Modified Q18: Is there a difference in how pain behavior testing should be conducted in mice with aging-related OA vs other types of OA models?

Rachel E. Miller, Kelsey H. Collins, Adam Konopka, Richard Loeser, Tristan Maerz, Anne-Marie Malfait, Carla R. Scanzello

Recommendation: YES. Naturally occurring osteoarthritis (OA) with age can affect multiple joints while other models are most often unilateral affecting, a single joint. These differences are important to consider when measuring pain-related behaviors. The slow temporal development of pain-related behaviors in aging models makes blinding of the experimenter more difficult. While some types of behavioral changes can be found in all models of OA including knee hyperalgesia, hind paw mechanical allodynia, and changes in spontaneous locomotion, other behavioral tests such as static weight-bearing cannot be performed in spontaneous aging models because both knees are at equal risk of developing OA. In addition, for assays monitoring functional limb use, the variables that are affected may change across the animal's lifespan due to a variety of factors. Therefore, validation is required to link a particular variable to pain.

Level of Evidence: Moderate

Delegate Vote:

Rationale:
A PubMed search was conducted using the search terms: ((behavior) OR (pain) OR (hyperalgesia) OR (allodynia) OR (locomotion) OR (mechanical sensitization)) AND (osteoarthritis) AND (mice) AND (pain) AND ((spontaneous) OR (aging) OR (ageing) OR (age-associated)), which yielded 136 results. These search results were manually curated and papers reporting behavioral changes associated with spontaneous aging in mice were summarized. These results were supplemented with additional web searches to identify papers reporting mechanical sensitization in aged mice and other mouse models of OA.

Unilateral vs. Systemic Models

Different types of osteoarthritis (OA) mouse models have been developed to incorporate risk factors relevant to OA disease pathology, including aging, prior joint injury, and obesity [1, 2]. Unilateral mouse models intervene in one joint, typically the knee, to induce OA in that joint. Systemic models, such as those that seek to model aging, result in increased structural damage risk in both knees and also the spine. In general, systemic models require lengthier time courses than unilateral induced models to develop appreciable structural damage, and the onset and progression of structural damage can often occur with greater heterogeneity. To address these issues, sometimes these models are combined such that a unilateral injury is superimposed on a systemic model, such as aging + surgery in one knee [3] or high fat diet + surgery in one knee [4].

Behavioral Phenotypes Observed in Aging Mice

Assays used to test for mechanical sensitization include hind paw mechanical allodynia or knee mechanical hyperalgesia, and these tests have been used for both unilateral and systemic mouse models of OA[5-22]. Lower mechanical withdrawal thresholds in the hind paw have been observed in naïve mice by the age of 18-20 months [23, 24] relative to younger mice, but other studies assessing earlier time points did not find this (9-18 months) [25, 26]. A study examined the effect of monoiodoacetate (MIA) injection into the knees of mice of different ages and found that 22-month old mice did not develop as much mechanical allodynia during the initial inflammatory phase (0-10 days), while the late phase of MIA-induced mechanical allodynia was comparable between age groups [27]. Lower response thresholds to pressure applied to the knee joint have also been reported in 16-20-month old mice compared to younger mice, indicative of greater knee hyperalgesia [23, 28, 29]. Temperature sensitization can be assessed by hot plate or Hargreave's test for thermal sensitivity or by cold plate or acetone test for cold sensitivity. Mouse models of aging have reported cold sensitivity by using the acetone test [26, 30].

Other types of tests evaluate changes in limb use, such as grip strength, burrowing, and the Rotarod test. Changes in these measures may be affected by muscle weakness, axial stretching, or other functional debilitation in addition to pain associated with OA development. Grip strength has been shown to decrease

with age in mice [23, 25, 31]. Overall spontaneous activity levels assessed using systems such as LABORAS, BlackBox, AnyMaze, or other activity monitoring platforms have been shown to decrease with age [25, 32-34], which may be due to multiple factors, not solely OA joint pain. Notably, it remains unclear which specific spontaneous activity-related parameters are most closely associated with OA pain, and high heterogeneity in activity patterns across mice makes the implementation of these assessments challenging. Similarly, for gait, dynamic weight-bearing, or other assays monitoring functional limb use, the variables that are affected may change depending on the model and across the animal's lifespan. For example, as mice age, they also gain weight. This can affect the way they ambulate and should be considered for dynamic or gait assessments, as it can make it more difficult to resolve isolated limb changes. One study demonstrated that paw area can change with age in a treadmill-based gait assay without being linked to pain [35]. Another study showed that mice can adapt their strategy to perform vertical climbing tasks with age to overcome potential functional limitations [36]. Therefore, validation is required to link a particular variable to pain.

Considerations for Behavioral Testing in Aging Mouse Models

Pain-related behavioral changes have been shown to develop in all types of OA mouse models [2, 37], including sensitization as well as changes in spontaneous joint use. While many best practices for behavioral testing apply to all models [2, 38], there are some considerations regarding the type of test performed and the approach to testing that should be adjusted based on the model. For example, the approach to experimenter blinding should be considered differently in the case of systemic aging models compared to unilateral models. In the case of evaluating the effect of age on OA pain, groups of mice at different ages can be compared, but it may be challenging to blind the experimenter to age if the overt appearance of the animal changes or if cohorts of mice are tested as they become available. For studies where a variable such as a treatment or genetic knock-out is being tested, mice at the same age should be compared in a blinded manner [5, 13]. Because it has been described that pain-related behaviors are different with age, all control groups should always be age-matched. Further, assessments of pain-related behaviors across age or experimental groups should be conducted in parallel and by the same investigator to minimize confounding variables.

Another challenge for evoked sensitization assays is that it can be more difficult to distinguish what is an individual animal's way of responding to a mechanical stimulus. In the case of a unilateral model, the contralateral uninjured limb is useful to calibrate an animal's normal response in mechanical sensitization assays to rule out responses due to stress or anxiety. In the case of aging models, this is not possible because both hind limbs are at equal risk of developing OA. Validation that the behavior is reversible in response to an analgesic can help to demonstrate that the behavior is not solely due to a stress or anxiety response in the animal. Observing similar magnitude responses in both hind limbs as well as across testing days within a period of days-weeks for a particular animal is another approach to validate a pain-relevant measurement in aging models, as a pain response should not change within a short period of time.

Finally, in the case of assays assessing limb use, hind limb weight-bearing asymmetry measured using a static incapacitance meter is not possible in models where both limbs are at equal risk of developing OA (*e.g.* aging). Methods that measure dynamic weight bearing could be helpful as they can identify load shifting not only from side-to-side, but from hindlimbs to forelimbs and the tail [22, 39], but this still requires more validation in aging OA models. Additionally, because models of aging result in not only spontaneous OA but also other co-morbidities, intra-articular delivery of an analgesic as an experimental control may help to directly link a given outcome measure to OA pain in a specific limb, as opposed to being caused by pain in other body parts, functional deficit, muscle weakness, neuropathy, stress, anxiety, or other reasons. Tracking the development of the behavior and relating the change to degree of structural damage development is another strategy to link the behavior to spontaneous OA pain [23, 25, 32].

1. Alves-Simões M. Rodent models of knee osteoarthritis for pain research. Osteoarthritis Cartilage. 2022;30(6):802-14. Epub 20220206. doi: 10.1016/j.joca.2022.01.010. PubMed PMID: 35139423.
2. Malfait AM, Little CB, McDougall JJ. A commentary on modelling osteoarthritis pain in small animals. Osteoarthritis Cartilage. 2013;21(9):1316-26. doi: 10.1016/j.joca.2013.06.003. PubMed PMID: 23973146; PMCID: PMC3903124.
3. Loeser RF, Olex AL, McNulty MA, Carlson CS, Callahan MF, Ferguson CM, Chou J, Leng X, Fetrow JS. Microarray analysis reveals age-related differences in gene expression during the development of

osteoarthritis in mice. Arthritis Rheum. 2012;64(3):705-17. doi: 10.1002/art.33388. PubMed PMID: 21972019; PMCID: PMC3269534.
4. Collins KH, Lenz KL, Pollitt EN, Ferguson D, Hutson I, Springer LE, Oestreich AK, Tang R, Choi YR, Meyer GA, Teitelbaum SL, Pham CTN, Harris CA, Guilak F. Adipose tissue is a critical regulator of osteoarthritis. Proc Natl Acad Sci U S A. 2021;118(1). doi: 10.1073/pnas.2021096118. PubMed PMID: 33443201; PMCID: PMC7817130.
5. Adamczyk NS, Ishihara S, Obeidat AM, Hoffman DB, David BT, Ren D, Miller RJ, Malfait A-M, Miller RE. FM-dye inhibition of Piezo2 relieves mechanically evoked pain in mouse models of acute inflammatory and osteoarthritic knee pain. British Journal of Pharmacology.n/a(n/a). doi: https://doi.org/10.1111/bph.70306.
6. Bergman RF, Lammlin L, Junginger L, Farrell E, Goldman S, Darcy R, Rasner C, Obeidat AM, Malfait A-M, Miller RE, Maerz T. Sexual dimorphism of the synovial transcriptome underpins greater PTOA disease severity in male mice following joint injury. Osteoarthritis and Cartilage. 2024;32(9):1060-73. doi: https://doi.org/10.1016/j.joca.2023.07.012.
7. Collins KH, Lenz KL, Welhaven HD, Ely E, Springer LE, Paradi S, Tang R, Braxton L, Akk A, Yan H, Zhang B, Wu X, Atkinson JP, Oestreich AK, June RK, Pham CTN, Guilak F. Adipose-derived leptin and complement factor D mediate osteoarthritis severity and pain. Science Advances. 2025;11(17):eadt5915. doi: doi:10.1126/sciadv.adt5915.
8. Courties A, Olmer M, Myers K, Ordoukhanian P, Head SR, Natarajan P, Berenbaum F, Sellam J, Lotz MK. Human-specific duplicate CHRFAM7A gene is associated with more severe osteoarthritis and amplifies pain behaviours. Annals of the Rheumatic Diseases. 2023;82(5):710-8. doi: 10.1136/ard-2022-223470.
9. Geraghty T, Ishihara S, Obeidat AM, Adamczyk NS, Hunter RS, Li J, Wang L, Lee H, Ko FC, Malfait A-M, Miller RE. Acute systemic macrophage depletion in osteoarthritic mice alleviates pain-related behaviors and does not affect joint damage. Arthritis Research & Therapy. 2024;26(1):224. doi: 10.1186/s13075-024-03457-9.
10. Liu H, Binoy A, Ren S, Martino TC, Miller AE, Willis CRG, Veerabhadraiah SR, Bons J, Rose JP, Schilling B, Jurynec MJ, Zhu S. Regulation of Chondrocyte Metabolism and Osteoarthritis Development by Sirt5 Through Protein Lysine Malonylation. Arthritis Rheumatol. 2025;77(9):1216-27. Epub 20250508. doi: 10.1002/art.43164. PubMed PMID: 40176311; PMCID: PMC12354001.
11. McCollum MM, Larmore M, Ishihara S, Ng LCT, Kimura LF, Guadarrama E, Ta MC, Vien TN, Frost GB, Scheidt KA, Miller RE, DeCaen PG. Targeting the tamoxifen receptor within sodium channels to block osteoarthritic pain. Cell Reports. 2022;40(8):111248. doi: https://doi.org/10.1016/j.celrep.2022.111248.
12. Obeidat AM, Ishihara S, Li J, Adamczyk NS, Lammlin L, Junginger L, Maerz T, Miller RJ, Miller RE, Malfait A-M. Intra-articular sprouting of nociceptors accompanies progressive osteoarthritis: comparative evidence in four murine models. Frontiers in Neuroanatomy. 2024;Volume 18 - 2024. doi: 10.3389/fnana.2024.1429124.
13. Obeidat AM, Wood MJ, Adamczyk NS, Ishihara S, Li J, Wang L, Ren D, Bennett DA, Miller RJ, Malfait AM, Miller RE. Piezo2 expressing nociceptors mediate mechanical sensitization in experimental osteoarthritis. Nat Commun. 2023;14(1):2479. Epub 20230429. doi: 10.1038/s41467-023-38241-x. PubMed PMID: 37120427; PMCID: PMC10148822.
14. Wang L, Ishihara S, Li J, Miller RE, Malfait A-M. Notch signaling is activated in knee-innervating dorsal root ganglia in experimental models of osteoarthritis joint pain. Arthritis Research & Therapy. 2023;25(1):63. doi: 10.1186/s13075-023-03039-1.
15. Miller RE, Ishihara S, Bhattacharyya B, Delaney A, Menichella DM, Miller RJ, Malfait AM. Chemogenetic Inhibition of Pain Neurons in a Mouse Model of Osteoarthritis. Arthritis Rheumatol. 2017;69(7):1429-39. Epub 20170602. doi: 10.1002/art.40118. PubMed PMID: 28380690; PMCID: PMC5501168.
16. Miller RE, Ishihara S, Tran PB, Golub SB, Last K, Miller RJ, Fosang AJ, Malfait A-M. An aggrecan fragment drives osteoarthritis pain through Toll-like receptor 2. JCI Insight. 2018;3(6). doi: 10.1172/jci.insight.95704.
17. Ishihara S, Miller RE, Malfait AM. Assessment of Knee Hyperalgesia in Mice using Pressure Application Measurement. J Vis Exp. 2025(220). Epub 20250613. doi: 10.3791/68480. PubMed PMID: 40587399; PMCID: PMC12237626.
18. Ishihara S, Obeidat AM, Wokosin DL, Ren D, Miller RJ, Malfait AM, Miller RE. The role of intra-articular neuronal CCR2 receptors in knee joint pain associated with experimental osteoarthritis in mice.

Arthritis Res Ther. 2021;23(1):103. Epub 20210407. doi: 10.1186/s13075-021-02486-y. PubMed PMID: 33827672; PMCID: PMC8025346.
19. Miller RE, Kim YS, Tran PB, Ishihara S, Dong X, Miller RJ, Malfait AM. Visualization of Peripheral Neuron Sensitization in a Surgical Mouse Model of Osteoarthritis by In Vivo Calcium Imaging. Arthritis Rheumatol. 2018;70(1):88-97. Epub 20171201. doi: 10.1002/art.40342. PubMed PMID: 28992367; PMCID: PMC5745281.
20. Miller RE, Tran PB, Das R, Ghoreishi-Haack N, Ren D, Miller RJ, Malfait AM. CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. Proc Natl Acad Sci U S A. 2012;109(50):20602-7. Epub 20121126. doi: 10.1073/pnas.1209294110. PubMed PMID: 23185004; PMCID: PMC3528555.
21. Malfait AM, Ritchie J, Gil AS, Austin JS, Hartke J, Qin W, Tortorella MD, Mogil JS. ADAMTS-5 deficient mice do not develop mechanical allodynia associated with osteoarthritis following medial meniscal destabilization. Osteoarthritis Cartilage. 2010;18(4):572-80. Epub 20091221. doi: 10.1016/j.joca.2009.11.013. PubMed PMID: 20036347.
22. Burt KG, South SM, Adamczyk NS, Nguyen V, Ishihara S, Zhou C, Sharp KL, Kim SY, Rapp AE, Hu B, Murphy LA, Hung LY, Herbert D, Baker JF, Mauck RL, Griffin TM, Malfait AM, Miller RE, Scanzello CR. CD14 plays a critical role in pain and inflammation across multiple models of post-traumatic osteoarthritis. bioRxiv. 2025. Epub 20250605. doi: 10.1101/2025.06.02.657478. PubMed PMID: 40502022; PMCID: PMC12157692.
23. Geraghty T, Obeidat AM, Ishihara S, Wood MJ, Li J, Lopes EBP, Scanzello CR, Griffin TM, Malfait AM, Miller RE. Age-Associated Changes in Knee Osteoarthritis, Pain-Related Behaviors, and Dorsal Root Ganglia Immunophenotyping of Male and Female Mice. Arthritis Rheumatol. 2023;75(10):1770-80. Epub 20230714. doi: 10.1002/art.42530. PubMed PMID: 37096632; PMCID: PMC10543384.
24. Weyer AD, Zappia KJ, Garrison SR, O'Hara CL, Dodge AK, Stucky CL. Nociceptor Sensitization Depends on Age and Pain Chronicity. eneuro. 2016;3(1):ENEURO.0115-15.2015. doi: 10.1523/eneuro.0115-15.2015.
25. Kerr GJ, To B, White I, Millecamps M, Beier F, Grol MW, Stone LS, Séguin CA. Diet-induced obesity leads to behavioral indicators of pain preceding structural joint damage in wild-type mice. Arthritis Res Ther. 2021;23(1):93. Epub 20210322. doi: 10.1186/s13075-021-02463-5. PubMed PMID: 33752736; PMCID: PMC7983381.
26. Millecamps M, Tajerian M, Naso L, Sage HE, Stone LS. Lumbar intervertebral disc degeneration associated with axial and radiating low back pain in ageing SPARC-null mice. PAIN. 2012;153(6).
27. Ogbonna AC, Clark AK, Malcangio M. Development of monosodium acetate-induced osteoarthritis and inflammatory pain in ageing mice. Age (Dordr). 2015;37(3):9792. Epub 20150514. doi: 10.1007/s11357-015-9792-y. PubMed PMID: 25971876; PMCID: PMC4430498.
28. Kundu P, Wise B, Norris E, James M, Zhang Y, Jonason J, Buckley M, Lee W. 3D Light Sheet Microscopy Reveals Aging Osteoarthritis-associated Joint-innervated Nerve Remodeling in Mouse Knee Joints. bioRxiv. 2026. Epub 20260110. doi: 10.64898/2026.01.09.698640. PubMed PMID: 41542522; PMCID: PMC12803232.
29. Carmon I, Zecharyahu L, Elayyan J, Meka SRK, Reich E, Kandel L, Bilkei-Gorzo A, Zimmer A, Mechoulam R, Kravchenko-Balasha N, Dvir-Ginzberg M. HU308 Mitigates Osteoarthritis by Stimulating Sox9-Related Networks of Carbohydrate Metabolism. J Bone Miner Res. 2023;38(1):154-70. Epub 20221127. doi: 10.1002/jbmr.4741. PubMed PMID: 36350089; PMCID: PMC10098743.
30. Vincent K, Mohanty S, Pinelli R, Bonavita R, Pricop P, Albert TJ, Dahia CL. Aging of mouse intervertebral disc and association with back pain. Bone. 2019;123:246-59. Epub 20190329. doi: 10.1016/j.bone.2019.03.037. PubMed PMID: 30936040; PMCID: PMC6549718.
31. Millecamps M, Czerminski JT, Mathieu AP, Stone LS. Behavioral signs of axial low back pain and motor impairment correlate with the severity of intervertebral disc degeneration in a mouse model. Spine J. 2015;15(12):2524-37. Epub 20150831. doi: 10.1016/j.spinee.2015.08.055. PubMed PMID: 26334234.
32. Costello KE, Guilak F, Setton LA, Griffin TM. Locomotor activity and gait in aged mice deficient for type IX collagen. J Appl Physiol (1985). 2010;109(1):211-8. Epub 20100401. doi: 10.1152/japplphysiol.00056.2010. PubMed PMID: 20360435; PMCID: PMC2904201.
33. Shoji H, Takao K, Hattori S, Miyakawa T. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. Mol Brain. 2016;9:11. Epub 20160128. doi: 10.1186/s13041-016-0191-9. PubMed PMID: 26822304; PMCID: PMC4730600.

34. Tran T, Mach J, Gemikonakli G, Wu H, Allore H, Howlett SE, Little CB, Hilmer SN. Male-Female Differences in the Effects of Age on Performance Measures Recorded for 23 Hours in Mice. J Gerontol A Biol Sci Med Sci. 2021;76(12):2141-6. doi: 10.1093/gerona/glab182. PubMed PMID: 34171083.
35. Poulet B, de Souza R, Knights CB, Gentry C, Wilson AM, Bevan S, Chang YM, Pitsillides AA. Modifications of gait as predictors of natural osteoarthritis progression in STR/Ort mice. Arthritis Rheumatol. 2014;66(7):1832-42. doi: 10.1002/art.38616. PubMed PMID: 24623711; PMCID: PMC4271671.
36. St Amant J, Michaud J, Hinds D, Coyle M, Pozzi A, Clark AL. Depleting transforming growth factor beta receptor 2 signalling in the cartilage of itga1-null mice attenuates spontaneous knee osteoarthritis. Osteoarthr Cartil Open. 2023;5(4):100399. Epub 20230812. doi: 10.1016/j.ocarto.2023.100399. PubMed PMID: 37649532; PMCID: PMC10462827.
37. Miller RE, Malfait AM. Osteoarthritis pain: What are we learning from animal models? Best Pract Res Clin Rheumatol. 2017;31(5):676-87. Epub 20180426. doi: 10.1016/j.berh.2018.03.003. PubMed PMID: 30509413; PMCID: PMC6284232.
38. Sadler KE, Mogil JS, Stucky CL. Innovations and advances in modelling and measuring pain in animals. Nat Rev Neurosci. 2022;23(2):70-85. Epub 20211126. doi: 10.1038/s41583-021-00536-7. PubMed PMID: 34837072; PMCID: PMC9098196.
39. Tétreault P, Dansereau MA, Doré-Savard L, Beaudet N, Sarret P. Weight bearing evaluation in inflammatory, neuropathic and cancer chronic pain in freely moving rats. Physiol Behav. 2011;104(3):495-502. Epub 20110517. doi: 10.1016/j.physbeh.2011.05.015. PubMed PMID: 21620878.