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The Role of Peripheral Nociceptive Neurons in the Pathophysiology of Osteoarthritis Pain

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Abstract Knee osteoarthritis is characterized by progressive damage and remodeling of all tissues in the knee joint. Pain is the main symptom associated with knee osteoarthritis. Recent clinical and pre-clinical studies have provided novel insights into the mechanisms that drive the pain associated with joint destruction. In this narrative review, we describe current knowledge regarding the changes in the peripheral and central nervous systems that occur during the progression of osteoarthritis and discuss how therapeutic interventions may provide pain relief.

Osteoarthritis Is a Major Cause of Chronic Pain

Osteoarthritis (OA) is a painful disease of the synovial joint, most commonly affecting knees, hips, hands, and facet joints of the spine. Knee OA affects 27 million people in the USA [1], and the lifetime risk of developing symptomatic knee OA

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is estimated to be ~45 % [2]. Aging, prior joint injury, obesity, gender, and genetics are among the leading risk factors for OA. The major symptoms of knee OA include pain and related functional impairment [3, 4]. In early stages of the disease, specific activities trigger sharp pain in a predictable fashion [5]. As the disease progresses, the pain becomes chronic and is described as constant dull/aching pain in combination with unpredictable episodes of acute pain. Options for symptom management include both pharmacologic and non-pharmacologic treatments [6], but the effectiveness of these treatments remains limited [7]. The inability to control pain is the major reason for total joint replacement [8], and OA is the principal diagnosis associated with total knee replacement [9].

OA represents failure of the synovial joint as an organ [10]. The radiographic manifestations of OA (joint space narrowing, subchondral bone sclerosis, and osteophytes) represent the late stage of a joint remodeling process that has often been going on for many years and is characterized by progressive articular cartilage degradation, sclerosis of the subchondral bone, remodeling at the joint margins leading to osteophyte growth, low-grade synovitis, and meniscal damage in the knee [10]. These remodeling processes result in profound changes in the biochemical milieu of the joint and in the joint mechanics, which may contribute to generation and maintenance of pain. Tissue remodeling probably also involves changes in joint innervation, which may also affect pain pathways. As in all forms of chronic pain, sensitization of the nervous system contributes to persistent OA pain, and there is abundant evidence that this sensitization is driven by the joint/periphery in most patients [11]. In this narrative review, we describe what is known from both human and pre-clinical studies regarding changes in innervation and vascularization of the normal versus the OA joint and possible factors that may contribute to the development of peripheral and central sensitizations, underlying persistent OA pain. We will

focus specifically on knee OA, since both the clinical and the pre-clinical literatures largely focus on this joint.

Animal Models of OA Pain

A variety of animal models is used for the study of OA and associated pain, and each model has advantages and disadvantages (for review [12–14]). For the purposes of this review, we will focus on rodent models. Rats are often chosen for behavioral studies due to the fact that they are easier to train, while mice offer the opportunity to create transgenic models.

OA pain models may be divided into two main groups: chemically induced models and surgically induced models. The two most common pain-related behavioral changes reported include mechanical allodynia of the hind paw, defined as a painful sensation induced by a non-noxious stimulus, and deficits in weight-bearing on the affected limb. Other behavioral tests indicative of pain are in development, including gait analysis, knee hyperalgesia, conditioned placement preference, spontaneous locomotion activities, temperature hypersensitivity, and burrowing [13, 15].

The most commonly used model for the study of OA-associated pain in rodents is induced by intra-articular (IA) injection of monosodium iodoacetate (MIA), which results in chondrocyte death. This causes acute inflammation and aggressive development of joint damage over the course of 3–4 weeks, often accompanied by mechanical allodynia and weight-bearing deficits [16, 17]. It has been suggested that the early, transient synovial inflammation observed in MIA-treated rats may be the primary cause of initial pain in these animals [16], whereas later, more persistent pain has been correlated with changes in joint histology [18].

Another chemically induced model that is gaining popularity is the collagenase-induced instability model [19]. IA injection of collagenase results in damage to collagen type I containing structures such as tendons, ligaments, and menisci, resulting in joint instability [19]. In the mouse, this induces medial and lateral cartilage destruction and subchondral bone sclerosis over a period of 6 weeks [19] and includes moderate levels of synovitis [20], perhaps providing a model for a more inflammatory subset of OA. Joint damage is associated with weight-bearing deficits in mice [21] and with mechanical allodynia and thermal hyperalgesia in rats [22].

Surgical models of OA include the rat medial meniscal tear (MMT) model, where transection of the medial collateral ligament followed by cutting of the medial meniscus at its narrowest point leads to progressive degenerative cartilage changes over 3–6 weeks [23]. Joint damage is accompanied by mechanical allodynia and weight-bearing deficits [24]. According to one side-by-side comparison, the rat MMT model displays more inflammation, osteophyte formation, and weight-bearing asymmetry than the MIA model [25].

Destabilization of the medial meniscus (DMM) is currently the most commonly used mouse model of OA and is performed by severing the medial meniscotibial ligament, causing mild instability of the knee [26]. The slowly progressive nature of this model (typically monitored up to 8 to 16 weeks after surgery) makes it well suited for studying OA pathophysiology and molecular mechanisms [27–29]. In addition to cartilage damage primarily on the medial side of the joint, pathological features include subchondral bone thickening, osteophytes, and low-grade synovitis [30]. As in the previous models, both mechanical allodynia [28, 29] and weight-bearing deficits have been reported [27]. Mechanical allodynia develops over the first 4 weeks after DMM but not sham surgery and is maintained through 16 weeks [28, 29], while weight-bearing deficits first develop by 10–12 weeks after surgery [27]. Locomotion deficits (decreased distance traveled, rearing, and climbing upside down) have also been detected in this model beginning 8 weeks after surgery [27, 29].

The partial meniscectomy mouse model is induced by freeing the medial meniscus from the attachment to the margin of the tibial plateau and removing approximately half of the medial meniscus (approximately 1 mm of tissue) [31]. The collateral ligament is left intact. In contrast to the DMM model, female mice may be used. This surgery results in progressive cartilage destruction over a period of 12 weeks; synovitis has not been assessed. Mice developed persistent paw pressure hypersensitivity, cold hypersensitivity, and knee pressure hypersensitivity by week 5 after surgery and significant mechanical allodynia by week 8 [31]. Statistically significant weight-bearing deficits were not detected, but a significant amount of variation was present in both the sham and partial meniscectomy groups perhaps reflecting the difficulty in measuring this behavior in mice.

Peripheral Sensitization

Overview of Neuroanatomy

The knee joint is innervated by both sensory and sympathetic peripheral nerve fibers [32, 33]. The cell bodies of the sensory neurons that innervate the knee joint are located in the dorsal root ganglia (DRG), primarily levels L3 to L5. These pseudo-unipolar neurons extend branches to the peripheral structures such as the knee joint and also extend branches centrally to the dorsal horn of the spinal cord, where the first synapse is made with interneurons or with supraspinally projecting neurons, carrying pain signals to the higher regions of the neuraxis where they are ultimately experienced by the conscious brain.

The pain-sensing sensory neurons, or nociceptors, are small-diameter unmyelinated C-fibers (slow conduction) or medium-diameter thinly myelinated Aδ-fibers (fast

conduction). Nociceptors have been sub-divided based on their ability to respond to particular types of stimuli [34••]. Traditionally, C-fiber nociceptors were separated into two sub-types, peptidergic or non-peptidergic, based on their ability to produce the neuropeptides, substance P and calcitonin gene-related peptide (CGRP). In addition, peptidergic neurons were attributed with expression of TrkA, a receptor for nerve growth factor (NGF). Non-peptidergic neurons were identified by their ability to bind isolectin IB4, to express the c-Ret neurotrophin receptor that is targeted by glial-derived neurotrophic factor (GDNF), and to express the purinergic receptor P2X3. It has since been discovered that in fact multiple distinct types of nociceptors can be defined, with one sub-type expressing both peptidergic and non-peptidergic markers [34••]. Ongoing work is focused on determining which subsets of neurons mediate mechanisms underlying touch, itch, and pain, and little is known regarding which subsets of neurons are important for driving pain associated with disease, including OA.

Tissue Damage Mediators of Pain

Acute or "protective" pain is stimulated through direct transduction of strong chemical, thermal, or mechanical stimuli into action potentials in order to generate a very fast response with the goal of protecting the peripheral tissue from injury [35]. Tissue injury, including the tissue remodeling characteristic of OA [36], produces a different biochemical milieu in joints than the set of molecules that facilitates normal acute pain responses. In OA, these injury products may include classical inflammatory molecules such as prostaglandins and bradykinins, as well as cytokines and chemokines. All of these molecules have been shown to excite pain-sensing sensory neurons (nociceptors) (for review [35, 37, 38]). In addition, recent evidence suggests that damage-associated molecular products (DAMPs), associated with inflammation in OA [39], also may directly excite sensory neurons [40–44]. As a result of continued stimulation by these tissue injury products, peripheral nociceptors may become sensitized, meaning that the threshold for activation is reduced.

Animal models of OA enable molecular and cellular characterization of peripheral sensitization associated with changes in pain-related behaviors. Recent reviews have focused on the role of cytokines, neuropeptides, and cannabinoids in the development of peripheral sensitization in OA [38, 45, 46]. Therefore, here, we will focus on describing the potential role of NGF, sodium channels, and non-neuronal cells to peripheral sensitization in OA.

NGF signaling has emerged as an analgesic target for a variety of diseases [47], including OA [48], and preliminary clinical trial results are encouraging despite some issues with adverse effects in a subset of patients. Peripheral inflammation has been shown to increase NGF expression in the inflamed

tissue [47], and gene expression levels of NGF and its high-affinity receptor, TrkA, are upregulated by human OA chondrocytes [49]. Retrograde transport of NGF to the DRG can cause altered gene expression, which can result in increased neuropeptide (substance P and CGRP) and ion channel production and can even cause non-peptidergic neurons to switch to a peptidergic phenotype [47]. In rat MIA and rat MMT, the TrkA receptor was upregulated in both ipsilateral and contralateral DRG neurons by the time that persistent pain behaviors present [50]. In murine DMM, increased NGF mRNA levels were found in the knee joints in the persistent phase stage (16 weeks after surgery), and systemic administration of TrkAd5, a human soluble NGF receptor, resulted in reversal of weight-bearing deficits at this time point [51]. IA injection of NGF into the knee joint of rats following induction of OA by either MMT surgery or MIA injection resulted in increased and sustained weight-bearing deficits compared to NGF effects in control knees [50]. Secondary mechanical allodynia of the hind paw was not further reduced following IA NGF injection in OA rats [50]. Supporting these findings, IA injection of NGF increased firing of spinal neurons in response to knee extension but not in response to noxious stimulation of the hind paw in MIA-injected rats [52]. Together, these studies suggest that NGF may play a role in peripheral sensitization in OA.

Previous studies in non-OA models have demonstrated that mechanical hyperalgesia stimulated by NGF appears to be a result of direct action on peripheral nerves as opposed to regulation of local cytokine production and other inflammatory processes that may be involved in joint destruction [53–55]. Similar results have been noted in the rat MIA model. Prophylactic treatment of rats with indomethacin prior to MIA injection was able to prevent TrkA upregulation in the DRG and reduce sensitivity to IA injection of NGF, but it had no effect on synovitis or joint swelling [50]. In another study, a single systemic dose of an anti-NGF antibody 3 days after MIA injection resulted in decreased gait deficits by day 35, despite the fact that treatment did not affect joint swelling and development of macroscopic cartilage lesions [56•]. The relative contributions of the two different NGF receptors, TrkA and p75, have not been fully elucidated in the context of OA pain.

Voltage-gated sodium channels expressed on nociceptors serve to generate and propagate action potentials in response to painful stimuli, and altered expression and sensitivity of these channels have been linked to chronic pain [57]. A recent study has begun to address the role of sodium channels in OA, by targeting voltage-gated sodium channels (Na$_v$) 1.7 and 1.8 in the MIA rat model [58•]. Both Na$_v$1.7 and Na$_v$1.8 are expressed by nociceptors and have been identified on both the peripheral and central terminals of these neurons. Two weeks after induction of MIA, spinal administration of ProTxII, a tarantula toxin that blocks Na$_v$1.7, or A-803467, an inhibitor of Na$_v$1.8, resulted in decreased responses of wide

dynamic range spinal dorsal horn neurons in response to mechanical and thermal stimuli applied to the hind paw [58•]. There was no effect on sham rats. In addition, systemic (s.c.) or intraplantar administration of A-803467 resulted in a decreased response by spinal neurons. This supports a previous study demonstrating the ability of an IA injection of A-803467 to inhibit the firing of knee joint afferents in response to noxious rotation of the joint and to inhibit mechanical allodynia and weight-bearing deficits in rats 14 days after induction of OA via MIA injection [59]. Together, these studies suggest that targeting peripheral sodium channels may be an effective treatment option in treating persistent OA pain.

Finally, another aspect of peripheral sensitization may include the infiltration and activation of inflammatory cells within the DRG itself. This process may help to promote the persistence of pain [60]. In the DMM model, macrophage (F4/80-positive cells) infiltration into the DRG correlated with the development of persistent pain behaviors [29]. In addition, in the antigen-induced arthritis model, macrophage infiltration into the DRG was significant 3 days after induction and these cells expressed markers consistent with macrophages activated by TNF-α, a sub-type of M1 macrophages that can produce factors supporting neuronal activation but do not promote cell death [61]. Further work is needed to understand whether reversing these inflammatory processes in the DRG results in pain relief.

Innervation and Vascularization Changes in the Osteoarthritic Knee

As outlined above, the majority of inflammatory mediators found in OA joints have the ability to stimulate knee-innervating nociceptors and many types of nociceptors may be present in the joint. Therefore, a better understanding of the innervation of the normal joint and the changes that occur during the course of OA may lead to new analgesic targets.

A pioneering study performed by two orthopedic surgeon brothers determined which intra-articular tissues elicited pain within a normal knee [62]. One brother arthroscopically palpated the intra-articular knee tissues of the other brother without intra-articular anesthesia. Entry into the intra-articular space through the anterior synovium and fat pad resulted in severe pain. Palpation of the suprapatellar pouch, capsule, and the medial and lateral retinacula (connective tissue surrounding the patella) with low forces resulted in moderate to severe pain. Higher forces were required to elicit moderate to severe pain from the tibial and femoral insertion sites of the cruciate ligaments. Likewise, medium-to-high force resulted in slight to moderate discomfort upon probing of the anterior and posterior horns of the meniscus. These observations are in line with the innervation of the normal knee, in which sensory nerves have been identified in the synovium, ligament and

tendon insertion sites, outer meniscus, and subchondral bone [63], while normal cartilage is aneural and avascular.

Few studies have compared the sensory innervation in human OA and age-matched healthy joints. Vascular changes have been described in more detail, including in human OA and age-matched healthy joints, and may contribute to OA pathogenesis [63]. Since new blood vessel growth and nerve growth share common mechanisms, it has been posited that nerve growth may accompany angiogenesis in OA (for review [63]).

Neovascularization of the non-calcified cartilage just above the osteochondral junction is associated with perivascular sensory (substance P- and calcitonin gene-related peptide (CGRP)-positive) and sympathetic (C-flanking peptide of neuropeptide Y (CPON)-positive) innervation [64]. Vascularization of the osteochondral region has been correlated with cartilage damage [65] and with subchondral bone marrow replacement by fibrovascular tissue [66] in OA. This process is associated with upregulated expression of angiogenic factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) in the subchondral tissues and highly algogenic nerve growth factor (NGF) expression within vascular channels [66]. Sensory and sympathetic innervation was also observed within the marrow cavities of tibial osteophytes [64].

One study examined vascular penetration and nerve growth in human menisci, comparing menisci from post-mortem knees with a high versus a low degree of macroscopic tibiofemoral cartilage damage [67]. Knees with high degree of chondropathy showed greater vascular density near the fibrocartilage junction (defined as the region dividing the outer third from the inner meniscus) [67]. CGRP-positive sensory nerve fibers were often present alongside blood vessels, and increased numbers of nerves were identified in the outer region of menisci from the severe cartilage damage group compared to the mild cartilage damage group [67]. This study did not include pain measures, but the results are consistent with MRI studies demonstrating increased meniscal pathologies in symptomatic versus asymptomatic subjects [68, 69].

Changes in the innervation of the anterior and posterior cruciate ligaments in OA have not been described in depth to date, and conflicting results have been reported. One study compared posterior cruciate ligaments taken from 22 total knee arthroplasty patients and three normal post-mortem patients of a similar age and found no significant differences regarding the distribution of sensory nerve fibers (staining for neurofilament protein, S-100 protein, epithelial membrane antigen, and vimentin) [70]. Another study comparing posterior cruciate ligaments from nine total knee arthroplasty patients to five normal post-mortem or amputation patients of similar age observed a significant reduction in the percentage area occupied by sensory nerves (gold chloride staining of free nerve endings) in the ligaments from osteoarthritic patients [71].

No published studies comparing the innervation of the synovium between OA and normal human subjects were identified. One study examined synovial tissues taken from 13 knee joints during total knee replacement surgery (symptomatic with K/L stage 3 or 4) with a range of synovitis scores [72]. Looking at the correlation between nerve density (protein gene product (PGP) 9.5-positive, pan-neuronal marker) and synovitis score, it was found that increasing synovitis resulted in decreased nerve density close to the synovial lining, but in deeper layers, the nerve fiber density remained constant with increasing synovitis score [72]. Another study examined changes in vascularity in synovium and found that endothelial cell proliferation, particularly at locations distant from the synovial lining, increased with increasing synovitis in OA but was not related to levels of osteochondral angiogenesis or to cartilage damage severity [65]. Finally, one study examined nerve growth factor (NGF) expression in the synovium. NGF-positive fibroblasts and macrophages were observed in areas of the synovial lining and sublining where blood vessels were also present, and the fractional areas positive for these cells were elevated in the synovium of advanced OA patients compared to non-OA controls [73••]. This study also demonstrated that advanced symptomatic OA is associated with synovitis, synovial NGF immunoreactivity, changes in chondrocyte morphology, and loss of cartilage surface integrity as compared with the advanced asymptomatic OA group [73••]. Together, these studies suggest that increasing synovitis levels in end-stage OA may contribute to pain through vascular and nerve growth in the deeper synovial layers and through the production of proalgesic products, particularly NGF, by synovial fibroblasts and macrophages.

In animal models of OA, two studies have examined innervation changes in the knee, using the collagenase-induced instability mouse model. Buma et al. originally reported that, 5 weeks after induction, there were deficits in substance P- and CGRP-positive innervating fibers, at specific locations around the cruciate ligaments and in the synovium and other soft tissues around the patella [74]. Recently, Murakami et al. performed a study examining changes in innervation of the synovium 1–4 weeks after collagenase injection [75]. Synovitis peaked 1–2 weeks after injection and remained elevated compared to contralateral knees 3–4 weeks after injection. Interestingly, PGP 9.5-positive nerve fiber density was decreased 1–2 weeks after injection but returned to normal levels by 3–4 weeks. Likewise, CGRP-positive nerve fiber density was decreased 1 week after injection, whereas no change was observed in the density of substance P-positive nerve fibers. Three studies reported that non-peptidergic nerve fibers (IB4-positive) are present at very low levels in the rat knee joint (1.5 % or less of all cells retrogradely labeled by IA injection), but all of these studies looked at colocalization of IB4 with

retrograde label in the dorsal root ganglion cell bodies instead in the joint structures themselves [76–78]. In addition, one study reports the presence of tyrosine hydroxylase (TH)-positive neurons in the deep layers of the normal rat synovium, but it is not clear from this study whether this represents the sympathetic neuron population [79] or the sensory neuron population known to express TH [80]. Overall, there is a need for further characterization of the different nociceptor sub-types in the knee and how the location and types of nerves change in OA.

Central Sensitization

OA pain may be driven by abnormal excitability in the pain pathways of both the peripheral and central nervous systems. Intense, repeated, or prolonged input from peripheral nociceptors can also lead to central sensitization [81], defined as an amplification of neuronal signaling within the central nervous system (CNS) that elicits pain hypersensitivity [82]. Therefore, this represents a state where normally innocuous stimuli may now be interpreted as painful.

The causes leading to central sensitization are multifaceted. Following peripheral sensitization, it is known that primary afferent-mediated transmission to neurons in the dorsal horn of the spinal cord becomes strengthened by a variety of mechanisms [83]. For example, sustained sensory input from nociceptors originating in the periphery interrupts descending inhibition of spinal dorsal horn neurons, which results in a reduction in their excitation threshold and allows for potentiation of pain signals [84].

Characterizing changes in clinical measures of sensitization in OA is a burgeoning area of research. In particular, determining whether central sensitization may play a role in chronic OA pain is important for informing therapeutic strategies. Clinical measures of sensitization include pressure pain threshold (PPT) testing, sensitivity mapping, mechanical temporal summation, mechanical allodynia, and conditioned pain modulation [4, 82]. A recently published systematic review of pressure pain threshold (PPT) and heat pain threshold (HPT) studies suggests that sensitization in knee OA patients may be associated with symptom severity [85]. Another recent systematic review suggests that OA pain is associated with central sensitization in a subset of patients, particularly those people with chronic pain [86]. Two studies have demonstrated that following successful total knee arthroplasty, measures of sensitization are normalized [87, 88], indicating that continuous peripheral input is important for maintenance of sensitization in OA. Additional high-quality studies are necessary to better understand which subset(s) of patients, possibly in a particular stage of disease, may benefit from centrally acting therapeutics.

In general, the relationship between peripheral sensitization and central sensitization must be further explored in OA in order to determine whether or not reducing peripheral sensitization is a *sine qua non* for central sensitization.

Changes at the level of the spinal cord have only been investigated in the MIA model thus far; no surgical models have been studied. During the late phase of the MIA model (28 days post injection), when both mechanical allodynia and weight-bearing deficits are established, spinal neurons of MIA rats displayed increased excitability in response to mechanical stimulation of the hind paw compared to saline-treated rats [18]. In addition, studies have shown that in the spinal cord, there is a time-dependent activation of microglia (7–14 days post-MIA) followed by astrocyte activation (28 days post-MIA) [18, 89–91]. However, in the mouse MIA model, microgliosis was seen only at the later time point and astrocytosis was not observed [92]. Together, these spinal changes suggest that central sensitization contributes to the aberrant pain responses in the late pain stage of this model.

Analgesic testing in the MIA model further supports a central component for pain behaviors during the late stage of the model. Non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to reverse pain behaviors up to 14 days after a 1 mg MIA injection [16, 93, 94]. Gabapentin, which has both peripheral and central sites of action, is able to reverse behaviors during the late phase of the model (days 14–28) [93, 95]. Amitriptyline, which most likely acts on the spinal cord, is also effective in reversing behaviors during the late phase of the model (days 14–28) [93]. Finally, systemic treatment with minocycline or nimesulide was associated with a reduction in microglia and astrocyte activation in the dorsal horn and with an attenuation of mechanical allodynia in the late phase of the model [90].

Conclusions and Future Directions

Clinical and pre-clinical studies support the hypothesis that the tissue damage characteristic of osteoarthritis contributes to the development and maintenance of osteoarthritis pain. Future work should be directed at better understanding exactly how joint damage results in sensitization of the peripheral and central nervous systems. For example, within the joint itself, little is known regarding the receptors expressed by peripheral nerve termini under normal and pathological conditions. Additionally, few studies have directly compared the innervation of the normal joint to the OA joint using samples from age-matched samples in which symptoms and sensitization have been characterized in order to understand how this pathophysiological feature may be related to pain. Finally, both peripheral and central sensitization contribute to OA pain, but continued effort should be focused on understanding under what circumstances this sensitization can be reversed.

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