Understanding osteoarthritis pain through animal models

M. O'Brien, H.T. Philpott, J.J. McDougall

Departments of Pharmacology and Anaesthesia, Pain Management & Perioperative Medicine, Dalhousie University, Halifax, Canada.

Melissa O'Brien,*
Holly T. Philpott,*
Jason J. McDougall,

**Co-first authors*

Please address correspondence to:
Dr Jason J. McDougall,
Departments of Pharmacology and Anaesthesia, Pain Management and Perioperative Medicine,
Dalhousie University,
5850 College Street,
Halifax, Nova Scotia, B3H 4R2, Canada.
E-mail: jason.mcdougall@dal.ca

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ABSTRACT

Osteoarthritis (OA) is the most prevalent musculoskeletal disease worldwide. Chronic pain remains the foremost concern of OA patients and is poorly controlled by available pharmacotherapies. Current preclinical research, which aims to develop analgesics better suited for OA, is largely dependent on animal models and laboratory pain testing. This review summarises commonly used small animal models for studying experimental OA, including their benefits and limitations. Also discussed are a variety of validated methods for studying pain within these models.

Introduction

Osteoarthritis (OA) is a multifaceted musculoskeletal disease which affects up to 50% of people aged 65 and over worldwide (1). OA is characterised by the inability of a damaged joint to launch an effective healing response. The foremost concern of OA patients is the alleviation of chronic pain which is not well controlled by current pharmacotherapies. The first line drug therapy for OA pain are the non-steroidal anti-inflammatory drugs (NSAIDs). While these drugs are effective for acute pain, their analgesic capacity diminishes for the long-term treatment of OA pain while their risk of causing adverse side effects increases (2). The development of highly targeted analgesics for OA pain is crucial, but is hampered by poorly understood mechanisms of OA pain. To elucidate the pathogenesis of OA pain further, translatable animal models and valid pain assessment techniques are required.

Animal models of osteoarthritis

Spontaneous models

• *Dunkin Hartley spontaneous OA*

Mice, rabbits, guinea pigs, dogs, sheep, and horses naturally develop OA as they age. The most commonly studied of these animals is the Dunkin Hartley guinea pig which exhibits spontaneous OA that is remarkably similar to that in humans. The earliest changes in joint structure can be seen when animals are approximately 3-months old and by 12-months to 2 years severe medial degeneration of the knee joints are present (3).

The relationship between joint damage and nociception was elegantly explored in these animals using histopathology and electrophysiology where it was discovered that nociception did not correlate with joint damage in this model of OA (4). The benefit of using this model is the natural progression of OA with age, which is similar to the human condition. Limitations include the long experimental time needed for OA development which in turn elevates the cost of experimentation. The lethargic and obese nature of these animals also precludes them for effective pain behavioural assessment.

• *Obesity and arthritis*

Obesity has been identified as a major risk factor for OA of the knee, but has also been implicated in hip and hand OA (5, 6). Obesity is associated with a chronic inflammatory state with the overproduction of proinflammatory molecules. In addition, overloading the joints contributes to the pathogenesis and progression of OA in these animals. Griffin *et al*. examined various inflammatory, biomechanical, and osteoarthritic changes in mice that were fed a high-fat diet for 45 weeks. This high-fat diet induced greater OA changes such as increased proteoglycan loss, decreased musculoskeletal performance, and hyperalgesia to a thermal stimulus compared to control animals receiving a normal diet (7). These studies also revealed a correlation between severity of knee osteoarthritis and serum levels of leptin adiponectin, and IL-1α (7).

Often preclinical models do not integrate risk factors and comorbidities, but by using a diet-induced obesity model it

is possible to incorporate a comorbidity into the assessment making the model closer to recapitulating the human disease state. A limitation to the obesity model is that it takes several weeks to develop, which would again greatly increase the cost of these experiments. Additionally, these studies did not thoroughly assess pain as an outcome measure, and there is evidence to suggest that in humans as well as animals, that being obese lowers the sensitivity to painful stimuli (8, 9), therefore further characterisation of pain in the obesity model is desired.

Induced models

Chemically-induced OA is a robust and reproducible means of modelling the development of OA pathophysiology and pain. These models are often used in laboratory rats and mice where they facilitate timely and cost-effective experiments.

• *Monosodium iodoacetate (MIA)*

The monosodium iodoacetate (MIA) model is a rapidly developing model that allows investigators to focus on the pain aspect of OA pathophysiology. This model was first described approximately 35 years ago by Kalbhen in chickens (10). The structural integrity of the joint, specifically the cartilage, relies on homeostatic chondrocytes. Intra-articular injection of MIA, a chemical which inhibits glycolysis, disrupts chondrocyte homeostatic balance and produces cartilage degeneration and subsequent subchondral bone loss (11). The rapid development of OA pathology and pain in the MIA model typically occurs within 1–2 weeks and is dose-dependent (12, 13). In addition to joint degeneration and pain, the MIA model also generates acute and transient inflammation similar to the flares observed in some human OA patients. Following intra-articular MIA injection, the joint becomes hyperaemic, oedematous and there is an infiltration of circulating leukocytes (14, 15). By days 5–7 the inflammation subsides and remains at low levels throughout the subsequent development of MIA-induced OA. The early inflammatory flare is thought to drive joint degeneration and damage to the nerves innervating the joint, leading to chronic pain. The MIA model is most commonly used in small laboratory animals such as mice, rats, and guinea pigs and is most commonly injected into the knee, hip, and ankle joints. Furthermore, there have been a range of concentrations tested, the most common of which being 1–3mg (16). The MIA model has been widely used in the assessment of OA pain and OA-associated nerve damage (15, 17). Peripheral nerve damage markers have been observed as early as day 3 and upregulation remains at day 14 (18). Although the MIA model is ideal for assessing OA pain and peripheral neuropathy, a limitation of this model is that the structural histopathology of the joint itself is severe and does not recapitulate all of the physical features commonly associated with human OA.

• *Lysophosphatidic acid*

The inflammatory lipid lysophosphatidic acid (LPA) is elevated in the synovial fluid of OA joints and its concentration correlates with the severity of OA in humans (19). The LPA model of OA has recently been developed and characterised whereby intra-articular injection of LPA in rats leads to arthritis-like lesions including subchondral bone loss and cartilage fibrillation (unpublished data), demyelination of joint afferent fibres and pain. LPA produces robust nerve damage to joint afferents which models a subset of OA patients who experience neuropathic pain, making this model useful in identifying analgesics which can target those patients with this particular type of pain.

• *Surgically-induced osteoarthritis*

While OA is generally considered a disease of the elderly population due to daily wear and tear of the large weight bearing joints, there exists a subpopulation of patients who develop OA earlier in life due to traumatic joint injury (20). These types of injuries commonly occur in athletes, but can arise as a result of repetitive strain injuries or falls. Surgically-induced models of OA are commonly utilised in sheep, dogs, rabbits, rats and mice and aim to mimic post-traumatic osteoarthritis (PTOA) by inducing joint instability.

In rats, commonly used models include anterior cruciate ligament transection (ACLT), meniscectomy (partial (PMx) or complete (CMx)) and medial meniscal tear (MMT) or a combination of these insults (3, 21-24). Each of these models leads to joint instability, OA like lesions consisting of cartilage degradation, bone remodelling, and pain. Caution should be taken when comparing the results between surgically-induced OA and human injuries as there are inherent differences in the rate and sites of OA progression. This is to be expected since each model produces disparate changes in joint biomechanics and kinematics (25). Limitations of these models are the large numbers of animals recommended to account for variability in the severity of OA lesions and the time required for OA to develop.

In mice, the most common PTOA model is destabilisation of the medial meniscus (DMM). This model is induced by surgical transection of the meniscotibial ligament (26, 27) which causes mild to moderate OA lesions in the central weight bearing area of the joint. Lesions occur as early as two weeks post-surgery and increase in severity over time (28). In a study that compared the severity and development of PTOA in male and female mice, it was found that OA severity was significantly higher in males than females after DMM which pointed to a role of sex-hormones in PTOA progression (29).

The surgically-induced models of osteoarthritis recapitulate most of the histopathological features of human OA. Important considerations when using these models are the differences in loading and gait in humans (bipeds) compared to animals (quadrupeds) which could contribute to divergences in joint damage (25). A further limitation of PTOA models is that when assessing pain, it is unclear whether we are measuring changes in kinematics due to joint instability or actual pain. One particular benefit of using PTOA models, however, is the translatability of early intervention in animal studies to the clinic whereby a potential treatment could be administered relatively quickly after joint trauma.

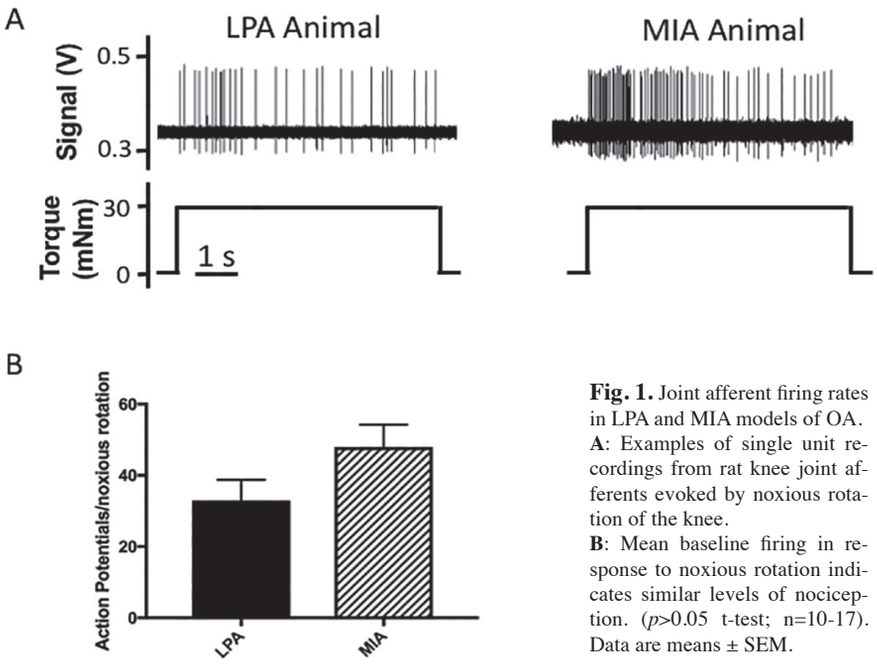

Fig. 1. Joint afferent firing rates in LPA and MIA models of OA. **A**: Examples of single unit recordings from rat knee joint afferents evoked by noxious rotation of the knee. **B**: Mean baseline firing in response to noxious rotation indicates similar levels of nociception. (p>0.05 t-test; n=10-17). Data are means ± SEM.

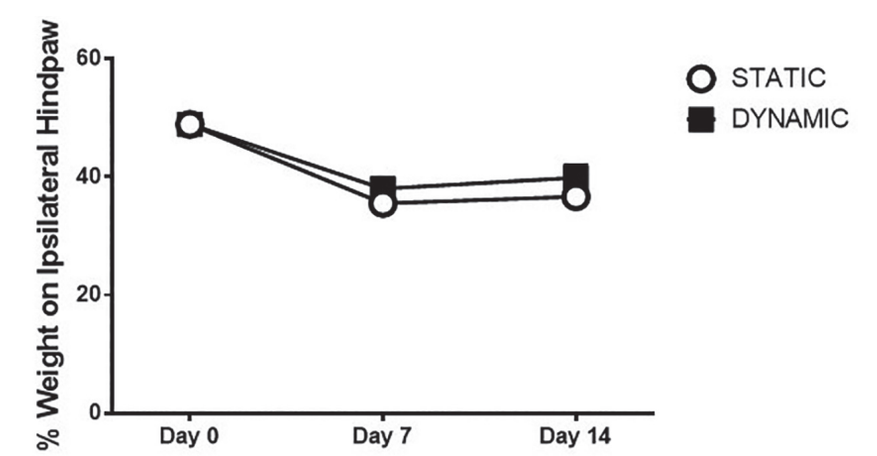

Fig. 2. Static *versus* dynamic weight bearing in OA rats. Data collected from the same cohort of animals, comparing static and dynamic weight bearing over 14 days during the development of MIA-induced OA. Both weight bearing tests produce similar outcomes, where static weight bearing is not significantly different from dynamic weight bearing (p>0.05, two-way ANOVA with Bonferroni's post hoc test; n=12-13). Data are means ± SEM.

Pain measurements in models of osteoarthritis

Electrophysiology

In vivo electrophysiology is a technically demanding yet powerful tool to measure the neuronal activity of nociceptive pathways. In OA, a combination of locally-released alogenic substances, joint degeneration, inflammation and nerve damage contribute to pain by sensitising peripheral afferent fibres. Recording the activity of peripheral nerves innervating OA joints and second order neurones in the dorsal horn of the spinal cord provide critical neurophysiological data to characterise neuronal activity and plasticity in OA.

Peripheral sensitisation of joint afferent fibres can be studied by single-unit recordings in a teased nerve preparation (4, 13). This type of recording has uncovered fundamental relationships between the movement of damaged joints and the firing of pain sensing nerves innervating these joints. Seminal studies found that nociceptive Aδ and C fibres express mechanogated ion channels such that rotation of the knee causes firing of these mechanosensitive primary afferents (30). In the Dunkin Hartley guinea pig model of spontaneous OA, it was discovered that structural damage does not correlate with nociceptor hyperactivity (4). This fundamental observation corroborates the clinical situation where the severity of joint destruction is not consistent with the level of pain reported by the patient (31). Recently, electrophysiological recording of joint afferents in the LPA model of neuropathic OA uncovered a decrease in nerve conduction velocity compared to control nerves confirming the demyelinating effect of LPA (19). In the MIA model, graded concentrations of intra-articular MIA draws a parallel graded sensitisation of joint afferents (13). Comparisons between the joint afferent activity in LPA and MIA models demonstrates a similar firing rate although the MIA model appears to be higher (Fig. 1) suggesting a greater degree of peripheral sensitisation in these animals. The major limitation of these types of electrophysiological experiments is the extensive training required for successful recording of joint single units.

Central sensitisation can be studied by recording the activity of spinal cord neurones. *In vivo* recordings from lamina V-VI of L4-L5 have been carried out in MIA-injected rats and demonstrated windup of dorsal horn neurones indicative of central sensitisation (17,20). In these experiments wide dynamic range neurones which have receptive fields on the hind paw rather than the knee joint itself were recorded. To the best of our knowledge, spinal nerves with a knee joint afferent input have not yet been recorded from. Spinal nociceptive reflexes have also been assessed in the MIA model by recording EMG activity in the biceps femoris muscle during plantar hind paw stimulation. Compared to control animals, spinal nociceptive reflex pathways are sensitised following the development of OA, suggesting the presence of central sensitisation in this model (32).

Spontaneous pain behaviour

• *Weight bearing and gait analysis*

OA patients with loss of joint function and pain often exhibit altered biomechanics and kinematics. Hindlimb

weight bearing and kinematic gait analysis are two types of joint function assessments which have been used to measure OA pain in animals. Hindlimb weight bearing can be measured by either static or dynamic approaches where the weight borne by an arthritic hindlimb is compared to the non-arthritic contralateral hindlimb. The static method of measuring hindlimb weight bearing involves extensive training of animals to stand upright with each hindlimb on an incapacitance force plate (14). The amount of weight borne on each hindlimb is averaged over 3–5 seconds. Dynamic incapacitance allows the measurement of hindlimb weight bearing in a freely moving animal. This technique obviates the need for extensive training and avoids any possibility of stress-related analgesia. Animals are placed in a Perspex chamber with a pressure sensitive floor and allowed to move freely for 3-4 minutes. Software allows for offline analysis of weight borne on each limb as well as the surface area of each paw (33, 34). Uneven weight bearing can be inferred as favouring the arthritic knee joint associated with pain. Both methods of assessing weight bearing reveal comparable levels of pain and are highly reproducible (Fig. 2).

Basic gait parameters such as joint angles and stride length can be quantified using automated kinematic equipment (*e.g.,* TreadScan, Catwalk) or kinematic analysis of treadmill running. In the automated systems, a high-speed camera placed under the animal records its footfalls as it walks across a transparent platform (35, 36). Kinematic methods involve placing reflective markers on specific anatomical landmarks on the animal's limbs. A high-speed camera then records joint displacement as the animal runs on a treadmill set at a constant speed (35, 36). There are some major limitations in interpreting the data generated by these techniques in small animals. For example, rodents are prey animals and tend to mask their pain to avoid becoming a target for predators. Additionally, it is difficult to determine if altered weight bearing or gait is due to pain or arising from abnormal joint biomechanics. These assessments should be paired with other pain tests to strengthen any changes in gait parameters.

Table I. Summary of animal models of OA and changes in pain outcome measures that have been reported in each model.

Model of OA	Species	Changes in Pain Assessments
Natural		
Spontaneous	Guinea pig	• Spontaneously active knee joint afferent fibres (4)
Obesity	Mouse	• Altered musculoskeletal function and gait (7)
Chemical		
MIA	Rat	• Sensitisation of knee joint afferent fibres (13) • Wind-up of dorsal horn neurones (central sensitisation) (17, 32) • Progressive secondary mechanical allodynia (15, 17) • Hindlimb weight bearing deficit (11, 14, 17, 38) • Conditioned place preference (38) • Vocalisation in response to knee bend test (48)
LPA	Rat	• Reduction in joint afferent conduction velocity (peripheral neuropathy) (18)
Surgical		
ACLT	Rat	• Altered hindlimb weight distribution (49) • Progressive secondary mechanical allodynia (49)
PMx	Mouse	• Vocalisation upon pressue applied to joint (50) • Secondary mechanical allodynia (50)
MMT	Rat	• Sensitisation of knee joint afferent fibres (51) • Secondary mechanical allodynia (24) • Progressive hindlimb weight bearing deficits (24)
DMM	Mouse	• Mechanical hyperalgesia (45) • Secondary mechanical allodynia (45)

• *Conditioned place preference testing*

The conditioned place preference (CPP) test has been used primarily to study reward behaviour, but can also be used to measure persistent pain (37-39). The assay is based on pairing positive (or negative) reinforcement with a specific context, so that animals are motivated to seek that context (or in the case of negative reinforcement animals will avoid the associated context) and this behaviour can be recorded. Briefly, an animal is placed in a three-chambered box and allowed to roam freely during an acclimation period during which the time spent in each chamber is recorded. Two of the chambers have distinct visual, tactile, and odor cues and the final chamber is devoid of stimulation. Animals are treated with a test analgesic and then restricted to one of the chambers before being returned to their cages. After an habituation to the test reagent/chamber animals are then treated with vehicle and placed in a different CPP chamber. If animals are in persistent pain they will seek out the "analgesia chamber"; however, if animals are not in pain they will not favour any chamber. CPP has been used to study pain in the MIA model of OA and shown that place preference occurs only in arthritic animals and not control animals, suggesting ongoing pain (38). Limitations of CPP is that it is difficult to analyse if animals have a biased preference of chamber prior to administering treatments and if the test drugs impair cognition as this is a learned assay (37). In addition, the fact that arthritic animals have a mobility impairment lessens the likelihood of them seeking out a specific chamber.

Evoked pain behaviour

• *von Frey Hair Algesiometry*

OA sufferers often experience varying and complex pain perception as their disease progresses. This includes pain at rest, pain remote from the injured joint (referred pain), and pain upon light pressure or touching of the skin (40), all of which can be deemed tactile hypersensitivity.

A von Frey hair is a filament used to measure tactile sensitivity and was invented by Maximilian von Frey in 1896. Von Frey filaments when placed

perpendicularly on a specific test area will elicit a specific bending force. Filaments of various bending forces may be used in the clinic to quantify tactile allodynia. Von Frey filaments can also be a useful diagnostic tool in preclinical animal experiments, where a withdrawal response is interpreted as indicating that the filament elicited pain. Most often, a set of filaments is used to measure hypersensitive areas by directly applying them to the injured area or by applying them away from the injured area (primary and secondary allodynia). There are two commonly used algorithms for determining the mechanical threshold, the up-down method created by Dixon (41) and adapted for pain tests by Chaplan (42), and the percent response method. The up-down method is the most commonly used, which requires a series of 4–8 responses used to calculate the withdrawal threshold. It should be noted that the range of bending forces should be chosen based on the type of pain wished to be assessed (*i.e.,* allodynia *vs*. hyperalgesia). Automated and electronic von Frey hair instruments have also been developed to avoid the potential for investigator bias and to reduce the number of required applications of filaments during a testing period, thereby reducing false positives or conditioning of the animal. The automated von Frey hair allows for the actual force, in grams, and withdrawal latency, measured in seconds, to be automatically detected and recorded (43). The electronic von Frey also allows investigators to record pain threshold automatically, easily, and accurately. These devices have uniform diameter rigid tips which eliminates the possibility of a false reading based on various tip diameters of the von Frey filaments.

• *Pressure application measurement*

The pressure application measurement (PAM) device is analogous to the clinically used pressure dolorimeter. The device consists of a pressure sensitive button strapped to the experimenter's thumb. Using the thumb and index finger to hold the animal's joint, a gradually increasing pressure is applied across the joint until the animal gives an indication of pain such as a squeak, an attempt to withdraw its hindlimb or freezing its whiskers (44, 45). The force at this endpoint is indicative of the mechanical threshold and is a measure of primary allodynia. A limitation of this test is the need to restrain the animal for testing; however, it is one of the few behavioural tests that measures nociception directly at the joint. Another limitation of the PAM technique is that the pressure must be applied to the joint at a constant rate so as to avoid any startle responses or activation of low threshold, dynamic afferents. In the mouse DMM model, PAM withdrawal forces did not correlate with cartilage degradation or early subchondral bone remodelling suggesting a disconnect between joint damage and this type of evoked pain (46).

• *Vocalisation*

Audible and ultrasonic vocalisations can be recorded in laboratory rats in response to evoked or spontaneous pain. Application of a noxious stimulus will likely result in an audible squeak which indicates nociception. The test animals may also emit ultrasonic vocalisations which signal the affective characteristics of the pain experience. These vocalisations can be recorded and analysed offline by specialised recording equipment and a bat sensor (44, 47). Animals will emit 22-kHz vocalisations in response to painful stimuli; however, interpretation of these chirps is complicated by the fact that this type of vocalisation can indicate a wide variety of aversive behavioural situations such as a response to nearby predators, startling noises, male-male aggression, anticipation of aversive stimuli, or when being handled by an unfamiliar experimenter.

Conclusions

Animal models are a useful approach to unravel the complexity of OA pain (Table I). One of the major criticisms of studying OA pre-clinically is the translatability of the animal models to the human condition. No model exists which fully recapitulates human OA and no pain test used in animals can fully capture the human experience. These issues complicate pre-clinical research in the OA field but standardisation of the conduct and reporting of preclinical research in OA with the use of the ARRIVE guidelines (52), will aid in providing better outcomes for translation into patient therapies. OA is a multifactorial disease and not all OA patients experience the same set of symptoms or rate of disease progression. This perpetuates an ongoing divide (or lack of consensus) in the research community about the driving factors which contribute to OA progression and pain. The use of multiple models to validate a novel therapy or target would allow for a better understanding of how these models relate to specific pathological changes seen in OA patients and how novel therapies can slow or prevent these changes. Furthermore, women are two-thirds more likely to develop OA than men and their reported pain is greater. Implementing the use of both male and female animals into pre-clinical OA research would help curb poor translatability and tease out these sex-specific differences in OA pathogenesis and pain sensation. Using several different approaches to measure pain allow for the assessment of multiple pain modalities, providing a better understanding of outcome measures received from a particular model as well as the changes in pain sensation and mechanisms over time. Only by understanding pain mechanisms using multiple animal models of OA can we hope to identify pharmacological and non-pharmacological targets to nullify this debilitating disease.

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