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*Overview*

# A Review of Strain and Sex Differences in Response to Pain and Analgesia in Mice

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**Pain and its alleviation are currently a highly studied issue in human health. Research on pain and response to analgesia has evolved to include the effects of genetics, heritability, and sex as important components in both humans and animals. The laboratory mouse is the major animal studied in the field of pain and analgesia. Studying the inbred mouse to understand how genetic heritable traits and/or sex influence pain and analgesia has added valuable information to the complex nature of pain as a human disease. In the context of biomedical research, identifying pain and ensuring its control through analgesia in research animals remains one of the hallmark responsibilities of the research community. Advancements in both human and mouse genomic research shed light not only on the need to understand how both strain and sex affect the mouse pain response but also on how these research achievements can be used to improve the humane use of all research animal species. A better understanding of how strain and sex affect the response to pain may allow researchers to improve study design and thereby the reproducibility of animal research studies. The need to use both sexes, along with an improved understanding of how genetic heritability affects nociception and analgesic sensitivity, remains a key priority for pain researchers working with mice. This review summarizes the current literature on how strain and sex alter the response to pain and analgesia in the modern research mouse, and highlights the importance of both strain and sex selection in pain research.**

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Pain is a prevalent disease state and public health crisis in humans worldwide.[70,97,105,124] In 2016, an estimated 20.4% of adults (50 million) in the United States suffered from chronic pain, with up to 8% (11 million) reporting pain lasting over 6 mo, resulting in substantial restriction of life activities, including employment.[26] Furthermore, pain has emerged as a primary symptom in both mental health and substance abuse treatment.[68]

The science of pain has now become a unique research field, and animal models have played a significant role in this advancement.[84] A categorical and bibliometric meta-trend analysis of the number of articles submitted to the journal *Pain* during the years 1975 to 2007 further demonstrates the use of animal models in pain research.[89] Approximately one-third of all the published work in *Pain* during this time used animal subjects, the majority of which included rodents (rats and mice) which were conscious during the studies.[84,89] During the past 2 decades of specific pain research, animal models have helped advance the hypothesis that genetics is a possible etiologic or confounding factor of pain.[58-60] We now know that pain and analgesia traits are heritable in both humans and mice.[59,75,81,91-93,102,108]

In humans, studies of the genetics of pain are somewhat limiting due to the large amount of resources needed and unique study design (for example, twin studies).[1,79] Rodent models have advantages over their human counterparts in genetic studies due to their small size and ease of use in larger investigations.[92] Rodent models are also advantageous due to their ability to provide better control over genotype, environment, and pain stimulus parameters.[59] Moreover, rodent models offer easier access to aging populations[113] and studies can be conducted over an animal's entire lifecycle.[38]

The preferred animal model for the study of genetic heritability in pain research is the laboratory mouse.[1,15,66,78,123] With its large number of inbred strains with known pedigrees, and the capacity to genetically modify the species, the laboratory mouse has become an important tool in the study of pain and analgesia.[1,59] The purpose of this review was to identify, describe, and summarize the current trends in pain research, relating to the strain and sex differences of common inbred strains of mice.

## Pain Terminology

Pain and nociception are 2 different terms. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".[3,68] Pain typically involves a noxious stimulus that activates nociceptors in the body to send signals to the central nervous system, where they are processed to generate multiple responses,[98] including a complex biopsychosocial pain experience.[68,71] The IASP defines nociception as "the neural process of encoding and processing noxious stimuli".[3] Nociception represents the nervous systems' processing of information, as generated by nociceptor activation. This information is processed at both spinal and supraspinal levels of the central nervous system, providing details about the noxious events.[98,117] The study of pain in the laboratory also includes terms such as allodynia (pain in response to a stimulus that does not usually provoke pain), and hyperalgesia (increased pain sensitivity from a stimulus that usually provokes pain).[3,44] To simplify the terms in this review, "pain" will be used to describe the overall process of

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nociception processing and response to noxious stimuli; while "nociception" will be referred to only in the discussion of specific laboratory methodology, which could include allodynia and/or hyperalgesia.

## The Genetics of Pain

**Background.** Early research on pain documented significant variability in the response to pain in both humans and animals.[78,91] This variability was hypothesized, and later confirmed, to have a genetic cause.[78] Much of this early pain research was developed in rodent models, with the laboratory mouse remaining the most studied animal species.[21,66,74] The mouse models that were initially used were bred specifically for nociception testing, and include the CXBK recombinant inbred strain, and the High Analgesia/Low Analgesia (HA/LA), and High Analgesia Response/Low Analgesia Response (HAR/LAR) mouse lines.[78] Although advantageous, these mouse lines were soon replaced with more common inbred mouse strains (for example C57BL/6 and 129) as specific phenotypes for pain were characterized.[85,87,91]

**Pain at the Gene Level.** Completion of the Human Genome Project (HGP) in 2003, and its subsequent mouse genome sequence, was a significant milestone in identifying, sequencing, and storing over 20,000 human genes.[37] Pain and analgesia traits are heritable in humans and mice.[85,91] Trait heritability refers to the portion of the overall variability that is due to inherited genetic factors[59] and is an essential component to identifying "pain genes." The heritability of a trait must be known before it can be identified at the gene level.[59] Several methods exist to determine if a trait is heritable, including selective breeding, genetic reference populations, and inbred strain differences, which will be the focus of this review. It is estimated that less than 50% of the total variability in a trait is due to genetic factors in humans and rodent pain models; however, this percentage can be increased with the appropriate mitigation of environmental factors.[48]

Mapping and manipulating the genes specific to pain has greatly improved the tool kit for the modern researcher. More recently, the Pain Genes Database[57] has been established to provide an interactive web-based browser specific for mouse genes. Through the use of this new database, several hundred genes germane to pain or analgesia in the mouse have been identified and cataloged for use in research.[57,78] The inclusion of functional genomics[67] and the ability to further exploit genetic information in "pain genes" has further advanced the study of pain and analgesia.

Similarly, the field of pain genetics has been advanced by the use of genetic linkage mapping to identify and establish the genomic position of the pain related trait.[59] By estimating the distance between the genomic loci, this technique can be used to map specific regions or quantitative trait loci (QTL) of the genome associated with a pain phenotype of interest. Using QTL to guide the homologous recombination breeding, the resulting genetic population provides an inexpensive path to the identification of potential pain gene candidates.[59,108]

**The Transgenic Knockout Mouse.** Transgenesis, homologous recombination, and CRISPR gene editing have allowed new genetic models to be developed, and the "pain genes" that have been identified to be further evaluated.[57,66] One review estimates that at least 60 publications per year are published demonstrating a significant behavioral pain phenotype resulting from the null mutation of at least one single gene.[57] Inclusion and summation of the myriad of knock-out strains specific to pain or analgesic response is beyond the scope of this review; however, the relationship to common inbred strains will be addressed in the following section.

***Common Inbred Mouse Strains.*** The inbred mouse remains the most studied species in heritability pain research.[92] Common inbred mouse strains have been thoroughly characterized, making their similarities and differences well known.[4] For instance, inbred strains can differ in behavior,[15,22] serotonin levels,[6,127] pigmentation,[125] immune system components,[118] and responses to both chemotherapeutic agents[131] and nicotine.[42] These advanced strain characterizations have been loosely called "strain surveys" and have utility in pain modeling for disease states such as endometriosis,[28] arthritis,[33] fibromyalgia,[11] and psychiatric disorders.[61]

The earliest strain surveys were completed on common strains from different vendors.[96] More recently, common inbred strains have been compared in nociception assays.[13] To date, several studies have been undertaken to survey the possible genetic contributions of strain to complex disorders, and these strain surveys are essential tools in providing researchers with a road map for selection of a strain.[22,96] Caution is warranted in generalizing from a mouse strain survey to the variation in human responses; however, this connection between the mouse and human genome suggests the strain screening to be a useful tool in research.[52]

**Differences in mouse strain relating to pain and analgesia.** Few reviews on mouse strains include a compilation of comparisons between strain and responses to nociception tests and/or analgesia.[19,69,75,81] The seminal work on this topic by Mogil and colleagues demonstrated that inbred mice differ in their responses to common nociceptive challenges.[92] Testing 11 inbred mouse strains (129/J, A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C58J, CBA/J, DBA2/J, RIIIS/J, and SM/J) against 12 common models of nociception (writhing, autotomy after hindlimb denervation, carrageenan hypersensitivity, formalin test, hot-plate test, Hargreaves' test of thermal nociception, Chung peripheral nerve injury model, tail-withdrawal test, and von Frey filament test of mechanical sensitivity), this laboratory demonstrated that rodent populations display large and heritable differences in both nociception and analgesic sensitivity.[92] Data from these common inbred mouse strains show that they react differently to nociception assays. Genetic variation between mouse strains and their response to nociception assays suggests that nociception in the mouse is heritable. Follow up strain screening studies[60,93] continued to expand the number of nociception assays that were used, ultimately revealing 5 major "clusters" or "types" of nociception and pain processing, including baseline thermal nociception, spontaneous response to chemical stimuli, thermal hypersensitivity, mechanical hypersensitivity, and afferent input-dependent hypersensitivity.

To further highlight the importance of strain variation on pain research, we conducted a literature search to investigate the connection between mouse inbred strain differences and performance on nociceptive assays relating to pain and analgesia. Table 1 through Table 3 provides the reader with a compilation of this search, summarized by the strain(s) studied. Inclusion in this table was limited to published work that compared at least 2 strains (or substrains of one strain) in similar testing methodology. Published literature was identified by the key search terms "mouse", "strain", "pain", "nociception", "analgesia" (PubMed). In addition to the strain breakdown found in Table 1 through Table 3, the following mouse models of pain and nociception have been characterized and validated using the strain screening approach: neuropathic pain,[39,110,134] inflammatory nociception,[87,134] cutaneous thermal nociception,[13,30,101,134] hot and

**Table 1.** Summary of literature (1998–2003) assessing common mouse strain differences relating to pain and analgesia.

| | Mogil 1998[85] | Mogil 1999[81] | Mogil 1999[92] | Mogil 1999[93] | Wan 2001[125] | Kest 2002[52] | Lariviere 2002[60] | Bon 2003[10] | Chesler 2003[19] | Kamp 2003[46] |
|---|---|---|---|---|---|---|---|---|---|---|
| 129/J | x | x | x | x | x | | | | | |
| 129P2/J | | | | | | | | | | |
| 129P3/J | | | | | | x | x | x | x | |
| 129Sv | | | | | | | | | | |
| 129S6 | | | | | | | | | | x |
| A/J | x | x | x | x | x | x | x | x | x | |
| A/HeJ | | | | | | | | | | |
| AKR/J | x | x | x | x | x | x | x | x | x | |
| BALB/c | | | | | | | | | | |
| BALB/cJ | x | x | x | x | x | x | x | x | x | |
| C3H/He | | | | | | | | | | |
| C3H/HeJ | x | x | x | x | x | x | x | x | x | |
| C3H/HeN | | | | | | | | | | |
| C57BL/6 | | | | | | | | | | x |
| C57BL/6J | x | x | x | x | x | x | x | x | x | |
| C57BL/6N | | | | | | | | | | |
| C57BL/10J | | | | | | | x | x | x | |
| C58/J | x | x | x | x | x | | x | x | x | |
| CBA | | | | | | | x | | | |
| CBA/CaCrl | | | | | | | | | | |
| CBA/J | x | x | x | x | x | x | | x | x | |
| CD1 | x | | | | x | | | x | | |
| DBA/2 | | | | | | | | | x | |
| DBA/2J | x | x | x | x | x | x | x | x | | |
| FVB | | | | | | | | | | |
| ICR | x | | | | x | | | | | |
| LP | | | | | | x | | | | |
| RIIIS/J | x | x | x | x | x | | x | x | | |
| SJL | | | | | | | | | | |
| SM/J | x | x | x | x | x | | x | x | x | |
| SW | | | | | | x | | | | |

**Table 2.** Summary of literature (2003–2009) assessing common mouse strain differences relating to pain and analgesia.

| | Levin-Arama 2003[64] | Neilan 2003[99] | Wilson 2003[128] | Wilson 2003[129] | Banik 2006[2] | Green 2006[36] | Liang 2006[66] | Mogil 2006[88] | Rigaud 2008[110] | Dickinson 2009[27] |
|---|---|---|---|---|---|---|---|---|---|---|
| 129/J | | | | | | | | | | |
| 129P2/J | | | | | | | | | | |
| 129P3/J | | | | x | | x | | | | |
| 129Sv | | | | | x | | x | | | |
| 129S6 | | | | | | | | | | |
| A/J | | | | x | | x | x | | | |
| A/HeJ | | | | | | | x | | | |
| AKR/J | | | | x | | x | x | | | |
| BALB/c | x | | | | | | | | | |
| BALB/cJ | | | | x | | x | x | | | |
| C3H/He | | | | | | | | | | |
| C3H/HeJ | | | | x | | x | x | | | |
| C3H/HeN | | | | | | | | | | |
| C57BL/6 | x | | | | x | | | | | |
| C57BL/6J | | x | | x | | x | x | x | x | |
| C57BL/6N | | | | | | | | | | |
| C57BL/10J | | | | | | | | | | |
| C58/J | | | | x | | x | | | | |
| CBA | | | | | | | | | | |
| CBA/CaCrl | | | | | | | | | | x |
| CBA/J | | | | x | | x | | | | |
| CD1 | | x | x | x | | | | | | |
| DBA/2 | | | | x | x | | | | | |
| DBA/2J | | | | | | x | x | | x | x |
| FVB | | | | | | | x | | | |
| ICR | x | | | | | | | | | |
| LP | | | | | | | | | | |
| RIIIS/J | | | | x | | x | | | | |
| SJL | | | | x | | | | | | |
| SM/J | | | | x | | x | | | | |
| SW | | | x | | | | | | | |

**Table 3.** Summary of literature (2010–2019) assessing common mouse strain differences relating to pain and analgesia.

| | Wijnvoord 2010[127] | Benedetti 2012[6] | Young 2014[134] | Miller 2015[73] | Moloney 2015[96] | Ono 2015[101] | Tajerian 2015[121] | Blennerhassett 2017[7] | Cho 2018[20] | Isami 2018[42] | Bryant 2019[13] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 129/J | | | | | x | | | | | | |
| 129P2/J | | | | | | | | | | | |
| 129P3/J | | | x | | x | | | | | | |
| 129Sv | x | | | | | | | | | | |
| 129S6 | | | | | | | | | | | |
| A/J | | | x | | x | | | | | x | |
| A/HeJ | | | | | | | | | | | |
| AKR/J | | | x | | | | | | | | |
| BALB/c | x | | x | | x | x | | x | | | |
| BALB/cJ | | x | | | | | | | | | |
| C3H/He | | | | | | | | | | | |
| C3H/HeJ | | | x | | | | | | | x | |
| C3H/HeN | | | | | x | | | | | | |
| C57BL/6 | | | | | | x | | | | | |
| C57BL/6J | x | x | x | | x | | x | | | x | x |
| C57BL/6N | | | | | | | | | x | | x |
| C57BL/10J | | | x | | | | | | | | |
| C58/J | | | x | | | | | | | | |
| CBA | | | | x | | | | | | | |
| CBA/CaCrl | | | | | | | | | | | |
| CBA/J | | | x | | x | | | | | | |
| CD1 | | | | | x | | | x | x | | |
| DBA/2 | | | | x | | | | | | | |
| DBA/2J | x | | x | | x | | | | | x | |
| FVB | | | | | x | | | | | | |
| ICR | | | | | | | | | | | |
| LP | | | | | | | | | | | |
| RIIIS/J | | | x | | | | | | | | |
| SJL | | | | | | | | | | | |
| SM/J | | | x | | x | | | | | | |
| SW | | | | | x | | | | | | |

cold nociception,[29,81] visceral nociception,[10,46,96] and scratching and itch behavior.[36,134]

Similarly, strain surveys have documented variation in the sensitivity to analgesics.[128,129] Given that strains of mice perform differently in various nociception assays,[134] one might assume that strains that are more sensitive to pain will be less responsive to analgesics. However, early strain surveys[86] demonstrated the opposite effect. Strains that were reactive to nociceptive assays were less responsive to morphine, and strains that were less responsive to nociceptive assays were more responsive to morphine.[75,86] These results suggest the potential for mouse strains to differ in pain and/or analgesia sensitivity primarily due to genetic mechanisms.[75]Sensitivity to analgesics may also be influenced by a wide variety of other factors including age,[35] sex,[83] and laboratory environmental factors (for example diet, housing, stress).[74,78,80] While the majority of strain surveys relating to analgesia centered around the evaluation of opioids (which will be covered in the "sex" section below), nonopioid analgesic responses also differed by strain, including electroacupuncture,[125] xylazine,[64] ketamine,[64] and over-the-counter medications (acetaminophen and NSAIDs).[128] Common mouse strains have also been surveyed for general anesthetic characterization and pain model development. For instance, heritable differences in respiratory drive and breathing pattern in C58BL/6, C3H/HeJ and B6C3F1/J strains of mice while under isoflurane anesthesia have been established.[40] Moreover, the effect of isoflurane anesthesia on the Mouse Grimace Scale in DBA and DBA/2 strains have been evaluated.[73]

The work discussed in this section has the potential to positively impact the welfare of laboratory mice by providing targeted analgesia by strain and ensuring that the selection of an analgesic agent is appropriate for the strain and model being studied. A point of discussion in the review of these examples is the need for improved and consistent measures of pain in mice. Sources have demonstrated the variability in the assessment of rodent pain,[16] especially at the strain level.[128,129] The evaluation of contemporary methods of pain assessment has now been documented in laboratory animal literature.[20,73,100,112] These adjuncts to typical nociception assays will enhance the body of literature and provide a more holistic and consistent approach to the assessment of rodent pain and analgesia.

**C57BL/6 versus 129.** The common use of knockout or transgenic mice in biomedical pain research requires the interpretation of the genetic background of the resulting strain. The genetically modified strain is almost always a mixture of alleles from 2 different strains, usually 129 or C57BL/6.[62]

Of particular interest to pain researchers are the significant nociceptive and pain model differences identified between C57BL/6 and 129 inbred strains.[13,15,58,69,85,92,93] (Table 1 through Table 3.) Significant differences between these 2 strains have also been demonstrated for sensitivity to analgesia.[123,128,129] For example, early strain surveys demonstrated the C57BL/6 strain as one of the most sensitive inbred strains when tested on a battery of nociceptive assays.[92] In the same survey, the C57BL/6 and 129 strains demonstrated significantly different responses to 8 of 12 nociceptive assays.[92] Other laboratories have also established the nociceptive sensitivity of the C57BL/6 strain in inflammatory pain but not neuropathic or visceral pain conditions.[62] Subsequent work on strain differences have highlighted the importance of substrain characterization, especially for the C57BL/6 and 129 strains.[56] For example, the C57BL/6J and C57BL/6N substrains vary in response to ethanol preference, conditioned fear, and pain sensitivity.[15,56,69] This work is notable because these strains are generally used as the default genetic background for null mutations.[66,67]

***Sex Differences in Response to Pain and Analgesia.*** Pain research has evolved from debating whether sex differences in the sensitivity to pain and analgesia exists to now recognizing the importance of these differences and how to understand their effects in human and animal models.[38,95,103,126]

In humans, females are overrepresented in complex disease states such as autoimmune disorders and chronic pain.[17,119] Differences between men and women regarding pain perception, tolerance and behavior, as well as the prevalence in seeking medical attention for the treatment of pain, are well known.[104] Pain studies in humans have also documented that variability exists in response to analgesia by sex, and that a portion of these variabilities are likely to have a heritable etiology.[17] Historically, these variations in sex response to pain and analgesia and heritability have been studied independently;[77] however, contemporary pain research suggests that both play a fundamental role and should be studied concurrently.[26,77,90]

In the mouse, sex differences have been demonstrated to significantly affect the response to nicotine,[43] Down's Syndrome,[8] neuoimmunity,[114,115] obesity,[113] diabetes,[113] aging,[113] cardiovascular health,[72] liver disease,[72] and cancer research.[72]

Several anatomic/physiologic factors come into play when interpreting sex differences in regard to pain and analgesia. For example, adult male rodents have greater percentage of body fat than females[38] while the opposite is true for humans. This sexual dimorphism can affect the distribution of highly lipophilic drugs and influence analgesia potency, and duration of action. Similarly, metabolism, immune response, activity level, and response to analgesics all have a sex difference.[2,8]

Sex differences to pain and analgesia in inbred mice are likely the result of genetic background, making variability by strain and sex important research considerations.[9,76] However, the majority of animal subjects in pain research are overwhelmingly male,[109] which can lead to a bias in interpreting studies of animal pain.[82]

In a recent review[83] of a 10-y history in the journal *Pain*, nearly 80% of publications included only male subjects. Conversely, 8% of the studies used only female subjects. Given that a greater percentage of clinical pain conditions affect women over men, and the growing supportive research on this topic, all pain research be performed in both sexes, and when only one can be used, it should be female.[38]

The above point was further punctuated within the research community in 2014, when the United States National Institutes of Health (NIH) issued a requirement that grant applicants use both sexes of model organisms in their work.[21] To date, strong justification from the scientific literature or preliminary data must be included in an application seeking to study only one sex[21] to reduce the male bias of the study.[104]

Examples of sex variation in common inbred mouse models of pain and analgesia include: morphine sensitivity,[25,32,34,41,45,50,51,54,95] NMDA receptor antagonism,[14] Kappa receptor sensitivity,[18] response to nicotine,[43,107,120] femoral cancer pain,[39] immune system,[114] and sleep disruption.[111] In the evaluation of the sex variation studies in inbred mice described above, a portion of the research is conflicting, suggesting that in addition to sex variation, gonadal hormones should be evaluated.[82,119]

**Role of Gonadal Hormones.** Animal studies have demonstrated variable results regarding the role of gonadal hormones on pain sensitivity and analgesic efficacy.[24,104] Gonadal hormones are produced by the ovaries (estrogens and progestins) and testes (androgens),[24] and pain researchers are primarily interested in the modulating effects of estradiol and testosterone.[24] The exact role

of gonadal hormones in pain are not well understood,[45] however they are suspected to affect pain and analgesia either activationally (in adulthood) or organizationally (in development).[5,24]

A common method to evaluate gonadal hormones and their research effect is to include female subjects at different stages of the estrous cycle.[38] In humans, sex hormones have been shown to influence pain sensitivity, pain threshold, and pain tolerance and vary with stage of the menstrual cycle.[126] In mice, estrous cycle has been demonstrated to impact pain and symptom severity on a multiple sclerosis model;[106] however, the value of testing female mice at different stages of the estrous cycle for pain research is still debatable.[38,119] While testing mice for pain during the entire estrous cycle is interesting; it can be prohibitive due to the large numbers of animals needed to adequately power the analysis.[83] In addition, the task of assigning the correct stage of estrus requires extra handling and vaginal cytology, which could confound the study.[38] To improve research reproducibility, it would be useful for the study design to include the evaluation of the stage of estrous. An advancement in this approach is the use of gonadectomy with or without hormone depletion and/or replacement.[38]

Gonadectomy can be performed via surgery (most common approach) or via "chemical castration." The surgical method, although useful in identifying the effects of hormones on pain and analgesia in animals, has drawbacks including alteration of pain thresholds and sensitivity to analgesic agents, and the disruption of normal hormonal feedback loop, potentially causing both males and females to have elevated or depressed circulating hormones.[38] The most common method of using gonadectomy in female mouse models of pain and analgesia is to provide hormone replacement (that is estradiol) after gonadectomy. While this is a popular approach, factors such as strain differences, inconsistent dose range, and age differences, can introduce variations in results.[119]

**The Role of Sex-Based Factors Specific to Opioids.** Similar to the concept of sex differences in nociception, studies have also shown a relationship between sex differences and sensitivity to analgesics.[23] The largest body of work on this subject involves opioid analgesia, specifically morphine, whose action is defined as a μ receptor agonist.[50-54] Both humans and animals display variable responses to morphine sesitivity,[54] and some of this variability may be attributable to sex.[51] Review of early pain research specific to mice documents that male and female mice differ in their sensitivity to morphine;[50,76] however, the lack of consistent findings makes a broad statement of strain and morphine sensitivity problematic.[54] Rather, it is now generally believed that the variability in male and female mice to the effects of morphine are likely the result of several mechanisms, including strain differences.[14,23,32,41,51,76]

Multiple studies have documented that sex affects opioid analgesia,[25] but the exact mechanism is unknown and is likely due to multiple factors. In mice, opioids can be more potent in male compared with female mice when given systemically,[23,76] which may be due to the variable degree of morphine tolerance existing between male and females.

In addition to differences in sensitivity, analgesic responses to opioid classes also demonstrate a sex predilection (for example κ opioid analgesics).[23,47] κ-Opioid analgesia has been further evaluated on the effects of detromethorphan (DEX) N-methyl-d-aspartate (NMDA) antagonist,[47,77] and melanocortin-1-receptor[94] abilities to potentiate analgesia, especially in females.[47,77]

## Other factors

The nature of this broad literature review does not allow for detailed consideration of all of the factors proposed to influence the effect of strain and/or sex on the response to pain and/or analgesia in the mouse. However, several potential factors have been discussed in this review. One potentially influential factor in the study of mouse pain could be age. In humans, gender differences in pain sensitivities emerge during adolescence, with females experiencing an increase during puberty while their male counterparts remain stable.[63] In rodents, the impact of advancing age on the biologic systems responsible for experiencing pain have not been fully established.[132,133] Unfortunately, the majority of the studies reviewed for this manuscript did not include age as a factor in the study design. Another factor that could affect the response of mice to pain and/or analgesia is the source of the strain used. This review has identified and tabulated the strains surveyed based on the information and nomenclature included in the study design of each reference (Table 1 through Table 3). In many cases, the referenced manuscripts did not include the substrain or the source of the strain(s) of mice. To more clearly understand the effects that age or source of strain could have on mice in relation to pain research, future study designs must provide comprehensive detail on animal subjects and their background.

## Conclusions

The use of mice in the study of pain and analgesia will likely continue, although should be enhanced to include updated modalities in human medicine (for example imaging, genetics).[66,84] Studies examining nociception assays have established performance differences within common mouse strains,[92,93,128,129] many of which can also be influenced by sex. Similarly, surveys of common mouse strain have been used to evaluate the differences of genes and sex on the sensitivity to analgesia.[128,129] Collectively, this review establishes that over the past several decades, genetic heritability and sex are well documented to influence the experience of pain and response to analgesia in the laboratory mouse; however, the mechanisms of action and relationship to complex interactions are still under investigation.

**Refinements Needed.** Rice and colleagues[109] articulately describe key enhancements needed by the research community regarding animal models of pain and lists the choice of animal as one of the key factors for reducing experimental bias. These concepts, punctuated by the call to reduce the overwhelming male-dominated bias,[109] and to minimize the effects of laboratory environmental factors,[80] remain some of the hallmark goals for animal models of pain. Ensuring research transparency and minimizing method bias in the laboratory animal community[16] will, in addition, improve the pain and analgesia related work.

To assess this concept in the laboratory animal literature, a survey of the last 5 y of literature published by AALAS journals[122] (*JAALAS*, and *Comparative Medicine*) revealed that roughly half of the submissions relating to mouse anesthesia and/or analgesia included both male and female subjects.[12,31,49,64,100,116,130] In only one case[55] was a justification for single-sex study based on published literature included in the Materials section. The deficiency in dual sex inclusion underscores the need to improve the study design of all laboratory animal studies, specifically those relating to pain and analgesic response.

Recent work in the laboratory animal community provides new tools to assess both pain and analgesia in mice. Evaluation of nest building behaviors,[112] species-specific cage side assessments,[100] and the Mouse Grimace Scale[20] are all examples of novel methods for assessing pain. These methods can be used to characterize strain and sex differences and should be considered for use in order to obtain accurate assessment of pain and analgesic agents in future studies.

Reproducibility of pain research relating to animals can only be achieved when these complex interactions are accurately included in the study design. This review was undertaken to solidify these important concepts and provide a workable summary for the laboratory animal community to reference.

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