# Chapter 6
# Sex Differences in Pain with Emphasis on Neuroimmune Interactions


**Xin Luo, Jasmine Ji, and Ru-Rong Ji**

**Abstract** To date, the mechanisms underlying how neuroimmune interactions contribute to sex dimorphism of chronic pain remain elusive. Although women suffer from chronic pain at greater rates than men, the current mechanistic understanding of chronic pain has been predominantly derived from the study of male animals. As such, a greater emphasis will be needed to investigate female-specific signaling mechanisms in chronic pain. These efforts will improve our understanding of sex dimorphism in chronic pain and improve the ability of pain medicine address specific patient backgrounds in the future. In this chapter, we will discuss pain-related sex differences in neural, immune, and glial mechanisms, with a focus on sex dimorphism in neuroimmune interactions.

**Keywords** Females · IL-17 · IL-23 · Macrophages · Males · Microglia · Sex dimorphism · Spinal cord · T cells · TRPV1

X. Luo (✉)
Center for Translational Pain Medicine, Department of Anesthesiology, Duke University, Durham, NC, USA

Guangdong-Hong Kong-Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence, Southern Medical University, Guangzhou, China

J. Ji
Wellesley College, Boston, MA, USA

R.-R. Ji
Center for Translational Pain Medicine, Department of Anesthesiology, Duke University, Durham, NC, USA

Departments of Cell Biology and Neurobiology, Duke University, Durham, NC, USA



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## 6.1 Introduction

Since the introduction of the National Institutes of Health Revitalization Act in 1993 clinical studies have been required to include female participants. However, no such requirement currently exists for preclinical research, due in part to perceived complications of hormone fluctuations related to menstruation in female animals, and in part due to the belief that results seen in male animals could be generalized across sexes (Mogil 2021; Navratilova et al. 2021; Sorge and Totsch 2017). Such biases are particularly pronounced in neuroscience, where nearly five times as many male single-sex studies have been conducted versus female single-sex studies (Beery and Zucker 2011). This discrepancy in research design poses a significant problem in chronic pain studies, as clinical statistics indicate that women are more likely to suffer from chronic pain conditions, experience more severe levels of pain, and have weaker responses to medical treatment for pain when compared with men. Clinical studies by research teams from around the world, including from the United States (Dahlhamer et al. 2016), Canada (Shupler et al. 2019), the European Union (Langley 2011), and China (Wu et al. 2019) have recognized that sex dimorphism present in chronic pain. Women are at higher risk for chronic pain conditions such as neuropathic pain, chronic fatigue syndrome, interstitial cystitis, and fibromyalgia (Fillingim et al. 2009; Maixner and Humphrey 1993; Navratilova et al. 2021). In addition, women with chronic pain conditions often report pain that is more severe, frequent, and longer lasting than men with the same conditions, and may also suffer from more chronic pain-related disability than men (Unruh 1996). Moreover, some studies have also suggested that women may not experience as much opioid-induced pain relief as men do, though further research is necessary to confirm these results (Fillingim et al. 2009).

In any case, the evidence of sex dimorphism in chronic pain is abundant, yet the mechanisms underlying this phenomenon remain largely unclear, which has been a major impediment toward translating research findings and increasing failure rates in clinical trials (Mogil 2012). Recent studies suggest that chronic pain is not only controlled by neuronal sensitization in the peripheral sensitization (PNS) and central sensitization (CNS), but it is also driven by immune cells and glial cells (Chen et al. 2018a, b; Chiu et al. 2012; Grace et al. 2014; Inoue and Tsuda 2018; Ji et al. 2013, 2016, 2019; Salter and Stevens 2017). In this chapter, we will discuss pain-related sex differences in neurons, immune cells, and glial cells, with specific focus on the sex dimorphism in neuroimmune interactions.

## 6.2 Sexual Dimorphism in Neuronal Modulation of Pain

### *6.2.1 Sex Dimorphism in Capsaicin Response, TRPV1 Signaling, and CGRP Response*

Capsaicin receptor TRPV1 is specifically expressed by C-fiber nociceptors. Several reports suggest that females may show greater responses to capsaicin stimulation and TRPV1 activation versus males (Frot et al. 2004; Gazerani et al. 2005; Hartmann et al. 2015). Interestingly, estrogen (estradiol or E2) may not regulate *Tprv1* expression in sensory neurons (Diogenes et al. 2006), as no sex differences were found in the overall TRPV1 expression in mouse or human DRGs (Luo et al. 2021). RNAscope in situ hybridization revealed a significant sex difference in the size distribution of TRPV1 (or *Trpv1*)-positive neurons in mouse DRGs. As expected, we observed that TRPV1 is mainly expressed in small (cell area: 100–400 μm$^2$) and medium-sized neurons (400–600 μm$^2$) in mice of both sexes. However, while percentages in other size categories remained comparable, female DRGs showed a significantly greater percentage of TRPV1-positive neurons than males in the 100–200 μm$^2$ size category. While further research is required to identify the role of these neurons in female-dominant pain, it is possible that TRPV1-positive neurons in the 100–200 μm$^2$ size category in females may be regulated by estrogen, thus contributing to chronic pain in females. Furthermore, we found that optogenetic blue light stimulation of ChR2-expressing nociceptors in the hind paw of TRPV1-ChR2 transgenic mice, in which ChR2 is selectively expressed in TRPV1-expressing neurons, produces greater levels of pain behavior (indicated by the mouse licking and flinching in response to light stimulation) in females than males (Ji et al. 2021).

Additionally, estrogen was shown to regulate TRPV1 signaling. After a brief 10 min incubation, estrogen prevented TRPV1 desensitization in dissociated sensory neurons (Payrits et al. 2017). Estrogen was found to facilitate TRPV1 agonist-induced mechanical hyperalgesia in ovariectomized (OVX) mice, as well as ocular pain in OVX rats (Payrits et al. 2017; Yamagata et al. 2016). Furthermore, women were shown to have higher facial pain intensity and unpleasantness than men in response to topical capsaicin administration (Frot et al. 2004). Capsaicin was shown to induce mechanical pain via neurogenic inflammation that can release the neuropeptide calcitonin gene-related peptide (CGRP) (Warwick et al. 2019). It was found that intraplantar administration of a very low dose of capsaicin (50 ng) elicited mechanical pain only in female mice (Luo et al. 2021). Additionally, injection of a low dose of CGRP (1 pg) produced mechanical pain only in female rats as well (Avona et al. 2019).

### 6.2.2 *Prolactin (PRL) and Prolactin Receptor (PRLR)*

Prolactin (PRL) is a hormone produced in the anterior pituitary gland, and under inflammatory conditions, PRL may also be synthesized by cells outside of the pituitary gland. Prolactin was shown to modulate TRPV1 activity in female sensory neurons in an estrogen-dependent manner (Diogenes et al. 2006). PRL was also shown to regulate TRPA1 and TRPM8 in sensory neurons in a sex-dependent manner in inflammatory pain (Patil et al. 2013). Notably, prolactin produces female-specific pain signals via prolactin receptors expressed on nociceptors (Chen et al. 2020a, b, Patil et al. 2019). Prolactin receptors have two isoforms: a short isoform (PRLR-S) and a long isoform (PRLR-L). Homodimer signaling through PRLR-S heightens neuronal excitability and pain sensitization, while signaling through PRLR-L *protects* against the nociceptive effects of PRLR-S by interfering with PRLR-S signaling. Normally, PRLR-L expression is higher in female trigeminal ganglion neurons compared to males, but under pathological conditions, inverted PRLR-S upregulation and PRLR-L downregulation may result in heightened pain responses in females. For example, studies have shown that priming with hyperalgesia-promoting medications results in PRLR-L downregulation in female animals only. These prolactin signaling processes may therefore play an important role in stress-related pain conditions that are prevalent in females (e.g., chronic migraine). Females have higher serum prolactin concentrations compared to males, and stressful events (e.g., injury and trauma) further increase serum prolactin concentrations in females only. Studies have shown that priming with stressors can result in allodynia and PRLR-L downregulation in females, and inhibition of prolactin release was able to block stress-induced pain (Navratilova et al. 2021).

In addition, sex differences have also been shown in the regulation of nociceptor translatomes. In Nav1.8-positive neurons (presumably nociceptors), it was found that 66 genes whose messenger RNAs were sex differentially actively translated. Among the notable genes in the nociceptor translatome, prostaglandin PGD2 synthesizing enzyme (PTGDS) is enriched in female mouse DRG (Tavares-Ferreira et al. 2022).

## 6.3 Sex Differences in Glial and Immune Modulation of Pain

A growing body of research considers neuroimmune interactions an important biological cause for sex dimorphism in chronic pain (Chen et al. 2018b; Mogil 2012, 2020; Rosen et al. 2017; Sorge and Strath 2018). This section will focus on the current knowledge and research advancements concerning the roles of immune cells, including microglia, macrophages, T cells, B cells, and astrocytes in the sex dimorphism of chronic pain (Table 6.1).

**Table 6.1** Immune and glial cell signaling molecules with sex dimorphic functions in pathological pain

| Signaling pathway | Experimental approaches | Sex preference | Pain models | References |
|---|---|---|---|---|
| Microglia | | | | |
| p38 | Pharmacology | Male | CCI, HP | Taves et al. (2016), Luo et al. (2018), Paige et al. (2018) |
| TLR4 | KO, pharmacology | Male | SNI, CFA, CRPS | Sorge et al. (2011), Huck et al. (2021) |
| HMGB1 | Pharmacology | Male | | Agalave et al. (2021b) |
| Caspase-6 | KO, pharmacology | Male | Formalin | Chen et al. (2018a, b) |
| Mu opioid receptor | KO | Male | Morphine analgesia | Reiss et al. (2022) |
| PI3K/Akt | Pharmacology | Male | Incision | Xu et al. (2019) |
| $P2X_4R$ | KO, pharmacology | Male | SNI, CCI | Sorge et al. (2015), Paige et al. (2018) |
| BDNF | KO | Male | SNI | Sorge et al. (2015) |
| Macrophage | | | | |
| TLR9 | KO, pharmacology | Male | CIPN | Luo et al. (2019) |
| CSF1 | KO | Male | SNI | Yu et al. (2020) |
| HMGB1 | Pharmacology | Male | CAIA | Rudjito et al. (2021) |
| IL-23/ IL-17A | KO, pharmacology, electrophysiology, $Ca^{2+}$ imaging, Optogenetics | Female | CIPN, CCI, DN, Formalin | Luo et al. (2021), Ji et al. (2021) |
| T cells | | | | |
| PPARα | Pharmacology | Male | SNI | Sorge et al. (2015) |
| PPARγ | Pharmacology | Female | SNI | Sorge et al. (2015) |
| Astrocyte | | | | |
| eIF4E | KO | Male | CIPN | Agalave et al. (2021a) |

Abbreviations: *KO* knockout, *CCI* chronic constrictive injury, *HP* hyperalgesic priming, *SNI* spared nerve injury, *CFA* complete Freund's adjuvant, *CRPS* complex regional pain syndrome, *CIPN* chemotherapy-induced peripheral neuropathy, *CAIA* collagen antibody-induced arthritis, *DN* diabetic neuropathy

### *6.3.1 Sex Dimorphism in Microglial Signaling*

In multiple chronic pain models, microglial reactions, such as morphological changes, microgliosis, and microglial proliferation, exhibit no sex differences in the spinal cord. For example, spinal levels of $Iba1^+$ and/or $CX3CR1^+$ microglia increase in both male and female animals in neuropathic pain models of spared nerve injury (SNI) (Sorge et al. 2015) and chronic constrictive injury (CCI) (Taves et al. 2016). However, several lines of evidence have identified male-specific microglial function in multiple chronic pain conditions, using transgenic and pharmacological methods, including selective microglial activation, selective depletion of microglia, general

inhibition of microglial function with microglial inhibitors, and specific inhibition of microglial signaling pathways (Sorge et al. 2015).

Selective activation of spinal microglia by intrathecal clozapine-*N*-oxide (CNO), using a chemogenetic approach called designer receptor exclusively activated by a designer drug (DREADD), elicits mechanical allodynia in male but not female rats and mice (Grace et al. 2018; Saika et al. 2020). Moreover, CCI-induced allodynia was attenuated by intrathecal CNO in male rats intrathecally transfected with Gi (inhibitory) DREADDs (Grace et al. 2018).

Despite the estrous cycles in females, male and female rodents develop comparable baseline pain sensitivities under normal conditions and also exhibit comparable pain hypersensitivity under pathological conditions, showing no sex differences. However, intrathecal injections of microglial inhibitors (e.g., minocycline) produce male-specific antiallodynic effects in pathological pain models of spared nerve injury (SNI, Sorge et al. 2015), formalin-induced acute inflammatory pain (Chen et al. 2018a), CCI (Chen et al. 2018a; Mapplebeck et al. 2018; Taves et al. 2016), complex regional pain syndrome (CRPS) (Guo et al. 2019), and collagen antibody-induced arthritis (CAIA) (Fernandez-Zafra et al. 2019).

For instance, 7 days after inducing the SNI, minocycline, a nonselective inhibitor of microglia, was intrathecally injected into both male and female mice and their pain thresholds were tested over the next 2 h. It was found that while the minocycline dose-dependently reversed mechanical hypersensitivity in males, the inhibitor was actually ineffective in reversing mechanical hypersensitivity in females regardless of the dose. A similar experiment that tested inflammatory pain instead of neuropathic pain, yielded similar results (Sorge et al. 2015). However, because minocycline has other functions besides suppressing glial function at high doses, the researchers of this study carried out additional experiments to confirm that microglia are necessary to induce mechanical allodynia in males (Sorge et al. 2015). In one experiment, microglia in mice of both sexes were briefly depleted via an intrathecal injection of sporin toxin conjugated with macrophage antigen complex-1 (MAC1). Four hours after injection, microglial levels were similarly deleted in both males and females. This resulted in a noticeable reversal of mechanical allodynia in males, but once again, the treatment had no effect on female mechanical pain levels. The researchers soon found that $P2X_4$ receptor ($P2X_4R$), an ATP receptor induced in microglia after nerve injury, is also required for SNI-induced allodynia in male animals (Sorge et al. 2015).

In a mouse model of fibromyalgia, intramuscular injections of acidic saline into the gastrocnemius produce mechanical allodynia in both male and female mice. Notably, intracerebroventricular minocycline only reduces pain in male mice in the late-stage fibromyalgia, suggesting that brain microglia may contribute to fibromyalgia in a sex- and stage-dependent manner (Ueda et al. 2020). In both male and female adult mice, neonatal priming incisions can cause long-term alteration into adult life of somatosensory function and enhance the second injection-induced pain response, which is reversed by minocycline treatment in early life, but only in male animals (Moriarty et al. 2019).

Numerous studies indicate that several microglial signaling pathways including toll-like receptor 4 (TLR4), p38 MAP kinase, caspase-6, mu opioid receptor, phosphatidylinositol 3-kinase (PI3K)/Akt, $P2X_4$ receptor (P2X4R), and brain-derived neurotrophic factor (BDNF) contribute to chronic pain in a male-specific manner (Chen et al. 2018a, b; Mapplebeck et al. 2018; Sorge et al. 2015). Sorge et al. found that toll-like receptor 4 (TLR4), the activation of which results in inflammatory cytokine production, was involved in the production of mechanical allodynia in only males. Knowing that TLR4 is located on microglia, they suspected that microglia might be the reason for this sex difference. The group further suspected that perhaps microglia were not even necessary for the processing of pain in female mice (Sorge et al. 2011).

TLR4 recognizes lipopolysaccharide (LPS), one of the most potent activators of microglia (Xu et al. 2013). Intrathecal injection of LPS produces robust mechanical allodynia in male but not female mice, whereas systemic injection of LPS induces pain hypersensitivity in both sexes. Such male-selective pronociceptive effects of LPS are abolished by testosterone deficiency or *Tlr4* knockout. Concordantly, intrathecal injection of the TLR4 antagonist LPS-RS results in male-specific analgesia in mouse models of inflammatory pain (complete Freund's adjuvant, CFA) and SNI (Sorge et al. 2011). Using transgenic mice with inducible and selective depletion of the *Tlr4* gene in myeloid-lineage cells ($Cx3cr1^{Cre\text{-}ERT2\text{-}eYFP}/Tlr4^{fl/fl}$), the role of microglial TLR4 signaling was examined in the tibial fracture model of postoperative pain. *Tlr4* conditional knockout (cKO), induced before the tibial fracture surgery, alleviated mechanical allodynia and spontaneous pain only in male mice and not in female mice. However, *Tlr4* cKO, induced *after* surgery, partially reduced mechanical allodynia in both sexes. These findings suggest that microglial TLR4 may play an essential role in the transition from acute pain to chronic pain (Huck et al. 2021).

p38 is a member of the mitogen-activated protein kinases (MAPK) family and primarily expressed by microglia in the spinal dorsal horn, which contributes to pathological pain processing (Ji and Suter 2007). In the mouse CCI model, nerve injury induces more p38 activation (indicated by p38 phosphorylation) in the spinal cord in males, as opposed to $CX_3CR1^+$ microglia in females. Of note, intrathecal injection of the p38 inhibitor skepinone reduced CCI-induced mechanical allodynia only in male mice, whereas systemic or perineural injection of skepinone produced analgesia in both sexes, suggesting that the peripheral and central p38 pathways may have separate mechanisms (Taves et al. 2016). Intrathecal injection of p38α antisense oligonucleotides (ASO) causes a nearly 50% reduction in spinal p38α mRNA levels, which attenuates CCI-induced mechanical allodynia in male but not female animals (Luo et al. 2018). In the mouse hyperalgesic priming model, the p38 inhibitor skepinone prevented the development of persistent mechanical allodynia produced by interleukin-6R and prostaglandin $E_2$ (PGE2), but only in male animals (Paige et al. 2018).

Microglia-derived BDNF is crucial for intercellular communication between microglia and neurons in pain signaling (Coull et al. 2005). Selective depletion of *Bdnf* in CX3CR1$^+$ microglia ($Cx3cr1^{Cre\text{-}ER}/Bdnf^{fl/fl}$) does not affect microgliosis in

the spinal dorsal horn in male and female mice. However, *Bdnf* cKO attenuates SNI-induced mechanical allodynia only in male animals (Sorge et al. 2015).

High mobility group box 1 protein (HMGB1) is a pathogenic mediator of various diseases and injured states, and it is an endogenous agonist of TLR4. Intrathecal injection of disulfide HMBG1 increases spinal levels of Iba1$^{+}$ microglia and promotes mechanical allodynia in both male and female mice with no sex differences. Interestingly, selective depletion of *Tlr4* in microglia prevents disulfide HMBG1-induced mechanical allodynia only in male *LysM*$^{Cre}$*/TLr4*$^{fl/fl}$ mice (*Tlr4* cKO), suggesting that the mechanisms underlying disulfide HMBG1-mediated pain may be sex dimorphic (Agalave et al. 2021b).

Neuron-derived Caspase-6 can regulate microglial function in chronic pain (Berta et al. 2014). Intrathecal injection of caspase-6 produces mechanical allodynia in male but not female mice. Accordingly, the caspase-6 inhibitor ZVEID or *Caspase6*$^{-/-}$ knockout exhibits male-dominant analgesia against formalin-induced pain hypersensitivity (Berta et al. 2016).

P2X receptors are ligand-gated ion channels, which are activated by extracellular ATP. Microglial $P2X_4R$ drives pain hypersensitivity in pathological conditions (Beggs et al. 2012). In the mouse SNI model, surgery upregulates *P2rx4* gene expression in the spinal dorsal horn of male but not female mice. Intrathecal injection of the P2X4R inhibitor TNP-ATP produces analgesia only in male SNI-injured mice (Sorge et al. 2015). In the rat CCI model, surgery promotes $P2X_4R$ expression and function in spinal dorsal horn microglia of males but not females. Additionally, intrathecal injection of TNP-ATP alleviates mechanical allodynia only in male but not female CCI-injured rats. Moreover, adoptive transfer of ATP-stimulated primary cultured microglia from male but not female rats produced mechanical allodynia in male naïve rats (Mapplebeck et al. 2018). In the mouse hyperalgesic priming model, the P2X4R inhibitor TNP-ATP prevented persistent mechanical allodynia induced by the IL-6R/PGE2 pathway in male but not female mice (Paige et al. 2018).

The mu opioid receptor (MOR) is encoded by OPRM1 gene and mediates opioid-induced analgesia and side-effects. Using *Cx3cr1*$^{CRE}$*/egfp-Oprm1-mCherry* reporter mice, MOR was found to be expressed in about 40% of spinal microglia across male and female animals (Maduna et al. 2019). Intrathecal injection of minocycline improved morphine analgesia in male but not female rats (Posillico et al. 2015). Selective depletion of *Oprm1* in microglia (*Oprm1* cKO) alleviated morphine analgesic tolerance in the hot plate test in male but not female *Cx3cr1*$^{CRE}$*/Oprm1*$^{fl/fl}$ mice (Reiss et al. 2022).

Additionally, a recent transcriptional profiling study revealed 10 major subtypes of microglial cells (Masuda et al. 2019). It is unclear though, which microglia subtypes have sex-dimorphic roles in chronic pain. As microglial activation and microgliosis occur in spinal dorsal horn of both sexes in chronic pain (Sorge et al. 2015), we cannot exclude the possibility that some aspects microglial signaling may be female specific in pain processing, as has been demonstrated in males. Moreover, the male-selective role of microglia in pain may be site dependent.

Microglia may play a role in females under conditions not mentioned above. Female microglia have been shown to enhance neuropathic pain in mice and rats

after spinal cord injury and in rats with bone cancer (Chen et al. 2012; Hains and Waxman 2006; Yang et al. 2015). It is well known that females require more morphine than males for comparable levels of analgesia. A recent study reported that female mice exhibit greater microglial activation in the periaqueductal gray (PAG) than male mice under naïve and LPS-priming conditions. Intra-PAG inhibition of microglial activation by minocycline can reverse such sex dimorphism in morphine analgesia (Doyle et al. 2017).

### *6.3.2 Sex Dimorphism in Macrophage Signaling*

Macrophages overall do not exhibit sex-dimorphic changes in response to chronic pain. In the chemotherapy-induced peripheral neuropathy (CIPN) model, the chemotherapy agent paclitaxel increases DRG levels of F4/80$^{+}$ macrophages in both male and female mice (Luo et al. 2019). Using the macrophage Fas-induced apoptosis (MAFIA) transgenic mouse line, the Basbaum Lab showed that systematic administration of the apoptosis inducer AP20187 causes a significant loss of CX3CR1$^{+}$ macrophages in lumbar DRGs but not in spinal cord microglia. Of note, macrophage depletion attenuates SNI-induced mechanical allodynia in both male and female mice (Yu et al. 2020).

Emerging studies indicate that male and female macrophages may utilize signaling distinct from microglia to modulate chronic pain. In macrophages of male animals, signaling through Toll-like receptor (TLR9), colony stimulating factor-1 (CSF1) and HMGB1 (Luo et al. 2019; Yu et al. 2020) play major roles in chronic pain, and in female animals the IL-23/IL-17A axis has a particularly important function (Luo et al. 2021).

Neuronal colony-stimulating factor 1 (CSF1) can regulate macrophage function through its receptor CSF1R (Chitu et al. 2016). Depletion of *Csf1* in DRG sensory neurons ($Adv^{CRE}/Csf1^{fl/fl}$) prevents the development of neuropathic pain in mice (Guan et al. 2016). Neuronal depletion of *Csf1* reduces SNI-induced CX3CR1$^{+}$ macrophage expansion in DRGs of male but not female $Adv^{CRE}/Csf1^{fl/fl}$ mice (Yu et al. 2020).

In the collagen antibody-induced arthritis (CAIA) model, intraarticular injection of collagen antibodies causes mechanical allodynia and increases *Hmgb1* gene expression in both male and female mice. Intraarticular injection of disulfide HMGB1 produces mechanical allodynia and promotes joint expression of proinflammatory cytokines (*Tnf, Il1b, Il6, and Ccl2 mRNA*) in male but not female mice. In primary macrophage cultures, disulfide HMGB1 causes greater release of TNF, IL-6 and CXCL1 in male macrophages versus female macrophages. When minocycline is coinjected into the joint, there is a reversal of disulfide HMGB1-induced pain hypersensitivity in male but not female mice, suggesting an involvement of myeloid-derived cells (e.g., macrophages). Moreover, selective depletion of *Tlr4* in LysM+ myeloid-derived cells abolishes disulfide HMGB1-induced joint pain hypersensitivity in male but not female $LysM^{Cre}/Tlr4^{fl/fl}$ mice,

suggesting HMGB1-mediated pain signaling may require macrophage expression by macrophages in male animals (Rudjito et al. 2021).

Male-specific TLR9 signaling has also been implicated in the development of neuropathic pain (Luo et al. 2019). In immune cells, TLR9 is localized to endolysosomes and senses endocytosed single-stranded DNA-containing CpG motifs derived from bacterial DNA (Krieg 2002). Intraplantar injection of the TLR9 agonist ODN1826 induces mechanical allodynia in both male and female mice. However, in a mouse model of chemotherapy-induced peripheral neuropathy (CIPN), TLR9 inhibition by ODN2088 or *Tlr9* mutation reduced mechanical allodynia in male but not female mice (Fig. 6.1). Of note, in both female and male mice, adoptive transfer of paclitaxel-activated macrophages induced potent and persistent mechanical allodynia, which is reversed by *Tlr9* mutation only in males. In primary macrophage cultures, paclitaxel treatment promotes TNF and CXCL1 production, which is blocked by *Tlr9* mutation only in male cells. These results suggest a predominance of TLR9 signaling in male macrophages. Additionally, T cell deficiency enables antinociceptive effects of ODN2088 in female nude mice, suggesting an involvement of T cells in regulating TLR9 pathways in females (Fig. 6.1) (Luo et al. 2019).

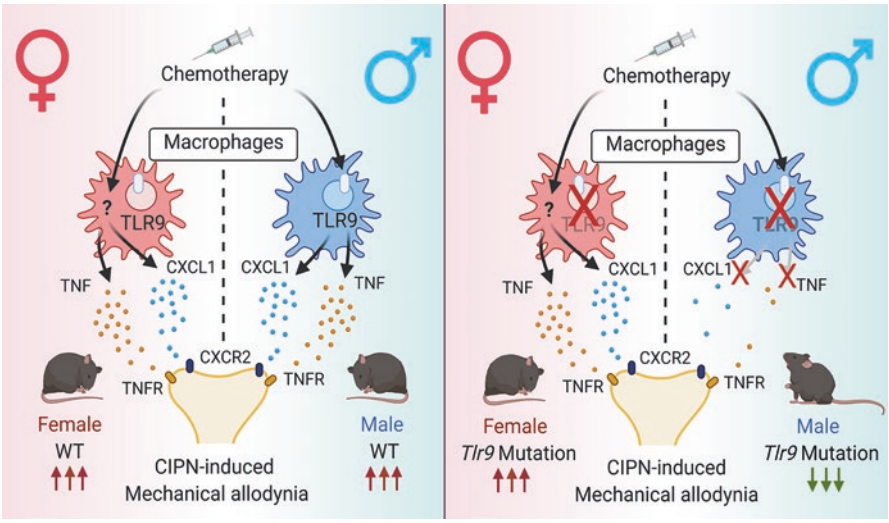


**Fig. 6.1** Schematic of macrophage TLR9 signaling in WT and *Tlr9* mutant mice in CIPN. (**a**) Macrophage infiltration and activation is induced by PTX in DRGs of mice in both males and females. Release of TNF and CXCL1 from macrophages in DRGs, as well as subsequent binding to TNFR1/R2 and CXCR2 on sensory neurons, is likewise promoted by PTX. These actions drive mechanical allodynia in CIPN mice by causing hyperexcitability in nociceptive DRG neurons. (**b**) Mechanical allodynia elicited by PTX is attenuated by blocking TLR9, downregulating release of PTX-induced TNF and CXCL1 release from macrophages in male, but not female mice. In addition, T- and B-cell-deficient female mice may use TLR9 signaling in CIPN instead. (Reproduced from Luo et al. (J Neurosci 2019) with permission)

### 6.3.3 Sex Dimorphism in T Cells, B Cells, and Astrocytes

T cells may primarily affect pain processing in females (Sorge et al. 2015). Several studies demonstrate a female-dominant T cell reaction in chronic pain at different sites. In the mouse SNI model, females exhibit higher levels of $CD4^+$ and $CD8^+$ T cells in the blood compared to males (Sorge et al. 2015). In the mouse CCI model, females exhibit a higher infiltration of $CD3^+$ T cells in the sciatic nerve than males (Vacca et al. 2021). In the mouse partial sciatic nerve injury (PSNL) model, females exhibit a higher ratio of certain populations of T cells in DRGs than males (Lopes et al. 2017). Furthermore, several studies indicate T cell involvement in the sex dimorphism of pain. Peroxisome proliferator activated receptor (PPAR) is a transcription regulator; its subtypes, PPARα and PPARγ, are selectively expressed in male and female T cells respectively. The PPARα inhibitor GW6471 produces analgesia against SNI-induced mechanical allodynia in male mice, whereas the PPARγ inhibitor GW9662 does so in females.

These sexually dimorphic roles of PPAR subtypes require sex hormones. Moreover, T cell-deficient mice (nude and *Rag1*$^{-/-}$) display normal mechanical allodynia in the SNI model. Notably, T cell-deficiency can restore the analgesic effects of intrathecal minocycline in females with SNI or CFA, and adoptive transfer of wildtype splenocytes render female *Rag1*$^{-/-}$ mice insensitive to minocycline in the CFA model (Sorge et al. 2015). In another study, female mice exhibit less sensitivity to morphine analgesia, with females showing a right-shift of the morphine dose–response curve compared to males. T cell deficient (nude) mice of both sexes show reduced morphine analgesia with no sex differences. Notably, adoptive transfer of male WT $CD4^+$ T cells restores greater morphine analgesia in nude mice than female WT $CD4^+$ T cells, suggesting a sex dimorphic role of T cells in regulating morphine analgesia (Rosen et al. 2019).

Recent studies have pointed toward a sex dimorphic role of B cells in chronic pain. In the mouse PSNL model, males exhibit a higher ratio of $CD19^+$ B cells in DRG tissues than females (Lopes et al. 2017). In the tibial fracture pain model, WT mice developed mechanical allodynia in both sexes, which was attenuated by B cell deficiency (muMT) only in male animals. Of note, such effects of B cell deficiency can be blunted by treating the animals with serum collected from male but not female mice with tibial fracture (Guo et al. 2019).

Several studies show that astrocytes exhibit no sex dimorphism in their response to and functioning in chronic pain. Spinal levels of $GFAP^+$ and/or Connexin-$43^+$ astrocytes increase in mice of both sexes in chronic pain models of CCI (Chen et al. 2018a), CAIA (Fernandez-Zafra et al. 2019), and CIPN (Agalave et al. 2021a). Moreover, in mouse models of acute inflammatory pain, intrathecal injection of the astroglial toxin L-AA reduced formalin-induced spontaneous pain in both male and female animals. Spinal inhibition of astrocyte-selective pathways, such as JNK and Connexin-43, also produces analgesia against formalin-induced pain in both male and female animals (Chen et al. 2018a).

However, studies also indicate that astrocytes may have sex dimorphic roles in chronic pain. Several glial toxins have been used to study glial cells by inhibiting their function. While minocycline mainly targets microglia, fluorocitrate preferentially inhibits astrocytes, and propentofylline may inhibit both microglia and astrocytes (Ji et al. 2019; Sweitzer et al. 2001). Sorge et al. showed all these inhibitors reduced neuropathic pain predominantly in males (Sorge et al. 2015). During puerperium, female rats exhibit a lack of astrocyte activation following spinal nerve ligation compared to their normal female counterparts (Gutierrez et al. 2013). In the CAIA model, spinal astrocytic inhibition by pentoxifylline alleviates mechanical allodynia only in male mice (Fernandez-Zafra et al. 2019). Eukaryotic translation initiation factor 4E (eIF4E) is a cytosolic regulator of mRNA translation. Interestingly, *eif4e* mutation reduces CIPN-induced $GFAP^+$ astrocyte reaction only in males (Agalave et al. 2021a). Thus, astrocytes may only show sex dimorphism under specific pain conditions.

## 6.4 IL-23/IL-17 Axis-Mediated Neuroimmune Interactions Exhibit Multilevel Sex Differences in Females

Interleukin 23 (IL-23) is a proinflammatory cytokine and belongs to the interleukin 12 (IL-12) family. IL-23 is released by antigen-presenting cells such as dendritic cells and macrophages (Gaffen et al. 2014). It was found that intraplantar injection of IL-23 elicits mechanical allodynia in female mice but not in male mice (Luo et al. 2021). Moreover, loss of IL-23 function, whether through $Il23^{-/-}$ or $Il23r^{-/-}$ knockout, or by treatment with the IL-23R antagonist P2305, can reduce CIPN-induced mechanical allodynia in female but not male mice. Additionally, intraplantar P2305 produces female-specific analgesia in pathological pain models of CCI, diabetic neuropathy, and formalin-induced acute inflammatory pain. These results suggest a female-specific role of the IL-23/IL-23R axis in pain processing (Luo et al. 2021).

Depletion of macrophages, but not T cells, reverses IL-23-induced pain in females, demonstrating a macrophage-dependent mechanism. In both male and female recipient mice, adoptive transfer of paclitaxel-activated macrophages produces comparable levels of mechanical allodynia. However, this allodynia effect is reversed by *Il23* or *Il23r* deficiency only in females, suggesting a female-specific role of the macrophage IL-23/IL-23R axis in pain. It was also found that IL-23-induced pain requires $TRPV1^+$ C-fiber nociceptors. Interestingly, $Ca^{2+}$ imaging and electrophysiological evidence indicates that IL-23 does not act on these nociceptors directly. Instead, another proinflammatory cytokine, IL-17A, is required for IL-23-induced pain and it can directly activate nociceptors. In primary macrophage cultures, IL-23 treatment causes higher production of IL-17A in female cells compared to male cells. Intraplantar administration of IL-17A produces female-dominant mechanical allodynia in mice, which also requires TRPV1. Consequently, IL-17A

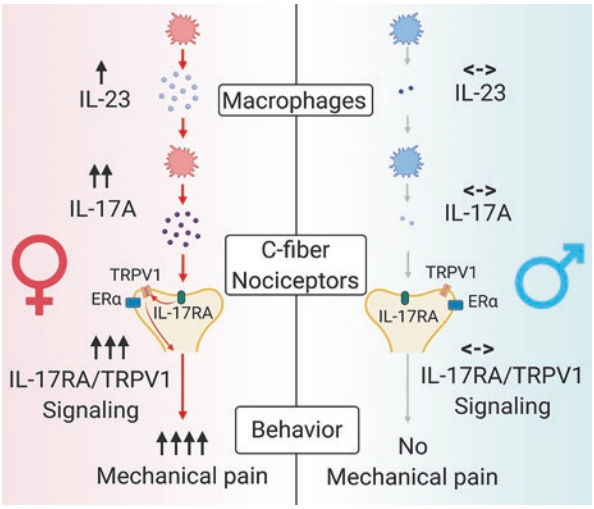


**Fig. 6.2** Estrogen/estrogen receptor (ER) signaling in neurons promotes female-dominant pain mediated by IL-23/IL-17, which may explain the female-specific modulation of mechanical pain by the IL-23/IL-17A/TRPV1 axis. Notably, both macrophage and nociceptive signaling have sex-dimorphic characteristics. (Reproduced from Luo et al. (Neuron 2021) with permission)

evokes $Ca^{2+}$ influx and potentiates action potential firing in a female-dominant manner. These results indicate that IL-17A functions as a downstream effector of the IL-23/IL-23R axis in pain modulation. The TRPV1 agonist capsaicin also produces female-dominant mechanical allodynia in mice (Luo et al. 2021).

Notably, the link between IL-17A receptor (IL-17RA) and TRPV1 in DRG sensory neurons is essential to female-specific pain mediated by the IL-23/IL-17A axis, which is regulated by estrogen receptor α (ERα). In situ hybridization studies indicate that male and female mice exhibit comparable ratios of $Il17ra^{+}/Trpv1^{+}/Er\alpha^{+}$ neuron subsets in DRGs. However, selective depletion of *Erα* in $TRPV1^{+}$ neurons ($Trpv1^{CRE}/Er\alpha^{fl/fl}$) diminishes IL-23, IL-17A, and capsaicin-evoked mechanical allodynia in females, suggesting that estrogen signaling is an essential component of IL-23/IL-17A-mediated pain in females (Fig. 6.2). We also observed that in human DRG tissues, ERα but not ERβ shows sex dimorphism, where females have significantly higher expression levels of Erα expression (Luo et al. 2021).

Using an optogenetic approach in which ChR2 is expressed in TPRV1+ nociceptors, we found blue light stimulation induced greater pain in females. We also found that intraplantar IL-23 injection can potentiate blue light-induced pain, but this only occurs in female mice and not in male mice (Ji et al. 2021).

## 6.5 Summary and Future Directions

The incidence of chronic pain in women is higher than men. Women also suffer disproportionately from inflammatory diseases associated with pain such as fibromyalgia, osteoarthritis, chronic migraine, and autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus). Because scientists have mainly used male rodents due to built-up inertia within the research field and the fact that male rodents do not have cyclical hormonal fluctuations like female rodents do, sex-related differences and sex dimorphism in pain were poorly understood until very recently. As the previous chapters have shown, immune and glial cells are crucial to the modulation of pain. This chapter has highlighted several glial and immune cell types and how they contribute to sex dimorphism in chronic pain (Table 6.1). Notably, the mechanisms of most sex-dimorphic immune pain molecules have predominantly been characterized in male animals. As such, further studies will be necessary to investigate novel female-specific pain signaling pathways in these immune cells across various chronic pain conditions. These efforts will improve our understanding of sex dimorphism in chronic pain to improve the treatment of pain for patients in the future.

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