Q1: Should we define a standard minimum number/list of pain-dependent behavioral outcomes that constitute a robust/complete/reliable pre-clinical study (*i.e.,* that test different pain types/mechanisms? (*e.g.*, von Frey, PAM, weight-bearing, gait analysis, spontaneous behavior etc.)?

Anne-Marie Malfait, Christopher Little, Meagan Makarczyk, Rachel E. Miller

Response: YES.

Level of Evidence: High

Rationale: A PubMed search (25th Feb 2026) for "osteoarthritis AND animal models" *vs.* "osteoarthritis AND animal models AND pain" returned 6,136 and 1,384 results respectively, suggesting that approximately 20% of OA studies in animal models incorporate pain-related behaviors as an outcome. Of these 1,384 papers, approximately 500 used rodent models. We manually searched these 500 papers to list the types of pain-related behaviors that are commonly used, and we evaluated how these pain-dependent behaviors might correspond to translationally relevant pain behaviors in patients with OA.

Types of pain-dependent behaviors in experimental OA models in rodents - Several techniques to assess pain associated with experimental OA have been reported in recent years, often borrowed from the pain field at large and adapted to inform researchers on mechanisms underlying chronic joint pain and the effects of long-term joint damage on pain-related behaviors in the animal. Pain-dependent behaviors commonly assessed in mice and rats with experimental OA fall broadly into three categories: (1) Evoked behavioral responses in response to a noxious or non-noxious stimulus; (2) Non-reflexive tests to assess spontaneous ongoing pain; (3) Behavioral assessments of affective components to pain[1-3].

Translational value of these assays: Human pain equivalent and mechanistic insight

1. Evoked behavioral responses include: joint hyperalgesia (an exaggerated response to a painful/noxious mechanical stimulus applied to the affected joint (most commonly the knee[4] but also reported in models of ankle[5] and hip[6; 7] arthritis); mechanical allodynia in the hindpaw (a response to a non-noxious stimulus applied with a von Frey fiber to a site distant from the affected joint, for example the footpad); thermal allodynia (response to a hot or cold stimulus applied to a distant site, usually the footpad or dorsum of the foot).

Human equivalent: Quantitative sensory testing (QST) in large cohorts of patients with knee OA has revealed somatosensory abnormalities[8], showing they respond to lower forces applied to the affected knee, as well as sites away from the joint. Sensitization measures have been associated with OA knee pain severity - for example, temporal summation was significantly associated with greater odds of pain during walking and stair climbing[9]. One particular QST finding that robustly distinguishes subjects with symptomatic OA from controls is a reduction in pressure pain threshold (PPT). There are very few reports of altered sensitivity to hot or cold stimuli in the literature[10; 11].

Mechanistic insight: Joint hyperalgesia is indicative of peripheral sensitization at the OA-affected site, while mechanical allodynia in the hindpaw is indicative of peripheral and/or central sensitization as mechanisms contributing to the initiation and maintenance of chronic OA pain. Importantly, lowered PPTs to mechanical stimuli applied to the knee in older adults with or at risk for knee OA are predictors of persistent knee pain[12], and higher pain sensitization (by PPTs and temporal summation) was associated with a higher likelihood of unpredictable pain[13], suggesting that prevention or reduction of sensitization may be a strategy to prevent or ameliorate persistent knee pain in OA. In mice with experimental OA, knee hyperalgesia is an early onset behavior, preceding behaviors that are more indicative of ongoing pain[14].

2. Non-reflexive test to assess spontaneous ongoing pain in experimental OA in rodents include static and dynamic weightbearing[15; 16], burrowing/nesting behavior[17; 18], grip strength[19], gait analysis[20], activity-based assessments and locomotion[21; 22], conditioned place preference[23], and facial expressions[24]. State-of-the-art methods for facial grimacing have been validated for acute pain but not for persistent pain and warrant continued investigation with whole-body machine learning methods.

Human equivalents: Human patients can be asked about the localization, severity, and characteristics of pain (*e.g.*, sharp, dull, throbbing). The various assays in rodents aim to capture different aspects of the human pain experience. For example, weightbearing asymmetry between the hindlegs can serve as an indicator for weightbearing pain but can only be assessed in mice with unilateral experimental OA.

Mechanistic insights: Patients living with OA often exhibit abnormal movement patterns primarily to altered joint kinematics, and pain likely also contributes to this. In animals, it is difficult to interpret whether gait changes are due to OA pain or to altered joint biomechanics. Specifically, gait changes in rodents can be very subtle, since prey animals tend to disguise gait deficiencies. Gait changes in higher order mammals with OA (*e.g.,* dogs and cats) are more pronounced than in rodents[2], making these animals better suited for kinematic studies. One way to deduce whether a behavior is pain-driven is to investigate the response to a painkiller, determining whether the reduction of

locomotion is pain *vs.* function driven[22]. Furthermore, the response to the class of drug can start to offer mechanistic insights into the mechanisms driving the pain (*e.g.*, response to gabapentin *vs.* NSAIDs)[25].

3. Affective components and sequelae of chronic pain can also be measured, such as anxiety and depression-associated behaviors, as well as sleep. Assays in rodents include elevated plus maze[26], sleep behaviors[27], anhedonia measurements[28], and the forced swimming test to assess anxiety[28; 29]. Here again, it should be considered that these behaviors often require a functional locomotive system and thus might be confounded by functional deficits associated with OA.

Human equivalents: Chronic pain can affect sleep and cognitive function, and anxiodepressive co-morbidity has been observed in chronic pain states such as diabetic neuropathy. People who suffer from painful OA may suffer from depression and mental health issues. A recent meta-analysis including 49 studies with a total of 15,855 participants, concluded that "one-fifth of people with osteoarthritis experience symptoms of depression and anxiety[30]. These psychological factors can also affect post-operative total knee replacement outcomes, in which the presence of presurgical subcortical brain factors can relate to postsurgical persistence of OA pain[31].

Mechanistic insights: Assessment of these types of tests can be used to determine the relationship between chronic pain and affective disorders. In conjunction with molecular and functional assays in the central nervous system (CNS) and fMRI, this can start to provide insight into mechanisms of these processes in the brain[32]. Very few studies to date have done this in rodents, for example chronic pain has been associated with changes in thalamic connectivity[32]. Other studies have found that supraspinal astrocyte activation might be a significant mechanism underlying anxiety-augmented pain behavior as indicated by elevated levels of glial fibrillary acidic protein in pain detecting regions of the brain[33].

Pain-structure correlations- Incorporating pain-dependent tests in experimental OA enables description of overall OA disease. Mechanistic insights can be obtained from studying the type of behavior, time of onset, response to interventions, and correlation between specific pain-dependent behaviors and pathological features at different timepoints in progressive experimental OA[34; 35]. Correlations can be made between specific behaviors and synovitis, cartilage damage, bone remodeling, and neuronal sprouting. Additionally, due to the heterogeneous nature of OA, the selection of OA-induction method and timepoint are important considerations. The OA induction methods result in an OA endpoint, but the pathological mechanisms to get there may vary, resulting in different pain outcomes[14]. This is related to the structural progression of OA- joint inflammation (synovitis) typically occurs at an earlier timepoint compared to bone remodeling, a significant contributor to pain[36; 37].

Recommendations for the use of these assays

Since the different types of assays listed all test different aspects of pain-related behaviors, the type of assay included in a specific experiment ought to be guided by the aim of the experiment: which mechanism is the experiment aiming to study, or which type of drug is the experiment aiming to test? There is also the practical consideration that not all types of behavioral assays can be tested on the same group of mice, since this is stressful on the animals[38; 39]. For a study incorporating pain assessments, the goal of the study should be defined (mechanistic, drug testing), and primary endpoints should be defined *a priori*. Rigor should be defined by mechanistic relevance and internal consistency, not the number of endpoints – for example, include knee hyperalgesia as a measure of early onset sensitization and weightbearing as late-onset measure of ongoing pain may provide a good overall assessment of the model. Although structural changes do not necessarily denote pain, joint structure assessment is often recommended to correlate structural and pain phenotypes.

1. Neugebauer V, Han JS, Adwanikar H, et al. 2007. Techniques for assessing knee joint pain in arthritis. Mol Pain 3:8.
2. Malfait AM, Little CB, McDougall JJ. 2013. A commentary on modelling osteoarthritis pain in small animals. Osteoarthritis Cartilage 21:1316–1326.
3. Mogil JS. 2022. The history of pain measurement in humans and animals. Front Pain Res (Lausanne) 3:1031058.
4. Ishihara S, Miller RE, Malfait AM. 2025. Assessment of Knee Hyperalgesia in Mice using Pressure Application Measurement. J Vis Exp.
5. Chen Y, Willcockson HH, Valtschanoff JG. 2009. Vanilloid receptor TRPV1-mediated phosphorylation of ERK in murine adjuvant arthritis. Osteoarthritis Cartilage 17:244–251.
6. Kanno K, Suzuki-Narita M, Kawarai Y, et al. 2022. Analgesic effects and arthritic changes following tramadol administration in a rat hip osteoarthritis model. J Orthop Res 40:1770–1777.

7. Kawarai Y, Orita S, Nakamura J, et al. 2020. Analgesic Effect of Duloxetine on an Animal Model of Monosodium Iodoacetate-Induced Hip Osteoarthritis. J Orthop Res 38:422–430.
8. Arant KR, Katz JN, Neogi T. 2022. Quantitative sensory testing: identifying pain characteristics in patients with osteoarthritis. Osteoarthritis Cartilage 30:17–31.
9. Aoyagi K, Liew JW, Farrar JT, et al. 2022. Does weight-bearing versus non-weight-bearing pain reflect different pain mechanisms in knee osteoarthritis?: the Multicenter Osteoarthritis Study (MOST). Osteoarthritis Cartilage 30:545–550.
10. Wright A, Benson HAE, Will R, et al. 2017. Cold Pain Threshold Identifies a Subgroup of Individuals With Knee Osteoarthritis That Present With Multimodality Hyperalgesia and Elevated Pain Levels. Clin J Pain 33:793–803.
11. Moss P, Knight E, Wright A. 2016. Subjects with Knee Osteoarthritis Exhibit Widespread Hyperalgesia to Pressure and Cold. PLoS One 11:e0147526.
12. Carlesso LC, Segal NA, Frey-Law L, et al. 2019. Pain Susceptibility Phenotypes in Those Free of Knee Pain With or at Risk of Knee Osteoarthritis: The Multicenter Osteoarthritis Study. Arthritis Rheumatol 71:542–549.
13. Carlesso LC, Law LF, Wang N, et al. 2022. Association of Pain Sensitization and Conditioned Pain Modulation to Pain Patterns in Knee Osteoarthritis. Arthritis Care Res (Hoboken) 74:107–112.
14. Miller RE, Malfait AM. 2017. Osteoarthritis pain: What are we learning from animal models? Best Pract Res Clin Rheumatol 31:676–687.
15. McNamee KE, Burleigh A, Gompels LL, et al. 2010. Treatment of murine osteoarthritis with TrkAd5 reveals a pivotal role for nerve growth factor in non-inflammatory joint pain. Pain 149:386–392.
16. Obeidat AM, Wood MJ, Adamczyk NS, et al. 2023. Piezo2 expressing nociceptors mediate mechanical sensitization in experimental osteoarthritis. Nat Commun 14:2479.
17. Wodarski R, Delaney A, Ultenius C, et al. 2016. Cross-centre replication of suppressed burrowing behaviour as an ethologically relevant pain outcome measure in the rat: a prospective multicentre study. Pain 157:2350–2365.
18. Bryden LA, Nicholson JR, Doods H, et al. 2015. Deficits in spontaneous burrowing behavior in the rat bilateral monosodium iodoacetate model of osteoarthritis: an objective measure of pain-related behavior and analgesic efficacy. Osteoarthritis Cartilage 23:1605–1612.
19. Geraghty T, Obeidat AM, Ishihara S, et al. 2023. Age-Associated Changes in Knee Osteoarthritis, Pain-Related Behaviors, and Dorsal Root Ganglia Immunophenotyping of Male and Female Mice. Arthritis Rheumatol 75:1770–1780.
20. Chan KM, Bowe MT, Allen KD. 2023. Recommendations for the analysis of rodent gait data to evaluate osteoarthritis treatments. Osteoarthritis Cartilage 31:425–434.
21. Sambamurthy N, Zhou C, Nguyen V, et al. 2018. Deficiency of the pattern-recognition receptor CD14 protects against joint pathology and functional decline in a murine model of osteoarthritis. PLoS One 13:e0206217.
22. Miller RE, Tran PB, Das R, et al. 2012. CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. Proc Natl Acad Sci U S A 109:20602–20607.
23. Liu P, Okun A, Ren J, et al. 2011. Ongoing pain in the MIA model of osteoarthritis. Neurosci Lett 493:72–75.
24. De Rantere D, Schuster CJ, Reimer JN, et al. 2016. The relationship between the Rat Grimace Scale and mechanical hypersensitivity testing in three experimental pain models. Eur J Pain 20:417–426.
25. Knights CB, Gentry C, Bevan S. 2012. Partial medial meniscectomy produces osteoarthritis pain-related behaviour in female C57BL/6 mice. Pain 153:281–292.
26. Lillywhite A, Woodhams SG, Goncalves SV, et al. 2021. Anxiety enhances pain in a model of osteoarthritis and is associated with altered endogenous opioid function and reduced opioid analgesia. Pain Rep 6:e956.
27. Silva A, Andersen ML, Tufik S. 2008. Sleep pattern in an experimental model of osteoarthritis. Pain 140:446–455.
28. Laranjeira IM, Apolinario E, Amorim D, et al. 2024. Baccharis dracunculifolia DC Consumption Improves Nociceptive and Depressive-like Behavior in Rats with Experimental Osteoarthritis. Foods 13.
29. Mlost J, Bialon M, Kedziora M, et al. 2024. Network analysis of monoamines involved in anxiety-like behavior in a rat model of osteoarthritis. Pharmacol Rep 76:72–85.
30. Stubbs B, Aluko Y, Myint PK, et al. 2016. Prevalence of depressive symptoms and anxiety in osteoarthritis: a systematic review and meta-analysis. Age Ageing 45:228–235.

31. Barroso J, Branco P, Pinto-Ramos J, et al. 2023. Subcortical brain anatomy as a potential biomarker of persistent pain after total knee replacement in osteoarthritis. Pain 164:2306–2315.
32. Lehtinen P, Stenroos P, Salo R, et al. 2026. Functional MRI following sensory stimulation in rat monosodium iodoacetate model of osteoarthritis pain as a tool for drug therapy discovery. Neuroimage 325:121670.
33. Burston JJ, Valdes AM, Woodhams SG, et al. 2019. The impact of anxiety on chronic musculoskeletal pain and the role of astrocyte activation. Pain 160:658–669.
34. Nwosu LN, Mapp PI, Chapman V, et al. 2016. Blocking the tropomyosin receptor kinase A (TrkA) receptor inhibits pain behaviour in two rat models of osteoarthritis. Ann Rheum Dis 75:1246–1254.
35. Rashid MH, Theberge Y, Elmes SJ, et al. 2013. Pharmacological validation of early and late phase of rat mono-iodoacetate model using the Tekscan system. Eur J Pain 17:210–222.
36. Zaki S, Smith MM, Little CB. 2021. Pathology-pain relationships in different osteoarthritis animal model phenotypes: it matters what you measure, when you measure, and how you got there. Osteoarthritis Cartilage 29:1448–1461.
37. Ivanavicius SP, Ball AD, Heapy CG, et al. 2007. Structural pathology in a rodent model of osteoarthritis is associated with neuropathic pain: increased expression of ATF-3 and pharmacological characterisation. Pain 128:272–282.
38. Imbe H, Iwai-Liao Y, Senba E. 2006. Stress-induced hyperalgesia: animal models and putative mechanisms. Front Biosci 11:2179–2192.
39. Riehl L, Kress M, Kalpachidou T. 2025. Repeated testing and housing conditions impact complex mouse behaviors independently of surgical interventions. Brain Res Bull 232:111598.