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Supplementary information

Innovations and advances in modelling and measuring pain in animals

In the format provided by the authors and unedited

Supplementary Methods

Using the MeSH search terms "pain AND (mouse OR rat)", and restricting results to full text articles, we identified 6,054 preclinical pain papers published between 2016-2020 in the PubMed database. The journal *Pain* published the most papers using these criteria (292 papers, 4.8% of total; Supplementary Data). Only 7 other journals published more than 100 manuscripts using the same search criteria, and the impact factor of those journals, according to Clarivate Analytics *Journal Citation Reports,* was at least 2 points lower than that of *Pain.* Owing to its relative dominance in preclinical pain publishing, complemented by its subject specificity and international contributor list, *Pain* was therefore selected as the representative journal for our analyses described below. We acknowledge that the extent to which these analyses accurately represent preclinical pain practice is arguable considering these inclusion criteria.

To perform the analyses, the methods and results sections of all primary research papers published in the journal *Pain* (ISSN: 1872-6623) from 2016–2020 (Vol. 157, No. 1 through Vol. 161, No. 12) that featured (although not necessarily exclusively) the testing of pain sensitivity of live, behaving (non-anesthetized) non-human animals (n=360 papers) were opened and inspected. For the purposes of defining trends over time, similar papers from the years 2010 (Vols. 148–151), 2000 (Vols. 84–89), 1990 (Vols. 40–43), and 1980 (Vols. 8–9) were also examined. Only studies in which the pain state was induced by the experimenter at a defined time were included in our detailed analysis; thus excluded were a few studies involving ongoing clinical pain in companion animals of undetermined duration. This search strategy yielded a total of 506 papers, featuring 541 combinations of species-specific and etiology-specific (see below) entries.

The following information, either as reported in the manuscript text or as gleaned from provided graphs, was coded: species, strain, sex, age (or weight), supplier, parameters of the light-dark cycle in which the animals were housed, food supplied, the etiology of the induced pain state (see below for categories), the pain-producing assay(s) used, dose/volume of complete Freund's adjuvant or carrageenan injections, habituation times (to the testing room itself on the day of testing), pain measures used, the body part in which pain was induced (abdominal, back, forepaw, hind paw, joint, or orofacial), and testing days (in cases where this varied by experiment, the most extensive set was used). In papers featuring more than one species or more than one etiology, data were collected separately for each. From these data, the total number of measures, total number of post-baseline testing days, and maximum duration of testing were calculated. In those studies featuring von Frey testing, the von Frey method used was coded, and baseline withdrawal threshold (from control or wildtype groups) was estimated from the graph. These data in their entirety are presented in the Supplementary Data.

The following assay etiologies were defined:

Acute: assays in which pain would be expected to last from seconds to less than 24 hours

Cancer: assays involving experimental induction of tumors (bone cancer, lung cancer, oral cancer, pancreatic cancer, and skin cancer)

Disease: assays modeling disease states not included in another category (chronic post-ischemic pain model of complex regional pain syndrome, disc puncture model of back pain, destabilized medial meniscus model of osteoarthritis,

ischemia/reperfusion model of complex regional pain syndrome, intraspinal nerve growth factor model of back pain, and the introduction of a ureteral calculus)

Headache: assays modeling headache (the introduction of "inflammatory soup", interleukin-6, or nitroglycerin to the dura, and mild traumatic brain injury)

Inflammatory: assays featuring inflammation of a body part (acrolein exposure, adjuvant-induced arthritis, collagen antibody-induced arthritis, carrageenan, carrageenan/kaolin, complete Freund's adjuvant, experimental autoimmune prostatitis, endothelin, interleukin-6, T cell receptor transgene KRN and MHC class 11 molecule A(g7) (K/BxN), knee monosodium urate, monoiodoacetate, mucositis, pancreatitis via dibutylin dichloride, thoracotomy, tumor necrosis factor a, turpentine, ultraviolet B radiation, and zymosan)

Itch: assays producing itch and measuring scratching behavior (atopic dermatitis, dry skin induction, and injection of compound 48/80, chloroquine, or imiquimod)

Musculoskeletal: assays modeling musculoskeletal pain conditions (eccentric exercise, acidic saline plus fatigue, activity-induced muscle stimulation, repetitive tasks, running wheel exercise, sustained mouth opening, or the model described in Sluka et al., *Pain* 106:229-239, 2003)

Neuropathic: assays featuring nerve damage (injection of bortezomib, cisplatin, collagenase into the thalamus, gp120, human simian virus-1, indinavir, lysophosphatidic acid into the joint, oxaliplatin, paclitaxel, resiniferatoxin, streptozotocin, or vincristine; chronic constriction injury [CCI]; facet capsule stretch injury; "Gazelius model"; hyperhomocysteinemia; inflammation of the dorsal root ganglion; nerve axotomy; nerve crush; nerve hemisection; nerve ligation; nerve stimulation; nerve transection or partial transection; partial sciatic nerve ligation [PSNL]; perineural invasion; spinal cord injury [SCI]; spared nerve injury [SNI]; and, spinal nerve ligation [SNL])

Nociplastic: assays modeling nociplastic (previously known as functional or idiopathic) pain disorders (chronic mild stress model of fibromyalgia, injection of catechol-*O*-methyltransferase inhibitors, experimental autoimmune encephalitis model of multiple sclerosis, early life stress model of fibromyalgia, maternal stress model of fibromyalgia, reserpine model of fibromyalgia, and water avoidance stress model of fibromyalgia)

Postoperative: assays modeling postoperative/postsurgical pain (arthrotomy, incision, and ovariectomy)

Trauma: assays involving experimenter-induced trauma (bone fracture, impact injury, and mild traumatic brain injury [with testing of hind paw])

Visceral: assays involving pain delivered to visceral organs (bladder cystitis, bladder distention, colitis, colon obstruction, colonic distention, endometriosis, mustard oil into colon, and transurethral zymosan)

Pain measures were grouped into categories with respect to stimulus type and behavioral response complexity (see Supplementary Data), to assess trends over time. The stimulus type categories were defined as follows:

Chemical: measures involving reactions to the injection of a noxious and/or inflammatory chemical substance into a body part (acetic acid, benzalkonium chloride, capsaicin, dynorphin, endothelin, formalin, hypertonic saline, lipopolysaccharide, mustard oil, pruritogens (various), substance P, or yeast)

Cold: measures of cold pain sensitivity (acetone test, cold-plate test, or cold-water immersion of the paw or tail)

Electrical: measures of responses to electric stimulation of tissue (electric shock to face, hind paw, muscle, or tail)

Heat: measures of heat pain sensitivity (hot-plate test, infrared radiation, laser exposure, paw or tail immersion in hot water, paw-withdrawal from radiant heat (Hargreaves') test, Peltier devices, tail-withdrawal from radiant heat, thermal gradient test, thermal probes)

Mechanical: measures of mechanical sensitivity (adhesive removal test, brush test, cotton swab, grip force, paw pinch, pin prick, place-escape avoidance paradigm [PEAP], Randall-Selitto test, tail pressure, toe spreading reflex, visceromotor response, von Frey test, or various measures of weight bearing)

Spontaneous: measures of spontaneous/ongoing pain not involving experimenter-delivered evoking stimuli (autotomy, behavioral ethogram, burrowing, conditioned place avoidance to pain, conditioned place preference to analgesia, "dolognawmeter", electromyography, facial expressions [Mouse Grimace Scale], facial grooming, LABORAS system, locomotor activity, meal patterns, nesting, nocifensive behaviors [paw fluttering, grooming, lifting, licking, rubbing, shaking, wiping], operant procedures, real-time place aversion, vocalization [audible or ultrasonic], or wheel running)

Behavioral response complexity categories were defined as follows:

Low: reflexive behaviors

Medium: nocifensive behaviors and other behavioral responses that do not necessarily involve advanced cognitive decision-making (facial expressions, grip force, scratching, vocalizations, weight bearing)

High: behaviors involving decision making and/or classically or operantly conditioned responses (adhesive removal test, autotomy, behavioral ethograms, conditioned place avoidance to pain, conditioned place preference to analgesia, "dolognawmeter", LABORAS-measured behaviors, locomotor activity, meal patterns, nesting, operant procedures, PEAP, real-time place aversion, thermal gradient, and wheel running)

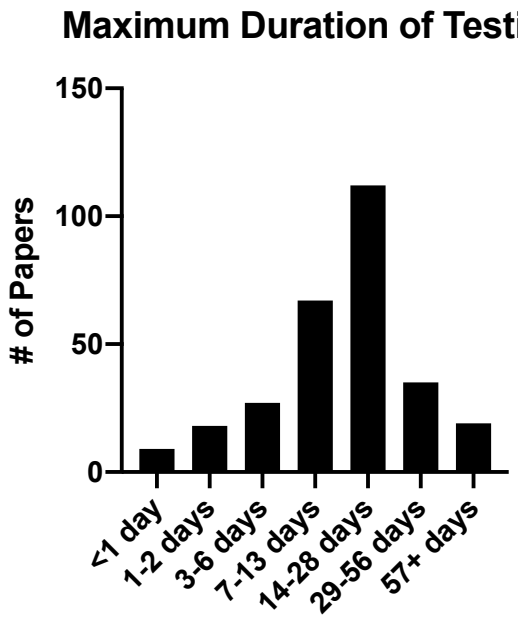
References

Sluka, K.A. *et al.* Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC 1. *Pain* 106, 229-239 (2003).

Supplementary Figure 1

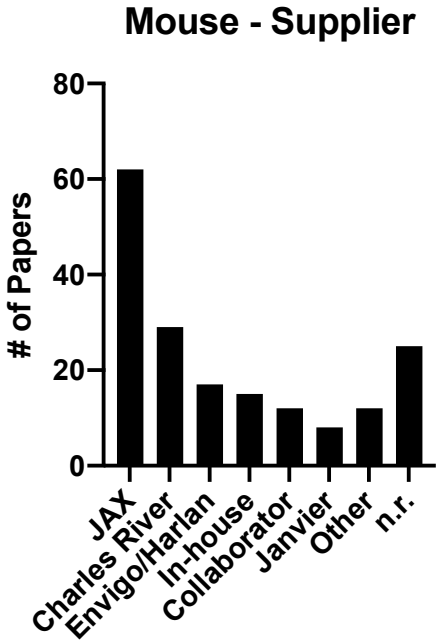
Age of mice utilized in pain studies. Age range of mice at beginning and end of behavior experiments published in *Pain* between 2016-2020.

Supplementary Figure 2:



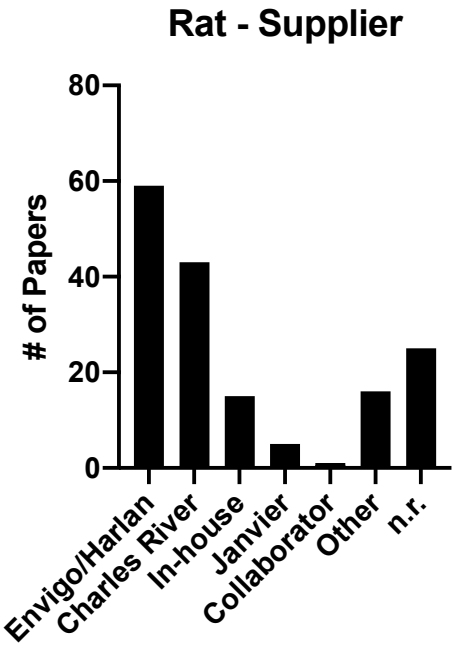
Time frame range for preclinical pain studies. Distribution of time periods included in preclinical experiments published in *Pain* between 2016-2020.

Supplementary Figure 3



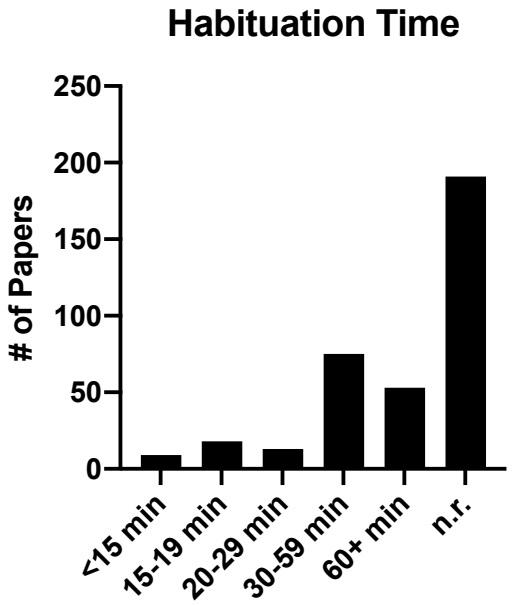
Source of mice utilized in pain studies. Distribution of mouse suppliers reported in preclinical experiments published in *Pain* between 2016-2020; n.r., not reported.

Supplementary Figure 4:



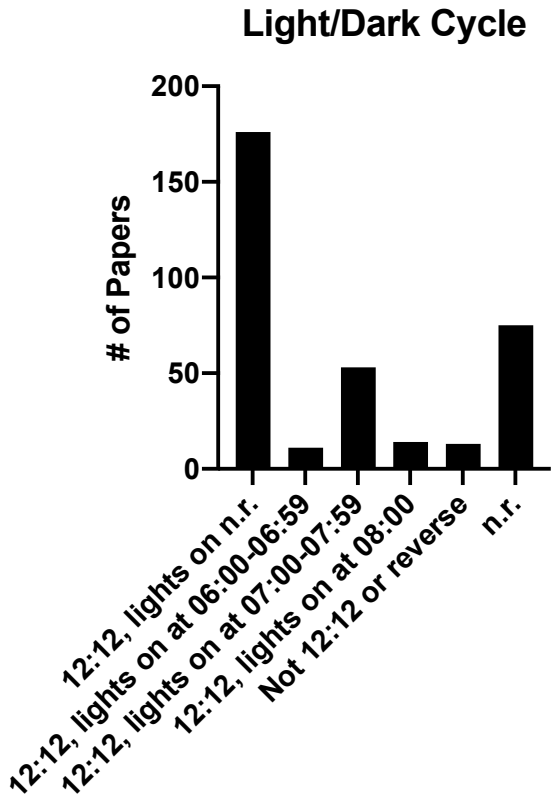
Source of rats utilized in pain studies. Distribution of rat suppliers reported in preclinical experiments published in *Pain* between 2016-2020; n.r., not reported.

Supplementary Figure 5



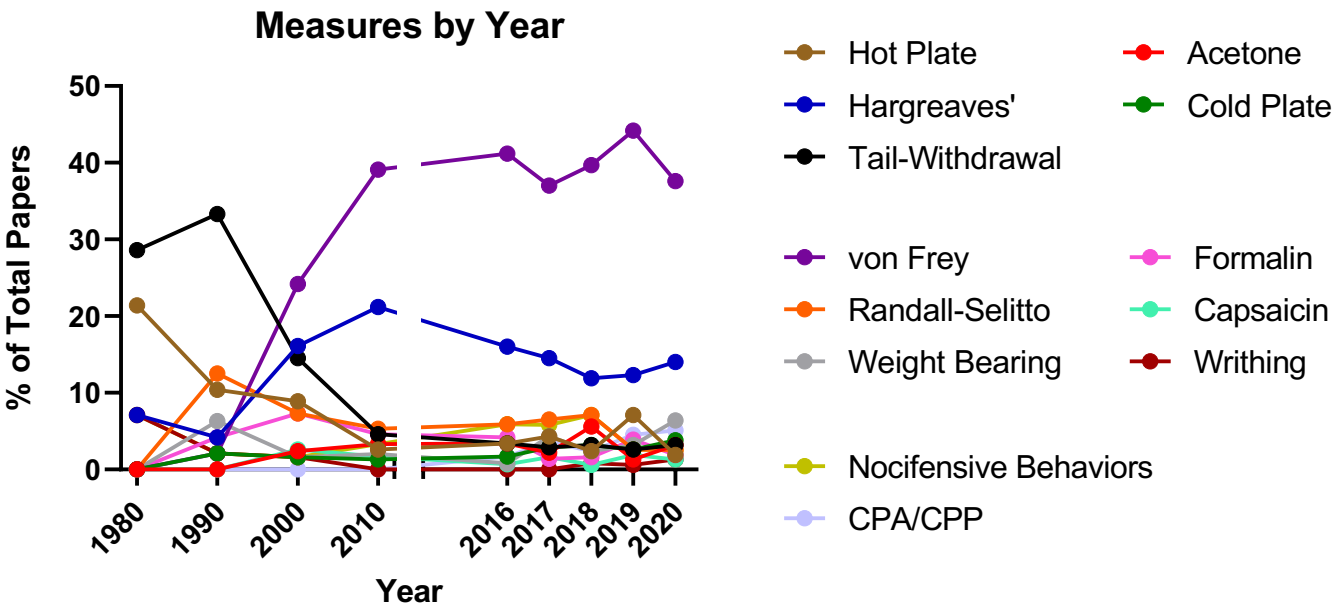
Habituation time ranges utilized in pain studies. Combined distribution of rat and mouse habituation times reported in preclinical experiments published in *Pain* between 2016-2020; n.r., not reported.

Supplementary Figure 6:



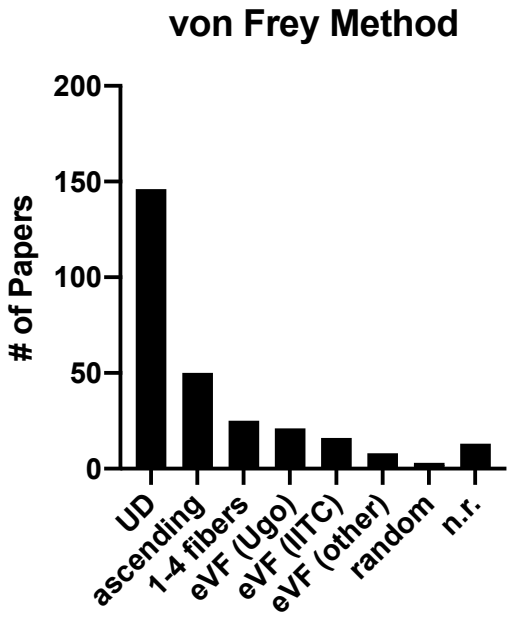
Light/dark cycle periods utilized in pain studies. Distribution of light/dark cycles reported in preclinical experiments published in Pain between 2016-2020; n.r., not reported.

Supplementary Figure 7:



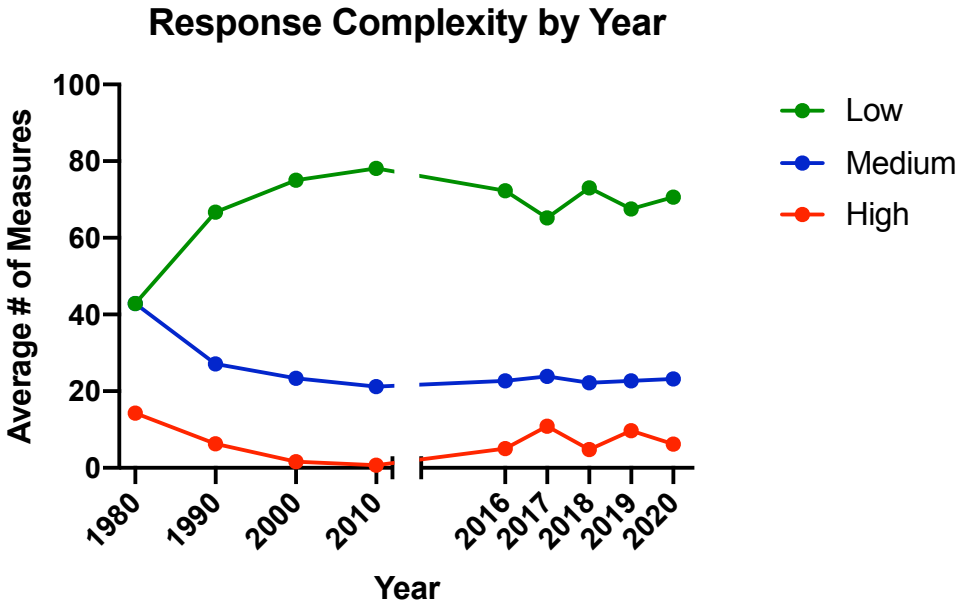
Pain measurement utilization over time. Frequency of measure utilization reported in preclinical experiments published in *Pain* in listed years, presented as percentage of total papers; CPA/CPP, conditioned place aversion/conditioned place preference.

Supplemental Figure 8:



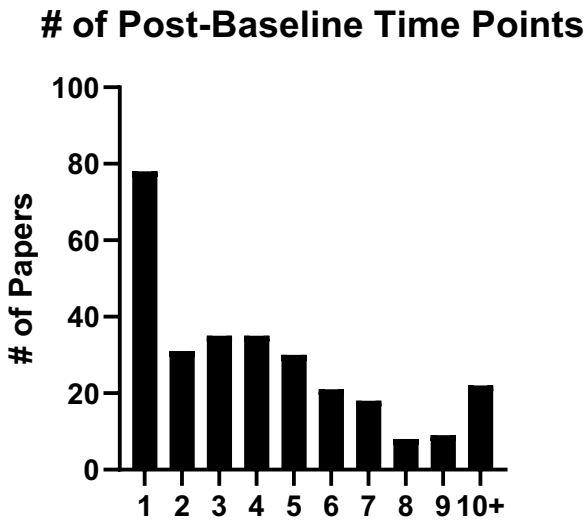
von Frey methods utilized in pain studies. Distribution of von Frey methods reported in preclinical experiments published in *Pain* between 2016-2020; UD: up/down, eVF: electronic von Frey, n.r.: not reported.

Supplementary Figure 9



Pain measurement response complexity over time. Frequency of low (*e.g.,* reflexive), medium (*e.g.,* nocifensive, involuntary), or high (*e.g.,* CPP/CPA) complexity responses reported in preclinical experiments published in *Pain* in listed years. The Supplementary Data contains complexity classifications for all measures included in this panel.

Supplementary Figure 10



Frequency of data collection in pain studies. Distribution of number of time points reported in preclinical experiments (post baseline) published in *Pain* between 2016-2020.