarmola, Y.Y. Shah, E.H. Lakes, Y.C. Pacheco, D.F. Xie, J. Dobson, K.D. Allen, Use of magnetic capture to identify elevated levels of CCL2 following intra-articular injection of monoiodoacetate in rats, Connect. Tissue Res. 61 (2020) 485–497, https://doi.org/10.1080/03008207.2019.1620223.
[59] G. Moschetti, G. Amodeo, M.S. Paladini, R. Molteni, G. Balboni, A. Panerai, P. Sacerdote, S. Franchi, Prokineticin 2 promotes and sustains neuroinflammation in vincristine treated mice: Focus on pain and emotional like behavior, Brain Behav. Immun. 82 (2019) 422–431, https://doi.org/10.1016/J.BBI.2019.09.012.
[60] G. Moschetti, G. Amodeo, D. Maftei, R. Lattanzi, P. Procacci, P. Sartori, G. Balboni, V. Onnis, V. Conte, A. Panerai, P. Sacerdote, S. Franchi, Targeting prokineticin system counteracts hypersensitivity, neuroinflammation, and tissue damage in a mouse model of bortezomib-induced peripheral neuropathy, J. Neuroinflammation 16 (2019) 89, https://doi.org/10.1186/S12974-019-1461-0.
[61] S. Franchi, P. Sacerdote, A. Panerai, The prokineticin system: an interface between neural inflammation and pain, Neurol. Sci. 38 (2017) 27–30, https://doi.org/10.1007/S10072-017-2875-Z.
[62] R. Lattanzi, R. Miele, Versatile Role of Prokineticins and Prokineticin Receptors in Neuroinflammation, Biomedicines 9 (2021) 1648, https://doi.org/10.3390/BIOMEDICINES9111648.
[63] K. Noda, B. Dufner, H. Ito, K. Yoshida, G. Balboni, R.H. Straub, Differential inflammation-mediated function of prokineticin 2 in the synovial fibroblasts of patients with rheumatoid arthritis compared with osteoarthritis, Sci. Rep. 11 (2021) 18399, https://doi.org/10.1038/S41598-021-97809-Z.
[64] H. Ito, K. Noda, K. Yoshida, K. Otani, M. Yoshiga, Y. Oto, S. Saito, D. Kurosaka, Prokineticin 2 antagonist, PKRA7 suppresses arthritis in mice with collagen-induced arthritis, BMC Musculoskelet. Disord. 17 (2016) 387, https://doi.org/10.1186/S12891-016-1243-0.
[65] M. Turati, S. Franchi, M. Crippa, L. Rizzi, L. Rigamonti, P. Sacerdote, S.D. Gatti, M. Piatti, G. Galimberti, D. Munegato, G. Amodeo, R.J. Omeljaniuk, G. Zatti, A. Torsello, M. Bigoni, Prokineticin 2 and cytokine content in the synovial fluid of knee osteoarthritis and traumatic meniscal tear patients: preliminary results, J. Clin. Med. 12 (2023) 4330, https://doi.org/10.3390/JCM12134330.
[66] G. Amodeo, S. Niada, G. Moschetti, S. Franchi, P. Savadori, A.T. Brini, P. Sacerdote, Secretome of human adipose-derived mesenchymal stem cell relieves pain and neuroinflammation independently of the route of administration in experimental osteoarthritis, Brain Behav. Immun. 94 (2021) 29–40, https://doi.org/10.1016/J.BBI.2021.03.011.
[67] M.M. Muley, E. Krustev, A.R. Reid, J.J. McDougall, Prophylactic inhibition of neutrophil elastase prevents the development of chronic neuropathic pain in osteoarthritic mice, J. Neuroinflammation 14 (2017) 168, https://doi.org/10.1186/S12974-017-0944-0.
[68] M. Thakur, W. Rahman, C. Hobbs, A.H. Dickenson, D.L.H. Bennett, Characterisation of a peripheral neuropathic component of the rat monoiodoacetate model of osteoarthritis, PLoS One 7 (2012) e33730, https://doi.org/10.1371/journal.pone.0033730.
[69] R. Lattanzi, D. Maftei, V. Marconi, F. Florenzano, S. Franchi, E. Borsani, L. F. Rodella, G. Balboni, S. Salvadori, P. Sacerdote, L. Negri, Prokineticin 2 upregulation in the peripheral nervous system has a major role in triggering and maintaining neuropathic pain in the chronic constriction injury model, Biomed Res. Int. 2015 (2015) 301292, https://doi.org/10.1155/2015/301292.
[70] S.P. Ivanavicius, A.D. Ball, C.G. Heapy, F.R. Westwood, F. Murray, S.J. Read, Structural pathology in a rodent model of osteoarthritis is associated with neuropathic pain: increased expression of ATF-3 and pharmacological characterisation, Pain 128 (2007) 272–282, https://doi.org/10.1016/J.PAIN.2006.12.022.
[71] J. Ferreira-Gomes, S. Adães, R.M. Sousa, M. Mendonça, J.M. Castro-Lopes, Dose-dependent expression of neuronal injury markers during experimental osteoarthritis induced by monoiodoacetate in the rat, Mol. Pain 8 (2012) 50, https://doi.org/10.1186/1744-8069-8-50.
[72] A.C. Ogbonna, A.K. Clark, C. Gentry, C. Hobbs, M. Malcangio, Pain-like behaviour and spinal changes in the monosodium iodoacetate model of osteoarthritis in C57Bl/6 mice, Eur. J. Pain 17 (2013) 514–526, https://doi.org/10.1002/J.1532-2149.2012.00223.X.
[73] J. Sun, X.H. Wang, F.H. Song, D.Y. Li, S.J. Gao, L.Q. Zhang, J.Y. Wu, D.Q. Liu, L. W. Wang, Y.Q. Zhou, W. Mei, Inhibition of Brd4 alleviates osteoarthritis pain via suppression of neuroinflammation and activation of Nrf2-mediated antioxidant signalling, Br. J. Pharmacol. 180 (2023) 3194–3214, https://doi.org/10.1111/BPH.16195.
[74] R. Raoof, C.M. Gil, F.P.J.G. Lafeber, H. De Visser, J. Prado, S. Versteeg, M. N. Pascha, A.L.P. Heinemans, Y. Adolfs, J. Pasterkamp, J.N. Wood, S. C. Mastbergen, N. Eijkelkamp, Dorsal Root Ganglia Macrophages Maintain Osteoarthritis Pain, J. Neurosci. 41 (2021) 8249–8261, https://doi.org/10.1523/JNEUROSCI.1787-20.2021.
[75] C. Martin Gil, R. Raoof, S. Versteeg, H.L.D.M. Willemen, F.P.J.G. Lafeber, S. C. Mastbergen, N. Eijkelkamp, Myostatin and CXCL11 promote nervous tissue macrophages to maintain osteoarthritis pain, Brain Behav. Immun. 116 (2024) 203–215, https://doi.org/10.1016/J.BBI.2023.12.004.
[76] G. Sideris-Lampretsas, M. Malcangio, Pain-resolving microglia, Science 376 (2022) 33–34, https://doi.org/10.1126/SCIENCE.ABO5592.
[77] M. Malcangio, Introduction for special issue on neuroimmune interactions in chronic pain, Pain Rep 6 (2021) E894, https://doi.org/10.1097/PR9.0000000000000894.
[78] C.J. Woolf, Q. Ma, Nociceptors–noxious stimulus detectors, Neuron 55 (2007) 353–364, https://doi.org/10.1016/J.NEURON.2007.07.016.
[79] E.S. Haight, T.E. Forman, S.A. Cordonnier, M.L. James, V.L. Tawfik, Microglial modulation as a target for chronic pain: from the bench to the bedside and back, Anesth. Analg. 128 (2019) 737–746, https://doi.org/10.1213/ANE.0000000000004033.
[80] D.R. Sagar, J.J. Burston, G.J. Hathway, S.G. Woodhams, R.G. Pearson, A. J. Bennett, D.A. Kendall, B.E. Scammell, V. Chapman, The contribution of spinal glial cells to chronic pain behaviour in the monosodium iodoacetate model of osteoarthritic pain, Mol. Pain 7 (2011) 88, https://doi.org/10.1186/1744-8069-7-88.
[81] J.J. Burston, A.M. Valdes, S.G. Woodhams, P.I. Mapp, J. Stocks, D.J.G. Watson, P. R.W. Gowler, L. Xu, D.R. Sagar, G. Fernandes, N. Frowd, L. Marshall, W. Zhang, M. Doherty, D.A. Walsh, V. Chapman, The impact of anxiety on chronic musculoskeletal pain and the role of astrocyte activation, Pain 160 (2019) 658–669, https://doi.org/10.1097/J.PAIN.0000000000001445.
[82] J. Sun, F.H. Song, J.Y. Wu, L.Q. Zhang, D.Y. Li, S.J. Gao, D.Q. Liu, Y.Q. Zhou, W. Mei, Sestrin2 overexpression attenuates osteoarthritis pain via induction of AMPK/PGC-1α-mediated mitochondrial biogenesis and suppression of neuroinflammation, Brain Behav. Immun. 102 (2022) 53–70, https://doi.org/10.1016/J.BBI.2022.02.015.
[83] M. Carcolé, S. Kummer, L. Gonçalves, D. Zamanillo, M. Merlos, A.H. Dickenson, B. Fernández-Pastor, D. Cabañero, R. Maldonado, Sigma-1 receptor modulates neuroinflammation associated with mechanical hypersensitivity and opioid tolerance in a mouse model of osteoarthritis pain, Br. J. Pharmacol. 176 (2019) 3939–3955, https://doi.org/10.1111/BPH.14794.
[84] S.M. Lockwood, D.M. Lopes, S.B. McMahon, A.H. Dickenson, Characterisation of peripheral and central components of the rat monoiodoacetate model of

Osteoarthritis, Osteoarthritis Cartilage 27 (2019) 712–722, https://doi.org/10.1016/J.JOCA.2018.12.017.
[85] Y. Lee, M. Pai, J.D. Brederson, D. Wilcox, G. Hsieh, M.F. Jarvis, R.S. Bitner, Monosodium iodoacetate-induced joint pain is associated with increased phosphorylation of mitogen activated protein kinases in the rat spinal cord, Mol. Pain 7 (2011) 39, https://doi.org/10.1186/1744-8069-7-39.
[86] D.R. Sagar, S. Ashraf, L. Xu, J.J. Burston, M.R. Menhinick, C.L. Poulter, A. J. Bennett, D.A. Walsh, V. Chapman, Osteoprotegerin reduces the development of pain behaviour and joint pathology in a model of osteoarthritis, Ann. Rheum. Dis. 73 (2014) 1558–1565, https://doi.org/10.1136/ANNRHEUMDIS-2013-203260.
[87] Y. Lin, L. Liu, H. Jiang, J. Zhou, Y. Tang, Inhibition of interleukin-6 function attenuates the central sensitization and pain behavior induced by osteoarthritis, Eur. J. Pharmacol. 811 (2017) 260–267, https://doi.org/10.1016/J.EJPHAR.2017.06.032.
[88] Y. Li, Y. Yang, J. Guo, X. Guo, Z. Feng, X. Zhao, Spinal NF-kB upregulation contributes to hyperalgesia in a rat model of advanced osteoarthritis, Mol. Pain 16 (2020), https://doi.org/10.1177/1744806920905691, 1744806920905691.
[89] E.S. Fu, Y.P. Zhang, J. Sagen, K.A. Candiotti, P.D. Morton, D.J. Liebl, J.R. Bethea, R. Brambilla, Transgenic inhibition of glial NF-kappa B reduces pain behavior and inflammation after peripheral nerve injury, Pain 148 (2010) 509–518, https://doi.org/10.1016/J.PAIN.2010.01.001.
[90] J.G. Luo, X.L. Zhao, W.C. Xu, X.J. Zhao, J.N. Wang, X.W. Lin, T. Sun, Z.J. Fu, Activation of spinal NF-κB/p65 contributes to peripheral inflammation and hyperalgesia in rat adjuvant-induced arthritis, Arthritis Rheumatol. 66 (2014) 896–906, https://doi.org/10.1002/ART.38328.
[91] V. Maletic, C.L. Raison, Neurobiology of depression, fibromyalgia and neuropathic pain, Front. Biosci. (Landmark Ed) 14 (2009) 5291–5338, https://doi.org/10.2741/3598.
[92] F. Radat, A. Margot-Duclot, N. Attal, Psychiatric co-morbidities in patients with chronic peripheral neuropathic pain: a multicentre cohort study, Eur. J. Pain 17 (2013) 1547–1557, https://doi.org/10.1002/J.1532-2149.2013.00334.X.
[93] O. Moriarty, B.E. McGuire, D.P. Finn, The effect of pain on cognitive function: a review of clinical and preclinical research, Prog. Neurobiol. 93 (2011) 385–404, https://doi.org/10.1016/J.PNEUROBIO.2011.01.002.
[94] C. Villemure, M.C. Bushnell, Mood influences supraspinal pain processing separately from attention, J. Neurosci. 29 (2009) 705–715, https://doi.org/10.1523/JNEUROSCI.3822-08.2009.
[95] E.E. Barcelon, W.H. Cho, S.B. Jun, S.J. Lee, Brain microglial activation in chronic pain-associated affective disorder, Front. Neurosci. 13 (2019) 213, https://doi.org/10.3389/FNINS.2019.00213.
[96] M. Kremer, L.J. Becker, M. Barrot, I. Yalcin, How to study anxiety and depression in rodent models of chronic pain? Eur. J. Neurosci. 53 (2021) 236–270, https://doi.org/10.1111/EJN.14686.
[97] K.R. Bailey, J.N. Crawley, J.J. Buccafusco, Anxiety-Related Behaviors in Mice, in: Methods of Behavior Analysis in Neuroscience, 2nd. edition, Boca Raton (FL): CRC Press/Taylor & Francis, 2009. https://pubmed.ncbi.nlm.nih.gov/21204329.
[98] T.R. Powell, C. Fernandes, L.C. Schalkwyk, Depression-related behavioral tests, Curr. Protoc. Mouse Biol. 2 (2012) 119–127, https://doi.org/10.1002/9780470942390.MO110176.
[99] B. Planchez, A. Surget, C. Belzung, Animal models of major depression: drawbacks and challenges, J. Neural Transm. (Vienna) 126 (2019) 1383–1408, https://doi.org/10.1007/S00702-019-02084-Y.
[100] C. La Porta, S. Andreea Bura, J. Llorente-Onaindia, A. Pastor, F. Navarrete, M. S. García-Gutierrez, R. De La Torre, J. Manzanares, J. Monfort, R. Maldonado, Role of the endocannabinoid system in the emotional manifestations of osteoarthritis pain, Pain 156 (2015) 2001–2012, https://doi.org/10.1097/J.PAIN.0000000000000260.
[101] M. Carcolé, D. Zamanillo, M. Merlos, B. Fernández-Pastor, D. Cabañero, R. Maldonado, Blockade of the sigma-1 receptor relieves cognitive and emotional impairments associated to chronic osteoarthritis pain, Front. Pharmacol. 10 (2019) 468, https://doi.org/10.3389/FPHAR.2019.00468.
[102] G. Batallé, L. Cabarga, O. Pol, The inhibitory effects of slow-releasing hydrogen sulfide donors in the mechanical allodynia, grip strength deficits, and depressive-like behaviors associated with chronic osteoarthritis pain, Antioxidants (Basel) 9 (2019) 31, https://doi.org/10.3390/ANTIOX9010031.
[103] G. Batallé, X. Bai, E. Pouso-Vázquez, G. Roch, L. Rodríguez, O. Pol, The recovery of cognitive and affective deficiencies linked with chronic osteoarthritis pain and implicated pathways by slow-releasing hydrogen sulfide treatment, Antioxidants (Basel) 10 (2021) 1632, https://doi.org/10.3390/ANTIOX10101632.
[104] B.D. Arbo, L.E. Schimith, M. Goulart dos Santos, M.A. Hort, Repositioning and development of new treatments for neurodegenerative diseases: Focus on neuroinflammation, Eur. J. Pharmacol. 919 (2022) 174800, https://doi.org/10.1016/J.EJPHAR.2022.174800.
[105] M.H. Rashid, Y. Theberge, S.J. Elmes, M.N. Perkins, F. McIntosh, Pharmacological validation of early and late phase of rat mono-iodoacetate model using the Tekscan system, Eur. J. Pain 17 (2013) 210–222, https://doi.org/10.1002/J.1532-2149.2012.00176.X.
[106] J. Cuitavi, J.V. Torres-Pérez, J.D. Lorente, Y. Campos-Jurado, P. Andrés-Herrera, A. Polache, C. Agustín-Pavón, L. Hipólito, Crosstalk between Mu-Opioid receptors and neuroinflammation: Consequences for drug addiction and pain, Neurosci. Biobehav. Rev. 145 (2023) 105011, https://doi.org/10.1016/J.NEUBIOREV.2022.105011.
[107] X. Wang, L.C. Loram, K. Ramos, A.J. De Jesus, J. Thomas, K. Cheng, A. Reddy, A. A. Somogyi, M.R. Hutchinson, L.R. Watkins, H. Yin, Morphine activates neuroinflammation in a manner parallel to endotoxin, PNAS 109 (2012) 6325–6330, https://doi.org/10.1073/PNAS.1200130109/-/DCSUPPLEMENTAL/SAPP.PDF.
[108] J. Zhou, R. Ma, Y. Jin, J. Fang, J. Du, X. Shao, Y. Liang, J. Fang, Molecular mechanisms of opioid tolerance: From opioid receptors to inflammatory mediators (Review), Exp. Ther. Med. 22 (2021) 1004, https://doi.org/10.3892/ETM.2021.10437.
[109] C.M. Cahill, A.M. Taylor, Neuroinflammation-a co-occurring phenomenon linking chronic pain and opioid dependence, Curr. Opin. Behav. Sci. 13 (2017) 171–177, https://doi.org/10.1016/J.COBEHA.2016.12.003.
[110] D. Reiss, T. Maduna, H. Maurin, E. Audouard, C. Gaveriaux-Ruff, Mu opioid receptor in microglia contributes to morphine analgesic tolerance, hyperalgesia, and withdrawal in mice, J. Neurosci. Res. 100 (2022) 203–219, https://doi.org/10.1002/JNR.24626.
[111] C.C. Chao, S. Hu, K.B. Shark, W.S. Sheng, G. Gekker, P.K. Peterson, Activation of mu opioid receptors inhibits microglial cell chemotaxis, J. Pharmacol. Exp. Ther. 281 (1997) 988–1004.
[112] P. Zhang, M. Yang, C. Chen, L. Liu, X. Wei, S. Zeng, Toll-like receptor 4 (TLR4)/opioid receptor pathway crosstalk and impact on opioid analgesia immune function, and gastrointestinal motility, Front. Immunol. 11 (2020) 1455, https://doi.org/10.3389/FIMMU.2020.01455.
[113] G. Ishikawa, Y. Nagakura, N. Takeshita, Y. Shimizu, Efficacy of drugs with different mechanisms of action in relieving spontaneous pain at rest and during movement in a rat model of osteoarthritis, Eur. J. Pharmacol. 738 (2014) 111–117, https://doi.org/10.1016/J.EJPHAR.2014.05.048.
[114] K. Nastić, U. Pecikoza, M. Labudović-Borović, J. Kotur-Stevuljević, A. Micov, A. Jovanović, M. Tomić, R. Stepanović-Petrović, The antidepressant drugs vortioxetine and duloxetine differentially and sex-dependently affect animal well-being, cognitive performance, cardiac redox status and histology in a model of osteoarthritis, Biomed. Pharmacother. 166 (2023) 115360, https://doi.org/10.1016/J.BIOPHA.2023.115360.
[115] S. Yoneda, E. Kasai, M. Matsuo, R. Tamano, Y. Sakurai, T. Asaki, M. Fujita, Duloxetine ameliorates the impairment of diffuse noxious inhibitory control in rat models of peripheral neuropathic pain and knee osteoarthritis pain, Neurosci. Lett. 729 (2020) 134990, https://doi.org/10.1016/J.NEULET.2020.134990.
[116] M.A. Greene, R.F. Loeser, Aging-related inflammation in osteoarthritis, Osteoarthr. Cartilage 23 (2015) 1966–1971, https://doi.org/10.1016/J.JOCA.2015.01.008.
[117] A.C. Ogbonna, A.K. Clark, M. Malcangio, Development of monosodium acetate-induced osteoarthritis and inflammatory pain in ageing mice, Age (Dordr.) 37 (2015) 9792, https://doi.org/10.1007/S11357-015-9792-Y.
[118] V.K. Srikanth, J.L. Fryer, G. Zhai, T.M. Winzenberg, D. Hosmer, G. Jones, A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis, Osteoarthritis Cartilage 13 (2005) 769–781, https://doi.org/10.1016/J.JOCA.2005.04.014.
[119] J.Y. Ro, Y. Zhang, C. Tricou, D. Yang, J.T. da Silva, R. Zhang, Age and sex differences in acute and osteoarthritis-like pain responses in rats, J. Gerontol. A Biol. Sci. Med. Sci. 75 (2020) 1465–1472, https://doi.org/10.1093/GERONA/GLZ186.
[120] É. Midavaine, J. Côté, S. Marchand, P. Sarret, Glial and neuroimmune cell choreography in sexually dimorphic pain signaling, Neurosci. Biobehav. Rev. 125 (2021) 168–192, https://doi.org/10.1016/J.NEUBIOREV.2021.01.023.
[121] R.E. Sorge, J.C.S. Mapplebeck, S. Rosen, S. Beggs, S. Taves, J.K. Alexander, L. J. Martin, J.S. Austin, S.G. Sotocinal, D. Chen, M. Yang, X.Q. Shi, H. Huang, N. J. Pillon, P.J. Bilan, Y. Tu, A. Klip, R.R. Ji, J. Zhang, M.W. Salter, J.S. Mogil, Different immune cells mediate mechanical pain hypersensitivity in male and female mice, Nat. Neurosci. 18 (2015) 1081–1083, https://doi.org/10.1038/NN.4053.