Q11: Which mechanistic endpoints should be incorporated in preclinical experiments to complement pain-related behavior assessment (*e.g.,* should we do electrophysiology, DRG expression/proteomics etc) to define pain phenotypes/endotypes?

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RESPONSE/RECOMMENDATION:
Researchers should incorporate endpoint evaluations to support a molecular, cellular, and behavioral understanding of OA pathology and pain. We do not prescribe a minimum number of endpoints, but endpoints that capture functional activity, such as electrophysiology, can be particularly informative; rigor should be defined by mechanistic relevance and internal consistency, not by the number of endpoints. Although structural changes do not necessarily denote pain, joint structure assessment is recommended to correlate structural and pain phenotypes.

LEVEL OF EVIDENCE:
Strong. Publishing mechanistic endpoint data to support behavioral observations has clear benefits, although animal behavior complexity means that no fixed number of analyses guarantees mechanistic insight. Given their role in the sensation of pain, researchers are encouraged to focus on sensory neuron function and associated central circuitry, but we note important roles of non-neuronal cells in modulating pain[1].

RATIONALE: Eligibility criteria:
PubMed searches (5th February 2026) for “osteoarthritis pain behavior AND rat” and “osteoarthritis pain behavior AND mouse” returned 446 and 259 results respectively, making full review of mechanistic endpoints infeasible. We therefore discuss commonly used methods and/or those enabling gain in strong mechanistic insight. We focus on mice and rats, the most used species in preclinical OA studies; separate questions address large animals (Q4) and companion animals (Q5). When reviewing mechanistic endpoints, there is also room for debate about the relevance of the animal model used to study OA vs. joint pain in general[2,3]. We have considered endpoints that could be used to assess pain mechanisms in OA models, rather than excluding any method not routinely incorporated into OA research to date. Although endpoint analysis could restrict discussion to methods only used postmortem, we have also considered longitudinal methods. Our aim is not to provide an exhaustive inventory, but to highlight categories that offer clear interpretive value.

Mechanistic endpoints:
1) Structural assessment of joints: Histological scoring of cartilage damage is the gold standard[4–7], but cannot be conducted in live animals, which precludes studying acute to chronic pain transition in the same animal. MRI imaging[8], μCT, and dual energy X-ray absorptiometry are also often used to measure bone mineral content. Features of synovitis have also been shown to correlate with symptoms pre-clinically and clinically[9–13]. However, structural joint damage and pain can also be uncoupled, with discordance notable in clinically meaningful subgroups, like people with obesity[14]. In addition to traditional immunostaining of sections, microscopy, spatial transcriptomics, and more sophisticated 3D visualization tools are actively being developed to provide further information on the innervation of the joint (see below)[15].

2) Electrophysiology: measures sensory neuron excitability. In vivo electrophysiology includes: i) recording from knee and bone afferents, which in OA models show hyperactivity to torque and mechanical stimulation[16–19], and ii) recording from spinal cord dorsal horn neurons, which exhibit hypersensitivity indicative of central sensitization in OA models[20]. In vitro dorsal root ganglia (DRG) sensory neuron recordings from OA rodents show enhanced voltage-gated ion channel activity[21,22]. Retrograde tracing of knee afferents enables recording of neurons innervating the OA joint, which exhibit hyperexcitability aligned with pain-related behaviors[23,24]. Thus, electrophysiology provides precise readouts of how neuronal activity changes as OA pain behavior changes, which can be combined with pharmacology and/or genetics to investigate specific molecular targets. Electrophysiology does however require bespoke equipment and specific training, which might limit feasibility of incorporation into experimental pipelines.

3) Ca^{2+}-imaging: measures neuronal activity via an action potential, Ca^{2+} flux through Ca^{2+} permeable ion channels, or intracellular Ca^{2+} mobilization via G protein-coupled receptors. Ca^{2+}-sensitive dyes and genetically encoded Ca^{2+} indicators enable higher throughput than electrophysiology, although automated electrophysiology of DRG neurons is feasible[25]. Knee-innervating neurons of OA mice show increased TRPV1 responsiveness[23], correlating with the role of $TRPV1^+$ neurons in rodent OA[26]. Similarly, increased responsiveness to molecules elevated in the OA joint has been observed in DRG neurons isolated from OA animals and those same molecules induce knee hypersensitivity[27–30]. In vivo Ca^{2+}-imaging of lumbar DRG demonstrates increased neuronal responsivity to mechanical paw/knee stimulation in OA[31]. Thus Ca^{2+}-imaging maps changes in neuronal activity to behaviors and represents a pragmatic balance between mechanistic insight and experimental scalability.

4) Multi-omic profiling of tissues: Western blot or immunohistochemistry are low-cost tools to identify single or few proteins of interest, but a variety of high-throughput, unbiased techniques, e.g. metabolomics, lipidomics, and RNA sequencing, can be leveraged to understand the multi-dimensional molecular changes in OA. Single cell datasets of human neurons[32], synovium[13] and infrapatellar fat pads[33] are available. Future directions include integrating cell- and tissue-datasets[34], including spatial -omics[35] to link cellular and tissue changes to pain behavior. In depth data analysis of omics data often requires specialized bioinformatic/computational knowledge, which may limit widespread adoption until the development of affordable, commercially available analysis programs.

5) Neuronal innervation: Subchondral bone, synovium and the joint capsule are innervated by sensory neurons[36–45] and are likely targets for mechanism-based OA therapies. Most work has focused on soft tissue innervation because of ease of experimental access[16,36,45–52], but changes in subchondral bone[53–56] innervation are increasingly recognized. Innovative methods, such as light-sheet microscopy, enable 3D visualization of these changes[54,57–59]. Increased subchondral bone innervation correlates with OA symptoms in clinical and pre-clinical studies[51,52,60]. Understanding the relationship between nerve structure and function changes across joint tissues will provide important mechanism-based insights into OA pain.

6) Neuroimmune interactions: Neuroimmune interactions in the DRG and spinal cord actively sustain OA pain independently of joint pathology, e.g. M1-like DRG macrophages accumulate and their depletion/reprogramming resolves persistent pain without affecting knee pathology, implicating immune cell state as a causal driver of pain[61–65]. Tools such as spectral flow cytometry, immunohistochemistry, and 3D tissues-clearing/light-sheet imaging provide quantitative, spatial, and temporal resolution of these mechanisms in DRG and spinal cord, providing complementary mechanistic insight into OA pain mechanisms beyond neuronal endpoints.

7) Activity mapping and circuit-level plasticity markers: Immediate-early gene (c-Fos) and kinase activation (pERK) in the spinal dorsal horn map nociceptive drive and central sensitization. pERK is particularly dynamic (seconds–minutes) and closely linked to central sensitization mechanisms, whereas c-Fos reports integrated activation over tens of minutes. Increased dorsal horn c-Fos positive cells have been observed in OA models[66–70]. Genetic activity tagging (e.g., TRAP/FosTRAP) can link circuit recruitment to behavior. Pain-associated neuropeptides released by primary afferents in the dorsal horn (e.g., CGRP, Substance P) are elevated in rodent models of OA[71,72], and reflect increased synaptic transmission[73]. Functional brain imaging can also correlate circuitry changes with pain behaviors[8,74–76], but like other in vivo imaging approaches requires expensive, specialized equipment, which presents a barrier to most researchers being able to incorporate it into their studies as a routine mechanistic endpoint.

Conclusion

While no single mechanistic endpoint fully captures the complexity of OA pain, combining behavior with at least one functional neuronal readout and one joint tissue–level measure provides a robust and interpretable framework for mechanistic studies. Endpoint selection should remain driven by the specific biological question, technical feasibility, and translational intent.

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