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Overview

Mouse Models of Osteoarthritis: A Summary of Models and Outcomes Assessment

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Osteoarthritis (OA) is a multidimensional health problem and a common chronic disease. It has a substantial impact on patient quality of life and is a common cause of pain and mobility issues in older adults. The functional limitations, lack of curative treatments, and cost to society all demonstrate the need for translational and clinical research. The use of OA models in mice is important for achieving a better understanding of the disease. Models with clinical relevance are needed to achieve 2 main goals: to assess the impact of the OA disease (pain and function) and to study the efficacy of potential treatments. However, few OA models include practical strategies for functional assessment of the mice. OA signs in mice incorporate complex interrelations between pain and dysfunction. The current review provides a comprehensive compilation of mouse models of OA and animal evaluations that include static and dynamic clinical assessment of the mice, merging evaluation of pain and function by using automatic and noninvasive techniques. These new techniques allow simultaneous recording of spontaneous activity from thousands of home cages and also monitor environment conditions. Technologies such as videography and computational approaches can also be used to improve pain assessment in rodents but these new tools must first be validated experimentally. An example of a new tool is the digital ventilated cage, which is an automated home-cage monitor that records spontaneous activity in the cages.

Abbreviations: CST, capacitance sensing technology; DVC, digital ventilated cage; MIA, mono-iodoacetate; OA, osteoarthritis; OARSI: osteoarthritis research society international; PWT, paw withdrawal time

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Osteoarthritis (OA) is a multidimensional health problem and a common chronic disease.[36] Functional limitations, the absence of curative treatments, and the considerable cost to society result in a substantial impact on quality of life.[76] Historically, OA has been described as whole joint and whole peri-articular diseases and as a systemic comorbidity.[9,111] OA consists of a disruption of articular joint cartilage homeostasis leading to a catabolic pathway characterized by chondrocyte degeneration and destruction of the extracellular matrix (ECM). Low-grade chronic systemic inflammation is also actively involved in the process.[42,92] In clinical practice, mechanical pain, often accompanied by a functional decline, is the main reason for consultations. Recommendations to patients provide guidance for OA management.[22, 33,49,86] Evidence-based consensus has led to a variety of pharmacologic and nonpharmacologic modalities that are intended to guide health care providers in managing symptomatic patients. Animal-based research is of tremendous importance for the study of early diagnosis and treatment, which are crucial to prevent the disease progression and provide better care to patients.

The purpose of animal-based OA research is 2-fold: to assess the impact of the OA disease (pain and function) and to study the efficacy of a potential treatment.[18,67] OA model species include large animals such as the horse, goat, sheep, and dog, whose size and anatomy are expected to better reflect human joint conditions. However, small animals such as guinea pig, rabbit, mouse, and rat represent 77% of the species used.[1,87] In recent years, mice have become the most commonly used model for studying OA. Mice have several advantageous characteristics: a short development and life span, easy and low-cost breeding and maintenance, easy handling, small joints that allow histologic analysis of the whole joint,[32] and the availability of genetically modified lines.[108] Standardized housing, genetically defined strains and SPF animals reduce the genetic and interindividual acquired variability. Mice are considered the best vertebrate model in terms of monitoring and controlling environmental conditions.[7,14,15,87] Mouse skeletal maturation is reached at 10 wk, which theoretically constitutes the minimal age at which mice should be entered into an OA study.[64,87,102] However, many studies violate this limit by testing mice at 8 wk of age.

Available models for OA include the following (Table 1): spontaneous naturally occurring OA (C57BL/6, BALB/c, STR/ort or genetically modified mice); chemically-induced (mainly mono-iodoacetate [MIA] injection); noninvasive (high fat diet or obesity-induced) consistent with the metabolic human OA;[32,111] physical activity and exercise induced OA; noninvasive mechanical loading (repetitive mild loading and single-impact injury); and surgically induced (meniscectomy models or anterior cruciate ligament transection). The specific model used would be based on the goal of the study.[7] For example, OA pathophysiology, OA progression, and OA therapies studies could use spontaneous,

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Table 1. Mouse models of osteoarthritis.

Models		Pros	Cons
Spontaneous	Wild type mice[7,9,59,67,68,70,72,74,80,85,87,115,118,119,120]	- Model of aging phenotype - The less invasive model - Physiological relevance: mimics human pathogenesis - No need for technical expertise - No need for specific equipment	- Variability in incidence - Large number of animals at baseline - Long-term study: Time consuming (time of onset: 4 -15 mo) - Expensive (husbandry)
	Genetically modified mice[2,7,25,40,50,52,67,72,79,80,89,120]	- High incidence - Earlier time of onset: 18 wk - No need for specific equipment - Combination with other models	- Time consuming for the strain development - Expensive
Chemical-induced	Mono-iodoacetate injection[7,11,46,47,60,66,90,91,101,128]	- Model of pain-like phenotype - To study mechanism of pain and antalgic drugs - Short-term study: Rapid progression (2-7 wk) - Reproducible - Low cost	- Need for technical expertise - Need for specific equipment - Systemic injection is lethal - Destructive effect: does not allow to study the early phase of pathogenesis
	Papain injection[66,67,120]	- Short-term study: rapid progression - Low cost	- Need for technical expertise - Need for specific equipment - Does not mimic natural pathogenesis
	Collagenase injection[7,65,67,98]	- Short-term study: rapid progression (3 wk) - Low cost	- Need for technical expertise - Need for specific equipment - Does not mimic natural pathogenesis
Non-invasive	High-fat diet (Alimentary induced obesity model)[5,8,43,45,57,96,124]	Model of metabolic phenotype No need for technical expertise No need for specific equipment Reproducible	Long-term study: Time consuming (8 wk–9 mo delay) Expensive
	Physical activity and exercise model[45,73]	Model of post traumatic phenotype No need for technical expertise	Long-term study: time consuming (18 mo delay) Expensive Disparity of results
	Mechanical loading models Repetitive mild loading models Single-impact injury model[7,16,23,24,32,35,104,105,106]	Model of post traumatic phenotype Allow to study OA development Time of onset: 8-10 wk post injury Noninvasive	Need for technical expertise Need for specific equipment Heterogeneity in protocol practices Repetitive anesthesia required or ethical issues
Surgical	Ovariectomy[114]		Contested.
	Meniscectomy model[7,32,63,67,87]	Model of post traumatic phenotype High incidence Short-term study: early time of onset (4 wk from surgery) To study therapies	Need for technical expertise Need for specific equipment Surgical risks Rapid progression compared to human
	Anterior cruciate ligament transection (ACLT)[7,39,40,61,48,67,70,87,126]	Model of posttraumatic phenotype High incidence Short-term study: early time of onset (3-10 wk from surgery) Reproducible To study therapies	Need for technical expertise Need for specific equipment Surgical risks Rapid progression compared to human
	Destabilization of medial meniscus (DMM)[7,32,39,40]	Model of post traumatic phenotype High incidence Short-term study: early time of onset (4 wk from surgery) To study therapies The most frequently used method	Need for technical expertise Need for specific equipment Surgical risks Rapid progression compared to human

Since all animal models have strengths and weaknesses, it is often best to plan using a number of models and techniques together to combine the results.

genetic, surgical, or noninvasive models. In addition, pain studies could use chemical models. Lastly, post-traumatic studies would use surgical or noninvasive models; the most frequently used method is currently destabilization of the medial meniscus,[32] which involves transection of the medial meniscotibial ligament, thereby destabilizing the joint and causing instability-driven OA. An important caveat for mouse models is that the mouse and human knee differ in terms of joint size, joint biomechanics, and histologic characteristics (layers, cellularity),[32,64] and joint differences could confound clinical translation.[10]

In humans, the lack of correlation between OA imaging assessment and clinical signs highlights the need to consider the functional data and the quality of life to personalize OA management. Clinical outcomes are needed to achieve 2 main goals: to assess the impact of the OA in terms of pain and function and to study the efficacy of treatments.[65] Recent reviews offer few practical approaches to mouse functional assessment and novel approaches to OA models in mice.[7,32,67,75,79,83,87,100,120] This review will focus on static and dynamic clinical as-

Table 2. Outcome options in mouse models of osteoarthritis

Test name	Techniques	Kind of assessment	Output	Specific equipment required
Static measurement				
Von Frey filament testing	Calibrated nylon filaments of various thickness (and applied force) are pressed against the skin of the plantar surface of the paw in ascending order of force	Stimulus- evoked pain-like behavior Mechanical stimuli - Tactile allodynia The most commonly used test	Latency to paw withdrawal and Force exerted are recorded	Yes
Knee extension test	Apply a knee extension on both the intact and affected knee or Passive extension range of the operated knee joint under anesthesia	Stimulus-evoked pain-like behavior	Number of vocalizations evoked in 5 extensions	None
Hotplate	Mouse placed on hotplate. A cutoff latency has been determined to avoid lesions	Stimulus-evoked pain-like behavior Heat stimuli- thermal sensitivity	Latency of paw withdrawal	Yes
Righting ability	Mouse placed on its back	Neuromuscular screening	Latency to regain its footing	None
Cotton swab test	Bringing a cotton swab into contact with eyelashes, pinna, and whiskers	Stimulus-evoked pain-like behavior Neuromuscular screening	Withdrawal or twitching response	None
Spontaneous activity				
Spontaneous cage activity	One by one the cages must be laid out in a specific platform	Spontaneous pain behavior Nonstimulus evoked pain Activity	Vibrations evoked by animal movements	Yes
Open field analysis	Experiment is performed in a clear chamber and mice can freely explore	Spontaneous pain behavior Nonstimulus evoked pain Locomotor analysis	Paw print assessment Distance traveled, average walking speed, rest time, rearing	Yes
Gait analysis	Mouse is placed in a specific cage equipped with a fluorescent tube and a glass plate allowing an automated quantitative gait analysis	Nonstimulus evoked pain Gait analysis Indirect nociception	Intensity of the paw contact area, velocity, stride frequency, length, symmetry, step width	Yes
Dynamic weight bearing system	Mouse placed is a specific cage. This method is a computerized capacitance meter (similar to gait analysis)	Nonstimulus evoked pain Weight-bearing deficits Indirect nociception	Body weight redistribution to a portion of the paw surface	Yes
Voluntary wheel running	Mouse placed is a specific cage with free access to stainless steel activity wheels. The wheel is connected to a computer that automatically record data	Nonstimulus evoked pain Activity	Distance traveled in the wheel	Yes
Burrowing analysis	Mouse placed is a specific cage equipped with steel tubes (32 cm in length and 10 cm in diameter) and quartz sand in Plexiglas cages (600 × 340x200 mm)	Nonstimulus evoked pain Activity	Amount of sand burrowed	Yes
Digital video recordings	Mouse placed is a specific cage according to the tool	Nonstimulus evoked pain Or Evoked pain	Scale of pain or specific outcome	Yes
Digital ventilated cage system	Nondisrupting capacitive-based technique: records spontaneous activity 24/7, during both light and dark phases directly from the home cage rack	Spontaneous pain behavior Nonstimulus evoked pain Activity-behavior	Distance walked, average speed, occupation front, occupation rear, activation density. Animal locomotion index, animal tracking distance, animal tracking speed, animal running wheel distance and speed or rotation	Yes
Challenged activity				
Rotarod test	Gradual and continued acceleration of a rotating rod onto which mice are placed	Motor coordination Indirect nociception	Rotarod latency: riding time and speed with a maximum cut off.	Yes
Hind limb and fore grip strength	Mouse placed over a base plate in front of a connected grasping tool	Muscle strength of limbs	Peak force, time resistance	Yes
Wire hang analysis	Suspension of the mouse on the wire and start the time	Muscle strength of limbs: muscle function and coordination	Latency to fall gripping	None (self -constructed)

Pain cannot be directly measured in rodents, so methods have been developed to quantify "pain-like" behaviors. The clinical assessment of mice should be tested both before and after the intervention (induced-OA ± administration of treatment) to take into account the habituation and establish a baseline to compare against.

sessment of OA using automatic and noninvasive emerging techniques (Table 2).

Outcomes Measured to Assess Symptomatic OA

OA signs in mice comprise complex interactions between pain and dysfunction. Because pain cannot be directly measured in rodents, methods have been developed to quantify "pain-like" behaviors.[30] Mice should be clinically assessed both before and after OA induction or administration of treatment both to account for habituation and to establish a baseline for subsequent comparisons.[79] All outcomes are summarized in the Table 2. All static testing is based on pain assessment. Static measurements refer primarily to an assessment off evoked pain. Dynamic measurements require a monitoring of the mouse activity, which can be spontaneous or evoked.

Static measurements. Static assessments of pain are one way to assess symptomatic OA. Rodents are particularly useful for statis measurements due to their small size and easy manipulation. However, in contrast to rats, mice are more active, which can make static assessments more difficult.[83]

One static test is the used of Von Frey filament to identify mechanical/tactile allodynia.[17] Calibrated nylon filaments of various thickness (and applied force) are pressed against the skin of the plantar surface of the paw in ascending order of force.[83,90] The stimulus should lead to a rapid withdrawal response. After a training and habituation period, latency to paw withdrawal and force exerted are recorded.[100] The lowest amount of force inducing a response is the paw withdrawal threshold (PWT), expressed in grams.[63] As in sham-operated mice, mice with a partial medial meniscectomy had a consistent decrease of the ipsilateral PWT at day 7 after surgery (from 0.56g to 0.45g).[61] However, as compared with sham-operated mice, PWT remained low until day 56 in mice with meniscectomy, after which it fell from 0.45g to 0.24g. The effect of OA induction leads to an increase of allodynia with a clear decrease of withdrawal threshold.[61] The Von Frey filament test sensitive with regard to analgesia testing in OA.[123]

The knee extension is another test used to assess pain in mice with OA.[56,90,100,127] Various techniques can be considered. The knee can be extended on both the intact and the affected side and the number of vocalizations, indicating discomfort, evoked in 5 extensions can be counted.[100] This is a relevant clinical measure because OA patients experience loss of knee range of motion that leads to discomfort.[67,97] A surgically induced OA model created by resection of the medial collateral ligament and the medial meniscus in 8 wk old C57BL/6 male mice showed a significant impairment of knee extension starting from the third month after surgery; this difference from the control group was maintained until the sixth month.[82] This study used passive extension range of the operated knee joint under anesthesia, measured the maximal and minimal angle of the mouse knee joint, and obtained results consistent with the Osteoarthritis Research Society International OARSI scoring, showing significant OA development in the operated mice.[82] The reduction of extension angle leads to static limitations and also affects dynamic results.

The hotplate is a thermal sensitivity test.[3,100] The latency of nociceptive reaction is measured by measuring PWT. After cruciate ligament transection, OA led to a longer PWT of mice on a hotplate, starting from 4 wk onward.[109] Eight weeks after surgery, the response time in operated mice was approximately 7 s as compared with 4 s in the sham group.

Another global reflexive static test is neuromuscular screening using the cotton swab test. A cotton swab that is brought into contact with eyelashes, pinna, and whiskers should induce a withdrawal or twitching response.[2] If not, reflexive behavior will be viewed as abnormal, indicating a behavioral characteristic of pain that can be associated with induced OA.

Righting ability can also be used as a general test The mouse is placed on its back and the time taken to regain upright posture is assessed and can be scored as normal, delayed, or abnormal.[2]

Dynamic measurements. ***Spontaneous activity.*** Biomechanical and functional assessments allow evaluation of the functional consequences of deficiencies or disabilities related to OA. Spontaneous cage activity can be measured automatically.[32] This LABORAS (Laboratory Animal Behavior Observation Registration and Analysis System) records objective normal activity and OA-induced changes in locomotion (distance), climbing (hanging from a wire cage), feeding, grooming, rearing (standing on hind legs), head shakes, and nest-making as measures of behavior in mice.[12,83,100] To use this system, cages must be arranged on a specific platform, which can be time-consuming, and the results can be skewed by handling the cages.[121] As compared with human observation, software converts mouse movement into a data set that can be obtained and analyzed easily.[122]

The open field test consists of locomotor analysis with paw print assessment.[27,109] The test is performed in an open space that mice can explore.[109] Distance traveled, average walking speed, and rest time are related to horizontal locomotor activity.[113] Some studies have assessed vertical locomotor activity by measuring rearing.[113] Chronic pain leads to withdrawal and hypo-activity. Even if human observation introduces subjectivity, spontaneous pain behavior may be more clinically relevant than is evoked pain.[83]

The gait analysis or catwalk test is a method to indirectly assess pain. Automated quantitative gait analysis requires special equipment to measure the intensity of the paw contact area.[32] OA causes gait changes and a smaller footprint area.[109] Gait analysis includes velocity, stride frequency and length, symmetry, and step width.[109] Parameters related to interlimb coordination can also be measured objectively. Pain can cause compensatory changes in joint load shifting, and the automated gait analysis system can assess body weight redistribution to a portion of the paw surface that is associated with pain.[2,95,100] Computerized gait analysis provides nonbiased pain assessment. Gait analysis should include spatiotemporal, kinetic, and kinematic parameters.[69]

Voluntary wheel running has been used to assess pain in rodent. Data collection is completely automated, and the experimenter is not in the room during the assessment.[26] However, multiple activity cages need individual randomization before the experiment.

Burrowing can also be evaluated. This has been done with the MIA model of OA to assess pain-related behavior and analgesic efficacy.[13] Burrowing is an innate behavior and is reduced in rodents experiencing pain.[13] Bilateral MIA injection in rats impairs burrowing behavior.[13] The burrowing method has 2 phases: first, social facilitation during which rats are placed in pairs in a cage for 2 h on 2 consecutive days with a measurement of the amount of sand burrowed, and second placing a rat alone in the cage for 30 min per day and determining the average amount burrowed over 3 d to provide a baseline value for burrowing. Burrowing evaluation could also be useful in mice but the method must be optimized for mice before it can be used experimentally.[121] Digital video recordings can also be used to

Figure 1. The digital ventilated cage system is an automated home-cage monitoring that continuously records spontaneous activity in the cages. The system can collect data from thousands of home cages simultaneously.

assess facial grimace in rats and mice, which is not specific to OA but can be used to assess pain.[71,88,116,117]

Automated home-cage monitoring provides activity information directly from the home cage using electrical capacitance sensing technology (CST) of an electronic board placed under the home cage.[55] The digital ventilated cage system (DVC) (Tecniplast S.p.A, Buguggiate (VA), Italy) records spontaneous activity using 12 electrodes spread as 3 × 4 grids in the cages. Electrodes continuously detect electrical capacitance every 250 ms during both light and dark phases directly from the home cage rack. This nondisruptive capacitive-based technique with several advantages including the reduction of animal handling and no need to set up an external data collection system.[55,99] The system can collect data from thousands of home cages simultaneously; benefits are 2-fold (Figure 1). First, the DVC system can analyze mouse behavior global cage activity over time. As compared with conventional video metrics, individually housed mice CST metrics are highly correlated for distance walked, average speed, occupation front, occupation rear, and activation density. Currently validated DVC metrics are animal locomotion index, animal tracking distance, animal tracking speed, and running wheel distance and speed or rotation. Animal locomotion index is correlated with the activity pattern. The DVC system can also be used to monitor animal welfare.[55] The system is can detect high activity levels that may signal aggression.[38] Another use for the DVC system is to analyze the bedding status by monitoring increasing moisture due to urine and water bottle leakage. The range of capabilities of this technology can provide research, husbandry and welfare indicators. DVC systems will likely become an essential tool for many laboratories in the future.

Challenged activity. Motor dysfunction and indirect pain can be assessed using challenged exercises, although learning and motivation can mask true functional effects. The rotarod test is based on the gradual and continued acceleration of a rotating rod onto which mice are placed (Figure 2). Data collected are the riding times (the amount of time a mouse stays on the rod before falling off) and the speed (from 4 to 40 rpm) at which a mouse falls off.[2,100,123] Mice are trained for 5 min at a constant speed of 4 rpm on the rotarod before the experimental trials begin. Each

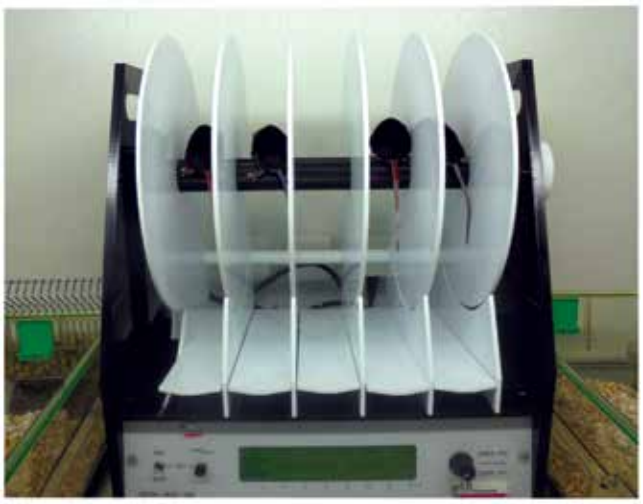

Figure 2. The rotarod test is based on a gradual and continued acceleration of a rotating rod onto which mice are placed. Data collected corresponds to the riding time (the amount of time each mouse stays on the rod before falling off or a passive rotation) and the speed (from 4 to 40 rpm).

trial has a maximum time of 5 min. The trial consists of testing mice for a number of consecutive days (typically 3) with a minimum of 30 min intertrial rest.[100,109] Rotarod creates a forced ambulation and involves motor coordination, balance, pain, sensorimotor skills, endurance, memory, and learning skills that could be limiting factors for the interpretation of the performance.[10,100,109] OA decreases time on the rotarod.[82,109] When cruciate ligament transection was performed on 8-wk-old male FVB/N mice and compared with the preoperative functional assessment, mice showed a postoperative motor dysfunction in rotarod analysis.[109] Those functional changes were linked with histologic grading by OARSI; functional decline should occur concomitantly with the cartilage degeneration.[109]

Hind limb and fore grip strength are also challenge exercises that are commonly used in OA studies.[2,29,79,93] Special equipment automatically measures the grip force of limbs of mice, including the peak force and time resistance. The mouse is

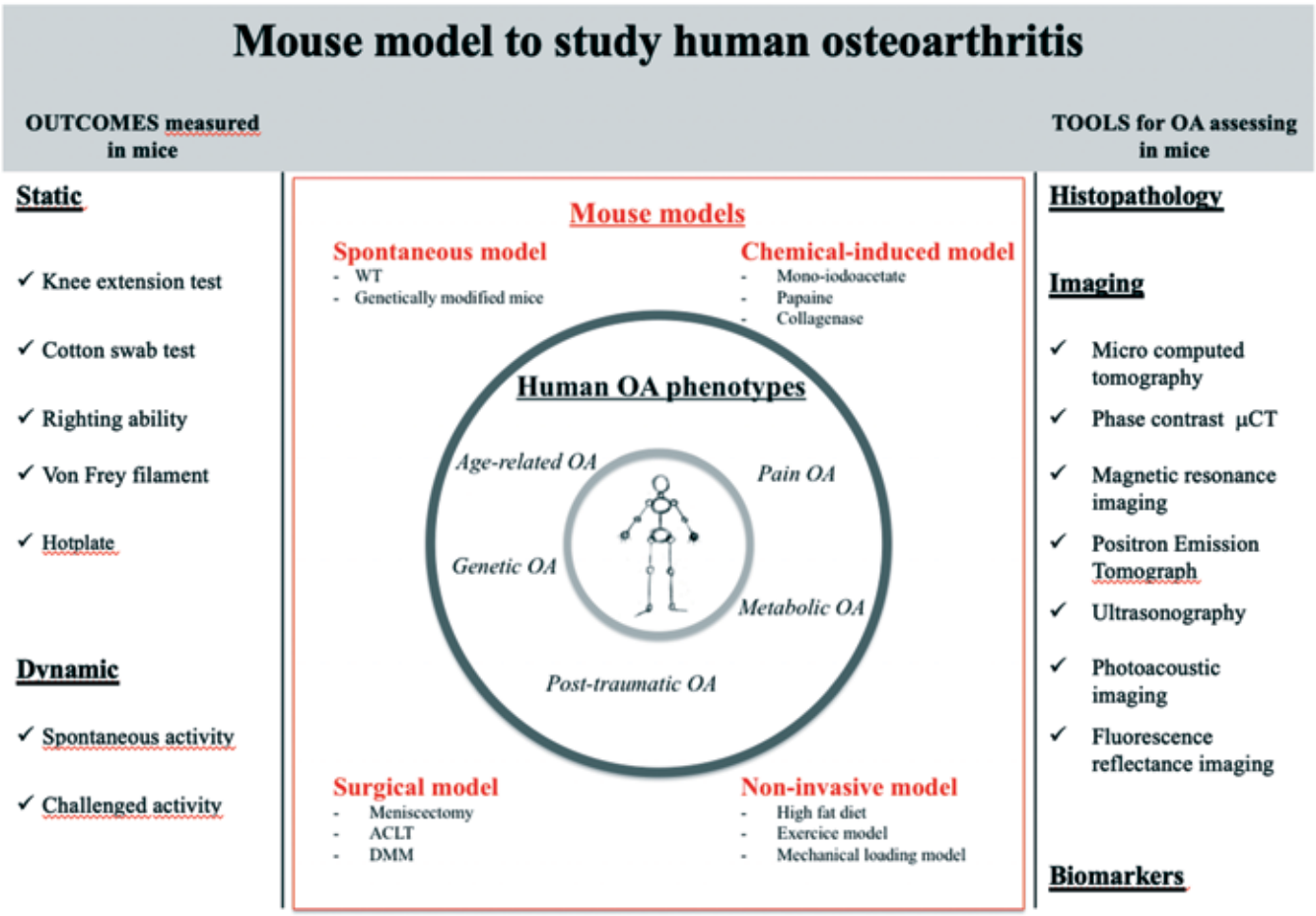

Figure 3. This figure shows the most recent data on all aspects of OA mouse models to support choice of the best model in terms of objective, pathophysiological pathway, surrogate markers of progression, imaging techniques, and functional assessment.

placed over a base plate in front of a grasping tool (T-shaped or trapeze-shaped), and data are recorded. Three successive trials administered on the same day test the maximum force applied as the mouse is pulled away from the attachment of the grasping tool,[2] allowing assessment of the effect of OA progression or pain medication on muscle strength. Wire hang analysis is another way to assess gripping ability, coordination and balance skills of mice. The measured parameter is the latency to fall.[109]

Discussion

Additional OA research is necessary to meet the health challenges of patients and OA researchers will continue to need innovative models and technology in their studies. Figure 3 shows outcomes and tools available to study mouse model of OA. An animal model should be isomorphic, homologous, and predictive. In principle, the animal model should have the same signs of disease as humans; the pathophysiology and response to treatment should be comparable. At least 3 to 5 OA human phenotypes have been described: age-related OA, posttraumatic-OA, metabolic OA, genetic, and pain OA.[9,107,111] However, interindividual heterogeneity is common among individuals with knee OA pain with regard to patient physical performance profiles.[28] Patient profiles based on physical performance and movement-evoked pain were also significantly different in psychologic and somatosensory function.[28] The identification of profiles supports the adjustment of the therapeutic plan to individual patients.

OA has a complex physiopathology, and one single animal model will not mimic all of the components of the human disease.[79,87,120] Some mouse models may replicate a phenotype without involving all the integrative pathways, and a perfect model does not exist. Therefore, the choice of rodent model must consider the objective of the study and the nature of the outcomes. Because all animal models have strengths and weaknesses, the use of several models and techniques considered together may provide the most useful results. For example, using a prey species as a model can be a weakness because prey animals such as rodents may not show obvious signs of pain[4,79] to order avoid attracting predators. A link between this masking behavior and prey position in the food chain is difficult to confirm with robust data. In addition, the behavioral and functional tests used in OA studies may cause mice stress.[6,14] Several assessments of pain and/or locomotion should be included in a study because mice may hide the signs of pain and distress. A summary of animal models used to study pain of OA with outcome measured longitudinally has been published to help promote use of the ARRIVE guidelines and achieve better cross-publication comparisons.[10,79,112]

The 3Rs principles of reduction, refinement, and replacement provide a framework for ethical decisions about using animals for scientific purposes.[53] Refinement should be a constant concern of researchers from the point of experimental design (by improving animal welfare with appropriate housing and handling procedures, minimizing suffering through pain treatment, and terminating animals that reach humane endpoints)[94] until the time of scientific publication (by reporting according

to the ARRIVE guidelines).[112] Consideration of analgesia and pain management is also important.[15] Both pharmacologic and nonpharmacologic measures can be used to manage pain in mice with OA. Depending on the procedure, pain medication ranging from general anesthesia to analgesics can be administered before and after surgery in surgical models of OA. In addition, refined surgical procedures can be developed. For example, the destabilization of medial meniscus model is less invasive and considered more homologous to OA in human.[39] Furthermore, assessment of modified surgical methods[41], such as refining surgical small rodent models of OA for joint pathology and pain, indicates that pain behavior is not always present despite significant histopathologic changes during disease progression.[40] This reflects the heterogeneity seen in human OA and therefore better mimics the human condition. Animals and human patients both experience pain as a symptom of OA.[62] Two arguments are used by animal care and ethical committees to promote the use of analgesia. The first is that refining surgical models of osteoarthritis in rodents by using analgesics alters the pain phenotype but does not alter the joint."[41] Second, not using pain alleviating drugs in mice might alter the comparability to humans because the majority of human patients with OA take medicine for pain.[43] We do not suggest that pain medication should be administrated in all OA studies, but consideration should be given to the issue and discussed both with ethical committees and within the OA scientific community.

Improved reporting of behavioral preclinical data will promote reproducibility.[58] Identifying appropriate humane endpoints is essential to avoiding unnecessary suffering in research animals.[14,94] Refinement could be achieved by using the earliest possible endpoints.[31] The description of endpoints in the methods of publications can be improved by adhering to the ARRIVE guidelines[112] and automated manuscript screening software can be used to detect inadequate reporting of methodology.[125] In addition, including veterinarians in the research design is important.[75]

Refinement could also benefit from the use of new imaging technologies for OA assessment.[84] Optimized for small animals, these imaging technologies include phase contrast μCT,[37,51] photoacoustic imaging,[19,81] and new fluorescent substrates.[54,110] These imaging methods can be used to assess morphologic structural changes or molecular disease activity and to track the progression of damage in longitudinal studies.[78] The ability to perform repeated observations on the same animal during disease progression can reduce both individual variation (due to using the same animal) and the number of animals needed (due to avoiding the need to terminate a statistically important number of animals at each interim time point). Finally, new technologies will allow nonlethal longitudinal monitoring of OA progression and support diagnosis, assessment of severity, and development of treatments, which are major concerns for the human disease.

Application of ethical principles also improves scientific knowledge and experimental outputs. Innovations, optimization of 3R rule, and scientific objectives are dynamically linked. The 3R's have a positive impact on the reproducibility, reliability, and translatability of data from animal studies.[77,103] Reporting of all the stimuli and conditions the animals experience is important because these features may interfere with disease development or intervention.[64] Pain and pain medications may also affect some data outcomes. These features are challenges in research fields like OA.

In conclusion, new techniques such as the automated digital cage system will be useful to record spontaneous activity from thousands of home cages simultaneously and to monitor lab conditions. This system will become an essential research tool that provides scientific data and saves time. Environmental factors such as season, humidity, housing cage, time of day, sex, order of testing cage mates, mouse genotype, and experimenter identity can affect outcomes.[20,21] New tools to improve pain assessment in rodents include videography and computational approaches but time will be required to assess these new tools.[34]

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