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Review

Rodent models of knee osteoarthritis for pain research

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SUMMARY

Osteoarthritis (OA) is a chronic degenerative joint disease and a leading cause of disability worldwide. Pain is the main symptom, yet no current treatment can halt disease progression or effectively provide symptomatic relief. Numerous animal models have been described for studying OA and some for the associated OA pain. This review aims to update on current models used for studying OA pain, focusing on mice and rats. These models include surgical, chemical, mechanical, and spontaneous OA models. The impact of sex and age will also be addressed in the context of OA modelling. Although no single animal model has been shown ideal for studying OA pain, increased efforts to phenotype OA will likely impact the choice of models for pre-clinical and basic research studies.

Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease and a leading cause of disability worldwide. OA most commonly affects knees, hands, hips, and feet in people over 40 and female[1,2]. OA prevalence and incidence varies between countries[3]. However, globally, the age-standardised prevalence rate of OA increased 9.3% between 1990 and 2017, and this is projected to increase as the global population ages[3]. The primary features of OA include pain, joint swelling, stiffness, decreased mobility, and bone enlargement[4]. Knee OA is estimated to affect 16% of the world population in individuals aged 15 and over[5]. Clinically, visible radiographic changes such as osteophytes and joint space narrowing are used to classify disease severity[6]. Nevertheless, it is pain that drives most sufferers to the clinic and is the most impactful on quality of life[7,8].

Pain is the main symptom of OA. Several articular and periarticular regions of the joint have been linked to OA pain. Namely, synovitis, subchondral bone marrow lesions, periarticular muscle dysfunction, and bursitis[9,10]. However, attempts to correlate radiographic changes in affected joints, physical function and pain have been met with challenges[11,12]. Furthermore, not all patients with OA experience pain[13]. Besides joint damage, other factors, such as psychological, social, and cultural factors, play a role in the disease severity and the likelihood of developing OA pain[14]. This heterogeneity between patients suffering from OA highlights the diverse mechanisms leading to OA pain and sets challenges for modelling the disease.

The first models of OA were developed to study structural aspects of OA[15]. For instance, to understand cartilage degeneration rather than the symptomatic aspects of OA. The need for animal models of disease stems from difficulties with human subjects. Experiments using human subjects can be complex logistically and ethically, are subjective, and data tends to be only collected towards later stages of the disease[16]. In contrast, OA models allow scientists to follow disease progression, and have been described in many species and *in vitro*[17]. However, mice are used primarily when developing new models because they are the primary species for transgenic animals. Transgene expression is a valuable tool in understanding pain pathways as it allows overexpression and knock out of proteins of interest[18]. Nevertheless, the use of rodent animal models comes with their own limitations. For instance, age and sex discrepancies (see other experimental factors), but also translational factors, such as articular cartilage physiology, weight bearing, and gait differences between rodents and humans[19,20]. Though, overall, animal models are an exceptional research tool to explore the molecular mechanisms surrounding OA development and progression.

Abbreviations: ACLT, Anterior cruciate ligament transection; CIOA, Collagenase-induced arthritis; DMM, Destabilisation of the medial meniscus; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; LABORAS, Laboratory Animal Behaviour Observation Registration and Analysis System; MRI, Magnetic Resonance Images; MNX, Medial meniscal transection; MIA, Monosodium iodoacetate; ML, Non-invasive mechanical loading; MLI, Menisco-ligament injury; NGF, Nerve growth factor; NSAID, Non-steroidal anti-inflammatory drug; OA, Osteoarthritis; ROR2, Receptor tyrosine kinase-like orphan receptor; TrkA, Tropomyosin receptor kinase A; TGF-β, Transforming growth factor beta.

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This review aims to discuss the current knowledge of rodent animal models of knee OA in the context of pain research, focusing on mice and rats. For reviews on pain in arthritis see[21–23] and reviews on non-rodent animal use in studying arthritis see[19].

Pain in knee osteoarthritis

In a healthy knee joint, pain is a protective mechanism. Pain is typically a consequence of twisting, exceeding the working range or intense pressure. Experimental testing in humans has revealed that pain can be evoked by noxious mechanical and chemical stimuli applied to ligaments and the fibrous capsule, but not by stimulation of the cartilage[24]. Painful stimuli are transmitted through predominantly unmyelinated C-fibers (~80%) innervating the joint capsule, ligaments, menisci, periosteum, and subchondral bone[25–28]. OA is regarded as a disease of the whole joint; although cartilage is initially damaged, the whole joint is affected long-term, including subchondral bone remodelling, osteophyte formation, innervation, and synovial changes[8].

Magnetic Resonance Images (MRI) and histological studies with patient samples have linked features associated with pain in OA. For example, bone marrow lesions and joint effusion with asymmetric weight-bearing[29–31], increased subchondral NGF expression, and angiogenesis[32]. In the early stages of OA, pain occurs sporadically with joint use that is relieved by rest. Nevertheless, as OA progresses, the pain no longer correlates with joint use, and it becomes constant even at rest and at night[23].

Chronic pain development in OA patients is heterogeneous. OA pain results from multiple factors that include peripheral and central sensitization, reduced descending inhibition, and atrophy of cortical areas[23]. Signs of central sensitization such as mechanical allodynia and referred pain have been described in OA patients[33]. In addition, evidence supports a neuropathic pain component[34–36], changes in joint innervation[28,37], and a strong neuroimmune correlate in OA pain[38]. Thus, pre-clinical pain assessment requires a range of experimental procedures to address this heterogeneity.

Pain assessment in OA models

Behavioural tests to assess pain in OA animal models have been broadly taken from established pain protocols[39] and have been addressed in the context of OA[40,41] (Table I). These include tests to evaluate mechanical thresholds to innocuous mechanical pressure (von Frey) on the hind paw as a measure to test secondary mechanical hyperalgesia; to test weight distribution asymmetry induced by pain (gait analysis, static and dynamic weight-bearing assay); to measure ambulatory pain (rotarod); to measure thermal hyperalgesia (hot/cold plate test)[39]; to measure movement-induced pain (grip strength test)[42]; to measure on-going pain (digging assay[43]), and to measure spontaneous pain (Laboratory Animal Behaviour Observation Registration and Analysis System, LABORAS[44,45]). Specific knee-related tests have also been described to assess primary mechanical hyperalgesia in rats, such as compression of the knee using a digital pressure paw Randall-Selitto instrument[42] and scoring vocalizations in the knee-bend test[46]. Furthermore, novel methods for measuring pain thresholds *in vivo* using machine learning and optogenetics (e.g.[47–49]) are promising tools in understanding OA pain processing. These methods when compared to classical behaviour tests provide objective data acquisition, high-speed throughput, and "remote touch" in freely moving animals. In addition to behavioural tests, *in vivo* electrophysiology and *in vivo* imaging experiments are also powerful tools to describe the neuronal activity of nociceptive pathways and will likely contribute to the mechanistic understanding of OA.

Models of osteoarthritis

Rodent models used to study OA associated pain can be broadly subdivided into surgical, chemical, mechanical, and spontaneous, according to the induction method.

Surgical models

Surgical models aim to destabilise the joint via the transection of ligaments or the removal of fibrocartilaginous tissue to induce progressive joint degeneration (Fig. 1 and Table II). Early models of OA involving surgical procedures were developed in guinea pigs and rats, such as the medial meniscal tear model (also referred as menisco-ligament injury, MLI)[50,51]. The first OA surgical models adapted to mice were by Visco and colleagues in 2003 and were further developed by Kamekura and colleagues in 2005[52,53](Table II). These models exhibited various speeds of OA progression and OA score depending on the severity of the joint instability[52].

Surgical models of OA most closely reflect post-traumatic human OA as they induce joint instability and alter the loading in the

Test	Assessment	Key reference
von Frey	Light touch perception threshold/Primary or secondary mechanical hyperalgesia	39
Gait analysis and Catwalk	Joint use/weight distribution	139,140
Static and dynamic weight-bearing	Joint use/weight distribution	42
Rotarod	Joint use/motor coordination	141
Hot/cold plate	Noxious thermal sensitivity	39
Grip strength	Joint use/strength	42
Digging assay	on-going pain	43
LABORAS	Spontaneous pain	44
Knee compression	Primary mechanical hyperalgesia	42
Knee bend	Joint use/primary mechanical hyperalgesia	46

Table I

Osteoarthritis and Cartilage

Assessment of pain and function of the knee joint. LABORAS, Laboratory Animal Behaviour Observation Registration and Analysis System

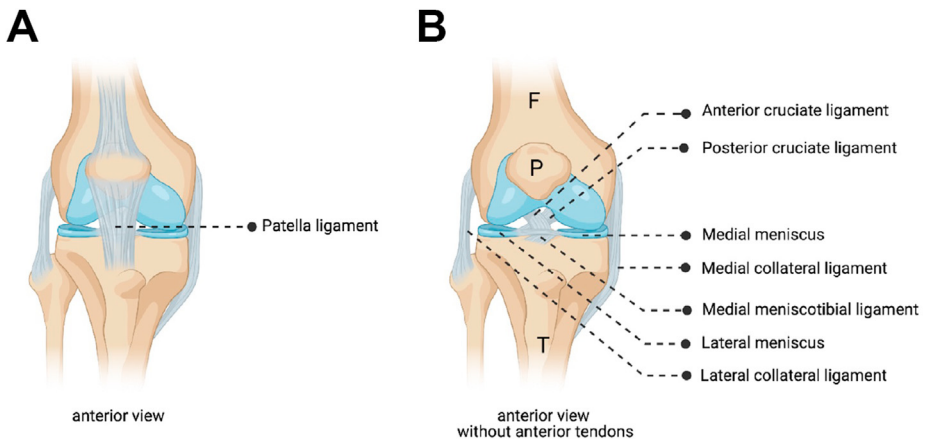

Fig. 1 Osteoarthritis and Cartilage

Simplified diagram of the knee joint. (A) Simplified diagram of the anterior view of the knee joint with the patella ligament. (B) Simplified diagram of the anterior view of the knee joint without anterior tendons. Structures altered in surgical OA models are labelled (F, femur; P, patella; T, tibia). Figure created using BioRender.com. Based on Kamekura et al. 2005[52].

knee. These models typically develop within weeks, whereas in human OA takes decades to develop[54]. They require strong surgical skills but deliver high reproducibility of time course. Notably, nociceptive behaviours may not be present despite significant changes in cartilage damage in line with clinical OA. Surgical models with functional changes associated with pain include medial meniscal tear, destabilisation of the medial meniscus (DMM), anterior cruciate ligament transection (ACLT) and medial meniscal transection (MNX) (Table II).

Medial meniscal tear/menisco-ligament injury

The Menisco-ligament injury (MLI) is induced by partial meniscectomy and medial collateral ligament transection (Fig. 1(B))[51]. In rats, this model induces rapid joint degradation within 7 days[51], increased synovial vascularization, cartilage damage, and osteophyte formation with characteristics of human OA[55]. In mice, MLI has also been shown to induce joint degradation 1 week post-surgery[53] with increased bone volume[56,57], which worsens for at least 20 weeks[56]. In a recent study exploring the therapeutic effect of blocking receptor tyrosine kinase-like orphan receptor (ROR2), the MLI model has been demonstrated to induce asymmetric weight-bearing from 7 weeks[58]. Moreover, a recent study exploring sex dysmorphism (discussed later in this review) further reproduced weight-bearing asymmetry, reported secondary mechanical hyperalgesia (von Frey) up to 12 weeks and transient thermal hypersensitivity within the first week, post-surgery[59]. Compared to other surgical models, MLI has a quicker onset of joint degeneration and pain phenotype (Table III), positioning MLI has a cost-effective OA pain model.

Destabilization of the medial meniscus

The DMM surgical model involves sectioning the medial meniscotibial ligament in the knee joint that anchors the medial meniscus to the tibial plateau [Fig. 1(B)][60–62]. The DMM induced instability leads to cartilage degradation from 2 weeks post-surgery and progressively expands to subchondral bone sclerosis and osteophyte formation[63,64]. Asymmetric weight-bearing has been reported to occur from 8[65], to 12 weeks post-surgery, which is reversed by celecoxib and morphine[63]. In a further study, mice developed secondary mechanical hypersensitivity (von Frey) from 4 weeks, maintained up to 16 weeks (Fig. 2)[45]. Research using the DMM model has revealed the key role metalloprotease, ADAMTS-5, in cartilage degradation[66]. In addition, genetic deletion of the protein kinase Cδ gene in mice inhibited OA pathogenesis induced by DMM but augmented knee joint OA-associated hypersensitivity to pain via NGF/TrkA axonal growth[67]. Furthermore, hyperalgesia in the DMM model has been associated with CCL2 - CCR2 signalling in the joint[45,68], and is attenuated by parathyroid hormone's subchondral bone remodelling[69].

Anterior cruciate ligament transection

In this surgical model of OA, the anterior cruciate ligament is transected (ACLT) [Fig. 1(B)]. First described in dogs[70], the ACLT model destabilizes the joint more severely than the medial meniscectomy model (see below)[15]. In humans, ACL rupture is a common sports injury that induces joint instability, increasing the risk of painful OA development 10–20 years later[71]. Rupture of the ACL as an OA model has also been described using a non-invasive mechanical loading (ML) on the knee joint with a force of 12 N (a method discussed later in Mechanical loading)[72]. One clear advantage of the ACL rupture model is the opportunity to investigate early responses to injury[73]. Using the surgical ACLT model in male mice, TGF-β contributed to subchondral bone degradation and its inhibition reduced the articular cartilage degradation. In addition, netrin-1 secreted by osteoclasts during subchondral bone remodelling increases CGRP-positive sensory neuron innervation and contributes to secondary mechanical hyperalgesia (von Frey)[37]. In male mice, secondary mechanical hyperalgesia was detectable from 1 week after ACLT surgery until the end of the study at week eight post-surgery[37]. In female mice, the ACLT model induces transient asymmetric weight-bearing 3 days post-surgery[74]. However, these behavioural effects were deemed too small by the authors for pharmacological intervention. Similarly, the ACL rupture model in male mice has been reported to induce secondary

Structure /Surgical model	Meniscal tear model/ Menisco-ligament injury (MLI)	Kamekura *et al.* 2005 Severe	Kamekura *et al.* 2005 Moderate	Kamekura *et al.* 2005 Mild	Kamekura *et al.* 2005 Medial	Destabilization of the medial meniscus (DMM)	Medial meniscal transection (MNX)	Anterior cruciate ligament transection (ACLT)
Anterior cruciate ligament	–	Transection	Transection	Transection	–	–	–	Transection
Patella ligament	–	Transection	–	–	–	–	–	–
Posterior cruciate ligament	–	Transection	–	–	–	–	–	–
Medial meniscus	Partial transection	Meniscectomy	Meniscectomy	–	Meniscectomy	–	Partial transection	–
Medial collateral ligament	Transection	Transection	–	–	Transection	–	–	–
Medial meniscotibial ligament	–	–	–	–	–	Transection	–	–
Lateral meniscus	–	Meniscectomy	–	–	–	–	–	–
Lateral collateral ligament	–	Transection	–	–	–	–	–	–
Key references	51	52	52	52	52	60–62	78	60

Table II

Osteoarthritis and Cartilage

The destabilization approach by transection of ligaments and meniscectomy in the various surgical models

mechanical hyperalgesia, transient changes in weight bearing[75], and knee hyperalgesia (Randall-Selitto)[76]. Thus, due to the limited pain reports using males and females, current evidence does not place ACLT as a robust model for OA pain research.

Meniscectomy and medial meniscal transection

Surgical removal of the meniscus, meniscectomy, in patients, increases the prevalence of radiographic OA and significantly increases knee pain within 21 years[77]. In rodents, the meniscectomy OA model has various approaches such as total, partial, unilateral, and bilateral MNX. Partial MNX is achieved by unilaterally cutting the medial meniscus. In mice, this model leads to osteoarthritic changes 4 weeks post-surgery, increased mechanical sensitivity from 8 weeks post-surgery measured by von Frey filaments[78], and altered weight-bearing from 7 weeks[65,79]. Partial MNX has been recently used in a proof of concept study where immunisation targeting NGF reduced asymmetric weight-bearing in mice[79].

Chemical models

Chemical-induced OA models involve an intraarticular injection of destabilising substances, typically via the patella ligament approach [Fig. 3(A)]. Models with a pain phenotype include the monosodium iodoacetate (MIA)-induced arthritis model, which is the predominant model, and the collagenase-induced arthritis (CIOA) model.

Monosodium iodoacetate

MIA is a cysteine peptidase inhibitor that targets glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in the glycolysis pathway[80,81]. Thus, MIA is a highly toxic compound. First used in rats and guinea pigs[82,83], the MIA-induced arthritis is characterised by a rapid onset, decreased function of the joint and associated pain[42]. To restrict the effects to the joint, MIA intra-articular injections (1 mg in 10 μl in mice)[84] are performed with minimal damage to the joint capsule to avoid leaking into the bloodstream. When injected, MIA induces cartilage degradation by suppressing the chondrocyte metabolism, which leads to cell death[81,84].

The histopathological progression of the MIA model reveals loss of articular cartilage and subchondral bone lesions[85]. Cartilage lesions in the knee joint have been reported as early as day one[86] and subchondral bone involvement from day seven post-injection[85]. Due to the joint space's avascular nature[87], MIA mainly targets chondrocytes. However, as the cartilage progressively degrades, it increases the loading on the subchondral bone and leads to bone lesions and remodelling[85,88]. Evidence suggests subchondral bone lesions are linked to clinical OA pain[30,31,89,90]. In the MIA model, secondary mechanical hyperalgesia is reported from 7 days post-injection up to 42 days in mice[91] [Fig. 3(C)] and up to 63 days in rats[92]. This hypersensitivity is susceptible to the non-steroidal anti-inflammatory drug (NSAID) diclofenac during the first days of the model[88]. However, inflamed knee diameter is unaffected by diclofenac[88], and weight-bearing imbalance is only affected by NSAIDs at day 14 post-MIA injection[93]. Further reports using conditioned place preference testing have also reported a neuropathic pain component in the MIA model and central sensitization[94].

The MIA model has been the model of choice in the pain field due to its demonstration of long-lasting hyperalgesia and weight-bearing asymmetry. However, transcriptional profiling and pathway analysis of articular cartilage showed a discrepancy between the MIA model in rats and clinical OA, with less than 4% of genes modulated overlapping[95]. Furthermore, extensive chondrocyte death and subchondral bone collapse are uncharacteristic of OA[85]. Nevertheless, the MIA model has been pharmacologically validated by commonly used therapeutic agents such as paracetamol, naproxen, rofecoxib, and diclofenac, all of which alleviated joint discomfort (as measured by weight-bearing) following MIA injection[88,93]. In addition, the MIA model has been used in pre-clinical studies for Nav1.8 inhibitors, A-893467 and VX-150, which led to phase IIa clinical trials[96,97].

Model	Species/ gender/typical age at induction	Onset of joint degeneration	Joint pathology	OARSI score reported	Onset of pain	Pain phenotype	Mechanisms	Pharmacological modulation	Possible OA phenotype	References
MLI	Mice and rats/ males and females*/10 weeks	7 days	Cartilage degradation, synovitis, osteophyte formation, increased vascularization, Increased bone volume	~6.5 (8 weeks)	1 week	Secondary mechanical hyperalgesia, transient thermal hypersensitivity, asymmetric weight-bearing	–	Inhibition of ROR2	Post-traumatic; malalignement	51,53,55,58,59,61
DMM	Mice/males and females*/8 weeks	14 days	Cartilage degradation, osteophyte formation, subchondral bone sclerosis	~4/7 (8 weeks), ~25 (12 weeks), ~12 (12 weeks)♀	3–4 weeks	Secondary mechanical hyperalgesia, Asymmetric weight-bearing	ADAMTS-5, PKCδ, CCL2-CCR2 signalling, parathyroid hormone	Celecoxib, morphine	Post-traumatic; malalignement	60–69
ACLT	Mice and rats/ males and females*/8 weeks	2–10 weeks	Cartilage degradation, osteophyte formation, subchondral bone degradation	15 (8 weeks), ~20 (12.8 weeks), 9.5 ± 1.96 (20 weeks)♀	3 days♀, 1 week	Secondary mechanical hyperalgesia, asymmetric weight-bearing (transient)♀	TGFβ netrin-1	–	Post-traumatic; malalignement	37,74,142
MNX	Mice and rats/ males/10 weeks	7 days (rats) 4 weeks (mice)	Cartilage degradation	~12 (8 weeks partial MNX), ~30 (12 weeks partial MNX), ~12.5 (18 weeks partial MNX), ~20 (12 weeks partial MNX)♀	7–8 weeks	Secondary mechanical hyperalgesia, asymmetric weight-bearing	–	Morphine, gabapetin, tramadol, anti-NGF (vaccine)	Post-traumatic; malalignement	61,65,78,79,143
MIA	Mice and rats/ males	1–7 days	Cartilage degradation, subchondral bone remodelling	–	1 week	Primary and secondary mechanical hyperalgesia, asymmetric weight-bearing	GAPDH, chondrocyte death	Diclofenac, paracetamol, naproxen, rofecoxib	Inflammatory	42,81,84,85,88,91–93,95,144
CIOA	Mice and rats/ males	1–4 weeks	Cartilage degradation, subchondral bone remodelling, osteophyte formation, joint swelling	–	1 week	Increased knee bending score, altered gait	Deterioration of tendons and ligaments	–	Inflammatory	98,99,102,103,122,145
ML	Mice/males/12 weeks	7 days	Cartilage degradation, synovitis, osteophyte formation, subchondral bone remodelling	~3 (6 weeks, 9N), ~5 (6 weeks, 11N)	1 week	Secondary mechanical hyperalgesia, asymmetric weight-bearing, altered gait	Cartilage degradation	Anti-NGF, diclofenac, gabapentin	Post-traumatic	109–111
ACL rupture	Mice/males and females*/12 weeks	2 weeks	Cartilage degradation, synovitis, osteophyte formation, subchondral bone remodelling	~30 (21 days, medial)	1–7 days	Secondary mechanical hyperalgesia, asymmetric weight-bearing (transient), knee hyperalgesia	–	HU308	Post-traumatic	72,73,75,76,146
SRT/ort	Mice/males/NA	Variable, high incidence (93%) from 17 months	Cartilage degradation	–	NA	No secondary mechanical hyperalgesia, no thermal hypersensitivity, no changes in vocalizations induced by knee compressions	–	–	Age-associated; malalignement	116,147,148
Col9a1 –/–	Mice/males/NA	3 months	Cartilage degradation	–	9 months	Secondary mechanical hyperalgesia, altered gait, no thermal hypersensitivity	Cartilage degradation	–	–	117,119

* All data refers to males unless noted with a ♀ symbol

Table III

Summary of the osteoarthritis (OA) models discussed in this review.

Collagenase

CIOA develops due to the destabilisation of the knee joint[98,99]. Intraarticular injections with collagenase from *Clostridium histolyticum bacterium* (type I, II, and VII) target tissues containing collagen type I such as tendons and ligaments[100]. It does not directly affect the articular cartilage, which is rich in collagen type II[101]. CIOA contrasts to the MIA chemical induction approach, which directly affects the chondrocytes in the joint. CIOA requires one injection containing 10–30 units[98,99,102,103]. This injection induces osteoarthritic-like progressive changes triggered by patellar dislocation, including osteophyte formation, subchondral bone trabecular structural changes, and cartilage loss[98,99,102,104,105]. As with the models mentioned above, most papers using the collagenase model use male mice as these are more prone to developing spontaneous OA[100]. Adães and colleagues (2014) further characterised this model in rats. They reported progressive OA-like structural changes from week four, transient joint swelling, and a pain phenotype from week one (knee bending score and gait analysis). The CIOA model has a rapid progression of joint degeneration, however, like other chemical models such as MIA, it is challenging to link the progression to clinical OA.

Mechanical loading models

The non-invasive ML model was first developed to study the impact of ML on the health of the joint[106]. Previous studies on the effects of loading had focused on *in vitro* models using chondrocyte preparations[107,108] and lacked the interaction of the surrounding tissues involved in joint health. The ML model induces reproducible lesions with repetitive loading in the articular cartilage that worsens over 3 weeks, promoting lesions characteristic of OA such as cartilage degradation, subchondral bone thickening, and osteophyte formation. The procedure involves positioning the mouse joint between two loading cups and applying 40 cycles of a force of nine N, six times three times per week, under isoflurane anaesthesia [Fig. 4(A) and (B)]. The key to the progressive nature of the model is the repetitive loading as a single loading does not trigger progressive lesions[106].

The ML model of OA pain is driven by cartilage degradation. Early ML studies revealed transient subchondral bone thickening in the contralateral joint and changes in gait[109]. In a further study the ML model was reported to induce a reproducible pain phenotype [Fig. 4(C) and (D)][110]. Mechanical hypersensitivity measured by von Frey and weight-bearing was significantly altered from 3 weeks post-loading. Importantly, there were no reports of fractures, and no hypersensitivity was detected within the first 2 weeks post-loading. In addition, treatment with anti-NGF, gabapentin or diclofenac, known to improve pain presentation in OA patients, were shown to reverse ML-induced hypersensitivity[110]. In a recent study, a high correlation between cartilage damage and mechanical hypersensitivity in the loaded knee joint was reported[111]. The cartilage damage was seen immediately post-loading on the ipsilateral joint and developed later on the contralateral joint, confirming earlier reports of contralateral joint involvement[106,109]. They also report a transient inflammatory period of synovitis before nociceptive behaviour detection. Taken together, the main advantages of this model are its reproducibility, low variability between mice, lack of surgery, and easy training of new experimenters. Hence, this firmly places ML as a model for progressive OA pain, which resembles post-traumatic OA in patients.

Spontaneous models

Spontaneous models of OA consist of genetically modified animals or those which naturally develop OA with ageing. In contrast to surgical or loading models, these models mimic the progression of non-traumatic OA and are thus regarded as more closely resembling human OA. For example, studies have shown that C57Bl/6J mice have a high incidence of OA (up to 80%) at the age of 18 months[112,113]. Furthermore, the SRT/ort mouse strain develops OA with a high incidence (93%) in male mice aged 17 months[114]. In the knee joint, around 85% of male SRT/ort mice develop OA in the medial tibial plateau. This effect is thought to be a consequence of the patella dislocating medially with age[114] and internal tibial torsion[115]. Yet only one study has explored the pain phenotype in the SRT/ort mouse and has reported no significant changes in pain sensitivity with ageing (and thus the development of OA) (Table III)[116]. Thus, spontaneous models might be a tool to study disease progression, however, no studies have shown a pain phenotype accompanying the OA-like joint degradation.

Genetic models used to investigate the pathophysiology of pain in OA include the type IX alpha 1 collagen col9a1 transgenic mice[117]. Mammalian articulate cartilage contains collagen types II, IX and XI in a network that surrounds chondrocytes[101]. Mutations in type II and type IX collagen have been associated with premature OA-like cartilage degradation and progressive development of OA features[117,118]. Col9a1$^{-/-}$ mice do not express type IX collagen, known to associate with type II collagen fibrils in articular cartilage[101], and develop premature OA-like joint degradation by 3 months of age[117]. At 9 months of age, Col9a1$^{-/-}$ mice have mechanical hyperalgesia (von Frey), altered gait, but no changes in thermal sensitivity (tail-flick test)[119].

Other experimental factors

Sex

Research has revealed a major discordance between human OA and animal models. Male mice and rats are more susceptible to OA-like joint degeneration than females across multiple OA models[59,120–122]. This 'protective' mechanism in female rodents is in stark contrast to human OA, where female patients are prevalent[1]. Ovariectomy, the surgical removal of the ovaries, has been shown to increase OA susceptibility in female mice. In contrast, orchiectomized males, the surgical removal of the testes, developed less severe OA than unoperated males[120]. Recent comparison studies using the DMM and partial meniscectomy surgical models in mice have shown that both males and females develop OA pain (secondary hyperalgesia and weight-bearing asymmetry)[65,121]. However, the damage in the knees of females (OARSI score[123]) was less severe compared to males by week 12 [Fig. 2(C)][65] and by week 20[121]. Further investigation revealed this is not due to altered activity or repair differences as previously suggested[120], but likely a heightened pain sensitivity by females that deters joint overuse.

Furthermore, microarray analysis depicted a distinct divergence in gene expression between sexes. Female mice had significant upregulation of neurotrophin genes *Gdnf* and *Nrtn*, whereas males had significantly higher *Ngf* and *Bdkrb1* at 10 weeks after partial meniscectomy. Thus, both female and male mice developed pain within the same timeline but expressed distinct neurotrophins[65]. In contrast, a recent study in rats using MIA showed that aged females, not males, had longer and more pronounced hyperalgesia[124]. Hence, demonstrating sex, species, and age differences in OA.

Age

Another important factor is the age at the time of OA induction. Not only nociceptor sensitization is different in younger

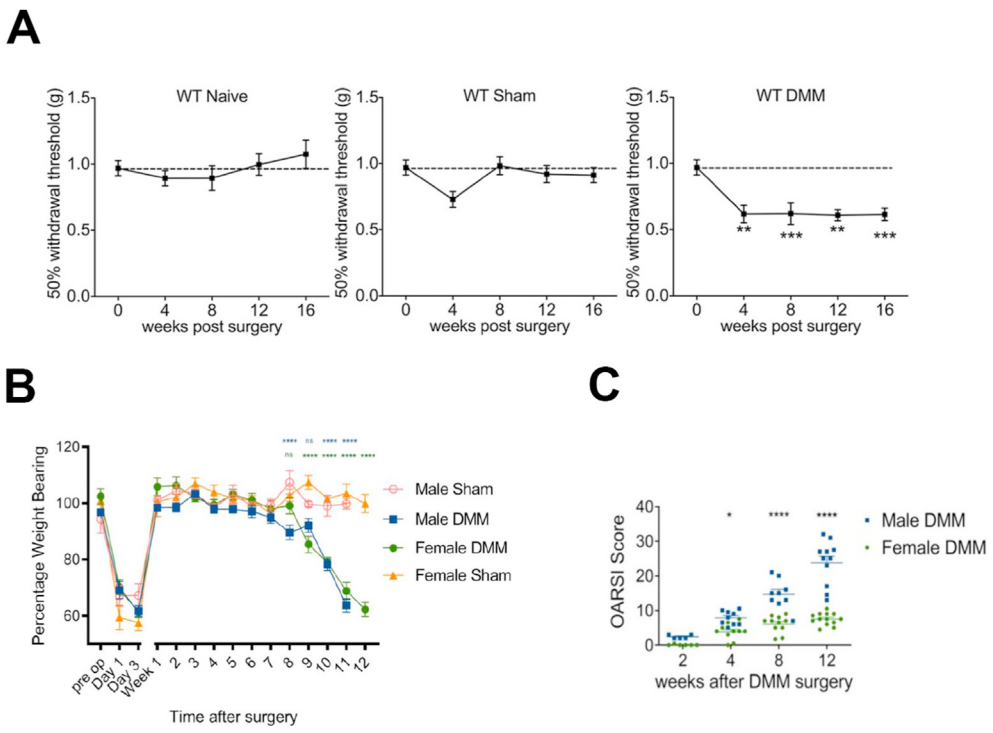

Fig. 2

Osteoarthritis and Cartilage

Representative examples of OARSI scores and phenotypic data of mice after the destabilization of medial meniscus (DMM). (A) Representative example of secondary mechanical allodynia following DMM induction measured with von Frey filaments. naïve (n = 7– 10), sham (n = 9), and DMM mice (n = 9–13), one-way ANOVA with Bonferroni's multiple comparison test, ** = P < 0.01, *** = P < 0.001 vs. week 0. Values given as the mean ± SEM. Reproduced with permission from Miller et al. 2012[45]. (B) Weight-bearing assay in male and female mice after DMM. Values given as the mean ± SEM and as a percentage of weight-bearing on the unoperated limb in male and female mice subjected to DMM or sham surgery. (n = 10 mice per group for DMM and 6 mice per group for sham surgery). **** = P < 0.0001 versus sham-operated mice, by mixed-effects analysis with Dunnett's post hoc test for multiple comparisons. Reproduced with permission from Loga et al. 2020[65]. (C) Osteoarthritis Research Society International (OARSI) score for operated knee joints of male (n = 34) and female (n = 40) C57BL/6 mice after DMM. Reproduced with permission from Loga et al. 2020[65].

animals[125], but also their ability to deal with joint insults[126]. C-fibre nociceptors, which primarily innervate the joint, have been shown to respond differently in aged mice (>77 weeks) compared to young mice (7-20 weeks) in skin preparations. In a complete Freund's adjuvant model (intraplantar injection), only younger mice showed sensitization to mechanical stimuli during acute measurements, and only young and not aged mice showed desensitization of C-fibres to mechanical force in chronic measurements[125]. Importantly both age groups showed pain behaviours during acute and chronic measurements. Thus, peripherally acting analgesics for chronic inflammatory conditions may be largely ineffective in aged populations in the later stages of the disease.

Moreover, Huang and colleagues (2017) have demonstrated age as a significant factor in OA progression using the DMM model. Cartilage degradation and subchondral bone erosion were more severe in older male mice (12 and 19 months) than younger males (4 months)[126]. In addition, Loeser and colleagues (2012) have shown that the fingerprint of upregulated and downregulated genes is different in 12-week-old vs 12-month-old animals in the DMM model from samples containing articular cartilage, subchondral bone, meniscus, and joint capsule. In the MIA model, hyperalgesia is more severe and lasts longer in older rats than young[124]. Functional magnetic resonance imaging studies using young (3–6 months) and old (20–24 months) rats further supported the impact of sex and age in chronic pain processing[127]. These studies strongly suggest that OA-like pain induced in the young is different from OA-like pain induced in older animals and that strategies to target pain in earlier stages of OA should be different from later stages of the disease where central sensitization is likely to be involved.

Pharmacological validation

Pharmacological validation of animal models must be done with caution. For instance, validation of animal models through the efficacy of NSAIDs may introduce a bias towards a subset of models. NSAIDs have shown modest efficacy in OA patients but have excelled in pre-clinical inflammatory models[128,129]. Furthermore, pre-clinical studies have been poor in predicting the outcomes of clinical studies, likely due to suboptimal animal models and measured outputs[130]. The pre-clinical models typically used young and normal weight-mice and post-traumatic models, whereas clinical trials recruit patients with late-stage OA associated with age and obesity[130].

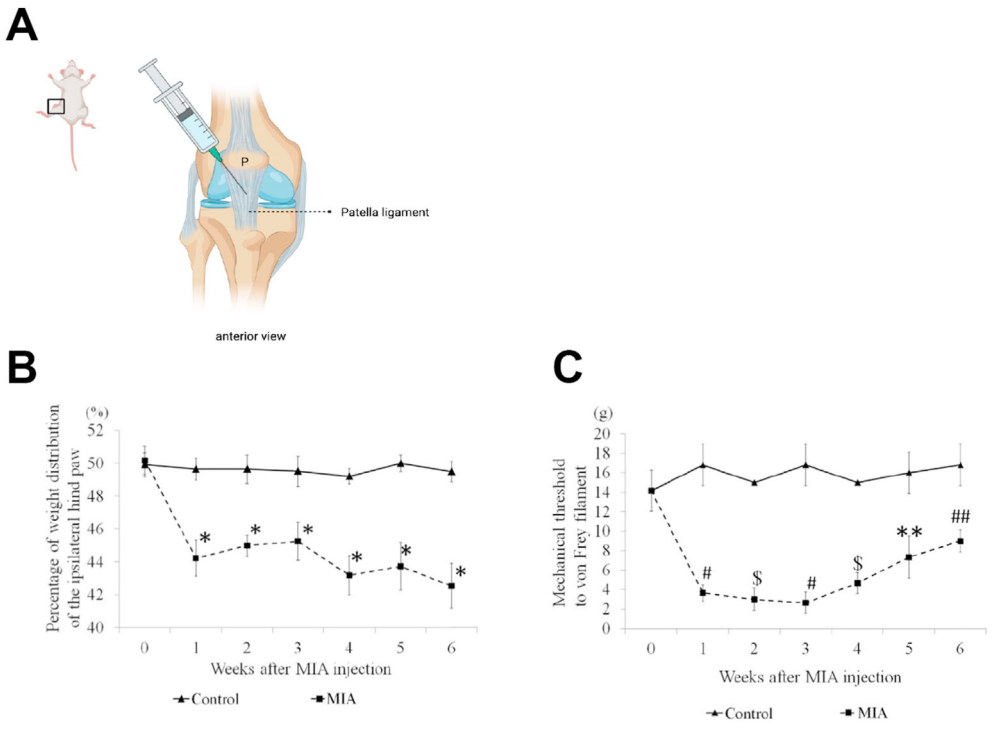

Fig. 3 Osteoarthritis and Cartilage

The monosodium iodoacetate (MIA) model of osteoarthritis. (A) Simplified diagram of the anterior view of the knee joint. MIA intraarticular injection is typically administered via the patella ligament (P, patella). Image created using BioRender.com. (B) Percentage of weight distribution of the ipsilateral hind paw in rats after MIA. (C) Mechanical threshold to von Frey filaments in rats after MIA. Values are mean ± 95% CI (n = 6 rats for each group). *P = 0.005; vs control. #P = 0.003; vs control. $P = 0.002; vs control. **P = 0.004; vs control. ##P = 0.006; vs control. Reproduced with permission from Aso et al. 2016[91].

OA phenotypes

Key to understanding the diverse mechanisms involved in OA pain development is to explore distinct clinical OA phenotypes. Phenotype classification has proved useful in other fields, such as heart failure, in basic research and clinical settings[131]. It has been previously described as a classification restricted to phenotypes that affect treatment or prevention decisions or phenotypes that are critical for understanding OA pathophysiology[132]. Multiple groups have detailed distinct OA phenotypes[132–135], including phenotypes on mechanism, prognostic, and treatment response subgroups[131]. In this review, the OA models are described according to the induction method, yet it is expected that the OA modelling will evolve as these phenotypes are validated in the clinic, and it may become more relevant to describe them according to OA phenotypes.

What is the ideal OA pain model?

The ideal model must be reliably induced, progressive within a suitable time frame, and have the characteristics of human OA[17]. For an ideal OA pain model, it is paramount that a pain phenotype develops with the progression of the disease and not due to the induction approach, as knee OA is characterised by a progressive development of pain associated with joint degradation[136]. Measures to standardise joint degradation assessment such as the OARSI histopathology initiative (OARSI score)[123] and ARRIVE guidelines for reporting animal research[137] facilitate discussion and research progress. Regarding OA pain, there is no such system. For instance, weight-bearing symmetry and gait analysis may generate translationally relevant data but are also in need of standardisation for accurate comparisons (see[41] for a review on the topic). Standardisation of pain measurements taken across different labs and models will further aid research. In addition, an increased understanding of OA phenotypes seen in patients with knee OA will aid pre-clinical research in adequately measure the relevant outputs. Hence, linking the OA phenotypes with models and pain measurements will likely advance the field.

In addition to OA phenotypes, existing rodent models of OA pain may be employed to address different stages or aspects of the disease (Table III). For instance, surgical models show a robust joint degradation by altering joint loading, chemical models trigger strong inflammatory responses, and naturally occurring models more accurately model disease progression. In OA pain research, chemical models may thus proportionate a greater understanding of joint inflammatory pain, whereas surgical and loading models an understanding of pain development with joint degradation seen in earlier stages of OA. When choosing a model, the researcher must consider the known modifying factors (age, sex, induction, and

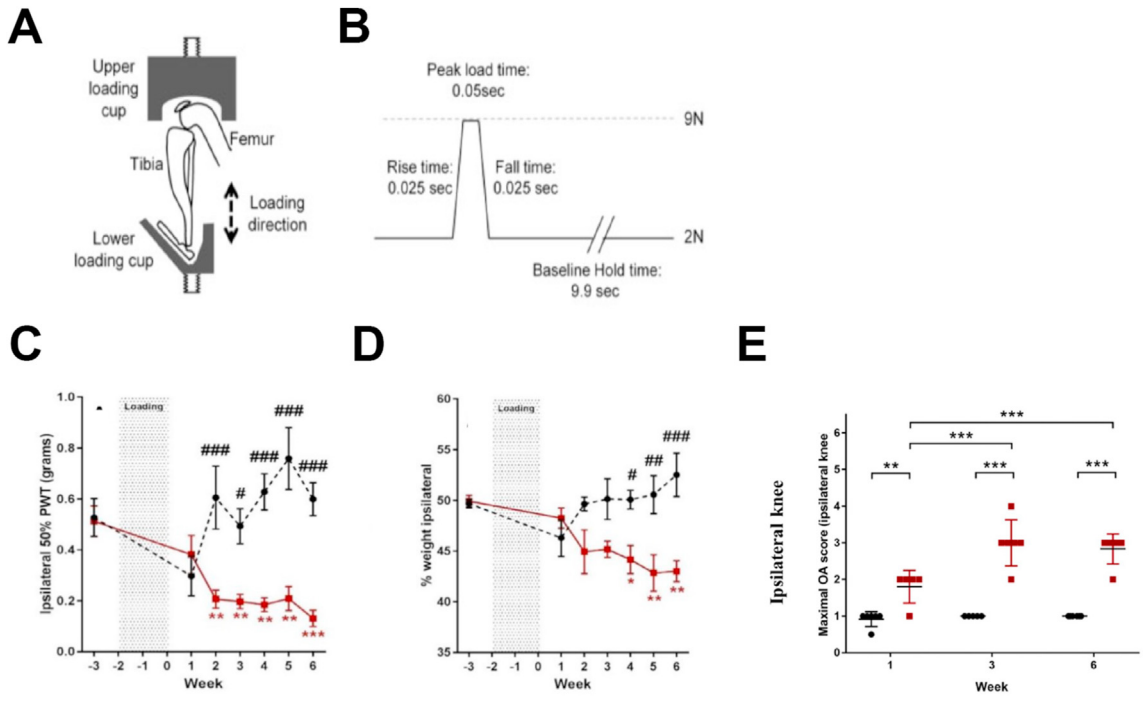

Fig. 4 Osteoarthritis and Cartilage

The non-invasive mechanical loading model of osteoarthritis. (A) Schematic representation of the position of the hind limb and loading direction when placed in the loading apparatus. (B) Diagram of a single cycle of applied load, showing hold and peak load magnitudes, rate of load application, and intervening peak and baseline hold times. Reproduced with permission from Poulet et al. 2011[106]. Representative data from mice after mechanical loading (C-E). (C) Mechanical hypersensitivity measured with von Frey filaments for non-loading control (black, n= 8) and 9 N loaded controls (red, n=8). Values are the mean ± SEM. # = P < 0.05; ### = P < 0.001, versus mechanical joint loading. ** = P < 0.01; *** = P < 0.001, versus baseline. (D) Weight-bearing depicted as % weight on loaded leg. Results were compared to those in non-loaded anesthetized controls (n = 8). Values are the mean ± SEM. # = P < 0.05; ## = P < 0.01; ### = P < 0.001, versus mechanical joint loading. * = P < 0.05; ** = P < 0.01, versus baseline. Reproduced with permission from ter Heegde et al. 2019[110]. (E) Maximal OA score. Severity of OA-like cartilage lesions following mechanical loading of the knee. 9N-loaded mice (red squares, n = 5-6), non-loaded isoflurane controls (black circles, n = 5-6). ** = P < 0.01, *** = P < 0.001. Values given as mean ± SD. Reproduced with permission from ter Heegde et al. 2020[111].

model mechanisms) according to the research question. Hence, the choice of the OA model will depend on the mechanism or type of human OA that needs to be replicated.

Concluding remarks

A variety of models can be useful to determine the different stages of OA and have been described for cartilage degradation[138]. While the current models of OA pain do not yet fully model the human disease, existing models resemble different stages or the progression of the disease. Difficulties also rely on our lack of basic understanding of OA pathophysiology; until we understand the underlying causes of this complex disease, animal models will only reflect some of the pathophysiology. OA pain is complex, and many recent studies and metanalyses have been published to close the gap on the possible OA phenotypes[132–135]. One model will not, alone, be the tool for advancing therapeutic interventions for patients suffering from this debilitating disease. Hence, as progress is made to phenotype OA in the clinic, models may be linked to specific OA phenotyping in humans and effectively link pre-clinical models to clinical trials.

Conflict of interests
No competing interests to declare.

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References

1. Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. Curr Opin Rheumatol 2018;30:160–7.
2. Allen KD, Golightly YM. State of the evidence. Curr Opin Rheumatol 2015;27:276–83.
3. Safiri S, Kolahi A-A, Smith E, Hill C, Bettampadi D, Mansournia MA, *et al*. Global, regional and national burden of osteoarthritis 1990–2017: a systematic analysis of the global

burden of disease study 2017. Ann Rheum Dis 2020;79:819–28.
4. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al.* Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039–49.
5. Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. EClinicalMedicine 2020;29–30:100587.
6. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502.
7. Hawker GA, Stewart L, French MR, Cibere J, Jordan JM, March L, *et al.* Understanding the pain experience in hip and knee osteoarthritis – an OARSI/OMERACT initiative. Osteoarthritis Cartilage 2008;16:415–22.
8. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet 2019;393:1745–59.
9. Hunter DJ, Guermazi A, Roemer F, Zhang Y, Neogi T. Structural correlates of pain in joints with osteoarthritis. Osteoarthritis Cartilage 2013;21:1170–8.
10. Hill CL, Gale DR, Chaisson CE, Skinner K, Kazis L, Gale ME, *et al.* Periarticular lesions detected on magnetic resonance imaging: prevalence in knees with and without symptoms: MRI detection of periarticular lesions in knee OA. Arthritis Rheum 2003;48:2836–44.
11. Neogi T, Felson D, Niu J, Nevitt M, Lewis CE, Aliabadi P, *et al.* Association between radiographic features of knee osteoarthritis and pain: results from two cohort studies. BMJ 2009;339:b2844.
12. Dieppe PA. Relationship between symptoms and structural change in osteoarthritis. What are the important targets for osteoarthritis therapy? Rev J Rheumatol 2004;70:50–3.
13. Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. J Rheumatol 2000;27:1513–7.
14. Neogi T. The epidemiology and impact of pain in osteoarthritis. Osteoarthritis Cartilage 2013;21:1145–53.
15. Bendele AM. Animal models of osteoarthritis. J Musculoskelet Neuronal Interact 2001;1(4):363–76.
16. Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci 2009;10:283–94.
17. Cope PJ, Ourradi K, Li Y, Sharif M. Models of osteoarthritis: the good, the bad and the promising. Osteoarthritis Cartilage 2019;27:230–9.
18. Maldonado R, Baños JE, Cabañero D. Usefulness of knockout mice to clarify the role of the opioid system in chronic pain. Br J Pharmacol 2018;175:2791–808.
19. Chakrabarti S, Ai M, Henson FMD, Smith EStJ. Peripheral mechanisms of arthritic pain: a proposal to leverage large animals for *in vitro* studies. Neurobiol Pain 2020;8:100051.
20. Bapat S, Hubbard D, Munjal A, Hunter M, Fulzele S. Pros and cons of mouse models for studying osteoarthritis. Clin Transl Med 2018;7.
21. Hu Y, Chen X, Wang S, Jing Y, Su J. Subchondral bone microenvironment in osteoarthritis and pain. Bone Res 2021;9:20.
22. Bharde S, Caxaria S, Dell'Accio F, Sikandar S. Update on pain in arthritis. Curr Opin Support Palliat Care 2021;15:99–107.
23. Eitner A, Hofmann GO, Schaible H-G. Mechanisms of osteoarthritic pain. Studies in humans and experimental models. Front Mol Neurosci 2017;10:349.
24. Dye SF, Vaupel GL, Dye CC. Conscious neurosensory mapping of the internal structures of the human knee without intra-articular anesthesia. Am J Sports Med 1998;26:773–7.
25. Schaible H-G, Grubb BD. Afferent and spinal mechanisms of joint pain. Pain 1993;55:5–54.
26. Pattison LA, Krock E, Svensson CI, Smith EStJ. Cell–cell interactions in joint pain: rheumatoid arthritis and osteoarthritis. Pain 2021;162:714–7.
27. Vincent TL. Peripheral pain mechanisms in osteoarthritis. Pain 2020;161:S138–46.
28. Obeidat AM, Miller RE, Miller RJ, Malfait A-M. The nociceptive innervation of the normal and osteoarthritic mouse knee. Osteoarthritis Cartilage 2019;27(11):1669–79, https://doi.org/10.1016/j.joca.2019.07.012.
29. Lo GH, McAlindon TE, Niu J, Zhang Y, Beals C, Dabrowski C, *et al.* Bone marrow lesions and joint effusion are strongly and independently associated with weight-bearing pain in knee osteoarthritis: data from the osteoarthritis initiative. Osteoarthritis Cartilage 2009;17:1562–9.
30. Zhang Y, Nevitt M, Niu J, Lewis C, Torner J, Guermazi A, *et al.* Fluctuation of knee pain and changes in bone marrow lesions, effusions, and synovitis on magnetic resonance imaging. Arthritis Rheum 2011;63:691–9.
31. Felson DT, Niu J, Guermazi A, Roemer F, Aliabadi P, Clancy M, *et al.* Correlation of the development of knee pain with enlarging bone marrow lesions on magnetic resonance imaging. Arthritis Rheum 2007;56:2986–92.
32. Aso K, Shahtaheri SM, Hill R, Wilson D, McWilliams DF, Walsh DA. Associations of symptomatic knee osteoarthritis with histopathologic features in subchondral bone. Arthritis Rheumatol 2019;71:916–24.
33. Suokas AK, Walsh DA, McWilliams DF, Condon L, Moreton B, Wylde V, *et al.* Quantitative sensory testing in painful osteoarthritis: a systematic review and meta-analysis. Osteoarthritis Cartilage 2012;20:1075–85.
34. Wylde V, Hewlett S, Learmonth ID, Dieppe P. Persistent pain after joint replacement: prevalence, sensory qualities, and postoperative determinants. Pain 2011;152:566–72.
35. Buvanendran A, Kroin JS, Della Valle CJ, Kari M, Moric M, Tuman KJ. Perioperative oral pregabalin reduces chronic pain after total knee arthroplasty: a prospective, randomized, controlled trial. Anesth Analg 2010;110:199–207.
36. Hochman JR, Gagliese L, Davis AM, Hawker GA. Neuropathic pain symptoms in a community knee OA cohort. Osteoarthritis Cartilage 2011;19:647–54.
37. Zhu S, Zhu J, Zhen G, Hu Y, An S, Li Y, *et al.* Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. J Clin Invest 2019;129:1076–93.
38. Geraghty T, Winter DR, Miller RJ, Miller RE, Malfait A-M. Neuroimmune interactions and osteoarthritis pain: focus on macrophages. PAIN Rep 2021;6:e892.
39. Minett MS, Quick K, Wood JN. Behavioral measures of pain thresholds. Curr Protoc Mouse Biol 2013;1:383–412.
40. Malfait AM, Little CB, McDougall JJ. A commentary on modelling osteoarthritis pain in small animals. Osteoarthritis Cartilage 2013;21:1316–26.
41. Jacobs BY, Allen KD. Factors affecting the reliability of behavioral assessments for rodent osteoarthritis models. Lab Anim 2020;54:317–29.
42. Marker CL, Pomonis JD. The monosodium iodoacetate model of osteoarthritis pain in the rat. Methods Mol Biol 2012;851:239–48, https://doi.org/10.1007/978-1-61779-561-9_18.
43. Chakrabarti S, Pattison LA, Singhal K, Hockley JRF, Callejo G, Smith EStJ. Acute inflammation sensitizes knee-innervating sensory neurons and decreases mouse digging behavior in a TRPV1-dependent manner. Neuropharmacology 2018;143:49–62.

44. Van de Weerd HA, Bulthuis RJA, Bergman AF, Schlingmann F, Tolboom J, Van Loo PLP, *et al.* Validation of a new system for the automatic registration of behaviour in mice and rats. Behav Process 2001;53:11–20.
45. Miller RE, Tran PB, Das R, Ghoreishi-Haack N, Ren D, Miller RJ, *et al.* CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. Proc Natl Acad Sci U S A 2012;109:20602–7.
46. Ferreira-Gomes J, Adães S, Mendonça M, Castro-Lopes JM. Analgesic effects of lidocaine, morphine and diclofenac on movement-induced nociception, as assessed by the Knee-Bend and CatWalk tests in a rat model of osteoarthritis. Pharmacol Biochem Behav 2012;101:617–24.
47. Schorscher-Petcu A, Takács F, Browne LE. Scanned optogenetic control of mammalian somatosensory input to map input-specific behavioral outputs. Elife 2021;10, e62026.
48. Jones JM, Foster W, Twomey CR, Burdge J, Ahmed OM, Pereira TD, *et al.* A machine-vision approach for automated pain measurement at millisecond timescales. Elife 2020;9:e57258.
49. Bohic M, Pattison LA, Jhumka ZA, Rossi H, Thackray JK, Ricci M, *et al.* Behavioral and nociceptor states of inflammatory pain across timescales in 2D and 3D. bioRxiv 2021;29, https://doi.org/10.1101/2021.06.16.448689.
50. Bendele AM. Progressive chronic osteoarthritis in femorotibial joints of partial medial meniscectomized Guinea pigs. Vet Pathol 1987;24:444–8.
51. Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L, Heitmeyer SA. Induction of osteoarthritis in the rat by surgical tear of the meniscus: inhibition of joint damage by a matrix metalloproteinase inhibitor. Osteoarthritis Cartilage 2002;10:785–91.
52. Kamekura S, Hoshi K, Shimoaka T, Chung U, Chikuda H, Yamada T, *et al.* Osteoarthritis development in novel experimental mouse models induced by knee joint instability. Osteoarthritis Cartilage 2005;13:632–41.
53. Clements KM, Price JS, Chambers MG, Visco DM, Poole AR, Mason RM. Gene deletion of either interleukin-1beta, interleukin-1beta-converting enzyme, inducible nitric oxide synthase, or stromelysin 1 accelerates the development of knee osteoarthritis in mice after surgical transection of the medial collateral ligament and partial medial meniscectomy. Arthritis Rheum 2003;48:3452–63.
54. Little CB, Hunter DJ. Post-traumatic osteoarthritis: from mouse models to clinical trials. Nat Rev Rheumatol 2013;9:485–97.
55. Mapp PI, Avery PS, McWilliams DF, Bowyer J, Day C, Moores S, *et al.* Angiogenesis in two animal models of osteoarthritis. Osteoarthritis Cartilage 2008;16:61–9.
56. Sampson ER, Beck CA, Ketz J, Canary KL, Hilton MJ, Awad H, *et al.* Establishment of an index with increased sensitivity for assessing murine arthritis: development OF a murine arthritis index. J Orthop Res 2011;29:1145–51.
57. Sampson ER, Hilton MJ, Tian Y, Chen D, Schwarz EM, Mooney RA, *et al.* Teriparatide as a chondroregenerative therapy for injury-induced osteoarthritis. Sci Transl Med 2011;3(101):101ra93.
58. Thorup A-S, Strachan D, Caxaria S, Poulet B, Thomas BL, Eldridge SE, *et al.* ROR_2 blockade as a therapy for osteoarthritis. Sci Transl Med 2020;12:eaax3063.
59. Temp J, Labuz D, Negrete R, Sunkara V, Machelska H. Pain and knee damage in male and female mice in the medial meniscal transection-induced osteoarthritis. Osteoarthritis Cartilage 2020;28:475–85.
60. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthritis Cartilage 2007;15:1061–9.
61. Welch ID, Cowan MF, Beier F, Underhill TM. The retinoic acid binding protein $CRABP_2$ is increased in murine models of degenerative joint disease. Arthritis Res Ther 2009;11:R14.
62. Maynard RD, Villani DA, Schroeder WG, Adams DJ, Zuscik MJ. Surgical induction of posttraumatic osteoarthritis in the mouse. Methods Mol Biol 2021;2230:91–103.
63. Inglis JJ, McNamee KE, Chia S-L, Essex D, Feldmann M, Williams RO, *et al.* Regulation of pain sensitivity in experimental osteoarthritis by the endogenous peripheral opioid system. Arthritis Rheum 2008;58:3110–9.
64. Fang H, Huang L, Welch I, Norley C, Holdsworth DW, Beier F, *et al.* Early changes of articular cartilage and subchondral bone in the DMM mouse model of osteoarthritis. Sci Rep 2018;8:2855.
65. Loga IS, Batchelor V, Driscoll C, Burleigh A, Chia SL, Stott B, *et al.* Does pain at an earlier stage of chondropathy protect female mice against structural progression after surgically induced osteoarthritis? Arthritis Rheumatol 2020;72:2083–93.
66. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma H-L, *et al.* Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 2005;434:644–8.
67. Kc R, Li X, Kroin JS, Liu Z, Chen D, Xiao G, *et al.* PKCδ null mutations in a mouse model of osteoarthritis alter osteoarthritic pain independently of joint pathology by augmenting NGF/TrkA-induced axonal outgrowth. Ann Rheum Dis 2016;75:2133–41.
68. Ishihara S, Obeidat AM, Wokosin DL, Ren D, Miller RJ, Malfait A-M, *et al.* The role of intra-articular neuronal CCR2 receptors in knee joint pain associated with experimental osteoarthritis in mice. Arthritis Res Ther 2021;23:103.
69. Sun Q, Zhen G, Li TP, Guo Q, Li Y, Su W, *et al.* Parathyroid hormone attenuates osteoarthritis pain by remodeling subchondral bone in mice. Elife 2021;10:e66532.
70. Pond MJ, Nuki G. Experimentally-induced osteoarthritis in the dog. Ann Rheum Dis 1973;32:387–8.
71. Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. Am J Sports Med 2007;35:1756–69.
72. Gilbert SJ, Bonnet CS, Stadnik P, Duance VC, Mason DJ, Blain EJ. Inflammatory and degenerative phases resulting from anterior cruciate rupture in a non-invasive murine model of post-traumatic osteoarthritis: inflammatory and degenerative changes IN post-traumatic osteoarthritis. J Orthop Res 2018;36:2118–27.
73. Hsia AW, Jbeily EH, Mendez ME, Cunningham HC, Biris KK, Bang H, *et al.* Post-traumatic osteoarthritis progression is diminished by early mechanical unloading and anti-inflammatory treatment in mice. Osteoarthritis Cartilage 2021;29:1709–19.
74. Ängeby Möller K, Aulin C, Baharpoor A, Svensson CI. Pain behaviour assessments by gait and weight bearing in surgically induced osteoarthritis and inflammatory arthritis. Physiol Behav 2020;225:113079.
75. Blaker CL, Zaki S, Little CB, Clarke EC. Long-term effect of a single subcritical knee injury: increasing the risk of anterior cruciate ligament rupture and osteoarthritis. Am J Sports Med 2021;49:391–403.
76. Rzeczycki P. Cannabinoid receptor type 2 is upregulated in synovium following joint injury and mediates anti-inflammatory effects in synovial fibroblasts and macrophages. Osteoarthritis Cartilage 2021;29(12):1720–31.
77. Roos H, Laurén M, Adalberth T, Roos EM, Jonsson K, Lohmander LS. Knee osteoarthritis after meniscectomy: prevalence of radiographic changes after twenty-one years, compared with matched controls. Arthritis Rheum 1998;41(4):687–93.

78. Knights CB, Gentry C, Bevan S. Partial medial meniscectomy produces osteoarthritis pain-related behaviour in female C57BL/6 mice. Pain 2012;153:281–92.
79. von Loga IS, El-Turabi A, Jostins L, Miotla-Zarebska J, Mackay-Alderson J, Zeltins A, *et al*. Active immunisation targeting nerve growth factor attenuates chronic pain behaviour in murine osteoarthritis. Ann Rheum Dis 2019;78:672–5.
80. Sabri MI, Ochs S. Inhibition of glyceraldehyde-3-phosphate dehydrogenase in mammalian nerve by iodoacetic acid. J Neurochem 1971;18:1509–14.
81. Dunham J, Hoedt-Schmidtt S, Kalbhent DA. Structural and metabolic changes in articular cartilage induced by iodoacetate. Int J Exp Pathol 1992;73(4):455–64.
82. Kalbhen DA. Chemical model of osteoarthritis–a pharmacological evaluation. J Rheumatol 1987;14:130–1.
83. Bendele AM, Hulman JF, Harvey AK, Hrubey PS, Chandrasekhar S. Passive role of articular chondrocytes in quinolone-induced arthropathy in Guinea pigs. Toxicol Pathol 1990;18:304–12.
84. Pitcher T, Sousa-Valente J, Malcangio M. The monoiodoacetate model of osteoarthritis pain in the mouse. J Vis Exp 2016;111:53746, https://doi.org/10.3791/53746.
85. Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicol Pathol 2003;31:619–24.
86. de Sousa Valente J. The pharmacology of pain associated with the monoiodoacetate model of osteoarthritis. Front Pharmacol 2019;10:974.
87. Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. Nat Rev Rheumatol 2012;8:390–8.
88. Fernihough J, Gentry C, Malcangio M, Fox A, Rediske J, Pellas T, *et al*. Pain related behaviour in two models of osteoarthritis in the rat knee. Pain 2004;112:83–93.
89. Felson DT, Chaisson CE, Hill CL, Totterman SMS, Gale ME, Skinner KM, *et al*. The association of bone marrow lesions with pain in knee osteoarthritis. Ann Intern Med 2001;134(7):541–9.
90. Driban JB, Price L, Lo GH, Pang J, Hunter DJ, Miller E, *et al*. Evaluation of bone marrow lesion volume as a knee osteoarthritis biomarker – longitudinal relationships with pain and structural changes: data from the osteoarthritis initiative. Arthritis Res Ther 2013;15:R112.
91. Aso K, Izumi M, Sugimura N, Okanoue Y, Ushida T, Ikeuchi M. Nociceptive phenotype alterations of dorsal root ganglia neurons innervating the subchondral bone in osteoarthritic rat knee joints. Osteoarthritis Cartilage 2016;24:1596–603.
92. Combe R, Bramwell S, Field MJ. The monosodium iodoacetate model of osteoarthritis: a model of chronic nociceptive pain in rats? Neurosci Lett 2004;370:236–40.
93. Bove SE, Calcaterra SL, Brooker RM, Huber CM, Guzman RE, Juneau PL, *et al*. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. Osteoarthritis Cartilage 2003;11:821–30.
94. Havelin J, Imbert I, Cormier J, Allen J, Porreca F, King T. Central sensitization and neuropathic features of ongoing pain in a rat model of advanced osteoarthritis. J Pain 2016;17:374–82.
95. Barve RA, Minnerly JC, Weiss DJ, Meyer DM, Aguiar DJ, Sullivan PM, *et al*. Transcriptional profiling and pathway analysis of monosodium iodoacetate-induced experimental osteoarthritis in rats: relevance to human disease. Osteoarthritis Cartilage 2007;15:1190–8.
96. Schuelert N, McDougall JJ. Involvement of Nav 1.8 sodium ion channels in the transduction of mechanical pain in a rodent model of osteoarthritis. Arthritis Res Ther 2012;14:R5.
97. Vertex announces treatment with the NaV1.8 inhibitor VX-150 showed significant relief of acute pain in phase 2 study 2018.
98. van der Kraan PM, Vitters EL, van de Putte LB, van den Berg WB. Development of osteoarthritic lesions in mice by 'metabolic' and 'mechanical' alterations in the knee joints. Am J Pathol 1989;135:1001–14.
99. van der Kraan PM, Vitters EL, van Beuningen HM, van de Putte LB, van den Berg WB. Degenerative knee joint lesions in mice after a single intra-articular collagenase injection. A new model of osteoarthritis. J Exp Pathol Oxf Engl 1990;71:19–31.
100. Van Osch GJVM, Van Der Kraan PM, Van Den Berg WB. Site-specific cartilage changes in murine degenerative knee joint disease induced by iodoacetate and collagenase. J Orthop Res 1994;12:168–75.
101. Hagg R, Bruckner P, Hedbom E. Cartilage fibrils of mammals are biochemically heterogeneous: differential distribution of decorin and collagen IX. J Cell Biol 1998;142:10.
102. Botter SM, van Osch GJVM, Waarsing JH, van der Linden JC, Verhaar JAN, Pols HAP, *et al*. Cartilage damage pattern in relation to subchondral plate thickness in a collagenase-induced model of osteoarthritis. Osteoarthritis Cartilage 2008;16:506–14.
103. Murakami K, Nakagawa H, Nishimura K, Matsuo S. Changes in peptidergic fiber density in the synovium of mice with collagenase-induced acute arthritis. Can J Physiol Pharmacol 2015;93:435–41.
104. van Osch GJ, van der Kraan PM, Blankevoort L, Huiskes R, van den Berg WB. Relation of ligament damage with site specific cartilage loss and osteophyte formation in collagenase induced osteoarthritis in mice. J Rheumatol 1996;23:1227–32.
105. van Osch GJ, van der Kraan PM, van Valburg AA, van den Berg WB. The relation between cartilage damage and osteophyte size in a murine model for osteoarthritis in the knee. Rheumatol Int 1996;16:115–9.
106. Poulet B, Hamilton RW, Shefelbine S, Pitsillides AA. Characterizing a novel and adjustable noninvasive murine joint loading model. Arthritis Rheum 2011;63(1):137–47.
107. Chowdhury TT, Arghandawi S, Brand J, Akanji OO, Bader DL, Salter DM, *et al*. Dynamic compression counteracts IL-1beta induced NOS and COX-2 expression in chondrocyte/agarose constructs. Arthritis Res Ther 2008;10:R35.
108. Kisiday JD, Jin M, DiMicco MA, Kurz B, Grodzinsky AJ. Effects of dynamic compressive loading on chondrocyte biosynthesis in self-assembling peptide scaffolds. J Biomech 2004;37:595–604.
109. Poulet B, de Souza R, Kent AV, Saxon L, Barker O, Wilson A, *et al*. Intermittent applied mechanical loading induces subchondral bone thickening that may be intensified locally by contiguous articular cartilage lesions. Osteoarthritis Cartilage 2015;23:940–8.
110. ter Heegde F, Luiz AP, Santana-Varela S, Chessell IP, Welsh F, Wood JN, *et al*. Non-invasive mechanical joint loading as an alternative model for osteoarthritic pain. Arthritis Rheumatol 2019;71(7):1078–88, https://doi.org/10.1002/art.40835.
111. ter Heegde F, Luiz AP, Santana-Varela S, Magnúsdóttir R, Hopkinson M, Chang Y, *et al*. Osteoarthritis-related nociceptive behaviour following mechanical joint loading correlates with cartilage damage. Osteoarthritis Cartilage 2020;28:383–95.
112. Lapveteläinen T, Nevalainen T, Parkkinen JJ, Arokoski J, Kiraly K, Hyttinen M, *et al*. Lifelong moderate running training increases the incidence and severity of osteoarthritis in the knee joint of C57BL mice: osteoarthritis and running training. Anat Rec 1995;242:159–65.

113. Wilhelm G, Faust R. Suitability of the C57 black mouse as an experimental animal for the study of skeletal changes due to ageing, with special reference to osteo-arthrosis and its response to tribenoside. Pharmacology 1976;14:289–96.
114. Walton M. Patella displacement and osteoarthrosis of the knee joint in mice. J Pathol 1979;127:165–72.
115. Naruse K, *et al*. Osteoarthritic changes of the patellofemoral joint in STR/OrtCrlj mice are the earliest detectable changes and may be caused by internal tibial torsion. Connect Tissue Res 2009;50(4):243–55.
116. Poulet B, Souza R, Knights CB, Gentry C, Wilson AM, Bevan S, *et al*. Modifications of gait as predictors of natural osteoarthritis progression in STR/ort mice. Arthritis Rheumatol 2014;66:1832–42.
117. Hu K, Xu L, Cao L, Flahiff CM, Brussiau J, Ho K, *et al*. Pathogenesis of osteoarthritis-like changes in the joints of mice deficient in type IX collagen. Arthritis Rheum 2006;54(9):2891–900.
118. Säämänen A-MK, Salminen HJ, Dean PB, De Crombrugghe B, Vuorio EI, Metsäranta MPH. Osteoarthritis-like lesions in transgenic mice harboring a small deletion mutation in type II collagen gene. Osteoarthritis Cartilage 2000;8:248–57.
119. Allen KD, *et al*. Decreased physical function and increased pain sensitivity in mice deficient for type IX collagen. Arthritis Rheum 2009;69(9):2684–93.
120. Ma H-L, Blanchet TJ, Peluso D, Hopkins B, Morris EA, Glasson SS. Osteoarthritis severity is sex dependent in a surgical mouse model. Osteoarthritis Cartilage 2007;15:695–700.
121. Hwang HS, Park IY, Hong JI, Kim JR, Kim HA. Comparison of joint degeneration and pain in male and female mice in DMM model of osteoarthritis. Osteoarthritis Cartilage 2021;29:728–38.
122. van Osch GJ. Induction of osteoarthritis by intra-articular injection of collagenase in mice. Strain and sex related differences. Osteoarthritis Cartilage 1993;1(3):171–7.
123. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse. Osteoarthritis Cartilage 2010;18:S17–23.
124. Ro JY, Zhang Y, Tricou C, Yang D, da Silva JT, Zhang R. Age and sex differences in acute and osteoarthritis-like pain responses in rats. J. Gerontol. Ser. A 2020;75:1465–72.
125. Weyer AD, Zappia KJ, Garrison SR, O'Hara CL, Dodge AK, Stucky CL. Nociceptor sensitization depends on age and pain chronicity(1,2,3). eNeuro 2016;3(1). ENEURO.0115-15.2015.
126. Huang H, Skelly JD, Ayers DC, Song J. Age-dependent changes in the articular cartilage and subchondral bone of C57BL/6 mice after surgical destabilization of medial meniscus. Sci Rep 2017;7:42294.
127. Da Silva JT, Tricou C, Zhang Y, Tofighbakhsh A, Seminowicz DA, Ro JY. Pain modulatory network is influenced by sex and age in a healthy state and during osteoarthritis progression in rats. Aging Cell 2021;20:e13292.
128. Laine L, White WB, Rostom A, Hochberg M. COX-2 selective inhibitors in the treatment of osteoarthritis. Semin Arthritis Rheum 2008;38:165–87.
129. Berge O-G. Predictive validity of behavioural animal models for chronic pain: animal models for analgesia. Br J Pharmacol 2011;164:1195–206.
130. Malfait A-M, Little CB. On the predictive utility of animal models of osteoarthritis. Arthritis Res Ther 2015;17:225.
131. Deveza LA, Nelson AE, Loeser RF. Phenotypes of osteoarthritis: current state and future implications. Clin Exp Rheumatol 2019;9.
132. Felson DT. Identifying different osteoarthritis phenotypes through epidemiology. Osteoarthritis Cartilage 2010;18:601–4.
133. Egsgaard LL, Eskehave TN, Bay-Jensen AC, Hoeck HC, Arendt-Nielsen L. Identifying specific profiles in patients with different degrees of painful knee osteoarthritis based on serological biochemical and mechanistic pain biomarkers: a diagnostic approach based on cluster analysis. Pain 2015;156:96–107.
134. Deveza LA, Melo L, Yamato TP, Mills K, Ravi V, Hunter DJ. Knee osteoarthritis phenotypes and their relevance for outcomes: a systematic review. Osteoarthritis Cartilage 2017;25:1926–41.
135. Vongsirinavarat M, Nilmart P, Somprasong S, Apinonkul B. Identification of knee osteoarthritis disability phenotypes regarding activity limitation: a cluster analysis. BMC Musculoskelet. Disord. 2020;21:237.
136. Glyn-Jones S, Palmer AJR, Agricola R, Price AJ, Vincent TL, Weinans H, *et al*. Osteoarthritis. Lancet 2015;386:376–87.
137. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the arrive guidelines for reporting animal research. PLoS Biol 2010;8:e1000412.
138. Poulet B. Models to define the stages of articular cartilage degradation in osteoarthritis development. Int J Exp Pathol 2017;98:120–6.
139. Ferreira-Gomes J, Adães S, Castro-Lopes JM. Assessment of movement-evoked pain in osteoarthritis by the knee-bend and CatWalk tests: a clinically relevant study. J Pain 2008 Oct;9(10):945–54.
140. Parvathy SS, Masocha W. Gait analysis of C57BL/6 mice with complete Freund's adjuvant-induced arthritis using the CatWalk system. BMC Musculoskelet Disord 2013 Dec;14(1):14.
141. Deacon RMJ. Measuring motor coordination in mice. J Vis Exp JoVE 2013 May 29;(75):e2609.
142. Zhen G, Wen C, Jia X, Li Y, Crane JL, Mears SC, *et al*. Inhibition of TGF-β signaling in mesenchymal stem cells of subchondral bone attenuates osteoarthritis. Nat Med 2013 Jun;19(6):704–12.
143. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, Duong LT. Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. Bone 2006 Feb;38(2):234–43.
144. Im H-J, Kim J-S, Li X, Kotwal N, Sumner DR, van Wijnen AJ, *et al*. Alteration of sensory neurons and spinal response to an experimental osteoarthritis pain model. Arthritis Rheum 2010 Jun 15;62(10):2995–3005.
145. Adães S, Mendonça M, Santos TN, Castro-Lopes JM, Ferreira-Gomes J, Neto FL. Intra-articular injection of collagenase in the knee of rats as an alternative model to study nociception associated with osteoarthritis. Arthritis Res Ther 2014;16(1):R10.
146. Christiansen BA, Anderson MJ, Lee CA, Williams JC, Yik JHN, Haudenschild DR. Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis. Osteoarthritis Cartilage 2012 Jul;20(7):773–82.
147. Evans RG, Collins C, Miller P, Ponsford FM, Elson CJ. Radiological scoring of osteoarthritis progression in STR/ORT mice. Osteoarthritis Cartilage 1994 Jun;2(2):103–9.
148. Kumagai K, Suzuki S, Kanri Y, Matsubara R, Fujii K, Wake M, *et al*. Spontaneously developed osteoarthritis in the temporomandibular joint in STR/ort mice. Biomed Rep 2015 Jul;3(4):453–6.