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Sex differences in neuroimmune and glial mechanisms of pain

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1. Introduction.

Therapeutic challenges.

The International Association for the Study of Pain (IASP) Task Force recently proposed a new definition of pain as an aversive sensory and emotional experience typically caused by, or resembling that caused by, actual or potential tissue injury [188]. Importantly, acute pain serves a critical Darwinian protective function: to initiate an escape response from noxious stimuli that in the future should be avoided for personal safety. However, chronic intractable pain is maladaptive and constitutes a widespread public health issue, significantly impairing quality of life and costing nearly $600 billion per year in the US alone [1]. Current efforts to develop novel pain therapeutics are guided by the following observations: 1) pain may arise from multiple mechanisms, and this complexity reflects the difficulty in achieving significant relief; 2) chronic pain states may reflect an important sex covariate in the development of the pain phenotype; and 3) there is a growing appreciation that secondary to tissue and nerve injury, elements of the immune system are recruited in a sex-dependent manner to influence the chronic pain phenotype. In the following sections, we will discuss aspects of these three points.

Categorization of pain phenotypes.

Mechanistically, pain states evolving into a chronic pain phenotype may be classified heuristically into four categories: **(1) Nociceptive pain** resulting from activation of high threshold sensory neurons (nociceptors); **(2) Inflammatory pain** resulting from persistent

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inflammation in soft tissue (viscera, fascia, muscle), joints (arthritis), or other specific tissues (*e.g.*, dental, meningeal, bone); **(3) Neuropathic pain** resulting from direct (trauma, compression, ischemia) or indirect (chemotoxins, radiation, or autoimmune attacks, as with paraneoplastic syndromes) injury to the peripheral afferent nerve or ganglia; or **(4) Dysfunctional/Centralized pain** occurring in the absence of a noxious stimulus, detectable inflammation, or structural damage to the primary afferent [228]. It should be noted that in accord with the IASP guidelines outlined above, these four categories are associated with the generation of an aversive state accompanied by changes not only in physiology (*e.g.*, blood pressure, hormone release [42]) but also in reward and cognition (*e.g.*, formation of a negative association leading to avoidance, development of a positive appetitive response to drugs that diminish the negative affect [169]). Chronic pain syndromes with neuropathic etiology are often challenging to manage as they tend to be refractory to treatment with anti-inflammatory drugs, and many patients report inadequate or variable relief from commonly employed first-line therapies such as anticonvulsants and antidepressants [239]. While it is informative to consider types of pain individually from a mechanistic standpoint, many chronic pain conditions represent multiple phenotypes expressed simultaneously. For instance, effective management of cancer pain may require several functionally distinct medications to target various underlying processes. Furthermore, there is increasing support for the assertion that acute pain states secondary to tissue injury may evolve into a chronic condition with peripheral and central neuropathic components [183].

Sex as a covariate in the evolution of chronic pain.

Evidence is accumulating in support of quantitative and qualitative sex differences in pain sensitivity and analgesia. Pain syndromes with high prevalence in humans – such as arthritis, temporomandibular disorder, migraine and fibromyalgia – disproportionately affect females, occurring with significantly greater incidence than in males [158; 194]. Such disparities largely have been attributed to genetic and hormonal differences between males and females in preclinical and clinical studies, as explored in depth by several elegant reviews [20; 53; 62; 73; 74; 158; 177]. Historically, most preclinical studies of pain hypersensitivity have focused on assessment of evoked behaviors in young adult male rats or mice. This approach largely derived from perceived challenges posed by evaluating effects of estrus cycle phases (which change every 4–5 days) on nociception and analgesic responsiveness [157]. Surprisingly, evidence suggests that variability associated with different stages of the estrus cycle is no greater than that occurring intrinsically in males [15]. There is a high failure rate of analgesic investigational new drugs in clinical trials, particularly for pain conditions with greater incidence in women [17]. Thus, elucidation of the molecular underpinnings of chronic pain states in females and the mechanisms underlying sex-dependent differences in pain signaling is critical for successful development of novel therapeutics [159]. Recent efforts are emphasizing inclusion of females and of spontaneous painful disease models such as osteoarthritis in companion animals to identify novel druggable targets for pain symptoms [111; 158], although the literature still remains biased toward males [159]. Recognizing this issue, the NIH specifically mandated that studies must employ both males and females unless there are organ-specific reasons to exclude one sex or the other. Several preclinical studies suggest that interactions between the immune and nervous systems contribute to sex differences in many chronic pain syndromes, and may serve as a source of novel drug targets

that are specific to either males, females or both [10; 40; 54; 133; 178; 195; 199; 206; 242]. In the present review, we provide a comprehensive synthesis of reported sex differences in neuroimmune mechanisms of pain hypersensitivity in rodent models, suggesting potential high-value targets to pursue for sex-specific treatments of chronic pain in men and women.

2. Assessment of nociception in rodent models

In humans, pain is difficult to assess reliably using objective clinical measures due to its highly subjective and individualized nature [45]. Thus, diagnosis of pain syndromes and subsequent evaluation of therapeutic efficacy relies heavily on patients' descriptions of their pain levels, features, and location. As non-verbal organisms (infants, rodents) lack this capacity, one endpoint that can be isolated and examined easily in behavioral models is nociception, or the neural process of encoding noxious stimuli constituting the sensory, non-affective component of pain (Figure 1). For excellent reviews of pain circuitry in development and adulthood, see Treede, Fitzgerald *et. al.*, and Basbaum and Fields [14; 77; 223]. Injury- or disease-induced pain hypersensitivity results from peripheral or central sensitization, or the increased responsiveness of nociceptive neurons in the peripheral (PNS) and central nervous systems (CNS) to normal or subthreshold primary afferent input [122], a process mediated by several mechanisms described herein. Pain hypersensitivity presents both in humans and in animals as allodynia, wherein stimuli that do not normally produce pain are perceived as painful, or hyperalgesia, a state of enhanced sensitivity to noxious stimuli that is often coupled with spontaneous (non-evoked) pain [196]. If present at the site of injury, hyperalgesia is considered as primary, while that which occurs in the surrounding area is termed secondary. The development of secondary hyperalgesia is attributed to central sensitization.

Classically, nociception in animals is measured as nocifensive (reflexive withdrawal) behaviors in response to evoked stimuli. However, the low probability of clinical success for candidate analgesic molecules based on evoked endpoints alone has sparked efforts to improve the face validity of preclinical paradigms of chronic pain [235]. Several groups have pursued various methods of also capturing affective and motivational components of the pain state in rodents as well as in larger animals [27; 79; 96; 131; 146; 162; 171], although these approaches are still undergoing refinement. In contrast to a spinally-organized nociceptive reflex, the perception and expression of pain unpleasantness depend on higher order functions in cognitive and limbic regions of the brain (Figure 2). Since the affective component of a pain state manifests as spontaneous as well as time-, species- and paradigm-dependent behaviors, there is no singular assay in existence that encapsulates the human experience of pain in an animal. Nonetheless, some testing approaches can capture specific aspects of emotional and motivational responses to noxious stimuli. When utilized concurrently with evoked measures, these methods may provide a stronger assessment of candidate analgesic drug efficacy in preclinical pain models. For example, rodents in pain display coping behaviors such as licking the site of injury or emitting ultrasonic vocalizations [96]. Context-dependent approach or avoidance responses following injury include conditioned place aversion (CPA) to a location linked with an aversive experience (*i.e.*, a pain state) and conditioned place preference (CPP) for a site in which the pain state is alleviated [170]. Similarly, operant paradigms for self-administration of analgesic drugs

display motivated and goal-directed behaviors to obtain relief from pain [90; 93], although interpretation of the results is complicated if the drug itself is intrinsically rewarding. Since depression often is co-morbid with chronic pain, incorporation of assays of depression-like behaviors comprised of both evoked measures, such as the forced swim test, and spontaneous assessments of anhedonia, including motivation for naturally reinforcing substances like sucrose [221].

3. Influence of sex on evoked and spontaneous nocifensive behaviors

Several rodent models of chronic pain states exhibit sex differences that parallel findings in many human disorders, with greater sensitivity to nociceptive stimuli in females (Table 1). For example, tactile allodynia is more pronounced and/or of longer duration in female rodents in nerve injury paradigms of Chronic Constriction Injury (CCI) [226; 227] (but see also [216]), partial Sciatic Nerve Ligation (pSNL) [51], Sciatic Nerve Ligation (SNL) [32; 210; 212], Intra-Articular Lysophosphatidic Acid- (IA LPA)-induced neuropathy [174] and Endoneurial injection of functionally active Myelin Basic Protein (MBP) fragment (84–104) [40]. Hyperalgesic priming, an age-dependent model of the acute to chronic pain transition characterized by the prolongation of hyperalgesia by repeated nociceptive insults, also exhibits sexual dimorphism. This paradigm elicits increased allodynia and facial grimacing in female compared with male rodents in a dural Calcitonin Gene-Related Peptide (CGRP) model of migraine [10]. Female mice also experience earlier onset of pain-related functional deficits with systemic Lipopolysaccharide (LPS) [106], IA Complete Freund’s Adjuvant (CFA)-Induced Arthritis [46], Muscle hyperalgesia [86], and Femoral Bone Cancer, correlating with faster progression of disease [64; 121]. Both mechanical and cold allodynia are more pronounced in female versus male mice in models of Multiple Sclerosis (Experimental Autoimmune Encephalitis, EAE) [185] and of Complex Regional Pain Syndrome (CRPS) [213].

In contrast, some studies report greater expression of pain hypersensitivity in males. For example, the development of allodynia following intrathecal (IT) LPS or the initiation of arthritis (K/BxN serum transfer- or intraplantar IPLT CFA-induced) is more pronounced in male mice [26; 205; 206; 242; 243], yet also see [22]. Similarly, males also exhibit increased allodynia versus females in the Spared Nerve Injury (SNI) model [97; 205; 206], yet also see [24]). The discrepancies in magnitude of allodynia reported by these studies may be explained in part by different strains of rodents or paradigms utilized. Evidence of sex differences also is emerging both in mice and in humans for the expression of cued pain-related fear memory mediated by limbic, mesolimbic, and cortical circuits [11; 153]. For example, context-dependent pain hypersensitivity is increased in males relative to females when tested by a male experimenter or when examined in an environment previously associated with an aversive tonic pain experience [145; 207].

In other injury paradigms, male and female rodents develop equivalent severity of evoked or spontaneous pain-like behaviors. Studies utilizing Collagen Antibody-Induced Arthritis (CAIA) [67], IA CFA-Induced Arthritis [66], Chemotherapy-Induced Peripheral Neuropathy (CIPN) [69], adult reincision following neonatal paw incision [165] and IPLT formalin [243] models all report allodynia of similar magnitude in males and females. Interestingly, despite

sex differences observed in allodynia during arthritis or following IT LPS, both male and female mice exhibit deficits in grip strength – a widely used rheumatology measure sensitive to analgesics and a frequently reported deficit known to correlate with pain in Rheumatoid Arthritis (RA) [106; 164]. Arthritis-induced declinations of functional measures such as locomotor activity and home cage wheel running also are observed in mice of both sexes [75; 104]. Both males and females exhibit post-surgical or arthritis injury-induced grimace behaviors as well as depressed nesting and burrowing [101; 102; 209]. Likewise, sucrose consumption and social exploration following systemic delivery of low dose LPS are transiently reduced [181] or unchanged [246] in both sexes. The effects of morphine on CPP and CPA during peripheral inflammation also are not significantly different between males and females [8; 92]. In spite of similar levels of pain-like behaviors in both sexes, it is important to note that the mechanisms underlying these behaviors sometimes differ in males versus females [26; 50; 89; 130; 149; 178; 195]. Thus, caution should be exercised when drawing conclusions about positive or negative effects of treatments for pain when males and females are not stratified [158].

Influence of gonadal hormones.

Sex differences in nociceptive thresholds and opioid analgesia depend largely on organizational effects of gonadal hormone status – that is, hormone action during critical periods of gestation. Specifically, neonatal exposure to testosterone appears necessary for the phenotype of decreased nociceptive sensitivity and increased morphine analgesia observed in adult males relative to females [23; 43; 114; 117]. Nonetheless, the presence of testosterone also can exert pronociceptive actions [205]. The acute, or activational, effects of estrogens on pain and analgesia are decidedly more complex. Activational effects can vary according to the type, level, stability and route of administration of estrogens, whether they are administered alone or in combination with progestins, as well as the nociceptive paradigm utilized and the chronicity of pain state. Particular caution should be exercised in the interpretation of studies in which supraphysiological doses of these hormones are administered [52; 53].

For example, systemic administration of estradiol decreases nociceptive behaviors in the second phase (10–60 minutes post-injection) of IPLT formalin-induced acute pain in gonadectomized male or female rats [81; 115; 140] and in nerve-injured intact mice [227]. In contrast, some chronic pain states that emerge days to weeks after injury or inflammation may be exacerbated by estrogens [25; 46] or are unaffected by hormones [12]. Mu Opioid Receptor (MOR)-mediated analgesia in cycling females also depends on the phase of the estrus cycle, as morphine potency is greatest in metestrus, diestrus and proestrus phases but is lowest during estrus [107; 219]. The intricate effects of estrogens are perhaps best illustrated by the observations that estradiol suppresses the *induction* yet facilitates the *expression* of hyperalgesic priming [68; 70; 103]. By contrast, progesterone appears mainly to serve a protective function, in that it mediates pregnancy-related analgesia [192] and attenuates hyperalgesia precipitated by IPLT CFA- or Carrageenan-induced monoarthritis [189; 214], excitotoxic spinal cord injury [84] and Peripheral Diabetic Neuropathy (PDN) [126]. For in-depth discussions of these processes, the reader is directed to several extensive reviews [20; 47; 52; 105; 147; 158; 234].

Influence of stress pathways.

Similarly, stress also exerts paradoxical analgesic and hyperalgesic effects that are sexually dimorphic and likely are mediated by estradiol and testosterone [99] as well as stress hormones of the Hypothalamic Pituitary Adrenal (HPA) axis such as Corticotrophin Releasing Factor (CRF), Adrenocorticotropic Hormone (ACTH), and glucocorticoids [80; 132]. Interestingly, antisense knockdown of spinal β2 adrenergic receptors attenuates CIPN in female but not male rats, while the inverse is observed following knockdown of spinal glucocorticoid receptors [69]. Following early life stress, female rats exhibit increased central amygdala CRF-mediated visceral pain hypersensitivity [184] and augmented expression of hippocampal Tumor Necrosis Factor alpha (TNFα) and IL-6 concomitant with greater SNL-induced allodynia [32]. These findings indicate sex-specific dependence on stress mediators of the sympathetic nervous system and the HPA axis in addition to gonadal hormones.

Influence of genetics.

In a rodent model of lumbar L5 radiculopathy, female Sprague-Dawley and Long-Evans but not Holtzman rats developed more severe mechanical allodynia than their male counterparts [116]. These findings are corroborated by the observation that L5 spinal nerve transection (SNT) produced greater allodynia in female versus male Sprague-Dawley rats, but no significant sex difference in Holtzman rats [57]. Swim stress-induced analgesia (SIA) is greater in female Wistar and Spontaneously Hypertensive (SHR) but not Lewis rats [230]. In contrast, SIA is enhanced in male C57BL/6 and Swiss Webster mice compared with isogenic females [160]. Similarly, morphine antinociception also is greater in several strains of male rats and mice, as reviewed in depth [161]. While allodynia is expressed in both sexes of CD-1 mice, it is evident in male but not female C57BL/6 mice in the destabilization of the medial meniscus (DMM) model of knee osteoarthritis (OA) [139]. Of note, substrain differences of C57BL/6J versus C57BL/6N mice in nociceptive behaviors are found following IPLT formalin, but not with IPLT CFA or CCI models [28]. QTL mapping in a cross of these strains uncovered a difference between B6J versus B6N, with thermal nociception being more pronounced in males. These observations indicate that rodent strain also should be considered when drawing conclusions about sex differences in pain hypersensitivity.

4. Sex-dependent neuroimmune mechanisms of pain hypersensitivity

Tissue damage or infection initiates an immune response that can ultimately lead to a chronic pain state. Acute inflammation serves a dual purpose in that a wide variety of mediators are secreted to prevent the organism from incurring further injury and to recruit peripheral immune cells for containing and repairing the damage. Historically, these factors have been characterized as either maladaptive “pro-inflammatory” or beneficial “pro-resolving”, and are released sequentially to promote active healing. Our current understanding of pathogen- or damage associated molecular pattern-induced inflammatory responses may best be described as an organized progression of interactions between immune cells. Accordingly, the secretion of factors by each cell type influences the timing and destination of chemotaxis by another cell type [19; 29; 82]. Under typical

circumstances, pain remains acute as the injury is repaired and inflammation is resolved, allowing the organism to resume homeostasis.

It is widely recognized that infiltrating as well as resident immune cells likely contribute to the transition from acute to chronic pain in instances where either the damage cannot be repaired or dysregulated inflammatory signaling continues even after the injury is resolved (see Figure 3 for definitions of immune cell types) [13]. For example, infiltrating neutrophils, macrophages and T-lymphocytes as well as activated Schwann cells and satellite cells secrete factors to communicate with resident astrocytes, microglia and oligodendrocytes in the CNS to release mediators that sensitize nociceptors. These processes in turn trigger adjacent glia and neurons to drive maintenance of hyperalgesia and allodynia [31; 98; 148; 155; 198; 200; 231; 247]. Among the molecules contributing to central sensitization are neurotransmitters (glutamate, ATP), peptide signals (cytokines, chemokines, neuropeptides), and bioactive lipids generated from cyclooxygenases (COX-1/2), 12/15-Lipoxygenases (12/15-LOX), and endocannabinoid system enzymes [87; 88; 108; 138; 236; 237; 241].

Emerging evidence supports a profound role for sex-specific immune responses that may underlie disparities in incidence of pain and other neurological disorders [18; 60; 62; 141; 186; 191; 208]. Due to specific challenges unique to each sex, the male and female immune systems have different requirements, with the female immune system specifically requiring the flexibility to allow for pregnancy without attacking the fetus or sperm required for procreation [187]. Consequently, females have larger populations of most immune cells, higher levels of immunoglobulins, and exhibit stronger responses to infection [110; 168]. Conversely in males, the Y chromosome contains multiple genes involved in epigenetic regulation of the immune system and susceptibility to autoimmune diseases [37]. While some neuroimmune interactions underly nociceptive processing in both males and females, some pain states exhibit clear sex-specific mechanisms that likely affect their responsiveness to current analgesics and adjuvant therapeutics [9].

Male-specific nociceptive mechanisms.

Chronification of pain states in males is believed to be facilitated largely by the innate immune system through neutrophil recruitment to the injury site [197] and to the spinal vasculature [156], along with CNS infiltration of monocytes and activation of microglial-neuronal crosstalk via several mechanisms [179] (Table 2). While significant spinal microgliosis is evident in both sexes of rodents following injury [3; 38; 206; 242], allodynia in males is believed to be mediated by several mechanisms including, but not limited to: stimulation of purinergic P2X4 receptors [142; 224] likely on CX3CR1- (Fractalkine receptor)-positive microglia [224; 248], phosphorylation of P38 Mitogen-Activated Protein (MAP) Kinase [100; 137; 165; 216] and release of cytokines such as Brain-Derived Neurotrophic Factor (BDNF) either from spinal microglia [206] or Dorsal Root Ganglion (DRG) nociceptors [166], acting on Tropomysin receptor kinase B (TrkB) receptors in spinal dorsal horn neurons. In a model of pain chronification, hyperalgesic priming with IT BDNF or IPLT Interleukin 6 (IL-6) is mediated by activation of spinal Dopamine D5 receptors (DRD5) in male but not female spinal neurons [149]. Furthermore, the NOD-like Receptor 3

(NLRP3) inflammasome drives IL-1β release likely from non-neuronal cells, leading to subsequent activation of neuronal Transient Receptor Potential Ankyrin 1 (TRPA1) in males but not females in a postoperative pain model of paw incision [50]. Likewise, in CCI or SNT paradigms of neuropathic pain, the cytokine TNFα mediates allodynia via spinal TNF Receptor 1 (TNFR1) only in male mice despite similar expression of allodynia in both sexes [56; 211]. TNFα and IL-1β are unchanged supraspinally in anterior cingulate cortex (ACC) following common peroneal nerve injury, indicating potential local release and site-specific involvement of these mediators. However, these studies were performed in a mixed-sex cohort of mice [135].

Thus, it is important to consider that male-specific involvement of macrophages and neuroimmune mediators in allodynia is likely dependent on the paradigm utilized, the activation of specific circuits (spinal versus supraspinal), hormone status, or be influenced by other factors such as age [94; 95; 129; 134] and strain [152; 202]. Injury-induced activation of Toll-Like Receptor 4 (TLR4) is a prominent example of this controversy. IT delivery of LPS or endogenous ligands (*e.g.*, High Mobility Group Box 1, HMGB1) and models of Peripheral neuropathy or RA elicit spinal TLR4-dependent allodynia that in some, but not all, cases is more pronounced in males than in females [2; 195; 205; 206; 240; 242; 243]. The observed reduction in responsivity of females to spinal TLR4 activation appears to be dependent on estrogen, as ovariectomy in conjunction with testosterone replacement restores expression of TLR4-mediated allodynia in CD-1 female mice to levels comparable to that observed in intact males [205]. Estrogen also attenuates LPS-induced inflammatory signaling and prevents expression of the proinflammatory phenotype of microglia during development [229; 232; 233]. Interestingly, the sex difference observed in spinal TLR4-mediated allodynia is absent when LPS is administered either at supraspinal (intracerebroventricular, ICV) or peripheral (IPLT) sites in uninjured CD-1 mice [205], or intramuscularly (IM) in a model of non-inflammatory acidic saline-induced muscle hyperalgesia [83]. In addition, systemic delivery of LPS produces pain hypersensitivity in both male and female Sprague-Dawley rats as neonates and as adults [21], correlating with decreased expression of *Oprm1* encoding MOR in the Periaqueductal Gray (PAG) [246] and IL-1β mRNA in the spinal cord, ventrolateral PAG and hippocampus [182]. These observations are consistent with the finding that intra-PAG LPS in rats significantly decreases morphine antinociception in both sexes [61]. Similarly, in both sexes of C57BL/6 mice, IPLT formalin-induced delayed tactile hypersensitivity is prevented by global deletion of TLR4 [243], and spinal blockade of HMGB1 reverses CAIA-induced mechanical allodynia [2].

Furthermore, in some paradigms, crosstalk between macrophages and sensory neurons contributes to allodynia in both sexes (Table 3). For example, IPLT Angiotensin II activates its receptors (AT2R) in peripheral Iba1(+) leukocytes, leading to TRPA1 transactivation in nociceptors concurrent with pain hypersensitivity in males and females [199]. IT delivery of BDNF elicits allodynia in both sexes of CD-1 mice [143], while IL-6 contributes to enhanced hyperalgesia in males and females following muscle injury [83] and peripheral inflammation [83; 217]. In a model of hyperalgesic priming for migraine, intracisternal (IC) IL-6-induced dural inflammation is BDNF-dependent in both male and female Sprague-Dawley rats [30]. K/BxN arthritis elicits time-dependent increases in spinal and circulating

TNFα in males and females, and IPLT delivery of TNFα produces spinal Transient Receptor Potential Vanilloid 1- (TRPV1)-dependent allodynia in both sexes [22; 66].

Female-specific nociceptive mechanisms.

Sustained allodynia in females is thought to derive in part from the adaptive immune system via activation and infiltration of Cluster of Differentiation 4 (CD4)+ T-lymphocytes to either central [206] or peripheral sites following nerve injury [91; 133] (Table 2). Interestingly, intra-sciatic (IS) injection of MBP(84–104) elicits T-cell migration to the DRG and spinal cord concurrent with tactile allodynia in female but not in male mice, in which T cells remain localized to the sciatic nerve [40]. Voluntary wheel running attenuates EAE-induced allodynia, correlating with reduced release of inflammatory cytokines from myelin-reactive T cells and attenuated DRG neuron excitability in female but not in male mice [154]. However, a female-specific role of the adaptive immune system remains to be clarified and may be paradigm- or strain-dependent. Several investigators have demonstrated that infiltrating CD4+ T-cells also contribute to tactile hypersensitivity following SNT or SNI in male Balb/c or C57BL/6 mice and Sprague-Dawley rats, respectively [34–36; 44; 49]. CD4+ T-cells mediate reduced formalin-mediated nociceptive sensitivity and increased morphine analgesia in male compared with female CD-1 mice [193]. In addition, T regulatory cells (Tregs) are essential for recovery from CCI-induced tactile allodynia via TNFα Receptor 2 (TNFR2) in both sexes [76].

Alternatively, other immune cells may be involved in female-specific mechanisms of neuropathic pain, as the number of mast cells is increased in lumbar spinal dura mater during Intradermal (ID) Capsaicin- or IPLT Carrageenan-induced inflammation [244] as well as on the side of the thalamus receiving nociceptive input following SNL [212], concurrent with allodynia in female but not in male rodents. Mast cells also mediate ID nitroglycerin-induced hyperalgesia, which is more pronounced in female rats [71]. However, paw incision- or CFA-induced activation of the mast cell receptor Mas-Related G Protein-Coupled Receptor b2 (Mrgprb2) elicits inflammation and pain hypersensitivity that is not different between male and female mice [85], so a female-specific involvement of mast cells may depend on the pain model utilized.

Microglia are believed to drive allodynia predominantly in males, yet several reports suggest that females can switch to a microglia-dependent pathway in some models of pain hypersensitivity when adaptive immune mechanisms are suppressed [89; 205; 206]. For example, microglial P2X7 is activated in females during IA Carrageenan- or Collagen-Induced Arthritis (CIA) [173; 218] yet not following IPLT CFA monoarthritis or pSNL- or SNI-induced nerve injury [41; 54]. Female-specific progesterone-dependent upregulation of Neuregulin-1 (NRG-1) in astrocytes has been observed in a model of experimental L5 lumbar radiculopathy, while exogenous spinal delivery of NRG-1 produces allodynia in both sexes [118; 119]. Mice heterozygous for NRG-1 express sex-specific reductions in serum cytokines in conjunction with increased hotplate latency, including IL-6, IL-8 and IL-10 in females and IL-1β in males [58]. However, IL-6 may also exert a female-specific protective effect, as female IL-6 deficient mice experience increased autotomy behavior following nerve injury [245]. Other mechanisms of nociception in females include inflammation-

induced activation of CNS DRD3 [130] or DRD1 receptors [149], Gamma Aminobutyric Acid Receptor subtype A ($GABA_A$) in the PAG [222] or spinal cord [78], and Prolactin receptors (PRLR) in sensory neurons [39; 178].

5. Future directions

Chronic pain affects up to 33% of the population and surpasses cancer, diabetes and heart disease in terms of societal burden [59]. Management of persistent pain is largely an exercise of trial and error, and the scarcity of viable treatment options places undue burden on the patient [183]. There is a considerable body of evidence demonstrating that an interaction between the nervous and immune systems underlies many pain syndromes at the molecular and cellular level. Since immune cells play a major role in the development of mood disorders [151], it is likely that they also may contribute to the averse emotional experience of pain. Most clinically relevant pain states have a tonic component that is not captured by standard evoked paradigms, so continued incorporation of spontaneous and functional measures of pain behaviors in preclinical studies will be critical for a deeper understanding of sex-related differences in chronic pain states and the future development of analgesics [109; 169; 171; 215]. Taken together, studies suggest that neuroimmune signaling events altered by injury, disease, or aberrant central nociceptive processing may serve as a rich resource of novel druggable targets and of predictive biomarkers suitable for patient stratification in trials [55] examining efficacy of potential pain therapeutics.

Given the challenges of developing safe and effective drugs reaching the CNS [201], one potential therapeutic avenue is to neutralize release of cytokines and chemokines released from circulating leukocytes and/or to intercept these cells before they cross the blood-brain barrier (BBB). Accordingly, the development of monoclonal antibody-based interventions targeting immune system mediators for the treatment of cancer and autoimmune diseases has grown exponentially over the past decade [136]. As our understanding of the role of specific immune cells in the development and maintenance of central sensitization continues to evolve, some of the newer-generation FDA-approved biologics may be repurposed for treating chronic pain either of peripheral origin via systemic delivery, or by routes of administration that bypass the BBB (*e.g.* intranasal or intrathecal). Alternatively, humanized single-domain antibodies could be harnessed as therapeutics, diagnostic agents or as delivery devices of small molecules targeting specific leukocytes owing to their stability, small size and low production cost [16]. Nonetheless, small molecules still lead the field in percent of FDA approvals in spite of challenges encountered with safety and tolerability profiles [167]. Ultimately, it is imperative for more preclinical and clinical pain studies to draw direct comparisons between males and females. Including sex as a biological variable will allow experts both to better predict which therapeutic strategies may be effective in each sex and to achieve true progress in the discovery of novel non-opioid analgesics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AT2R	Angiotensin 2 Receptor
BDNF	Brain-Derived Neurotrophic Factor
CGRP	Calcitonin Gene-Related Peptide
S100A8	Calgranulin
S100A9	Calgranulin B
CIPN	Chemotherapy-induced Peripheral Neuropathy
CCI	Chronic Constriction Injury
Cd11b	Cluster of Differentiation 11b
CD14	Cluster of Differentiation 14
CD2	Cluster of Differentiation 2
CD4	Cluster of Differentiation 4
CD40	Cluster of Differentiation 40
CD68	Cluster of Differentiation 68
CD8	Cluster of Differentiation 8
CAIA	Collagen Antibody-Induced Arthritis
CFA	Complete Freund’s Adjuvant
CRPS	Complex Regional Pain Syndrome
COX2	Cyclooxygenase 2
DMM	Destabilization of Medial Meniscus
dsHMGB1	disulfide High Mobility Group Box 1
DRD1	Dopamine Receptor D1
DRD3	Dopamine Receptor D3
DRD5	Dopamine Receptor D5
DRG	Dorsal Root Ganglion

ErbB	Epidermal Growth Factor receptor tyrosine-protein kinase erbB-2
ErbB4	Erb-B2 Receptor Tyrosine Kinase 4
EAE	Experimental Autoimmune Encephalomyelitis
CX3CR1	Fractalkine Receptor
$GABA_A$	Gamma Amino Butyric Acid receptor subtype A
Oprm1	Mu Opioid Receptor gene
GFAP	Glial Fibrillary Acidic Protein
CXCL1 = Groα	Growth Related Oncogene alpha
HMGB1	High Mobility Group Box 1
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IA	Intra-Articular
IC	Intracisternal
ICV	Intracerebroventricular
ID	Intradermal
IM	Intramuscular
IP	Intraperitoneal
IPLT	Intraplantar
IT	Intrathecal
LPS	Lipopolysaccharide
Ly6G	Lymphocyte antigen 6 complex locus G6D
LPA	Lysophosphatidic Acid
CXCL2	MIP2α: Macrophage Inflammatory Protein 2 alpha
MAPK	Mitogen-Activated Protein Kinase
Mrgbr2	Mas-Related G protein-Coupled Receptor B2
MCP1 = CCL2	Monocyte Chemoattractant Protein 1
MBP	Myelin Basic Protein
NRG-1	Neuregulin-1

NK1	Neurokinin receptor 1
NMDAR1	N-methyl D-Aspartate Receptor 1
NLRP3	Nod-Like Receptor Protein 3
P2X2	P2X purinoceptor 2
P2X3	P2X purinoceptor 3
P2X4	P2X purinoceptor 4
P2X7	P2X purinoceptor 7
pSNL	partial Sciatic Nerve Ligation
PAG	Periaqueductal Gray
KCC2	Potassium-chloride transporter member 5
PFC	Prefrontal Cortex
PRLR	Prolactin Receptor
RAMP1	Receptor Activity Modification Protein 1
SNP	Sodium Nitroprusside
SNI	Spared Nerve Injury
SNL	Spinal Nerve Ligation
SNT	Spinal Nerve Transection
TAC1	Tachykinin Precursor 1
TLR4	Toll-Like Receptor 4
TRPA1	Transient Receptor Potential Ankyrin 1
TRPV1	Transient Receptor Potential Vanilloid 1
TNFα	Tumor Necrosis Factor alpha
TNFR1	Tumor Necrosis Factor Receptor 1
TNFR2	Tumor Necrosis Factor Receptor 2

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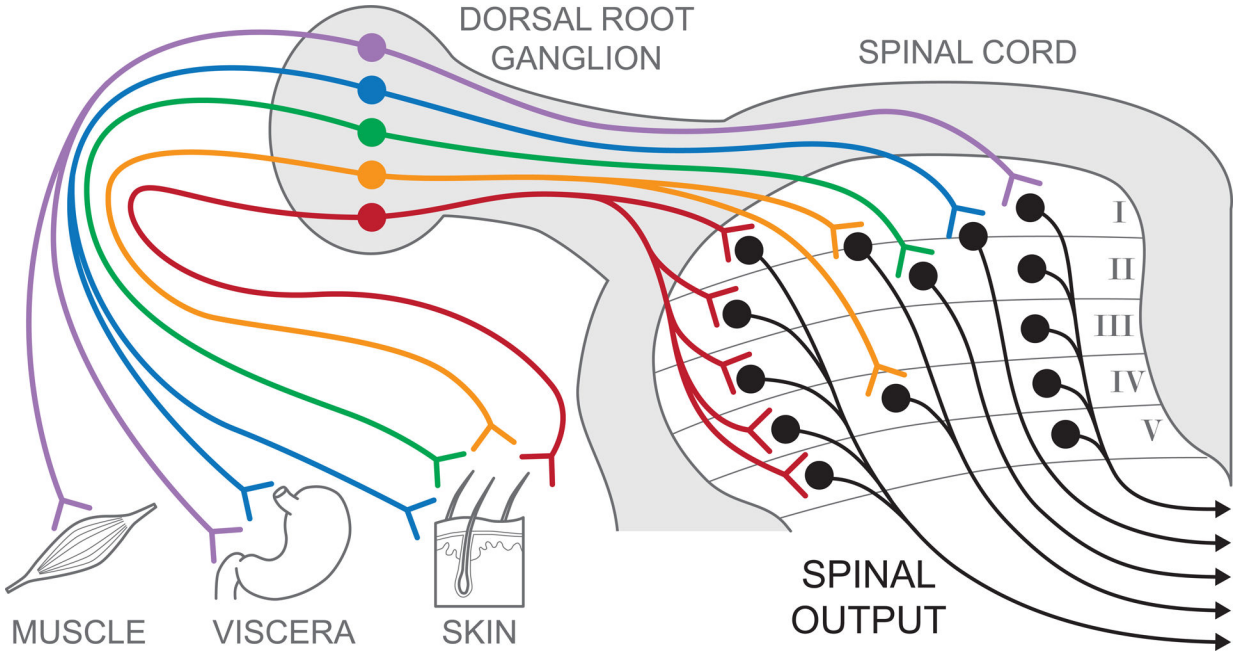

Figure 1. Nociceptive sensory primary afferent pathways.

Nociceptors specialized for detection of high-intensity mechanical, thermal and/or chemical stimuli originate in the sensory ganglia (dorsal root, trigeminal and nodose) of the peripheral nervous system generally possess small- to medium-diameter, thinly myelinated **Aδ fibers** or small-diameter unmyelinated **C fibers** and terminate predominantly in spinal superficial laminae I, II and V of the dorsal horn [180; 238]. Nociceptors can be described using the following categories: **Red,** Neurofilament H (NFH)+ **Aβ** large, high threshold mechanoreceptor (HTMR)/heat; **Orange,** peptidergic **Aδ** small/medium, HTMR/heat; **Green,** nonpeptidergic **C** small, HTMR/itch/chemical; **Blue,** peptidergic or nonpeptidergic **C** small polymodal or mechanoheat/cold; **Purple,** peptidergic **Aδ** small/medium HTMR or **C** small polymodal. In recent years, several subclassifications of nociceptors have been proposed, and the reader is directed to several excellent references for more detailed information on evolving designations of primary afferent sensory neuron subtypes: [48; 63; 120; 124; 125; 175; 220; 225].

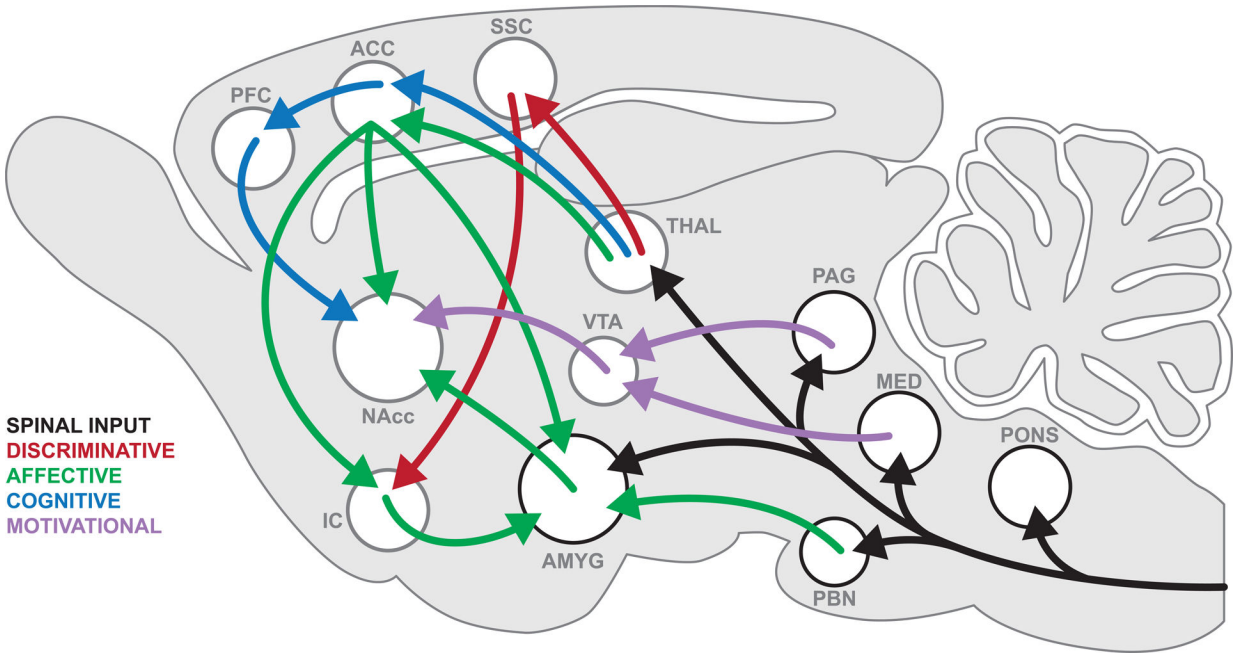

Figure 2. Supraspinal nociceptive circuits.

Nociceptive information transmitted via the **spinal cord dorsal horn** is communicated to the brain along several ascending pathways (merged together in black). Laterally projecting systems to the somatosensory (SSC) and insular cortices (IC) correspond to the classical somatosensory pathway, with a highly preserved body image mapped at several synaptic links. This tract mediates the **sensory/discriminative (red)** component of the pain phenotype. In contrast, medially projecting systems underlying the **affective (green)** and **motivational (purple)** aspects of pain have relatively crude somatosensory mapping and project to limbic structures appreciated for their roles in emotional responses such as the parabrachial nucleus (PBN), amygdala (AMYG), anterior cingulate cortex (ACC), nucleus accumbens (NAcc) and ventral tegmental area (VTA) [5; 33; 48; 150]. **Cognitive (blue)** interpretation of nociceptive information is mediated by the ACC, prefrontal cortex (PFC), and NAcc. Together, these structures contribute to pain processing by integrating information about its sensory, cognitive and affective/motivational components. The activity of ascending pathways is in turn regulated by descending facilitatory and inhibitory systems, which send projections down to the spinal cord mainly from the ACC or periaqueductal gray (PAG) by way of serotonergic neurons of the nucleus raphe magnus in the rostroventral medulla (MED) or via noradrenergic neurons in the locus coeruleus to modulate excitability of dorsal horn neurons [176].

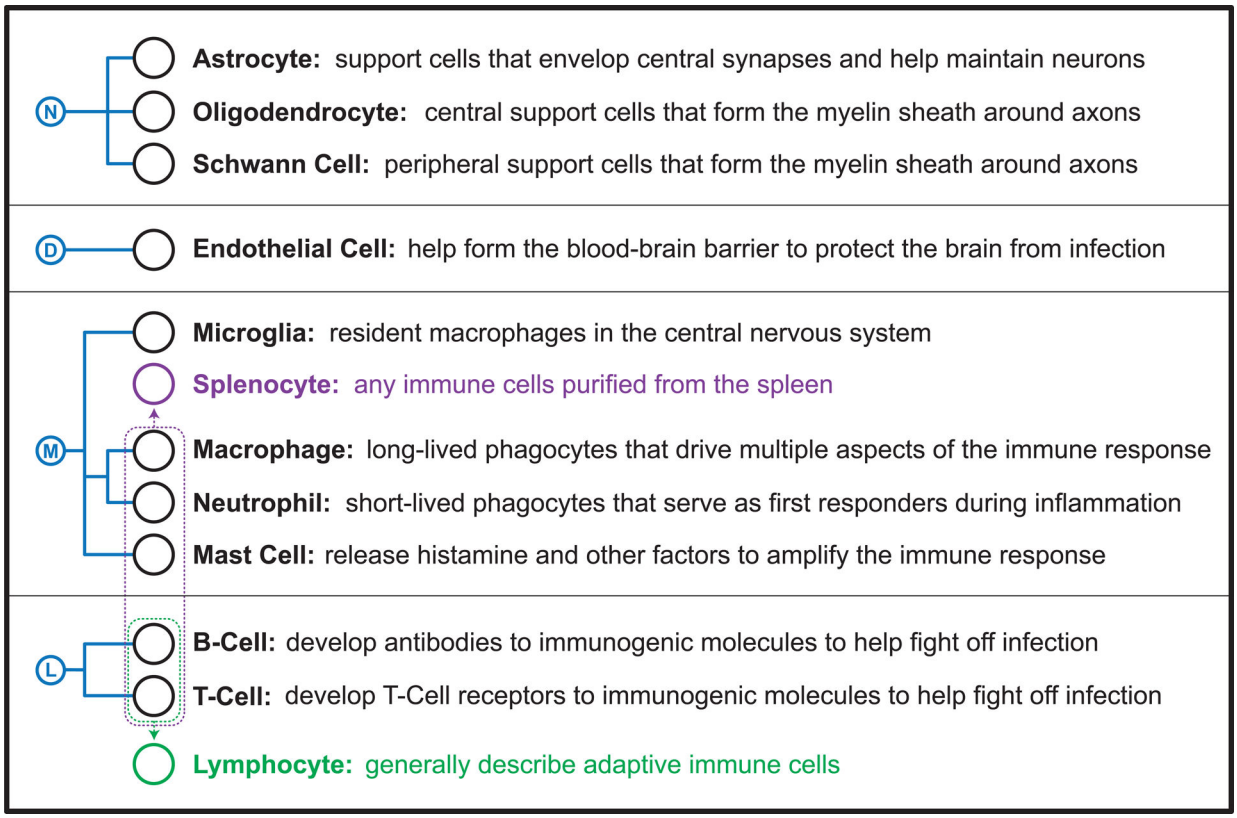

Figure 3. Immune and glial cells linked to sex differences in pain hypersensitivity.

Neuroimmune cells are generally derived from four different progenitor stem cell types (**Blue circles**): N, Neuroepithelial [112]; D, Mesodermal [72]; M, Myeloid [7]; L, Lymphoid [6]. Neuroepithelial cells can differentiate into Astrocytes [203; 204], Oligodendrocytes [190], or Schwann Cells [163]. Mesodermal cells can be programmed into Endothelial Cells [144]. Myeloid-derived cells include Microglia [128], Macrophages [123], Neutrophils [172], or Mast Cells [113] and are referred to as Splenocytes [127] (circled in **purple**). Lymphoid cells differentiate into B-Cells [4] and T-Cells [65], and collectively these cells are referred to as Lymphocytes (circled in **green**).

Table 1.

Rodent models exhibit sex-dependent differences in neuroimmune-mediated pain hypersensitivity

Sex	Model	Pain / Nociception	Rodent	REF
Females	Nerve Injury (SNI)	Tactile allodynia, withdrawal frequency	C57BL/6 mice	24
	Nerve Injury (SNL)	Tactile and cold allodynia	C57BL/6, SWR/J mice; Wistar rats	32, 210, 212
	Nerve Injury (CCI)	Tactile allodynia	CD-1 mice	226, 227
	Nerve Injury (L5 radiculopathy)	Tactile allodynia	Sprague Dawley, Long Evans rats	116
	Nerve injury (SNT)	Tactile allodynia	Sprague Dawley rats	57
	Nerve injury (pSNL)	Tactile allodynia	Sprague Dawley rats	51
	Systemic bacteremia (IP LPS)	Grip force	SWR/J mice	106
	Spinal TRL4 activation (IT LPS)	Grip force	SWR/J mice	106
	Serum-transfer arthritis (K/BxN)	Grip force	C57BL/6 mice	22
	Complete Freund’s Adjuvant (CFA)	Tactile allodynia	Lewis rats	46
	Fatigue-enhanced muscle insult	Muscle hyperalgesia	C57BL/6 mice	86
	Joint neuropathy (IA LPA)	Tactile allodynia	Wistar rats	174
	Femoral Bone Cancer	Limb use	Balb/c mice	64
	Complex Regional Pain Syndrome (CRPS)	Tactile allodynia	C57BL/6 mice	213
	Migraine (IC CGRP)	Tactile allodynia, Grimace	CD-1 mice; Sprague Dawley rats;	10
	Multiple Sclerosis (EAE)	Tactile and cold allodynia	C57BL/6 mice	185
	Autoimmune Demyelination	Tactile allodynia	C57BL/6 mice	40
Males	Complete Freund’s Adjuvant (CFA)	Tactile allodynia	Sprague Dawley rats	26
	Nerve injury (SNI)	Tactile allodynia	CD-1, C57BL/6 mice	97,205,206
	Context-dependent pain hypersensitivity	Tactile allodynia	CD-1 mice	145,207
	Osteoarthritis (DMM)	Tactile allodynia	CD-1, C57BL/6 mice	139
	Spinal TLR4 activation (IT LPS)	Tactile allodynia	CD-1, C57BL/6 mice	205,206,243
	Serum-transfer arthritis (K/BxN)	Tactile allodynia	C57BL/6J mice	242

Table 2.

Sex-dependent cellular and molecular neuroimmune mechanisms driving pain hypersensitivity

Sex	Model	Cell Type(s)	Mediator	Rodent	REF
Females	Nerve injury (CCI)	Schwann cells	MHC-II activation of helper Th-cells	129/FVB mice	91
		Microglia, Astrocytes	Phospho-P38 MAPK in spinal cord	CD-1 mice	226,227
	Nerve injury (SNI)	T-cells	CD4+ and CD8+ T-cell infiltration into lumbar spinal dorsal horn	CD-1 mice	206
	Nerve injury (SNL)	Mast cells	Increased mast cell infiltration into lumbar spinal dorsal horn	SWR/J mice	212
	Nerve Injury (pSNL)	T-cells	Not specified; T-cell infiltration into DRG	Sprague Dawley rats	49,133
	Nerve Injury (L5 radiculopathy)	Not specified	Spinal NRG1, ErbB4, and TAC1; Progesterone	Sprague Dawley rats	118,119
	Femoral bone cancer	Microglia; Not specified	TLR4, CD11b, CD14; Not specified	Balb/c mice; Sprague Dawley rats	64,121
	Migraine (IC nitroglycerin)	Mast cells, Endothelial cells	P2X3	Sprague Dawley rats	71
	Migraine (IC CGRP with priming)	Not specified	RAMP1, CLR, RCP	ICR mice; Sprague Dawley rats	10
	Fatigue-enhanced muscle insult	Lymphocytes	Lymphocyte migration to muscle	C57BL/6 mice	86
	Autoimmune Demyelination (IA MBP)	T-cells	T-cell migration into DRG and spinal cord; PLC in females	C57BL/6 mice	40
Males	Nerve injury (SNT)	Microglia	CD40+ spinal microglia interaction with infiltrating CD4+ T-cells	Balb/c mice	34,36
	Nerve injury (SNI)	T-cells	CD2+ cell migration into ipsilateral spinal dorsal horn; Th1-mediated	Sprague Dawley rats	49
			CD4+/CD8+ cell infiltration into ipsilateral spinal cord	C57BL/6 mice	44
		Neurons	TRPV1	C57BL/6 mice	44
		Microglia, Macrophages	TLR4, P2X4, BDNF, Phospho-P38 MAPK	CD-1, C57BL/6 mice; Sprague Dawley rats	142,205,206
	Nerve injury (SNL)	Microglia, Neurons	P2X4, Phospho-P38 MAPK	Sprague Dawley, Wistar rats	100,224
	Oral cancer	Neutrophils	Ly6G+ neutrophil migration to tumor	C57BL6 mice	197
	IPLT carrageenan	Neutrophils; Not specified	S100A8+ and S100A9+ neutrophil migration to spinal vasculature; gonadal hormones	Sprague Dawley, Fischer 344 FBNF1 rats	156,214
	Spinal TLR4 activation (IT dsHMGB1)	Microglia, Macrophages	Spinal TNF, IL-1b, CCL2, CxCl, CxCl2, Gfap, CD11b, females recover faster	C57BL/6 mice	2,3
	Spinal TLR4 activation (IT LPS)	Not specified	Spinal TLR4	CD-1, C3H/HEK, C3H/HeN, C57BL/6J, C57BL/10ScNJ, C57BL/10ScSnj mice	205,243

Sex	Model	Cell Type(s)	Mediator	Rodent	REF
	Serum-transfer arthritis (K/BxN)	Glia	Spinal TLR4 and TNFa, spinal TRPV1	C57BL/6 mice	22,242
	Chemotherapy-induced Peripheral Neuropathy	Not specified	Spinal TLR4, females recover faster	C57BL/6 mice	240

Table 3.

Sex-independent cellular and molecular neuroimmune mechanisms driving pain hypersensitivity

Model	Cell Type(s)	Mediator	Rodent	REF
Nerve injury (SNT)	Spinal glia	Spinal soluble TNF and IL-1b	Holzman rats	211
	Not specified	IL-6; allodynia F = M; autotomy behavior F > M	C57BL/6 × 129/BV/SV mice	245
	Microglia	CXCR1+ microglia	C57BL/6 mice	179
		C40+ spinal microglia interaction with infiltrating CD4+ T-cells	Balb/c mice	34,36
	T-cells	Cd4+ T-cell infiltration into ipsilateral spinal cord	Balb/c mice	35
Nerve injury (CCI)	Microglia	Phospho-P38 MAPK in males	CD-1 mice; Sprague Dawley rats	216
		CXCR1+ microglia	CD-1 mice; Sprague Dawley rats	216, 248
		P2X4+ microglia	Sprague Dawley rats	142
		TNFR1-induced NMDA1 activation in spinal cord, cortex	C57BL/6 mice	56
	Macrophages	CD68, Cd11b+ macrophages; tactile allodynia F = M	C57BL/6 mice	44
	T-cells	TNFR2 essential for recovery	C57BL/6 mice	76
Nerve injury (SNI)	Microglia	P2X7	Sprague Dawley rats	54
Nerve injury (pSNL)	Not specified	P2X7	C57BL/6 mice	41
Postoperative pain	DRG nneurons	PRLR in females	C57BL/6.129 mice; Sprague Dawley rats	178
	Microglia	Phospho-P38 MAPK in males	Sprague Dawley rats	165
	Macrophage, Mast cells, Neutrophils,	NLRP3-dependent IL-1b release in DRG and peri-incisional skin in males; NLRP3-independent in females	C57BL/6j, C57BL/6N/129 mice	50
	Mast cells	Mrgprb2	C57BL/6 mice	85
Complex regional pain syndrome (CRPS)	Microglia (males), T-cells (females), B-cells (both)	Spinal IL-6, NK1 in males; delayed adaptive immune response in females	C57BL/6J mice	89
IPLT Zymosan	Not specified	TLR2	CD-1 mice	205
IPLT CFA	Neurons	High-affinity tonic GABAA current in PAG	Sprague Dawley rats	222
	Not specified	Spinal TLR4, serum, TNFa, IL-1b, and IL-6 in males	CD-1 mice; Sprague Dawley rats	26,205
IPLT Formalin	Glia, Neurons (males); T-cells (females)	DRD1, DRD3 in females; DRD4 in males; BDNF, TLR4 in both sexes	CD-1, C57BL/6J, C57BL/6/129.P2 mice	130,149,193 202,243
Muscle Hyperalgesia	Macrophages	P2X4+ resident macrophages, TLR4, IL-6	C57BL/6 mice	83

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Model	Cell Type(s)	Mediator	Rodent	REF
IPLT TNFa	Not specified	Peripheral TRPA1, central TRPV1; mixed males and females	C57BL/6J.129.SvJ, CD-1 mice	66
IPLT Angiogensin II	Macrophages, Neurons	AT2R in skin macrophages transactivates TRPA1 in neurons	C57BL/6J, FVB/NJ mice	199
Hyperalgesic Priming	Neurons	Spinal DRD5 in males; DRG PRLR in females	C57BL/6J.129, C57BL/6J mice	149,178
IT BDNF	Microglia	Spinal KCC2 downregulation	CD-1 mice	143
IT NRG-1	Astrocytes, Neurons	Spinal ErbB	Sprague Dawley rats	118
IT LPS	Not specified	Spinal TLR4	SWR/J mice	106
ICV LPS	Not specified	TLR4	CD-1 mice	205
Intra-PAG LPS	Microglia	TLR4	Sprague Dawley rats	61
IPLT LPS	Not specified	TLR4	CD-1 mice	205
Systemic Bacteremia (IP LPS, neonatal)	Microglia, Not specified	Spinal TLR4, Spinal COX2; Downregulation of Oprm1 in PAG, PFC	Sprague Dawley rats	21,95,246
Migraine (Intracisternal IL-6)	Not specified	BDNF	Sprague Dawley rats	30
IA Carrageenan	Immune cells (in joint)	P2X2 and P2X3 in both sexes; P2X7 in females	Wistar rats	217,218
IA CFA	Not specified	TRPA1, TRPV1, TNFa	CD-1, C57BL/6J mice	66
Collagen antibody-induced arthritis (CAIA)	Glia, Neurons (spinal); Macrophages	Macrophage HMGB1/TLR4 in males; Nociceptor TLR4 in females	Balb/c mice	2,67,195