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Neuropathic Pain: Mechanisms, Sex Differences, and Potential Therapies for a Global Problem

Shahrzad Ghazisaeidi,[1,2,3,*] Milind M. Muley,[1,3,*] and Michael W. Salter[1,2,3]

[1]Program in Neurosciences & Mental Health, The Hospital for Sick Children, Toronto, Ontario, Canada; email: michael.salter@sickkids.ca

[2]Department of Physiology, University of Toronto, Toronto, Ontario, Canada

[3]University of Toronto Centre for the Study of Pain, Toronto, Ontario, Canada

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*These authors contributed equally to this article

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Keywords

neuropathic pain, sex difference, sex similarities, immune cells, neurons

Abstract

The study of chronic pain continues to generate ever-increasing numbers of publications, but safe and efficacious treatments for chronic pain remain elusive. Recognition of sex-specific mechanisms underlying chronic pain has resulted in a surge of studies that include both sexes. A predominant focus has been on identifying sex differences, yet many newly identified cellular mechanisms and alterations in gene expression are conserved between the sexes. Here we review sex differences and similarities in cellular and molecular signals that drive the generation and resolution of neuropathic pain. The mix of differences and similarities reflects degeneracy in peripheral and central signaling processes by which neurons, immune cells, and glia codependently drive pain hypersensitivity. Recent findings identifying critical signaling nodes foreshadow the development of rationally designed, broadly applicable analgesic strategies. However, the paucity of effective, safe pain treatments compels targeted therapies as well to increase therapeutic options that help reduce the global burden of suffering.

INTRODUCTION

Somatosensory system: a part of the sensory system associated with the perception of touch, pressure, position, temperature, movement, and pain that arises from the skin and muscles

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage" (1, p. 1976). Acute pain resolves once the underlying tissue damage has healed, in most cases, lasting less than three months. Despite being experienced as aversive, acute pain can be seen as an adaptive, protective response to threats of bodily integrity. In contrast to acute pain, chronic pain persists beyond the normal period of healing, even when tissue damage has resolved, and is maladaptive, serving no known biological function. Too often, the lack of effective treatments results in chronic pain becoming a devastating experience for the individual and their family and community. The global prevalence of chronic pain is estimated at approximately 20% of the population (2, 3), and the economic costs are greater than those of cancer, HIV, and cardiovascular diseases combined (4). As a result of an increasing understanding that chronic pain is due to persisting, pathological changes in the peripheral nervous system and/or central nervous system (CNS), chronic pain was recently recognized as a set of disease categories by the International Classification of Diseases of the World Health Organization (5).

The most recalcitrant type of chronic pain is neuropathic pain—pain that is caused by a lesion to or disease of the somatosensory system—which occurs in about 7–10% of the general population (6). Treating neuropathic pain remains challenging, mainly because the clinical translation of pharmacological agents has been limited, few new therapeutic options have become available, and the existing therapies are only partially effective and come with substantial negative side effects in a small proportion of the population. In most chronic neuropathic pain conditions, women show a higher prevalence of pain than men (reviewed in 7). Yet, in preclinical studies of pain, female animals have not been regularly incorporated, nor has sex been widely considered in clinical trials. The field of pain is not unique in this regard, but increased awareness in the scientific community and new mandates from research funding agencies have resulted in an upsurge of preclinical studies that incorporate sex as a biological variable (8, 9).

Recent work studying females in addition to males has revealed not only sex differences in underlying pain mechanisms but also sex similarities in signaling pathways. This work is built on a foundation of decades of prior work on neuropathic pain using models in male animals alone with the tacit assumption that sex differences in mechanisms did not exist or, if they did, were irrelevant. In this review, we discuss mechanistic pain differences and similarities with the goal of finding the basis for novel neuropathic pain therapies that can be effective in either sex.

NEUROPATHIC PAIN: DISORDERED NEURON–IMMUNE–GLIAL CELL INTERACTIONS

Neuropathic pain can arise from a lesion or damage in the peripheral somatosensory system caused by, for example, physical trauma or metabolic insults, or damage in the central somatosensory system as a result of stroke, trauma, or neurodegeneration. Due to the greater experimental tractability of the peripheral nervous system, better understanding of neuropathic pain mechanisms has come overwhelmingly from investigations of neuropathic pain hypersensitivity as a consequence of discrete lesioning of one or more peripheral nerves. Such peripheral nerve injury (PNI) drives waves of signaling changes in neurons, immune cells, and glial cells at the site of the lesion; in the dorsal root ganglia (DRG) (**Figure 1**), where the cell bodies of primary sensory neurons are located; in the dorsal horn of the spinal cord; and in the brain (10–12). PNI-induced alterations in the interplay between neurons, immune cells, and glia produce aberrant inputs from the periphery into the spinal cord and reconfigure processing in the somatosensory processing network in the dorsal horn such that there is pathological amplification of nociceptive

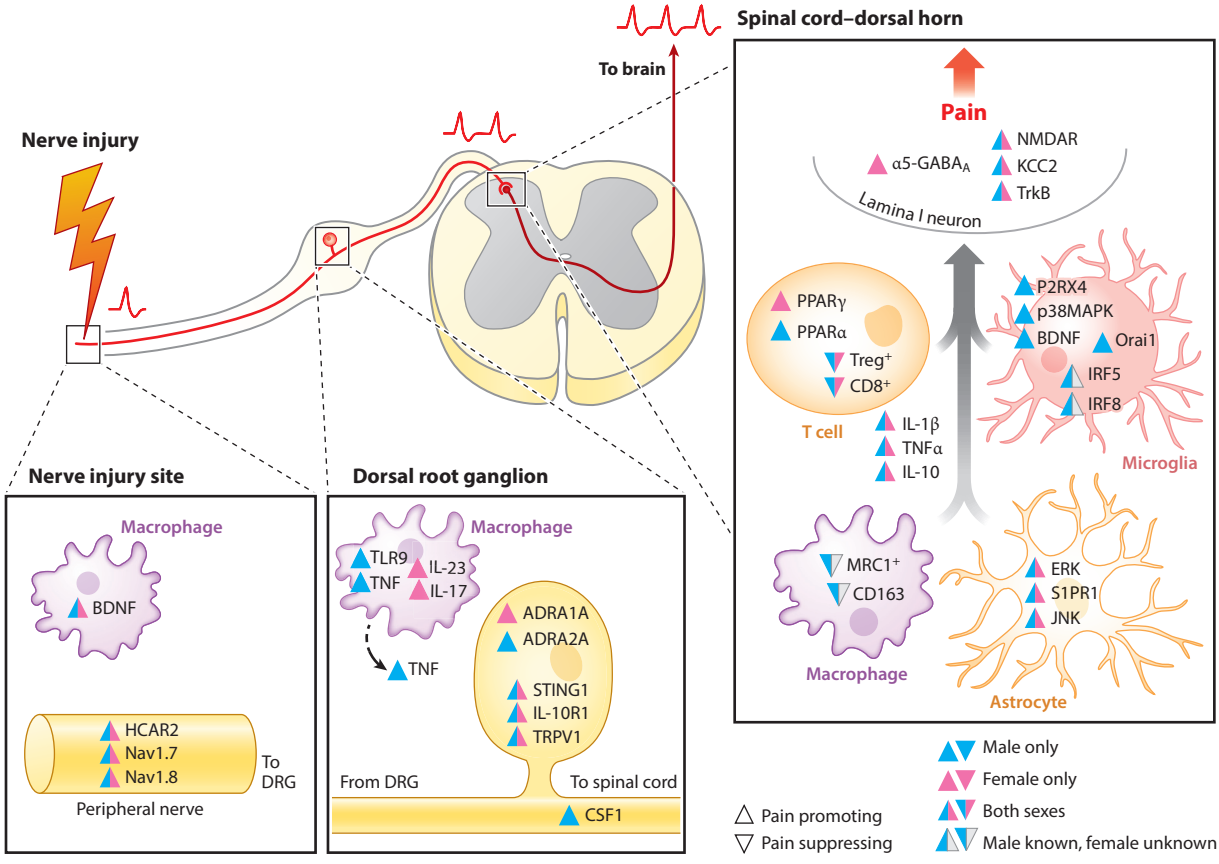

Figure 1

Sex differences and sex similarities in principal cellular and molecular mediators of neuropathic pain. The injured peripheral nerves transmit signals to the dorsal horn of the spinal cord, which transmits to the brain. Blue represents male-only involvement, pink represents female only, blue and pink together represent sex similarities, and blue with gray represents studies that were tested in male rodents only. Triangles pointing up indicate mediators that promote pain, whereas triangles pointing down indicate that mediators suppress pain effects. Plus signs signify a subpopulation of a specific cell type. Abbreviations: α5-GABA$_A$, gamma-aminobutyric acid A receptor, alpha 5; ADRA1A, adrenoceptor alpha 1A; ADRA2A, adrenoceptor alpha 2A; BDNF, brain-derived neurotrophic factor; CD163, macrophage-associated antigen; CD8$^+$, T cell expressing CD8; DRG, dorsal root ganglia; ERK, extracellular signal–regulated kinase 2; IL-10, interleukin 10; IL-10R1, interleukin 10 receptor; IL-17, interleukin 17; IL-1β, interleukin 1 beta; IL-23, interleukin 23; IRF5, interferon regulatory factor 5; IRF8, interferon regulatory factor 8; JNK, JUN N-terminal kinase; KCC2, neuronal K-Cl cotransporter; MRC1, macrophage mannose receptor 1–like protein 1; Nav1.7, voltage-gated sodium channel 1.7; Nav1.8, voltage-gated sodium channel 1.8; NMDAR, *N*-methyl-D-aspartate receptor; Orai1, calcium release–activated calcium channel protein 1; P2RX4, purinergic receptor P2X 4; p38MAPK, p38 mitogen-activated protein kinase; PPARα, peroxisome proliferator–activated receptor alpha; PPARγ, peroxisome proliferator–activated receptor gamma; S1PR1, sphingosine-1-phosphate receptor 1; STING1, stimulator of interferon response CGAMP interactor 1; TLR9, Toll-like receptor 9; TNF, tumor necrosis factor; Treg, regulatory T cell; TrkB, neurotrophic receptor tyrosine kinase 2; TRPV1, transient receptor potential cation channel subfamily V member 1.

activity and unmasking of crosstalk between nonnociceptive (nonpain-producing) and nociceptive (pain-producing) pathways (13). Together, these changes transform the output along nociceptive pathways from the spinal cord to the brain, with the aberrant activity leading to the cardinal symptoms of neuropathic pain: allodynia, that is, pain driven by normally nonpainful stimuli; hyperalgesia, that is, exaggerated pain in response to normally painful stimuli; and spontaneous pain in the absence of overt stimulation. Here we describe sex differences and similarities in major cell

Allodynia: pain due to a stimulus that does not normally evoke pain

MICROGLIA AND MACROPHAGE SUBPOPULATIONS THAT SUPPRESS PAIN HYPERSENSITIVITY

Microglia and macrophages are widely considered to be major drivers of neuropathic pain. However, recent findings are challenging this paradigm with discoveries of subpopulations of these immune cells that suppress rather than promote peripheral nerve injury–induced pain hypersensitivity. In the spinal cord, a subpopulation of microglia has been identified that express integrin αX (*Itgax*, also known as CD11c), which causes remission of pain hypersensitivity after spinal nerve injury in males and females (111) (**Figure 2**). $CD11c^+$ microglia appear in the dorsal horn after nerve injury. The key antiallodynia mediator released from these cells is IGF1. In the meninges, a subset of macrophages expressing CD206 (*MRC1*) proliferate upon injury and upregulate expression of the hemoglobin-haptoglobin scavenger receptor CD163, which, through release of IL-10, is proposed to suppress microglia activation and pain hypersensitivity (108). This finding was made using only males, and the potential role of $MRC1^+$ macrophages in females needs to be investigated. We anticipate that selectively boosting or pharmacologically mimicking the pain-relieving actions of these microglia and macrophage subpopulations may provide a basis for a viable approach for treating neuropathic pain.

Supplemental Material >

types involved in neuron-immune-glia interactions producing and regulating neuropathic pain hypersensitivity: microglia, macrophages, astrocytes, T cells, primary sensory neurons, and spinal dorsal horn neurons (**Figure 1**), all of which have been studied using mouse or rat models of neuropathic pain (**Supplemental Table 1**). Nonneuronal cells are widely seen as the main drivers of neuropathic pain hypersensitivity, but recent evidence points to a conceptual shift in that specific types of immune cells may, surprisingly, suppress or reverse pain hypersensitivity (see the sidebar titled Microglia and Macrophage Subpopulations that Suppress Pain Hypersensitivity).

MICROGLIAL CELLS: A PRINCIPAL LOCUS FOR SEX DIFFERENCES IN NEUROPATHIC PAIN

Microglia, the principal innate immune cells of the CNS, are increasingly known to be involved in developmental and physiological processes, as well as in CNS disease (14). These highly plastic cells adopt a variety of morphological and functional phenotypes to adapt to specific conditions within the CNS (14). Microglia in the spinal dorsal horn proliferate dramatically in response to injury to a peripheral nerve (15), but this proliferation is not in and of itself sufficient to produce pain hypersensitivity, as has been repeatedly shown. Rather, as was elucidated in numerous studies using only male animals, PNI-induced pain hypersensitivity is mediated by key signaling changes within spinal microglia (**Figure 1**): upregulation of the purinergic receptor P2X4 (P2X4R), activation of p38 mitogen-activated protein kinase (p38MAPK), and de novo synthesis and release of brain-derived neurotrophic factor (BDNF) (16, 17) (**Figure 2**). Factors released from primary afferents, such as colony-stimulating factor 1 (18), and other cell types (19) turn on the expression of *P2rx4* by a cascade of the transcription factors interferon regulatory factor 8 and interferon regulatory factor (20, 21). Interferon regulatory factor 5 binds directly to the *P2rx4* promotor, stimulating transcription and ultimately translation of P2X4Rs (22). These receptors are then trafficked to the surface of the microglia, where they are activated by ATP, which has been shown to be released by intrinsic neurons in the dorsal horn (23).

Hyperalgesia: increased pain from a stimulus that normally evokes pain

The robustness of the P2X4R-p38MAPK-BDNF pathway in males belied the fact that this pathway and indeed spinal microglial cells themselves are dispensable in females (24). Consistently across studies and research groups, manipulations aimed to inhibit microglia (22, 24–28) or to ablate these cells reverse PNI-induced mechanical hypersensitivity in male but not in female mice

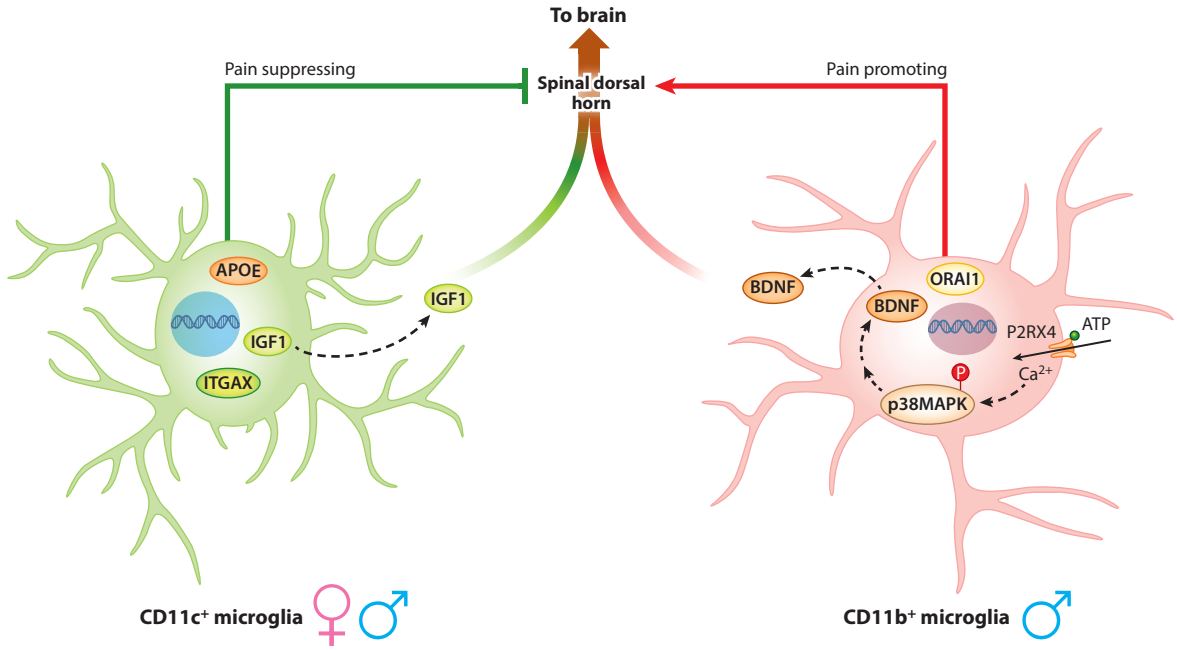

Figure 2

Dual role of microglial cells in pain hypersensitivity. Microglia expressing CD11b promote pain hypersensitivity in males, whereas microglia expressing CD11c suppress pain hypersensitivity in both sexes (see the sidebar titled Microglia and Macrophage Subpopulations that Suppress Pain Hypersensitivity). Abbreviations: APOE, apolipoprotein; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; IGF1, insulin-like growth factor 1; ITGAX, integrin subunit alpha X; ORAI1, calcium release–activated calcium channel protein 1; P2RX4, purinergic receptor P2X 4; p38MAPK, p38 mitogen-activated protein kinase.

or rats (22, 24). Although microglia are not required for PNI-induced pain hypersensitivity in females, the nerve injury does induce proliferation of microglia in the dorsal horn in females that is indistinguishable from that induced in males (24, 29).

Male-specific antiallodynia evoked by blocking P2X4Rs pharmacologically, originally observed with TNP-ATP (24), has also been elicited by using a function-blocking anti-P2X4R antibody (30). A single intraperitoneal dose of the anti-P2X4R antibody reversed mechanical allodynia for more than five weeks and prevented the development of anxiety- and depressive-like behaviors.

Inhibiting p38MAPK with intrathecal SB203580, an ATP-competitive p38MAPK inhibitor, produced male-specific antiallodynia (24), which was confirmed with another intrathecally administered ATP-competitive p38MAPK inhibitor, skepinone (29), and with depleting the p38α isoform of p38MAPK in the spinal cord (31). The antiallodynic effect of skepinone was observed 7 days after chronic constriction injury (CCI) but was lost by day 21 (29), suggesting a time-limited role of p38MAPK in males. In addition, intraperitoneal or perineural administration of skepinone reversed allodynia in both sexes. Therefore, p38MAPK sexual dimorphism appears to be exclusive to the spinal cord, while there is a sex-independent role for this kinase in the periphery at the site of nerve lesion.

Inhibiting BDNF signaling to its cognate receptor, TrkB, by Y1036 or TrkB-Fc reversed PNI-induced mechanical allodynia in males but not in females (24). Similarly, microglia-specific knockout of BDNF after allodynia had been established reversed this allodynia in males only. Furthermore, only in males did microglia-specific knockout of BDNF prior to the nerve injury

prevent the development of allodynia. These findings, first made in mice, have been replicated in rats (22). The lack of involvement of BDNF in females is not due to a lack of TrkB, as administering exogenous BDNF intrathecally causes mechanical allodynia in females to the same extent that this neurotrophin does in males (32). TrkB is engaged after the mechanistic switching observed in females that, when given testosterone, utilize the male P2X4R-p38MAPK-BDNF pathway (see **Supplemental Sidebar 1**).

Supplemental Material >

Male dependence on microglia involvement in PNI-induced hypersensitivity was confirmed recently in a study of the calcium release–activated calcium channel Orai1 (33). Deleting Orai1 impairs store-operated Ca^{2+} entry and the synthesis of inflammatory cytokines in spinal microglia from both sexes. However, PNI-induced hypersensitivity was suppressed only in male mice carrying a microglial-specific deletion of Orai1. Moreover, cytokines and the pathological enhancement of glutamatergic synaptic transmission are both reduced in the dorsal horn in male but not in female microglial-specific Orai1-null mice. Finally, the Orai1 channel blocker, CM4620, phenocopies the selective mitigation of allodynia in male but not in female mice. It is possible that Orai1 amplifies the P2X4R-mediated increase in intracellular calcium, as calcium is known to drive p38MAPK activation and BDNF transcription, translation, and release (34).

In addition to the necessity of microglia for mechanical allodynia, the sufficiency of activated microglia to evoke mechanical allodynia when delivered intrathecally was demonstrated in male animals (15). Recently, two studies used designer receptors exclusively activated by designer drugs (DREADD)-based technology to activate spinal microglia in mice by driving human Gq-coupled M3 muscarinic receptors (hM3Dq)-DREADD in microglia cells. Chemogenetic stimulation of spinal microglia cells with intrathecal clozapine-*N*-oxide produced mechanical allodynia in males but not in females (35, 36). Similarly, in mice carrying the CD68-hM3Dq construct, clozapine-*N*-oxide produced mechanical hypersensitivity exclusively in males (37). Thus, microglial cells appear both necessary and sufficient to induce pain hypersensitivity in male rodents.

MACROPHAGES: SEX-INDEPENDENT MEDIATORS OF NEUROPATHIC PAIN

Injury to peripheral nerves triggers infiltration of a diversity of immune cells at the site of nerve injury and also in the DRG, which contains the cell bodies of primary sensory neurons, the axons of which have been damaged (38, 39). Studies with male animals have long shown that macrophages infiltrate DRGs in response to nerve injury (40). Subsequently, a body of evidence from macrophage-ablation experiments implicated these infiltrating cells as being necessary for PNI-induced pain hypersensitivity in males (41, 42) and subsequently in females as well (43). The recruitment of macrophages from the bloodstream into the DRG appears to be through microRNA miR-21, which is packaged in exosomes and released from primary afferent cell bodies to be phagocytosed by macrophages (44). Macrophages in the DRG may drive mechanical rather than cold allodynia (45), consistent with activated macrophages being observed preferentially around injured large-diameter A-fiber sensory neuron cell bodies after sciatic nerve injury (46). These findings used males only, and whether the same observations will be seen in females is uncertain. For example, in chemotherapy-induced peripheral neuropathy (CIPN) produced by paclitaxel, males have been found to utilize macrophage Toll-like receptor 9 (47), whereas females utilize the IL-23/IL-17A/TRPV1 signaling axis in developing pain hypersensitivity (48). Thus, macrophages in males and females may use distinct pathways to modulate pain. Moreover, although macrophages may release a variety of mediators, such as cytokines or reactive oxygen species (49), it remains to be determined which affect the cell bodies of the primary afferents to drive pain hypersensitivity.

Macrophages accumulate at the site of lesion in peripheral nerves as well as in the DRG. In nerve transection studies, ablating circulating macrophages does not alter the development of pain hypersensitivity in either sex (43, 50). Recently, a role for macrophages at the site of a lesion in a peripheral nerve was identified in both males and females (50). A noncompressive, nontraumatic approach of apposing the nucleus pulposus of the intervertebral disc to the sciatic nerve was used and produced hypersensitivity to mechanical, cold, and heat stimuli. Hypersensitivity caused by nucleus pulposus application was not affected by inhibiting microglia. Rather, eliminating macrophages, either systemically or locally, prevented nucleus pulposus–induced pain hypersensitivity. Pain hypersensitivity was also prevented by genetically disrupting BDNF selectively in macrophages. Thus, macrophages recruited into a peripheral nerve can act locally to drive neuropathic pain hypersensitivity in both sexes (50, 51).

Tumor necrosis factor (TNF), a proinflammatory cytokine secreted by immune cells, including macrophages, has been implicated in neuropathic pain (52). A study investigated the involvement of TNF receptor 1 in pain hypersensitivity in male and female mice in a model of CCI-induced neuropathic pain. Pharmacological inhibition of soluble TNF receptor 1 (using XPro1595) accelerated recovery from neuropathic pain in males but not in females after animals had undergone CCI. Furthermore, male TNF receptor 1–null mice did not develop allodynia, but female mice developed allodynia that lasted for 4–5 weeks after CCI (53). These observations suggest sex-dependent pathophysiological involvement of TNF receptor 1 in the development of PNI-induced neuropathic pain. The identity of the cells expressing TNF receptor 1 and the source of TNF itself remain to be determined.

ASTROCYTES: MEDIATORS OF PAIN HYPERSENSITIVITY IN BOTH SEXES

Astrocytes are found abundantly within the CNS and have the capacity to form close contacts with synapses and modulate neuronal function (54–56). Astrocytes have been implicated in chronic pain (57, 58) in studies that used a variety of pharmacological interventions touted to be inhibitors of or toxins for these cells. Astrocytes within the spinal dorsal horn show increased immunofluorescence for the marker glial fibrillary acidic protein, but not for other markers such as ALDH1 (59), and shape changes starting about 7–14 days after PNI. The delay in these morphological changes and their persistence for long periods—tracking PNI-induced pain hypersensitivity—have given rise to the concept that astrocytes participate only in long-lasting neuropathic pain (60–62). Investigation of sex differences or similarities in astrocyte involvement, using both males and females, has been limited in comparison with those examining microglia or macrophages. An investigation of the involvement of astrocyte signaling in CCI-induced pain hypersensitivity in males and females tested the astrocyte toxin L-α-aminoadipate and inhibitors of the astrocyte signaling components connexin 43 (carbenoxolone), extracellular signal–regulated kinase (U0126), and c-Jun N-terminal kinase 12–17 days after nerve injury (27). All treatments reversed mechanical allodynia equally in both males and females, suggesting sex-independent involvement of spinal astrocytes in mediating pain hypersensitivity associated with traumatic nerve injury.

In addition, astrocytic sphingosine-1-phosphate receptor 1 (S1PR1) appears to be critical for the development and maintenance of PNI-induced neuropathic pain in males and females (63). Oral administration of S1PR1 antagonists and intrathecally delivered S1PR1-targeting small interfering RNA reversed CCI-induced mechanical allodynia without affecting basal nociception. Moreover, astrocyte-specific knockout of S1PR1 prevented the development of mechanical allodynia. The S1PR1-selective agonist SEW2871 drove mechanical allodynia through NOD-like receptor family pyrin domain–containing 3 inflammasome and IL-1β in naïve animals but not

in the astrocyte-specific S1PR1 knockout (64). Similarly, S1PR1 antagonism produced antiallodynia that was sex independent for paclitaxel-induced mechanical hypersensitivity, but was male specific for bortezomib-induced hypersensitivity (65). On a molecular level, reduction in pain hypersensitivity from acute S1PR1 inhibition was prevented by blocking IL-10, and IL-10 blockers rescued hypersensitivity in the astrocyte-specific S1PR1 knockout after PNI. The observation that PNI-induced mechanical allodynia was prevented at day 7 in astrocyte-specific knockouts suggests that astrocytes might participate in pain hypersensitivity earlier than suspected and prior to the morphological changes.

T CELLS: PLEIOTROPIC ROLES IN PAIN HYPERSENSITIVITY

PNI triggers T cell infiltration at the site of the nerve injury (66), DRG (67), leptomeninges, and spinal cord (68–70). While experimental findings on the roles and function of T cells may differ across studies, mice genetically lacking T cells have generally been found to have diminished PNI-induced pain hypersensitivity (reviewed in 71). T cells have been shown to communicate bidirectionally with nociceptors, macrophages (72), and microglial cells (73). There is evidence that T cells at the site of nerve injury (72) and in the DRG (45) coproduce pain hypersensitivity together with macrophages. In the spinal cord, T cells may contribute to PNI-induced mechanical hypersensitivity by releasing interferon gamma (74). As with other cell types, the extent to which mechanisms identified in males apply to females remains unclear.

In the spinal cord of females, it was found that PNI-induced mechanical allodynia is independent of microglia (see above). In females but not in males, the peroxisome proliferator–activated receptor gamma (PPARγ) agonist pioglitazone reversed allodynia induced by spared nerve injury (SNI), an animal model that involves transection of two branches (tibial and peroneal nerve, leaving sural intact) of the sciatic nerve. The effect of pioglitazone was blocked by the PPARγ antagonist GW9662 (24). The differential blockade of neuropathic pain hypersensitivity by pioglitazone in females compared to males has been independently confirmed (75, 76). In female mice that lack T cells and B cells (*Rag1*$^{-/-}$ and nude mice), pain hypersensitivity was found to be microglia-dependent, as in males (24). Adoptive splenocyte transfer to *Rag1*$^{-/-}$ mice restored the female microglia-independent pathway (see **Supplemental Sidebar 1**), suggesting that T cells actively suppress the microglia dependency in females. This suppression may be mediated by T regulatory cells (Tregs), as it has been found that colony stimulating factor 1, which activates microglia in males, is also responsible for expanding Tregs in female spinal cord meninges after nerve injury, and Tregs actively suppress microglia activation in females (77). This effect was confirmed in Treg-deficient female mice that displayed microglia-dependent pain hypersensitivity.

Supplemental Material >

CD8$^+$ T cells have also been found to be necessary for the resolution of CIPN induced by cisplatin, as hypersensitivity resolution is delayed in *Rag1*$^{-/-}$ mice that lack T cells and B cells (78). Moreover, the reconstitution of CD8$^+$ cells before chemotherapy treatment prevented the delay in both sexes. In addition, the adoptive transfer of cisplatin exposed CD8$^+$ T cells after chemotherapy treatment facilitated the resolution in both sexes (78). In addition, Tregs showed proresolution characteristics in neuropathy: One study using both sexes showed that nerve injury increased Treg infiltration at the injury site. Local Tregs suppressed pain hypersensitivity by inhibiting CD4$^+$ T cells (79). Similarly, another study showed that a TNFα receptor 2 (TNFR2) agonist resulted in pain-hypersensitivity attenuation in both sexes and revealed that Tregs are essential for this recovery (80).

The complex roles emerging for T cells and various subsets in driving or resolving pain hypersensitivity or in controlling the underlying cellular substrates may offer new disease-modifying strategies for therapeutic opportunities.

PRIMARY SENSORY NEURONS: DRIVING STIMULUS-EVOKED PAIN

Nociceptive sensory neurons perform an integral function of detecting and responding to damaging or potentially damaging mechanical, chemical, or thermal stimuli (81). These afferents readily sensitize, amplifying their input into the spinal dorsal horn to help focus the response to noxious stimuli and to prepare organisms to avoid noxious stimuli in the future (81). Nociceptors express voltage-gated sodium channels (e.g., Nav1.7, Nav1.8, Nav1.9) and voltage-gated calcium channels (e.g., N-type Cav2.2, and T-type Cav3.1–3) that are critical for the transduction and transmission of nociceptive signals (82–84). Voltage-gated sodium channels are targets of local anesthetic drugs that are highly effective in males and females, although few studies have specifically examined the potential of sex differences. There has been particular interest in developing subtype-specific analgesics. An investigation utilized an in silico structure-based similarity search approach to identify new compounds that possess Nav1.7 inhibitor activity. It found a potent Nav1.7 inhibitor, DA-0218, that exhibited an antiallodynic effect in males and females in a model of CIPN, suggesting pathophysiological involvement of the Nav1.7 channel in the development of neuropathic pain associated with CIPN in both sexes (85). However, the Nav1.8 inhibitor A-803467 was more efficacious in females than in males in preventing joint mechanosensitivity and secondary hyperalgesia after joint neuropathy (86), suggesting the involvement of these channels in the development of neuropathic pain hypersensitivity in both sexes. However, there may be a preferential activity in males versus females depending on the type of neuropathic pain.

Voltage-gated calcium channels contribute to pain transmission by modulating the release of pronociceptive mediators such as glutamate and substance P within the dorsal horn of the spinal cord (87). Several studies have demonstrated a contributory role for N- and T-type calcium channels in the development of experimental neuropathic pain after nerve injury (88–91). Additionally, it has been shown that targeting Cav3.2 T-type channels can prevent pain hypersensitivity associated with CIPN (92) and diabetic neuropathy (93). Blockade of N-type calcium channels can prevent chronic pain in patients who are refractory to opioid treatment (94). However, preclinical studies examining the role of voltage-gated calcium channels in neuropathic pain are dominated by the use of male animals only and largely do not include sex as a biological variable.

A recent detailed investigation assessed the involvement of a stimulator of interferon response cGAMP interactor-1 (STING1), an endoplasmic reticulum protein, in regulating nociception (95). Activation of STING1, using a synthetic or natural agonist, produced analgesia in models of paclitaxel-induced CPIN, bone cancer pain, and PNI-induced neuropathic pain (SNI and CCI). These effects were observed in both males and females, suggesting sex-independent involvement of STING1. Upon assessing the expression of STING1 within the DRG, it was observed that 60% of DRG sensory neurons expressed STING1. The study also observed an increase in the levels of interferon-α (IFN-α) and IFN-I receptor component Ifnar1 in DRG neurons. Mice lacking *Ifnar1* selectively in sensory neurons exhibited consistent pain hypersensitivity. Furthermore, activating IFN-I/IFNAR signaling suppressed sodium channel and calcium channel activity (95). However, it has been observed that STING1 can be expressed in macrophages, microglia, and the spinal cord after PNI and spinal cord injury (96, 97), raising the possibility that the effects of STING1 may go beyond its actions with DRG sensory neurons.

LAMINA I DORSAL HORN NEURONS: A MAJOR OUTPUT AND POINT OF MECHANISTIC CONVERGENCE

A major output for nociceptive information from the spinal cord to the brain is the projection neurons in lamina I of the spinal dorsal horn (**Figure 1**). Multiple lines of evidence implicate these neurons as critical in neuropathic pain hypersensitivity (98, 99). As described above, in males the

key molecule for signaling from microglia is BDNF (100). BDNF, through activating TrkB, evokes two major changes in lamina I neurons: disinhibition and facilitated excitation (17). Disinhibition is the result of TrkB-stimulated downregulation of the function and expression of the potassium chloride cotransporter KCC2 (98, 100), which in the adult CNS maintains low intracellular Cl^- levels by driving chloride extrusion (101). KCC2 expression and activity are necessary for effective gamma-aminobutyric acid A receptor– and glycine receptor–mediated fast inhibitory synaptic transmission (98). A consequence of the loss of KCC2 is elevated intracellular [Cl^-], which then decreases the effectiveness of such fast inhibition, that is, disinhibition (98). TrkB signaling in lamina I neurons enhances excitation by potentiating synaptic *N*-methyl-D-aspartate receptor (NMDAR) currents (102). Activating TrkB leads to stimulation of the nonreceptor tyrosine kinase, Fyn, which phosphorylates the GluN2B subunit of NMDARs, thereby potentiating receptor activity. Suppression of striatal enriched phosphotyrosine phosphatase (STEP) contributes to the activation of Fyn (102) and phosphorylation of NMDARs (103). Combined disinhibition and potentiated excitation produces a state of hyperexcitability in lamina I neurons and in the dorsal horn network that unmasks normally silent low-threshold peripheral inputs to these neurons and thereby increases the transmission of nociceptive information to the brain.

As neither BDNF nor microglia is a necessary intermediary of neuropathic pain hypersensitivity in females (see above), it was questionable as to whether suppression of KCC2 and/or facilitation of NMDARs contributes to neuropathic pain hypersensitivity in females. However, PNI-induced downregulation of KCC2 occurs in both sexes, and enhancing Cl extrusion reverses PNI-induced mechanical hypersensitivity in males and females (32). Moreover, the NMDAR antagonist amino-phosphonovaleric acid reverses PNI-induced pain hypersensitivity equivalently in both sexes (24). Thus, in neuropathic pain hypersensitivity, downregulation of KCC2 and enhancement of NMDAR function appear to be sex-independent mechanisms in lamina I neurons by which BDNF, in males, and a still unknown mediator or mediators in females converge to amplify and transform the output of these neurons to the brain along a pathway that normally signals nociception.

TRANSCRIPTOMICS IN NEUROPATHIC PAIN: IMPLICATIONS FOR THERAPY

Large-scale transcriptomic screening studies of bulk tissue messenger RNA (mRNA) or, increasingly, studies of single cells or single nuclei hold the promise of characterizing pathological pathways and identifying novel, potential therapeutic targets in an unbiased manner. Numerous studies have investigated gene expression profiles of tissues involved in neuroimmune interactions—peripheral nerve, DRG, or spinal cord—in neuropathic pain and were initially conducted in males but increasingly compare males and females (**Table 1**). The main focus of these studies has been to characterize biological processes and pathways that are altered following nerve injury and to determine whether there are sex differences. Below, we describe studies that have gone beyond this focus to reveal new therapeutic opportunities for neuropathic pain.

A striking example of the identification of a therapeutically tractable biochemical pathway began with microarray analysis of DRG mRNA after sciatic nerve transection (104). Two of the three enzymes in the synthesis cascade of 6(*R*)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4)—GTP cyclohydrolase and sepiapterin reductase—were found to be upregulated (105). Levels of BH4 were increased in primary sensory neurons and infiltrating macrophages in the DRG (106). Inhibiting BH4 synthesis pharmacologically by targeting GCH1 (105) or sepiapterin reductase (106) reversed mechanical hypersensitivity. In addition to identifying a pharmacological target and intervention for neuropathic pain, a haplotype of *GCH1* was found to be protective against pain following surgery for persistent radicular low-back pain (105).

Table 1 Transcriptomic studies on experimental neuropathic pain that included both sexes

Pain model	Species	POD	Sample	Tissue	Single cell	Data availability	Reference
SNL	Mice	8	FACS-sorted cells	SC, DRG	No	GSE100035	124
MBP	Mice	7	Tissue RNA	SN, DRG, SC	No	GSE34868	125
SNI	Mice	3, 14, 150	Sorted microglia	SC	Yes	GSE162807	110
CCI, CIPN	Mice	7	Isolated microglia	SC, supraspinal[a]	No	GSE162807	126
SNI	Mice	7	Tissue RNA	DRG, SC, brain, blood	No	GSE111216	127
CCI	Rats	14	Tissue RNA	DRG	No	GEO100122	128
SNI	Rats	7	Tissue RNA	DRG, SC	No	NA	129
SNI	Mice, rats	7	Tissue RNA	SC	No	NA	112

[a]Supraspinal areas used include the medial prefrontal cortex, hippocampus, amygdala, posterior thalamus and S1 cortex relating to the hindlimb, and the periaqueductal gray and rostroventral area.

Abbreviations: CCI, chronic constriction injury; CIPN, chemotherapy-induced peripheral neuropathy; DRG, dorsal root ganglia; FACS, fluorescent-activated cell sorter; MBP, 84–104 myelin basic protein–induced hypersensitivity; NA, not available; POD, postoperative days after surgery; SC, spinal cord; SN, sciatic nerve; SNI, spared nerve injury; SNL, sciatic nerve ligation.

By profiling mRNAs bound to ribosomes using translating ribosome affinity purification, the translatome in DRG Nav1.8 expressing nociceptors revealed 66 mRNAs whose levels differed between the sexes (107). After finding that one of the transcripts, *Ptgds*, which encodes the enzyme that catalyzes the conversion of PGH_2 to PGD_2, was much more highly expressed in females than in males, and reasoning that many analgesic drugs target prostaglandins and their synthetic pathways, Tavares-Ferreira et al. (107) focused on this enzyme. PTGDS protein was found to be expressed broadly in primary afferent cell bodies in the DRG, and the levels of the enzyme and of PGD_2 were much higher in females than in males. Administration of the PTGDS inhibitor, AT-56, evoked pain behaviors in both sexes, but males were 10 times more sensitive to AT-56 than were females. Thus, females were relatively protected against pain evoked by PTGDS inhibition, likely because of higher enzyme levels and PGD_2.

Single-cell technology is a powerful tool for detecting cell subpopulations and has been used to investigate nerve injury-induced changes in male mice only (108, 109). A recent study focused on isolated microglial cells from the spinal cord of males and females at three different time points (day 3, day 14, and 5 months) after SNI (110). In microglia from healthy, naïve animals, six clusters of microglia subpopulations were identified. After SNI, three additional clusters, including two proliferative microglia and one male-specific cluster, were detected. Sex differences were detected in the microglia and were more robust at day 3 post-SNI compared to the later time points, when male and female microglia transcriptomes exhibited much greater similarity. At day 3 post-SNI, microglia from males had more differentially expressed genes (DEGs) than did those from females, and only a small proportion of the DEGs were common between the sexes. At later times, the most highly upregulated transcript in both sexes was apolipoprotein E (*ApoE*), whose mRNA level did not change in the acute stage. Correspondingly, Apoe protein levels in microglia were low at day 3 post-SNI but significantly increased at both later time points. *APOE* polymorphisms in humans were found to be associated with chronic pain, and single-cell RNA sequencing of microglia from human spinal cord microglia revealed a subpopulation of cells with a transcriptional signature of microglia associated with disease. *Apoe* is highly expressed in $Cd11c^+$ microglia, the subset shown to produce pain resolution in both sexes (see the sidebar titled Microglia and Macrophage Subpopulations that Suppress Pain Hypersensitivity) (111) (**Figure 2**). Therefore, we speculate that APOE might be involved in pain resolution and be a therapeutic opportunity for neuropathic pain.

Transcriptome: the set of all RNA transcripts, including coding and noncoding, in a tissue population of cells or in a single cell

Studies of PNI-induced transcriptomic changes are often focused on sex differences, but many of the DEGs are similar between the sexes. A cross-species, cross-sex study investigating spinal cord transcriptomes after SNI from mice and rats of both sexes revealed an overall positive correlation between the sexes in both species (112). The PNI-induced transcriptional reprogramming imputed commonalities, as well as differences, in cellular pathways and gene regulation. The SNI pain interactome for the proteins encoded by the DEGs identified new signaling nodes conserved in both sexes and both species. Interrogating the interactome with the Drug Gene Interaction Database identified approved medications that could modulate key nodes within the network. The top hit from this analysis was fostamatinib, which targets the nonreceptor tyrosine kinase Syk. Administrating the active metabolite of fostamatinib, R406, intrathecally significantly reversed SNI-induced pain hypersensitivity in both sexes (112).

In summary, transcriptomic technology is a powerful tool for drawing a holistic image of pathology and proposing novel therapies or repurposing existing drugs that may not be discoverable without using unbiased, large-scale screening.

TOWARD TRANSLATION FROM MICE AND RATS TO HUMANS

In this review, we have highlighted mechanistic similarities and differences between neuroimmune signaling across both sexes in preclinical neuropathic pain models. However, clinically observed neuropathic pain is much more complex and includes significant interindividual differences that arise due to psychosocial and socioeconomic factors, as reviewed extensively elsewhere (reviewed in 113–115). These factors, in addition to the pathological mechanisms involved, have the potential to contribute to pain perception and the chronicity of pain response observed between men and women. Therefore, it can be advantageous to study certain factors in preclinical animal models as well. Furthermore, many patients with chronic pain experience comorbidities that are sexually dimorphic; examples include depression (116, 117), anxiety (118, 119), and obesity (120, 121). The presence of comorbidities can impact the treatment outcome for chronic pain patients; hence, it is important to conduct preclinical and clinical studies of the interaction between sex and comorbidities during chronic pain.

Chronic pain is classified (in the International Classification of Diseases 11 classification) into seven categories. In developing animal models and techniques, attempts have been made to recapitulate certain pathophysiological and behavioral aspects of different forms of chronic pain response in humans. However, in some of these models, the approach used to induce the disease or the techniques used to measure pain may not accurately represent the heterogeneity or the intensity of clinical pain. For example, SNI, an animal model utilized to study different aspects of nerve injury–induced neuropathic pain, produces significant allodynia in males and females. Furthermore, the preclinical techniques used to capture pain response heavily rely on behavioral changes, whereas humans, in most cases, can describe their pain intensity. Therefore, in humans, pain in men or women can range from mild to severe depending on the individual's perception and tolerance and the type of inciting injury. In this context, the preclinical model or the techniques are unable to recapitulate the complexity of the pain response that may be observed clinically. Therefore, it is beneficial to study clinical pain using multiple different preclinical models and pain assessment techniques to better mimic different aspects of the human disease. In addition, other variables that critically affect the chronic pain response are age, hormonal status, and diet; therefore, it is important to include these variables in preclinical research. For example, based on a study that looked at epidemiological data of patients with chronic pain from 17 countries, chronic pain is more prevalent at an older age and in older women compared to men (122). However,

preclinical pain research is primarily performed in young adult rodents and thus is not necessarily representative of chronic pain patients (123).

CONCLUSIONS AND FUTURE DIRECTIONS

The revelation that the neuroimmune basis for neuropathic pain is sexually dimorphic (24) raised wide interest as to whether, and if so how, the neuron-immune-glia interactions that drive pain hypersensitivity broadly differ between males and females. The ensuing years have seen a growing avalanche of studies that are illuminating the field and revealing new twists and turns. While much remains to be investigated, a consensus appears to be developing that mechanisms involving microglia and T cells, elements of the innate and adaptive immune systems, respectively, show considerable sex differences in the initiation, early, and later stages of neuropathic pain signaling. Mechanisms in macrophages, in the DRG or peripheral nerves, and in primary sensory neurons as well as dorsal horn neurons are, as a first approximation, sex independent. Undoubtedly, these sweeping generalizations will fail as more data become available, as already appears to be the case with involvement of microglia and T cells in the resolution of neuropathic pain. But understanding of whether, where, when, and how sex differences, or similarities, occur will need to continue to grow.

How to understand and rationalize the multiplicity, divergence, convergence, and apparent disorder in pain signaling remains a challenge. Perhaps this is not surprising given the immense survival value for organisms to detect, respond to, and learn from damage to the integrity of their bodies. Major components of immune and peripheral nervous systems are devoted to this. There is a need to function and adapt in an ever-changing external environment and in an internal milieu that can vary with hormone status, stress, developmental state, and age, to name just a few. Sex, in this context, is a key variable in an organism's nociceptive and immune systems.

In the context of sex differences and similarities in pain, the concept of degeneracy is useful and has important implications for therapeutics. In general, therapies that target one component of a degenerate system have limited likelihood of success because alterations in untargeted components can circumvent a therapeutic action. Several nodes that seem to show considerable degeneracy and thus are potentially prime therapeutic candidates have been identified in recent years: KCC2, GluN2B-containing NMDARs, APOE, and spleen tyrosine kinase. Expanded investigation will no doubt reveal more potentially targetable degenerate molecular nodes. Given the paucity of treatments for neuropathic pain, we cannot rely on these alone. We need in parallel a precision medicine–based approach to target less degenerate, critical nodes on the basis of sex, genotype, and other variables. Both approaches will require advances in molecular diagnostics, which is still in its infancy, if we have a hope of finding diverse, safe, effective, rationally designed treatments that can specifically be given to the right patient, at the right time, and in the right place.

SUMMARY POINTS

1. Neuron-immune-glia interactions play causative roles in giving rise to pain hypersensitivity after nerve injury, but the cellular and molecular signaling mechanisms may or may not differ between males and females.
2. The involvement of microglia and T cells in mediating pain hypersensitivity is sexually dimorphic, whereas macrophages, primary sensory neurons, and spinal dorsal horn neurons are involved in a sex-independent manner.

3. The use of transcriptomic analysis for studying neuropathic pain could be an unbiased, effective strategy to identify molecular mechanisms and better therapeutic targets in males and females.
4. To achieve better translation of preclinical findings into clinical settings, it is imperative to consider sex as a biological variable.
5. Finding degenerate molecular targets for treating neuropathic pain could be advantageous as this type of pain is a consequence of multiple simultaneous pathologic changes.

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