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Sex differences in pain: a tale of two immune cells

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Abstract

Substantial evidence has implicated microglia in neuropathic pain. After peripheral nerve injury, microglia in the spinal cord proliferate and increase cell-surface expression of the purinergic receptor P2X4. Activation of P2X4 receptors results in release of brain-derived neurotrophic factor, which acts on neurons to produce disinhibition of dorsal horn neurons which transmit nociceptive information to the brain. Disinhibition of these neurons produces pain hypersensitivity, a hallmark symptom of neuropathic pain. However, elucidating this microglia-neuronal signalling pathway was based on studies using only male rodents. Recent evidence has shown that the role of microglia in pain is sexually dimorphic. Despite similar microglia proliferation in the dorsal horn in both sexes, females do not upregulate P2X4Rs and use a microglia-independent pathway to mediate pain hypersensitivity. Instead, adaptive immune cells, possibly T cells, may mediate pain hypersensitivity in female mice. This profound sex difference highlights the importance of including subjects of both sexes in preclinical pain research.

Keywords: Microglia, T cells, Neuroimmune, Spinal cord

1. Introduction

Many chronic pain conditions, including neuropathic pain, have a marked female predominance.[6,14,38] Despite the higher prevalence of chronic pain in women, female animals are greatly underrepresented in preclinical pain research.[28] In 1993, the NIH Revitalization Act implemented mandatory inclusion of women in clinical research funded by the NIH. However, policies requiring equal representation of female subjects in NIH-funded preclinical research have only recently been developed and are expected to be fully implemented in late 2016.[8] A significant sex bias persists in neuroscience and much of biomedical research with a large majority of studies investigating only male subjects.[2] This bias is particularly prominent in preclinical pain research.[28] A review of articles published in this journal from 1995 to 2005 showed that 79% of studies used exclusively male rodents.[28] This bias has been attributed in part to a widely held assumption that estrous cyclicity introduces additional variability in data from female subjects, despite the variability of pain behavior being slightly, if not significantly, higher in male mice.[28] Profound sex differences in pain neurobiology have been reported, highlighting the importance of including female subjects in experiments.[18,29,30] The absence of female subjects in preclinical research means that fundamental differences between the sexes in pain neurobiology have been and will continue to be overlooked. This sex bias also diminishes the translational relevance of preclinical pain research because of inadequate representation of the clinical population.

2. Microglia-neuronal signalling pathway

Significant efforts have been made to improve understanding of the mechanisms underlying neuropathic pain. Spinal nociceptive processing is a neuronally mediated phenomenon, however maladaptive plasticity of this process, as a consequence of peripheral nerve injury (PNI), is profoundly influenced by non-neuronal immune cells.[41] The principal immune cells of the central nervous system (CNS) are microglia, accounting for approximately 10% of the total CNS cellular population.[1] Microglia have diverse roles within the CNS. They are the predominant activator of neuroinflammation,[35] act as the CNS' first responders, adopting reactive phenotypes in response to potential threats to its integrity[16,19] but also play an integral role in ensuring healthy CNS development and function.[31,32] Abundant evidence now also implicates microglia in rodent models of neuropathic pain.[10,42] After PNI, microglia in the spinal dorsal horn adopt a reactive phenotype that is a key cellular mediator in the maintenance of neuropathic pain.[3,42]

This function of microglia was confirmed by the discovery that P2X4 receptors (P2X4Rs), which within the CNS are specifically expressed by microglia, are integral in mediating pain hypersensitivity resulting from PNI in rodents.[42] After nerve injury, P2X4R expression on microglia is upregulated by increased activity of transcription factors IRF8 and IRF5.[25,26] IRF8 regulates IRF5 expression, which in turn directly regulates transcription of P2X4R.[25,26] Multiple lines of evidence have shown that P2X4Rs are both necessary and sufficient for pain hypersensitivity in male rodents. Pharmacological inhibition of P2X4Rs in the spinal cord reverses pain hypersensitivity in rats[42] and P2X4R-null mice do not develop pain hypersensitivity.[43] Similarly, *Irf5*-null mice do not show an upregulation in spinal P2X4Rs after PNI and display significantly attenuated hypersensitivity.[25] Spinal application of ATP-stimulated microglia produces pain behaviours in naive

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rodents similar to those seen following PNI, indicating sufficiency of P2X4+ microglia in producing hypersensitivity.[42]

ATP stimulation of microglial P2X4Rs induces calcium influx, activation of p38-mitogen-activated protein kinase (MAPK) and consequent synaptic release of brain-derived neurotrophic factor (BDNF).[40] BDNF acts on its cognate neuronal receptor, TrkB, to produce downregulation of the neuronal potassium-chloride transporter KCC2.[10,11] Disruption of chloride transport decreases $GABA_A$-mediated inhibition, producing a transformation in the functional output of lamina I projection neurons in the spinal cord, sufficient to produce allodynia and hyperalgesia.[17]

This key microglia-neuronal signalling pathway mediating PNI-induced pain hypersensitivity was based on experiments using primarily male rodents (eg, Refs. 10,11,25,26,42). It is now apparent that microglia do not contribute to pain hypersensitivity in female mice.[34] Instead, adaptive immune cells may perform an analogous role to microglia in females.[34]

3. Sexual dimorphism of spinal TLR4s in pain hypersensitivity

Evidence that the role of microglia in pain may be sexually dimorphic came with the discovery that spinal Toll-like receptor 4 (TLR4), specifically expressed on microglia in the CNS, contribute to pain hypersensitivity in male mice only.[21,33] TLR4s are involved in the innate immune response and detect bacterial membrane-derived lipopolysaccharide (LPS).[12] TLR4 has been implicated in pain pathology, with evidence that systemic and intrathecal administration of LPS produces hypersensitivity in rats[27,46] and nerve-injured TLR4 knockout mice exhibit decreased pain hypersensitivity.[5,37] However, these studies were largely performed in male animals. TLR4 mediation of pain hypersensitivity is sexually dimorphic; intrathecal LPS administration induces mechanical allodynia in male mice only and is ineffectual in females.[33] Interestingly, this sex difference is restricted to spinal application of LPS as administration to the brain or hind paw produces comparable hypersensitivity in both sexes. Furthermore, pain hypersensitivity from nerve injury is dependent on spinal TLR4s in males only.[33] Given the microglial specificity of TLR4s in the spinal cord, this evidence suggests a more extensive sexual dimorphism in the role of microglia in mediating PNI-induced hypersensitivity.

4. Females do not use microglia to mediate pain hypersensitivity

It had thus been shown that microglia, fundamental to neuropathic pain in males, are not involved to the same degree in female pain processing. Pharmacological disruption of glial functioning can influence PNI-induced hypersensitivity.[45] However, intrathecal application of several drugs that disrupt glial functioning, including minocycline, propentofylline, and fluorocitrate, produces dose-dependent reversal of hypersensitivity in male but not female mice.[34] Moreover, intrathecal injection of saporin toxin conjugated to macrophage antigen complex-1 (MAC-1), which specifically ablates microglia, attenuates pain hypersensitivity in males only.[34] Full confirmation came with a series of experiments in which mice generated with a microglial-specific conditional deletion of BDNF both prevented the maintenance of mechanical allodynia after PNI and reversed established hypersensitivity in male but not female

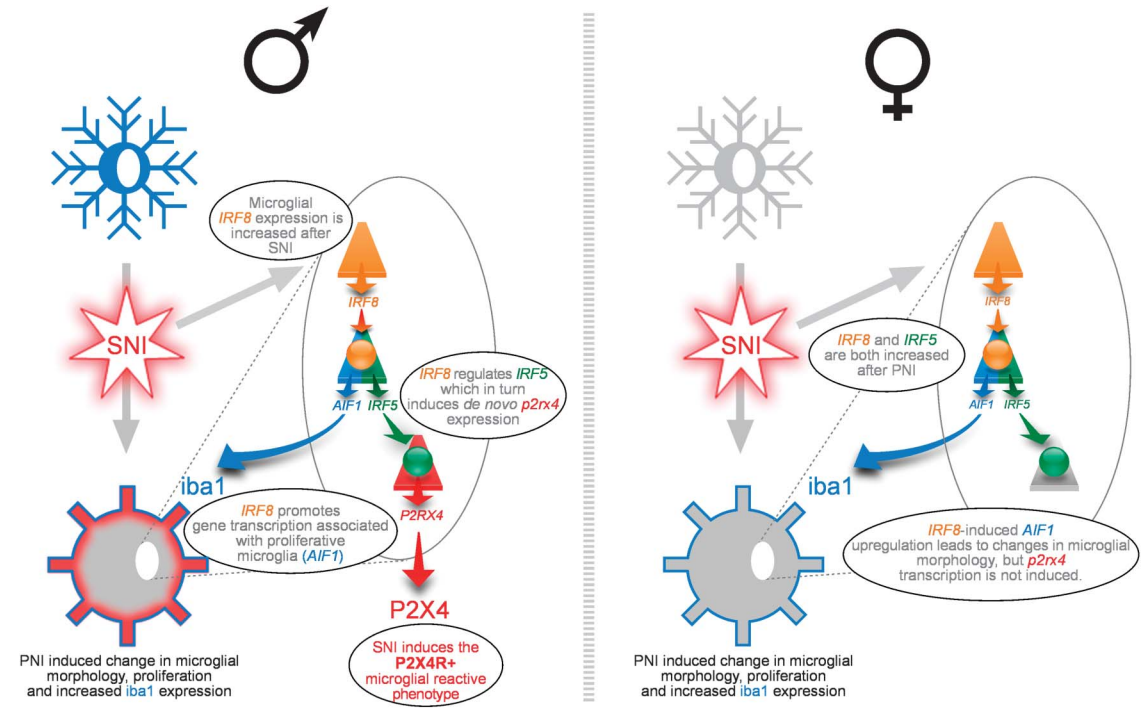

Figure 1. Transcriptional dissociation of *p2rx4* induction in male and female microglia. In male mice (left), P2X4R gene expression (p2rx4 [red]) is under the transcriptional control of interferon regulatory factors (IRFs). After SNI, IRF8 (orange) is upregulated and induces further gene expression changes, notably AIF1 (blue), involved in changes in microglial proliferation, morphology, and iba1 expression. It also increases IRF5 (green) expression. IRF5 binds to the p2rx4 promoter and directs transcription, which in males results in increased de nove expression and resultant increase in microglial P2X4Rs (shown in red). In females (right), IRF8 is upregulated, leading to the characteristic microgliosis in the affected spinal dorsal horn after SNI. IRF5 is also upregulated but fails to exert any transcriptional increase in p2rx4. Thus, the induction of p2rx4 is the point at which SNI-induced transcriptional changes in spinal microglia diverge, while other phenotypic changes associated with the spinal glial response to peripheral nerve injury, proliferative and morphological changes, remain intact in both males and females, and are therefore not sufficient for neuropathic pain.

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mice.[34] Together, these experiments indicate a microglial-independent pathway mediating pain hypersensitivity in females.

Consistent with a lack of microglial dependency in females, P2X4R involvement in PNI-induced hypersensitivity is a male-specific phenomenon.[34] Inhibition of spinal P2XRs reverses hypersensitivity in male but not female mice.[34] Inhibiting each component of the microglia-neuronal signalling pathway downstream of P2X4, including p38 MAPK and BDNF, shows the same sexual dimorphism: reversal of hypersensitivity in male mice with no effect in female mice. Divergence between the male and female signalling pathways seems to occur at the level of *p2rx4* upregulation after PNI. While *irf8* and *irf5* are similarly upregulated between the sexes after injury, *p2rx4* expression is upregulated in male mice only (**Fig. 1**). Why *irf5* fails to induce *p2rx4* transcription in females remains unclear. Microglial proliferation, morphology changes, and associated increases in surface markers after PNI are similar between males and females (**Fig. 1**).[34] IRF8, in addition to its role in P2X4R upregulation, is also critical in promoting these morphological and proliferative aspects of the injury-induced reactive phenotype of spinal microglia.[26] Therefore, increased *irf8* likely explains why the stereotypical dorsal horn microgliosis after PNI still occurs in females after PNI (**Fig. 1**).

5. Role of T cells in pain hypersensitivity in females

Female mice display equivalent levels of pain hypersensitivity as males after PNI.[34] Thus, there must be an alternative, microglial-independent, pathway mediating hypersensitivity in females. Adaptive immune cells, likely T cells, may be an integral component of this microglial-independent pathway. After PNI, T cells infiltrate the spinal cord and are implicit in mediating pain hypersensitivity.[7,9,36] B cells may also contribute to pain hypersensitivity in females; however, B cells do not infiltrate the spinal cord after nerve injury, at least in male mice.[7] B cells also do not seem to contribute to PNI-induced pain hypersensitivity in mice of unspecified sex.[9] Female mice lacking both T cells and B cells are able to switch to the microglial-dependent pathway to mediate hypersensitivity.[34] Reconstitution of adaptive immune cells by splenocyte adoptive transfer also activates a "switch" to a microglial-independent pathway (**Fig. 2**).[34] It is therefore likely that the absence of T cells is responsible for the switch between pathways, but a possible contribution of B cells cannot be definitively excluded. The exact role of T cells in mediating hypersensitivity in females is still to be defined.

Support for a T-cell role in females is the contribution of peroxisome proliferator-activated receptors (PPARs) α and γ in mediating pain hypersensitivity. PPARα and γ have been shown to have differential expression on T cells dependent on testosterone levels, with higher expression of PPARα and PPARγ in male and female mice, respectively.[47] PPARs are ligand-regulated transcription factors in the family of nuclear receptors with widespread functioning including modulation of inflammatory cytokines.[4] PPARs have been implicated in neuropathic and inflammatory pain and may also be able to modulate pain through nongenomic, transcription-independent mechanisms.[13] Intrathecal administration of pioglitazone, a PPARγ agonist, attenuates hypersensitivity in female mice only.[34] Conversely, administration of finofibrate, a PPARα agonist, attenuates pain hypersensitivity in males only.[34] Together, evidence supports a role for adaptive immune cells, likely T cells, in the mediation of pain hypersensitivity in female mice. However, in contrast with male mice, intrathecal administration of pioglitazone in male rats attenuates PNI-induced hypersensitivity.[15] This species difference highlights the need for further studies in males and females to determine whether the sexually dimorphic role of microglia is applicable across species.

6. Neuronal convergence of microglial-dependent and independent pathways

In addition to chloride transport disruption, facilitated glutamatergic excitation contributes to spinal neuronal hyperexcitability.[23] Facilitated excitation is principally produced by protein tyrosine kinase Src-mediated enhancement of NMDA receptor (NMDAR) functioning. Inhibition of Src binding attenuates PNI-induced hypersensitivity.[22] The role of NMDARs in pain hypersensitivity is not sexually dimorphic, as antagonism of spinal NMDARs with

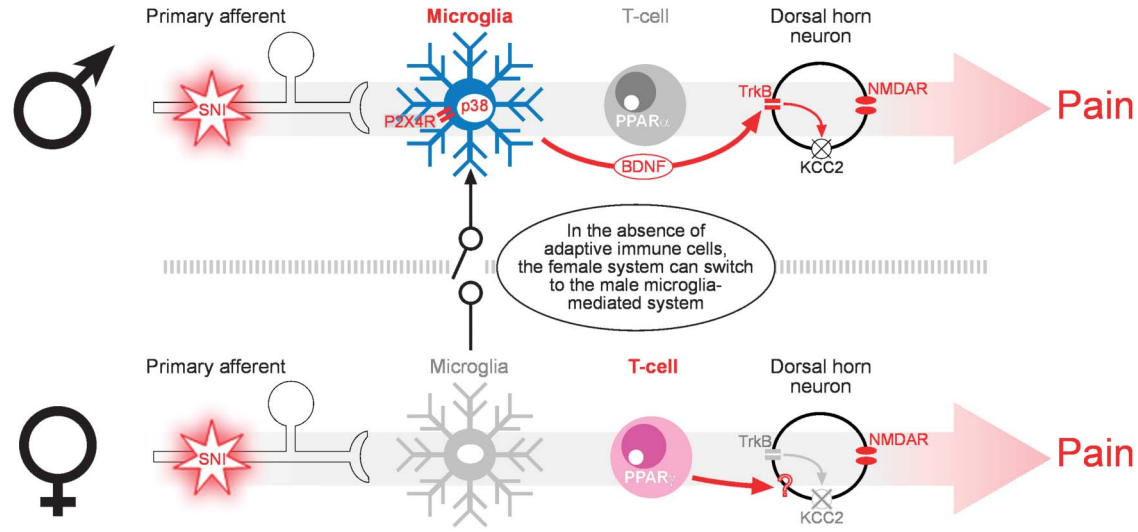

Figure 2. Male and female neuroimmune interactions in the spinal dorsal horn. Summary schematic of the cellular neuroimmune pathways mediating SNI-induced pain hypersensitivity in male and female mice. In male mice, peripheral nerve injury drives an increase in surface expression of microglial P2X4Rs. Activation of these receptors stimulates a p38-dependent synthesis and release of BDNF, which then binds to its neuronally located cognate receptor trkB. TrkB activation in turn leads to a downregulation of KCC2 and a subsequent disinhibition of spinal nociceptive circuitry. In female mice, microglial BDNF is not involved in the maintenance of neuropathic pain after SNI. Instead other neuroimmune interactions, notably involving T cells, drive pain hypersensitivity. The molecular signature of these interactions remains to be determined. In the absence of adpative immune cells, the female system is able to switch and adopt the microglial-dominant pathway.

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intrathecal APV reverses pain hypersensitivity after PNI in both sexes.[34] Thus, the microglial-dependent and independent signaling pathways mediating pain hypersensitivity seem to converge at the neuronal level. It is possible that neuronal changes in females are produced by inflammatory mediators released by adaptive immune cells in lieu of microglial BDNF. In fact, cytokines have been shown to produce NMDAR-enhancement by activation of Src family kinases.[44] Whether the role of KCC2 in mediating pain hypersensitivity is sexually dimorphic has yet to be determined.

7. Microglial proliferation is not an indicator of pain

Peripheral nerve injury induces microglial proliferation around the central terminals of peripherally damaged primary afferents in the dorsal horn of the spinal cord and the adoption of a specific reactive phenotype. Several molecules that are upregulated after nerve injury (eg, iba1 and CD11b) are widely used as markers to identify microglial proliferation. It is well established that microglial proliferation is not sufficient for pain hypersensitivity. For example, PNI in IRF5 knockout mice results in increases in Iba1 and CD11b indistinguishable from wild-type mice despite attenuated hypersensitivity.[25] Similarly, P2X4R-null mice (*P2rx4*$^{-/-}$) do not develop pain hypersensitivity after PNI in contrast with robust upregulation of microglial markers.[43] Dissociations between the presence of microglial proliferation and pain hypersensitivity have also been shown in P2Y12 receptor knockout mice (*P2ry12*$^{-/-}$) as well as mice lacking microglial BDNF (*Cx3cr1*^{CreER} × *loxP-Bdnf*).[34,39] Determining whether microglial proliferation is necessary for the presence of pain hypersensitivity has proven more difficult. The existence of a microglial-independent pathway mediating hypersensitivity in females provides evidence against this necessity. Given that it seems microglial reactivity is neither sufficient nor necessary for pain hypersensitivity, microglia proliferation should not be used to infer the presence of pain. However, the presence of a P2X4R+ microglial reactive phenotype may indicate that the microglia-dependent pathway is activated.

8. Discussion and summary

The status quo in the pain research field has long been the exclusion of female subjects in preclinical experiments. Recent research has illuminated the detrimental impact of this sex bias on our understanding of pain neurobiology. It is now known that the microglia-neuronal signaling pathway, which has been the focus of neuropathic pain research for well over a decade, is relevant only to male mice. The microglial-independent pathway mediating neuropathic pain hypersensitivity in females remains to be fully delineated. Given the profound sexual dimorphism in the neurobiology of neuropathic pain, it is imperative all future preclinical pain research consider the inclusion of both sexes as subjects.

As a result of preclinical research implicating microglia in neuropathic pain, there has been significant interest in using microglial inhibitors as a treatment for chronic pain. The failure of a clinical trial of propentofylline for treatment of postherpetic neuralgia cast doubt on whether microglia are relevant in chronic pain in humans.[20] A sexually dimorphic role of microglia in pain may have been a factor in the lack of efficacy in this trial. However, the veracity of translation of this sex difference to humans is not established. The first evidence for glial activation in patients with chronic pain was demonstrated recently using integrated positron emission tomography–magnetic resonance imaging.[24] Nevertheless, the presence of glial activation does not directly implicate microglia in chronic pain in humans. This is especially true given the dissociation between microglial reactivity and microglial involvement in pain in male vs female mice. Variables in addition to sex, eg, immune system functioning, could influence whether chronic pain is microglial dependent, highlighting the need for personalized medicine. Sex-specific drug development for chronic pain treatment may be necessary. Alternatively, drugs could be developed targeting convergent aspects of the microglial-dependent and independent pathways (eg, KCC2). If KCC2 involvement in pain is congruent between males and females, chloride extrusion enhancing drugs may prove effective in the treatment of chronic pain in both sexes.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Aguzzi A, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else? Science 2013;339:156–61.
[2] Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 2011;35:565–72.
[3] Beggs S, Trang T, Salter MW. P2X4R+ microglia drive neuropathic pain. Nat Neurosci 2012;15:1068–73.
[4] Berger J, Moller D. The mechanisms of action of PPARs. Annu Rev Med 2002;53:409–35.
[5] Bettoni I, Comelli F, Rossini C, Granucci F, Giagnoni G, Peri F, Costa B. Glial TLR4 receptor as new target to treat neuropathic pain: efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice. Glia 2008;56:1312–9.
[6] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. PAIN 2008;136:380–7.
[7] Cao L, DeLeo J. CNS-infiltrating CD4+ T lymphocytes contribute to murine spinal nerve transection-induced neuropathic pain. Eur J Immunol 2008;38:448–58.
[8] Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. Nature 2014;509:282–3.
[9] Costigan M, Moss A, Latremoliere A, Johnston C, Verma-Gandhu M, Herbert T, Barrett L, Brenner G, Vardeh D, Woolf C, Fitzgerald M. T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J Neurosci 2009;29:14415–22.
[10] Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 2005;438:1017–21.
[11] Coull JA, Boudreau D, Bachand K, Prescott SA, Nault F, Sik A, De Koninck P, De Koninck Y. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature 2003;424:938–42.
[12] Erridge C. Endogenous ligands of TLR2 and TLR4: agonists or assistants? J Leukoc Biol 2010;87:989–99.
[13] Fehrenbacher JC, Loverme J, Clarke W, Hargreaves KM, Piomelli D, Taylor BK. Rapid pain modulation with nuclear receptor ligands. Brain Res Rev 2009;60:114–24.

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[14] Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL III. Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain 2009;10:447–85.
[15] Griggs RB, Donahue RR, Morgenweck J, Grace PM, Sutton A, Watkins LR, Taylor BK. Pioglitazone rapidly reduces neuropathic pain through astrocyte and nongenomic PPARγ mechanisms. PAIN 2015;156:469–82.
[16] Hanisch U, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 2007;10:1387–94.
[17] Keller AF, Beggs S, Salter MW, De Koninck Y. Transformation of the output of spinal lamina I neurons after nerve injury and microglia stimulation underlying neuropathic pain. Mol Pain 2007;3:27.
[18] Kest B, Wilson SG, Mogil JS. Sex differences in supraspinal morphine analgesia are dependent on genotype. J Pharmacol Exp Ther 1999;289: 1370–5.
[19] Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci 1996;19:312–8.
[20] Landry RP, Jacobs VL, Romero-Sandoval EA, DeLeo JA. Propentofylline, a CNS glial modulator does not decrease pain in post-herpetic neuralgia patients: in vitro evidence for differential responses in human and rodent microglia and macrophages. Exp Neurol 2012;234:340–50.
[21] Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, Volpe JJ, Vartanian T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci U S A 2003;100:8514–19.
[22] Liu XJ, Gingrich JR, Vargas-Caballero M, Dong YN, Sengar A, Beggs S, Wang SH, Ding HK, Frankland PW, Salter MW. Treatment of inflammatory and neuropathic pain by uncoupling src from the NMDA receptor complex. Nat Med 2008;14:1325–32.
[23] Liu XJ, Salter MW. Glutamate receptor phosphorylation and trafficking in pain plasticity in spinal cord dorsal horn. Eur J Neurosci 2010;32:278–89.
[24] Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR, Hill E, Hsu S, Izquierdo-Garcia D, Ji RR, Riley M, Wasan AD, Zurcher NR, Albrecht DS, Vangel MG, Rosen BR, Napadow V, Hooker JM. Evidence for brain glial activation in chronic pain patients. Brain 2015; 138:604–15.
[25] Masuda T, Iwamoto S, Yoshinaga R, Tozaki-Saitoh H, Nishiyama A, Mak TW, Tamura T, Tsuda M, Inoue K. Transcription factor IRF5 drives P2X4R+ -reactive microglia gating neuropathic pain. Nat Commun 2014;5:3771.
[26] Masuda T, Tsuda M, Yoshinaga R, Tozaki-Saitoh H, Ozato K, Tamura T, Inoue K. IRF8 is a critical transcription factor for transforming microglia into a reactive phenotype. Cell Rep 2012;1:334–40.
[27] Meller S, Dykstra C, Grzybycki D, Murphy S, Gebhart G. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. Neuropharmacology 1994;33:1471–8.
[28] Mogil JS, Chanda ML. The case for the inclusion of female subjects in basic science studies of pain. PAIN 2005;117:1–5.
[29] Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF. Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. Neurosci Biobehav Rev 2000;24:375–89.
[30] Mogil JS, Sternberg WF, Kest B, Marek P, Liebeskind JC. Sex differences in the antagonism of swim stress-induced analgesia: effects of gonadectomy and estrogen replacement. PAIN 1993;53:17–25.
[31] Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR III, Lafaille JJ, Hempstead BL, Littman DR, Gan WB. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell 2013;155:1596–609.
[32] Salter M, Beggs S. Sublime microglia: expanding roles for the guardians of the CNS. Cell 2014;158:15–24.
[33] Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin JS, Ritchie J, Chanda ML, Graham AC, Topham L, Beggs S, Salter MW, Mogil JS. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neurosci 2011;31: 15450–4.
[34] Sorge RE, Mapplebeck JCS, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin J-S, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji R-R, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci 2015;18:1081–3.
[35] Streit WJ, Mrak RE, Griffin WS. Microglia and neuroinflammation: a pathological perspective. J Neuroinflammation 2004;1:14.
[36] Sweitzer SM, Hickey WF, Rutkowski MD, Pahl JL, DeLeo JA. Focal peripheral nerve injury induces leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. PAIN 2002; 100:163–70.
[37] Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci U S A 2005;102:5856–61.
[38] Torrance N, Smith BH, Bennett MI, Lee AJ. The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. J Pain 2006;7:281–9.
[39] Tozaki-Saitoh H, Tsuda M, Miyata H, Ueda K, Kohsaka S, Inoue K. P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. J Neurosci 2008;28:4949–56.
[40] Trang T, Beggs S, Wan X, Salter MW. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. J Neurosci 2009;29:3518–28.
[41] Tsuda M, Inoue K, Salter MW. Neuropathic pain and spinal microglia: a big problem from molecules in "small" glia. Trends Neurosciences 2005; 28:101–7.
[42] Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 2003;424:778–83.
[43] Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, Buell GN, Reeve AJ, Chessell IP, Rassendren F. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. J Neurosci 2008;28:11263–8.
[44] Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci 2003;23: 8692–700.
[45] Watkins L, Maier S. Glia: a novel drug discovery target for clinical pain. Nat Rev Drug Discov 2003;2:973–85.
[46] Wiertelak EP, Smith KP, Furness L, Mooney-Heiberger K, Mayr T, Maier SF, Watkins LR. Acute and conditioned hyperalgesic responses to illness. PAIN 1994;56:227–34.
[47] Zhang MA, Rego D, Moshkova M, Kebir H, Chruscinski A, Nguyen H, Akkermann R, Stanczyk FZ, Prat A, Steinman L, Dunn SE. Peroxisome proliferator-activated receptor (PPAR)alpha and -gamma regulate IFNgamma and IL-17A production by human T cells in a sex-specific way. Proc Natl Acad Sci U S A 2012;109:9505–10.

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