REVIEW

Gliarial contributions to visceral pain: implications for disease etiology and the female predominance of persistent pain

KN Dodds 1, EAH Beckett 1, SF Evans 2–3, PM Grace 2,4, LR Watkins 4 and MR Hutchinson 1,5

In the central nervous system, bidirectional signaling between glial cells and neurons ('neuroimmune communication') facilitates the development of persistent pain. Spinal glia can contribute to heightened pain states by a prolonged release of neuromodulatory signals that sensitize adjacent centrally projecting neurons. Although many persistent pain conditions are disproportionately common in females, whether specific neuroimmune mechanisms lead to this increased susceptibility remains unclear. This review summarizes the major known contributions of glia and neuroimmune interactions in pain, which has been determined principally in male rodents and in the context of somatic pain conditions. It is then postulated that studying neuroimmune interactions involved in pain attributed to visceral diseases common to females may offer a more suitable avenue for investigating unique mechanisms involved in female pain. Further, we discuss the potential for primed spinal glia and subsequent neurogenic inflammation as a contributing factor in the development of peripheral inflammation, therefore, representing a predisposing factor for females in developing a high percentage of such persistent pain conditions.

Translational Psychiatry (2016) 6, e888; doi:10.1038/tp.2016.168; published online 13 September 2016

FROM 'HYSTERIA' TO A MOLECULAR UNDERSTANDING OF FEMALE PAIN

Historical descriptions of chronic debilitating pain without obvious visible cause were originally restricted to females, and dated back over 2000 years to the era of renowned Greek physician Hippocrates (460–370 BC). Episodes of severe emotional and physical distress in women were diagnosed as 'hysteria', a condition attributed to the movement of the uterus outside of the pelvis (the 'wandering womb'). 1 Towards the end of the nineteenth century, the stigma surrounding female hysteria diminished owing to accumulating evidence that men could also suffer from persistent pain, work which was largely pioneered by Sigmund Freud (1856–1939). 2 Considering pain as sex-independent in this context, along with general medical advances from the mid-twentieth century, has contributed to an immense expansion in our understanding of the mechanisms underlying the development of persistent pain. Notably, this is now known to involve bidirectional signaling between neurons and glia within the central nervous system (CNS).

However, a key discrepancy that remains in the literature is the clear over-representation of females among patients with persistent pain. There is an almost unanimous consensus that women are not only more sensitive in detecting painful stimuli, but are also the predominant sex with the most common painful disorders. 3–6 This includes, but is not limited to, conditions associated with neuropathic pain, musculoskeletal pain (such as back pain, fibromyalgia, osteoarthritis and complex regional pain syndrome), orofacial pain (including temporomandibular joint pain), abdominal and pelvic pain (such as irritable bowel syndrome, painful bladder syndrome and dyspareunia) and headache/migraine. 5

Extensive epidemiological, clinical and experimental evidence implicates several biopsychosocial factors as contributing to the disparity in pain susceptibility across the sexes. 7 Despite this, a dichotomy exists in the pain research field at large, where the vast majority of preclinical studies have characterized pain models using male subjects only. 1 Moreover, evidence implicating neuroimmune signaling in the development of persistent pain has primarily been acquired using animal models of neuropathic and somatic inflammatory pain. This has included, but is not restricted to, muscle inflammation, spinal cord injury, peripheral nerve injury, arthritis, bone cancer and chemotherapy. Although many of these pathologies are important for understanding female pain, there is a lack of research into the large number of female-dominant conditions that stem from the viscera. Consequently, the specific biological mechanisms underlying the predisposition of females to persistent pain remain elusive.

It is possible that past research generalizing nociceptive mechanisms across the sexes has limited our approach in effectively treating female pain. Is it appropriate to assume that females process pain via identical mechanisms to males? Can we learn from, adapt and update aspects of the ancient Greek philosophy, by regarding female pain as a fundamentally distinct entity? And, to what extent do the sex-specific anatomical and neuroendocrine systems influence the heightened sensitivity of females to persistent pain?

To consider these questions, this review provides a summary of neuroimmune contributions, specifically those provided by astrocytes and microglia, to persistent pain signaling within the spinal

1 Discipline of Physiology, School of Medicine, University of Adelaide, Adelaide, SA, Australia; 2 Discipline of Pharmacology, School of Medicine, University of Adelaide, Adelaide, SA, Australia; 3 Pelvic Pain SA, Norwood, SA, Australia; 4 Department of Psychology and Neuroscience, Center for Neuroscience, University of Colorado Boulder, Boulder, CO, USA and 5 ARC Centre of Excellence for Nanoscale BioPhotonics, University of Adelaide, Adelaide, SA, Australia. Correspondence: KN Dodds, Discipline of Physiology, School of Medicine, University of Adelaide, Medical School North 416, Frome Road, Adelaide, SA 5005, Australia.

E-mail: kelsi.dodds@adelaide.edu.au

Received 2 June 2016; revised 14 July 2016; accepted 22 July 2016
cord. The concept that female sex hormones may modulate central neuroimmune signaling is then discussed, and that variations in these processes may have relevance for female-dominant pain conditions, as exemplified by several visceral inflammatory diseases. In addition, the dorsal root reflex is re-explored as a central driver of peripheral neurogenic inflammation, leading to the hypothesis that sensitized spinal glia might contribute to, and predispose, a subpopulation of females to persistent inflammatory pain.

**PERSISTENT PAIN ARISES FROM CENTRAL SENSITIZATION**

Pain is a complex, unpleasant sensory and emotional experience that arises in response to, or is described in terms of, tissue damage. Distinct from the well-established protective and adaptive functions of acute pain, pain persisting beyond tissue healing is maladaptive and serves no known physiological function. In contrast to acute pain, the mechanisms involved in the development and maintenance of persistent pain are not fully understood. One potential mechanism that has received detailed investigation is the process of ‘central sensitization’, whereby long-lasting molecular changes cause amplification of pain signaling by nociceptive neurons within the CNS. Central sensitization can include conditions of both hyperalgesia (heightened pain to a previously noxious stimulus) and allodynia (pain caused by a normally innocuous stimulus). It is now acknowledged that the development of central sensitization engages not only neuronal, but also glial processes. Hence, the following sections outline the rationale for considering persistent pain to be a ‘gliopathy’, in addition to the previously described ‘neuropathy’.

**GLIA AND THE TETRAPARTITE SYNAPSE SUPPORT THE MAINTENANCE OF CNS HOMEOSTASIS**

Glia are a non-neuronal, immune-like cell population that constitute the vast majority of cells within the CNS. They comprise satellite glial cells in the ganglia, and microglia, astrocytes and oligodendrocytes within the spinal cord and brain. The anatomical co-localization of astrocytes and microglia in the spinal cord, combined with pre- and postsynaptic neurons, forms a key site of interaction termed the ‘tetrapartite synapse’. Each cell within this functional unit reciprocally signals to another, contributing to a ‘neuroimmune communication’ that allows glia to respond rapidly to disruptions in neuronal signaling. The reactivity state and control of astrocytes and microglia is therefore critical in maintaining healthy CNS activity.

**DYSREGULATION OF HEALTHY GLIAL ACTIVITY CONTRIBUTES TO THE DEVELOPMENT OF PERSISTENT PAIN**

Following injury and aberrant nociceptive events, microglia and astrocytes increase their expression and secretion of various proinflammatory cytokines and chemokines. The stimulation of glial cells can occur by neurokinin products released as a result of tissue injury, or by neurotransmitters released from activated neurons. Many of the proinflammatory responses of glia are important in protecting against challenges that disrupt the homeostatic balance of the CNS, such as during the sickness response—a constellation of adaptive behaviors and physiological responses that promote recovery from illness. However, under certain conditions, glial reactivity is not advantageous and can instead be detrimental to neuronal function, such as during the manifestation of persistent pain.

In response to strong or persistent receptor stimulation, microglia switch from a surveillance state to an active response state, and astrocytes transition from a regulatory to reactive state. Under these circumstances, the release of proinflammatory mediators by glia can contribute to ongoing nociception, by inducing long-lasting plastic changes of synaptic connectivity that enhances the transmission of ascending nociceptive information. As such, glia and their products are sufficient to create exaggerated pain. This has been shown where intrathecal transfer of highly reactive microglia alone, or injection or induction of their proinflammatory products (such as interleukin (IL)-1β and tumor necrosis factor-α (TNFα)) into naive animals, can induce symptoms of neuropathic pain.1,2

The downstream effects of enhanced glial reactivity are strengthened by the fact that immune mediators, including those released by glia, are substantially more potent in modulating neuronal signaling compared with classical neurotransmitters on a per molecule basis. Glial proliferation, morphological changes and increases in protein expression can persist for months after initial injury, even beyond tissue healing. Moreover, proinflammatory mediators and glial-derived neurotransmitters can reciprocally stimulate glia in an autocrine and paracrine manner, thereby amplifying a positive feedback loop of unfavorable activity. How do glia become activated?

Glia function as a product of their microenvironment, and as such the types of receptors they express vary from site to site, and many receptors can be upregulated to make glia more ‘tuned’ to ongoing stimulation. Within the spinal cord, microglia are sensitive to ATP that binds to ionotropic (for example, P2X4 and P2X7) and metabotropic (for example, P2Y6 and P2Y12) purinergic receptors. Chemokine receptors, such as CX3CR1 (with CX3CL1/fractalkine as ligand) and CCR2 (activated by CCL2/MCP-1), also contribute to the microglial proinflammatory response, as well as receptors for the sensory neuropptide, calcitonin gene-related peptide (CGRP) and interferons (IFN), such as IFNy. Akin to microglia, astrocytes can respond to ATP via the surface expression of P2X7 (refs 35,36) and P2Y1 (refs 25,37) and can be stimulated by IFNy (ref 26), as well as a number of mediators released by microglia themselves, including TNFα and IL-18 (for reviews, see refs 11,42). There is also evidence that astrocytes express tachykinergic NK1 receptors, with substance P potentiating the IL-1β-mediated induction of IL-1β and prostaglandin E2 (PGE2) secretion from spinal cord astrocytes. Furthermore, a receptor family expressed by both glial cell types that has gained much recent attention, with regard to pain and immunity, are the Toll-like receptors (TLRs). TLRs allow glia to sense the presence of pathogen- or microbial-associated molecular products. Importantly, some receptor subtypes, such as TLR4, can additionally recognize endogenous ‘self’ warning molecules. Numerous putative ligands have been identified for these so-called damage-associated molecular patterns in the processing of pain, including high mobility group box 1 protein, heat-shock protein 90 (ref 49) and fibroactin.

What proinflammatory products do glia release upon activation? Glial-induced upregulation of proinflammatory signaling is achieved through the induction of gene expression by numerous second messenger-mediated pathways. This includes activation of transcription by phosphorylation of mitogen-activated protein kinases and nuclear factor-κB. Specifically, the mitogen-activated protein kinases implicated here are p38 in microglia, c-Jun N-terminal kinase in astrocytes and extracellular signal-regulated kinases (ERKs) in both glial cell types. The proinflammatory products subsequently released from microglia include IL-1β, IL-6, IL-18, TNFα, PGE2, nitric oxide and brain-derived neurotrophic factor, and IL-1β, IL-6, TNFα, IFNγ, CCL2, CXCL1, CXCL21 and MMP9 from astrocytes (for reviews, see refs 55–58). In addition, astrocytes can increase their release of gliotransmitters, such as ATP, glutamate and δ-serine.
GLIA ENHANCE EXCITATORY NOCICEPTIVE SIGNALING

Glia-derived proinflammatory mediators enhance nociceptive signaling in the spinal cord first by facilitating glutamatergic neurotransmission (Figure 1). IL-1β has been shown to increase presynaptic release of glutamate, ATP, TNFα, CCL2 and IFNγ increase postsynaptic N-methyl-D-aspartic (NMDA) and AMPA receptor currents.59–74 Postsynaptic neurons may further be excited by the release of glutamate from reactive astrocytes.75,76 TNFα can increase postsynaptic NMDA and AMPA-mediated activity by trafficking more receptor to the cell surface,77 and by increasing subsequent Ca2+ conductance through phosphorylation of neuronal ERK.78 In addition, IL-1β can induce SRC-1-mediated phosphorylation of the NRI subunit on NMDA.79,80 D-serine, a powerful neuromodulator released by reactive astrocytes, enhances depolarizing NMDA cation currents by binding to the NMDAR glycine site81. There is also a persistent decrease in astrocytic expression of GLAST and GLT-1,82,83 loss of function of these glutamate transporters causes an elevation in extracellular glutamate concentrations within the synapse.84,85 Thus, the resultant aberrant uptake and/or release of glutamate, as well as the enhanced activity of its postsynaptic receptors, can contribute to excessive nociceptive signaling reaching the brain.

In addition, increased exocytosis of ATP from reactive astrocytes86 can directly stimulate neuronal excitation87 or induce glutamate release from presynaptic neurons,87 an effect that is facilitated by the upregulation of purinoceptors, such as
GLIA ATTENUATE THE INHIBITION OF NOCICEPTIVE SIGNALING

Heightened glial activation can also induce disinhibition; that is, a loss of inhibitory signals within the CNS that usually suppress nociceptive transmission, such as GABA and glycine signaling (Figure 2). The activation of microglial TLR4 by lipopolysaccharide (LPS) in rodent spinal slices induces IL-1β release, which suppresses postsynaptic GABA receptor function through the activation of protein kinase C.\textsuperscript{101} IL-1β-induced protein kinase C activation also attenuates astrocytic GLT-1 activity, leading to increased glutamate within the synaptic cleft.\textsuperscript{101} This not only drives a sustained excitation of postsynaptic neurons, but also a deficiency in the supply of glutamine, which is metabolized from glutamate following its reuptake. Consequently, glutamate-glutamine cycle-dependent GABA synthesis by the presynaptic neuron is attenuated.\textsuperscript{102} Moreover, TNFα can prevent action potentials in inhibitory presynaptic neurons;\textsuperscript{103} IL-1β and IL-6 suppress postsynaptic GABA and glycine currents;\textsuperscript{10} and PGE2, CCL2 and IFNγ can attenuate postsynaptic electrical activity mediated by GABA or glycine.\textsuperscript{104–106} Thus, suppression of inhibitory influences within the spinal cord by glial-derived factors may exacerbate pain, by potentiating the transduction of nociceptive information.

FEMALE SEX HORMONES AND NEURONAL HYPOTHESES UNDERLYING THE SEXUAL DIMORPHISM OF PAIN

In addition to many pain syndromes having greater prevalence in females than males, other anecdotal evidence suggests that sex steroid hormones can have a direct influence on somatic and visceral persistent pain. In women, for instance, certain painful conditions typically occur during the menstrual years, and symptoms tend to fluctuate with the menstrual cycle.\textsuperscript{107,108} Symptom severity of several visceral pain conditions, such as irritable bowel syndrome, has been reported to decrease following menopause,\textsuperscript{109} and increase with hormone replacement therapy.
in postmenopausal women.\textsuperscript{109} Similarly, nociceptive stimuli in rodent visceral pain models are sensitive to both the changing steroid hormone levels throughout the estrous cycle,\textsuperscript{111–113} and during hormone supplementation following ovariectomy.\textsuperscript{114–116} Thus, it has been suggested that either elevated or fluctuating levels of sex hormones have a key role in exacerbating persistent pain.\textsuperscript{117}

However, the mechanisms underlying this modulation remain unclear and, to date, much of the research has focused on sex steroid-mediated alterations in neural activity and/or molecular targets expressed by neurons. For example, antagonism of neuronal NMDA receptors, often co-expressed with estrogen receptor α (ERα), can attenuate the visceromotor reflex to colorectal distension with greater potency in untreated ovariectomized rats, compared with those with estradiol replacement.\textsuperscript{118} Colorectal distension is correlated with an increase in PKA-mediated NMDAR NR1 subunit expression and phosphorylation in ovariectomized, estrogen-supplemented animals, compared with those not receiving estrogen.\textsuperscript{118} Furthermore, intrathecal administration of estrogen or an ERα-selective agonist can cause an increase in distension-evoked dorsal horn neuron pERK expression, and reverse the decrease in distension-evoked visceromotor reflex produced by ovariectomized rats.\textsuperscript{119}

DOES FEMALE SEX HORMONE MODULATION OF GLIAL REACTIVITY CONTRIBUTE TO THE FEMALE PREDOMINANCE OF PERSISTENT PAIN?

Despite our understanding of the tetrapartite synapse in facilitating nociceptive signaling, it is likely that the contribution of glia has not yet received sufficient attention with regard to the female susceptibility to persistent pain. Intriguingly, TLRs - which, as discussed previously, are one receptor family expressed by glia and have an important role in the immunological response to pathogenic stimuli—are well situated to serve as an important molecular target for persistent pain conditions. This is particularly true for hormonally regulated female pain, as estrogen appears to influence TLR4-mediated proinflammation and pain in various conditions. For instance, glucuronide metabolites (which typically have a longer half-life than the parent molecule) of estrogen cause potent activation of TLR4 \textit{in vitro}, correlating with enhanced mechanical allodynia in rats \textit{in vivo}.\textsuperscript{120} The proinflammatory response to LPS is potentiated by estrogen in female but not male neonatal microglia.\textsuperscript{121} Moreover, although adult hippocampal microglia from ovariectomized rats in ex vivo preparations show a downregulation in LPS-induced inflammation upon estrogen supplementation, IL-1β mRNA is potentiated when estrogen is administered \textit{in vivo}.\textsuperscript{121} Long-term estrogen exposure in ovariectomized mice promotes the expression of inflammatory mediators by CNS and peritoneal macrophages, in response to LPS activation \textit{in vivo}\textsuperscript{122} and \textit{ex vivo},\textsuperscript{123} respectively. Intravenous administration of LPS in humans induces a similar decrease in visceral and musculoskeletal pain thresholds, although intriguingly a much more pronounced increase in circulating levels of plasma TNFα and IL-6 was evidenced in females compared with males.\textsuperscript{124} A recent randomized control trial additionally showed that low-dose LPS was perceived to increase pain from supra-threshold noxious thermal stimuli in women only, and impaired conditioned pain modulation, a measure of endogenous pain inhibition.\textsuperscript{125}

Other studies have reported that TLR4-mediated responses are important in male but not female pain. Using LPS-induced (in TLR4 mutant mice)\textsuperscript{126} and spinal nerve ligation (in TLR4 knockout mice)\textsuperscript{127} models of pain enhancement, it was reported that mechanical allodynia is TLR4-dependent in males but TLR4-independent in females. Inhibition of spinal p38 MAP kinase has been effective in attenuating inflammatory and neuropathic pain in male, but not female mice.\textsuperscript{128} It has further been proposed that female pain is independent of microglia in a rodent model of mechanical allodynia, alternatively involving the recruitment of T cells.\textsuperscript{129} However, this argument bears further consideration given that males are comparable to females in the generation of autoimmune T cells, but the phenotype of regulatory T cells (Treg), which serve to suppress inflammatory processes, may be more aggressive in males.\textsuperscript{130}

Perhaps these opposing results mirror the highly complex, and well recognized, nature of estrogen being both a pronociceptive and antinociceptive hormone (see reviews in refs 131–135). Regardless, it is evident that the effects of female sex hormones on TLR4-mediated signaling are multifaceted and, given the range of receptors and pathways utilized by glia, highlight the need for research into neuroimmune mechanisms that may be specific to pain in females.

SOMATIC VERSUS VISCERAL PAIN

Persistent pain is a cardinal feature of chronic inflammation of peripheral tissues; thus, our increase in knowledge of neuroimmune signaling has led to investigations of the link between glia and persistent pain associated with inflammation. These data have been primarily acquired using animal models of neuropathic and somatic inflammatory pain, with considerably less attention given to pain arising from the viscera. Although there are many commonalities in the processing of somatic and visceral pain, there are also several important clinical distinctions (for reviews, see refs 136–138). For instance, pain cannot be evoked from all viscera; visceral pain is diffuse and poorly localized, owing to relatively few visceral afferents with extensive receptive fields; visceral pain can often be referred to remote locations, attributable to visceral and somatic afferent pathways converging into shared spinal levels; injury to the viscera does not necessarily cause pain; and intense motor and autonomic reflexes, such as nausea and muscle tension, usually accompany visceral pain. This aside, the fundamental mechanisms leading to the perception of somatic and visceral pain are similar, where enhanced activity from peripheral nociceptors activates ascending central pathways to the brain. Consequently, the involvement of neuroimmune signaling in persistent pain attributed to visceral inflammation has gained interest in the past few years.\textsuperscript{139}

NEUROIMMUNE CONTRIBUTIONS TO THE FEMALE PREDOMINANCE OF PAIN ASSOCIATED WITH INFLAMMATION OF THE PELVIC VISCERA

The viscera are also where sex divergences in pain processing become particularly intriguing, owing to the unique organization of the reproductive and pelvic anatomy in males and females. It has been estimated that women are at greater risk of developing persistent pain within the pelvis, currently affecting between 15 and 24% of women\textsuperscript{140,141} (versus 1.8–12% in men\textsuperscript{142,143}), including pain due to menstruation, intercourse, pregnancy and childbirth, and infection and inflammation via the vagina, cervix and uterus.\textsuperscript{144–146} Spinal microglia been found to contribute to pain in male animals with chronic prostatitis.\textsuperscript{146,147} To our knowledge, however, there are currently no comprehensive studies investigating glial contributions to pain associated with visceral diseases that have been restricted to, or with a substantial focus on, females. This alternative scope in research could reveal distinct female pain mechanisms that may be exploited to improve pain management. Potential neuroimmune contributions to three visceral conditions that have a greater prevalence in, or are exclusive to, females are discussed below: inflammatory bowel disease (IBD), painful bladder syndrome and endometriosis. These pathologies share several features of neuropathic pain and somatic inflammation, such as heightened neural activity, decreased pain thresholds and increased pain behavior, indicating that central neuroimmune
adaptations are probably taking place. This is supported by evidence demonstrating that experimentally induced IBD, cystitis or endometriosis can result in the sensitization of adjacent pelvic organs (for example, intestines, bladder and uterus).\textsuperscript{148–151} A similar phenomenon is observed clinically with the clustering of comorbidities in women with pelvic pain, such as patients with irritable bowel often presenting with viscero-visceral (for example, bladder or menstrual pain) or viscero-somatic (for example, pelvic muscle spasm, temporomandibular pain) complaints.

### Inflammatory bowel disease

IBD comprises ulcerative colitis and Crohn’s disease, both of which involve colonic inflammation; however, each has distinctive pathologic features.\textsuperscript{152} Although the prevalence of ulcerative colitis in males and females is generally similar, the female–male ratio of Crohn’s disease in adults is increased up to approximately 1.2–1.3 times.\textsuperscript{153,154} The studies on glia and IBD have utilized rodent models of di- or trinitrobenzene sulfonic acid-induced colitis, and potential differences between the sexes have not been analyzed.\textsuperscript{155–158} Nonetheless, marked increases in reactivity were described for microglia in the spinal cord and hippocampus.\textsuperscript{159} TLRs 1, 4, 5, 6, and activated satellite glia in the dorsal root ganglia.\textsuperscript{156} This is associated with an upregulation of TNFa levels,\textsuperscript{155,156} and closer apposition between satellite glial cells and primary afferent neurons in the dorsal root ganglia\textsuperscript{156} via enhanced neuron–glia gap junction coupling.\textsuperscript{158} Associated centrally derived hyperalgesia was assessed by various methods, including increased visceromotor reflex activity\textsuperscript{156} and abdominal withdrawal reflex\textsuperscript{157} to graded colonic distension. Intracerebroventricular,\textsuperscript{155} intrathecal or systemic\textsuperscript{156} minocycline or intrathecal administration of an anti-TNFa antibody\textsuperscript{157} attenuated the respective pain behaviors examined.

### Painful bladder syndrome

Contributions of neuroimmune overactivity to persistent pain have also been suggested in animal models of, and human patients with, painful bladder syndrome. Formally known as interstitial cystitis, painful bladder syndrome affects approximately 3–7% of adult females and 2–4% of males, encompassing a range of bladder disorders that involve persistent pelvic pain or discomfort, nonspecific urinary symptoms and often cystitis.\textsuperscript{159,160} In a preliminary study using pooled data from male and female cats with spontaneous feline interstitial cystitis, the fluorescent intensity and number of GFAP-immunopositive astrocytes in the S1 spinal cord dorsal horn was increased compared with healthy unaffected cats.\textsuperscript{161} In addition, it has recently been demonstrated that peripheral blood mononuclear cells from women with painful bladder have an increased proinflammatory response to TLR2 and TLR4 stimulation \textit{in vitro}.\textsuperscript{162} The magnitude of the proinflammatory response also positively correlated with the extent of pelvic and extra-pelvic pain, and the manifestation of comorbid conditions.\textsuperscript{163} This observation has great importance, as the TLR responsivity of peripheral blood mononuclear cells could serve as a neuroimmune biomarker for persistent pain,\textsuperscript{164} given the functional similarities between TLR signaling of immune cells in the periphery and in the CNS. Thus, the heightened TLR responsivity of peripheral immune cells in females with painful bladder syndrome may indicate that CNS sensitization involving neuroimmune modulation may be occurring in parallel, and remains to be explored further.

### Endometriosis

Endometriosis is an estrogen-dependent, chronic, inflammatory medical condition in women, defined as the presence of endometrial tissue in extra-uterine locations, and commonly associated with painful pelvic symptoms. It affects an estimated 5–10% women of reproductive age,\textsuperscript{165} and up to 60% women with persistent pelvic pain.\textsuperscript{166} Endometriosis-associated pain is thought to solely arise from the presence of lesions, yet pain symptoms attributed to the disease can occur in women with lesions removed,\textsuperscript{167} and the severity of experienced pain correlates poorly with the degree of lesions.\textsuperscript{168,169} Thus, it exemplifies all that is female, from the unique visceral anatomy to the complex hormonal interplay, and the long-standing association with unexplained persistent pain.

Given that the conditions mentioned above affect the visceral organs present in both sexes, studying endometriosis (and indeed other female-specific conditions, such as vulvodynia) may provide further insight into subpopulation adaptations of neuroimmune-mediated pain. Neural changes have been studied in detail,\textsuperscript{170,171} and it has been suggested that pain attributed to endometriosis is likely to involve neuronal processes leading to central sensitization.\textsuperscript{115,170,172,173} However, a potential role for glia has yet to be investigated. Accumulating evidence nevertheless demonstrates that there are alterations in peripheral immune function in endometriosis patients.\textsuperscript{174,175} LPS-stimulated peritoneal macrophages from women with endometriosis secrete significantly higher levels of proinflammatory cytokines (for example, IL-6 and TNFa) than non-diseased counterparts, an effect that can be attenuated by pre-treatment with a TLR4-neutralizing antibody.\textsuperscript{176} TLR4 mRNA transcript expression is increased up to sixfold in endometriosis lesions compared with eutopic endometrium,\textsuperscript{177} and TLR2 and TLR9 mRNA from peritoneal effusions are upregulated in endometriosis patients compared with healthy controls.\textsuperscript{178} It remains to be determined whether the increased TLR levels are owing to an upregulation of the receptors per immune cell, or recruitment of TLR-bearing cells to the diseased area. There is now also solid evidence from multiple lines of investigation that the development and maintenance of endometriosis involves atypical peritoneal macrophage activity.\textsuperscript{179,180}

Collectively, these data suggest that several alterations in neural, immune and neuroimmune functions exist in the female-predominant conditions of IBS, painful bladder and endometriosis. Studies that further investigate visceral disease-associated modifications in neuroimmune signaling are desirable. Such information would further our knowledge of persistent pain mechanisms, and may also identify a molecular basis of pain susceptibility in the subpopulation of females.

**DOES THE DORSAL ROOT REFLEX AND NEUROGENIC INFLAMMATION CONTRIBUTE TO THE DEVELOPMENT OF VISCERAL INFLAMMATORY CONDITIONS?**

Besides painful symptoms, many chronic inflammatory diseases present with visible tissue abnormalities and consequently a vast number of studies focus on characterizing and treating these lesions. However, attention has recently shifted to unraveling the complex molecular pathways that instead underlie disease etiology. This is particularly interesting in the example of endometriosis, which is generally attributed to the movement of menstrual debris through the fallopian tubes into the abdomino-pelvic cavity during menses (retrograde menstruation).\textsuperscript{181} Although it is estimated that approximately 90% women aged 15–49 years will exhibit retrograde menstruation,\textsuperscript{182} only around one in ten will develop endometriosis lesions. Similarly, in many patients, the onset of IBD follows a bout of gastroenteritis,\textsuperscript{183} yet not all individuals with gastroenteritis will develop IBD. Thus it seems other factors affect the likelihood of disease formation in subsets of patients, leaving them susceptible to developing disease compared with their peers.

It is well established that sensitized sensory nerves can initiate or exacerbate inflammatory conditions by the release of...
neuropeptides from peripheral nerve terminals, such as CGRP and substance P.\textsuperscript{184–186} This results in edema, immune cell infiltrate and other sequelae reminiscent of inflammation; hence has been termed neurogenic inflammation.\textsuperscript{187} The release of such peptides in the periphery is known to occur via two antidromic signaling mechanisms. Initially, there is strong local stimulation of peripheral nerve terminals at the site of disease, known as the ‘axonal reflex’. With increased afferent input, the central terminals of sensory neurons within the spinal dorsal horn may also be excited, leading to anterograde propagation of action potentials back to the periphery (the ‘dorsal root reflex’).\textsuperscript{188–190}

Centrally derived neurogenic inflammation via the dorsal root reflex contributes to pathology in several animal models of peripheral inflammation, mostly involving the skin\textsuperscript{191–196} and joints,\textsuperscript{187–199} but also colitis.\textsuperscript{200} Compared with control animals receiving infused saline, colonic tissues from rats stimulated with intrathecal SP to the lumbar spine showed increased protein expression of the proinflammatory cytokine, migration inhibitory factor, mucosal edema and lymphocyte infiltration, effects that were attenuated by intrathecal pre-treatment with an NK1-receptor antagonist. The efferent propagation of inflammation via central dorsal horn activation has also been supported in humans, by observations that relapses in ulcerative colitis have been associated with electrical stimulation of the spinal cord.\textsuperscript{201–203}

**Figure 3.** Possible involvement of centrally mediated neurogenic inflammation in the development of visceral inflammatory disease in the periphery: example for endometriosis. (1) During menstruation, endometrial debris passes both per vaginum and in a retrograde fashion through the fallopian tubes to the peritoneal cavity. (2) In certain women, the inflammatory events initiated by ectopic endometrial tissue activate sensory afferents innervating adjacent visceral structures, which transmit the noxious information to the spinal dorsal horn. In addition to exciting ascending neural signals projecting to the brain, afferent neurotransmitter release could potentially also activate spinal astrocytes and microglia, whose proinflammatory products contribute to the development of central sensitization and exaggerated pain (see Figures 1 and 2 for details). (3) Strong ongoing afferent stimulation associated with regular monthly menstruation and dysmenorrhea, as well as the excitatory environment created by reactive glia, may reciprocally activate the central terminals of sensory nerves. This can then induce the antidromic release of neuropeptides (such as SP and CGRP) at the peripheral site of disease (the ‘dorsal root reflex’). (4) The subsequent induction of neurogenic inflammation, including the release of cytokines (IL-1β and TNFα), PGE2 and nerve growth factor (NGF) from local immune cells, may then contribute to an environment that encourages the implantation of endometrial debris onto the peritoneum, and the development of endometriotic lesions (including the associated neovascularization and sprouted innervation). CGRP, calcitonin gene-related peptide; IL, interleukin; PGE2, prostaglandin E2; TNFα, tumor necrosis factor-α.
IL-1β-overexpression (peripheral projections, dorsal root ganglia and central projections), which also displayed spontaneous behavior indicative of pain. It was suggested that bidirectional crosstalk between the CNS and peripheral joints, via spinal IL-1β stimulation of sensory afferents to release CGRP in the periphery, may have a role in the exacerbation of inflammation and pain. Therefore, heightened spinal glial reactivity and proinflammatory signaling may contribute to ongoing peripheral inflammation, as well as enhancing pain by central sensitization.

This raises the interesting question as to whether centrally derived neurogenic inflammation, generated in part by neuroimmune signaling, contributes to the perpetuation of other inflammatory diseases. Indeed, neurogenic inflammatory processes have been implicated in the exacerbation of IBD, cystitis and endometriosis. In endometriosis, neurogenic inflammation is thought to create an optimal peritoneal environment for ectopic lesion formation in the visceral tissues. In this setting, enhanced afferent signaling in response to accumulating endometrial debris may facilitate lesion development by a positive feedback loop (Figure 3). Further research into the role of glia and the dorsal root reflex in the development of inflammation are recommended.

EARLY-LIFE STRESSORS AS CENTRAL GLIAL PRIMERS FOR VISCERAL INFLAMMATION

It is now realized that glia have the ability to be ‘primed’ by prior experience to over-respond to new immune challenges (a ‘two-hit hypothesis’). This is shown where laparotomy and intraperitoneal injection of LPS each individually cause modest increases in mechanical allodynia. However, alldynia is potentiated up to threefold when laparotomy and LPS are administered sequentially, with enhanced pain being associated with heightened microglial reactivity.

Many studies are currently investigating the impact of early-life stressors, such as maternal separation or injury, on long-lasting glial alterations in the adult. Such events can be the ‘first hit’ that primes glia to over-respond and be detrimental in restoring ‘second hit’ immune challenges later in life. Visceral hyperalgesia can be enhanced by early adverse events although associations with glia have thus far been described only for somatic pain. For instance, incisional surgery of the neonatal rat hind paw caused an increase in the intensity of microglial activation and expression within the dorsal horn that persisted into adulthood. This was associated with hyperalgesia following incisional surgery as an adult, and was prevented by intrathecal administration of minocycline at the time of adult injury. Thus, prospective studies comparing the root causes of sex-specific pain conditions may have important implications for both future pain prevention and treatment strategies.

As we unravel the molecular pathways involved in enhancing nociceptive transmission, this will provide opportunities for resultant drug discovery. New pharmacotherapies that aim to target glia to modulate their deleterious, proinflammatory contributions to pain are now steadily emerging.

Thus, it is likely that the future analgesic success of these agents will be highly dependent on the type of injury or disease, the selection of drug and dosing regimen, the route of delivery and the timing of treatment. With continued investigations, the neuroimmune system represents a key target to decrease the burden of persistent pain.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Mr Tavik Morgenstern for assistance with the generation of figures, and Emeritus Professor Roland Sussex for editorial review of the manuscript. This review was supported in part by funding from the University of Adelaide Joyner Scholarship in Medicine (to KND); the Pelvic Pain Foundation of Australia (SFE); a National Health and Medical Research Council CJ Martin Postdoctoral Fellowship (to PMG, ID: 1054091); a National Institutes of Health Grant (to LRW, ID: DE021966); and an Australian Research Council Fellowship (to MRH, ID: DP11010297).

REFERENCES

1. King H. Once upon a text: hysteria from Hippocrates. Hippocrates’ Woman: Reading the female body in Ancient Greece, 1st edn. Routledge: London, UK, 1998, pp 205–246.

2. Freud S, Freud A. Observation of a severe case of hemi-anaesthesia in a hysterical male (1886) and Hysteria (1888). The Standard Edition of the Complete Psychological Works of Sigmund Freud: Pre-Psycho-Analytic and Unpublished Drafts. Vintage Classics: London, UK, 2001, pp 23–34, 39–47.

3. Berkley KJ. Sex differences in pain. Behav Brain Sci 1997; 20: 371–380.
26 Inoue K. Purinergic systems in microglia.

23 Anderson CM, Bergher JP, Swanson RA. ATP-induced ATP release from astrocytes.

22 Shiga H, Tojima T, Ito E. Ca2+ signaling regulated by an ATP-dependent autocrine mechanism in astrocytes.

20 Beggs S, Currie G, Salter MW, Fitzgerald M, Walker SM. Priming of adult pain neurons.

19 Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH. Evidence for a role of heat shock protein-90 in toll like receptor 4 mediated pain processing.

18 Reddington RM, Priller J, Treichel J, Haas C, Kreutzberg GW. Astrocytes and microglia as potential targets for calcitonin gene related peptide in the central nervous system. Can J Pharmacol 1995; 73: 1047–1049.

17 Cady RJ, Glenn JR, Smith KM, Durham PL. Calcitonin gene-related peptide promotes cellular changes in trigeminal neurons and glia implicated in peripheral and central sensitization. Mol Pain 2011; 7: 94.

16 Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional neuroimmune interface.

13 Ren K, Dubner R. Activity-triggered tetrapartite neuron-glial interactions following peripheral injury. Purinergic Sign 2012; 8: 301–310.

12 Hu JH, Wu MY, Tao M, Yang JP. Changes in protein expression and distribution of spinal CCR2 in a rat model of bone cancer pain. Brain Res 2013; 1509: 1–7.

11 Yang WP, Yang J, Cui Y, Zhang J. Evidence for a role of heat shock protein-90 in toll like receptor 4 mediated pain processing.

10 Campbell JN, Meyer RA. Mechanisms of neuropathic pain. Neuro Sci 2005; 52: 77–92.

9 Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain.

8 Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB et al. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007; 132(Suppl 1): S26–545.

7 Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL. Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain 2009: 10: 447–485.

6 Mogil JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci 2012; 13: 859–866.

5 Mogil JS, Chanda ML. The case for the inclusion of female subjects in basic science studies of pain. Pain 2005; 117: 1–5.

4 Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB et al. Studying sex and gender differences in pain and analgesia: a consensus report. Pain 2007; 132(Suppl 1): S26–545.

3 Zeng JW, Liu XH, Zhang JH, Wu XG, Ruan HZ. P2Y1 receptor-mediated glutamate release from cultured dorsal spinal cord astrocytes. J Neurochem 2008; 106: 2106–2118.

2 Nagai Y, Miki H, Kim S, Kikuchi Y, Ono Y, Nishizawa Y. Tissue macrophages stimulate pain by g protein-coupled receptor 8 activation.

1 Translational Psychiatry (2016), 1–13

Glia and visceral pain

KN Dodds et al

1160.

135(S1): S26

J Pain 2011; 149: 147–153.

134: 438–449.

135: 576.

132: 1277–1280.

131: 404–417.

130: 572–576.

129:971–978.

128: 1377–1390.

127: 1534–1550.

126: 46–56.

125: 149–162.

124: 331–336.

123: 33–50.

122: 1576–1589.

121: 116–126.

120: 120–128.

119: 33–56.

118: 857–866.

117: 862–868.

116: 813–819.

115: 1353–1359.

114: 813–819.

113: 443–454.

112: 3074–3080.

111: 79–86.

110: 592–607.

109: 263–272.

108: 715–722.

107: 607–614.

106: 283–302.

105: 83–107.

104: 240–247.

103: 777–783.

102: 120–126.

101: 793–934.

100: 1534–1550.

99: 332–337.

98: 297–304.

97: 183–193.

96: 313–329.

95: 572–576.

94: 6–24.

93: 443–454.

92: 443–454.

91: 331–336.

90: 676–676.

89: 576–576.

88: 666–676.

87: 933–934.

86: 1354–1544.

85: 1802–1813.

84: 1682–1832.

83: 1576–1589.

82: 332–337.

81: 120–126.

80: 572–576.

79: 332–337.

78: 576–576.

77: 1534–1544.

76: 1802–1813.

75: 120–126.

74: 332–337.

73: 572–576.

72: 1354–1544.

71: 862–868.

70: 1534–1544.

69: 1802–1813.

68: 120–126.

67: 120–126.

66: 857–866.

65: 116–126.

64: 240–247.

63: 79–86.

62: 592–607.

61: 79–86.

60: 120–126.

59: 120–126.

58: 572–576.

57: 120–126.

56: 572–576.

55: 1534–1544.

54: 1802–1813.

53: 120–126.

52: 572–576.

51: 120–126.

50: 572–576.

49: 572–576.

48: 572–576.

47: 572–576.

46: 572–576.

45: 572–576.

44: 572–576.

43: 572–576.

42: 572–576.

41: 572–576.

40: 572–576.

39: 572–576.

38: 572–576.

37: 572–576.

36: 572–576.
Glia and visceral pain

Glia and visceral pain

Clark AK, Old EA, Malcangio M. Neuropathic pain and cytokines: current perspectives. J Pain Res 2013; 6: 803–814.

Mika J, Zychowska M, Popiolek-Barczyk K, Rovenska E, Przewlocka B. Importance of glial activation in neuropathic pain. Eur J Pharmacol 2013; 716: 106–119.

 fluoride acidic protein (GFAP) in lumbar spinal cord increases following a sciatic nerve injury and the spinal cord astrocytes is potentiated by substance P. J Neurochem 2006; 99: 524–536. 

Gassner M, Sandkuhler J. Induction of thermal hyperalgesia and synaptic long-term potentiation in spinal cord in addition to pain behavior. J Pain Res 2015; 8: 471–487.

Yan X, Weng HR. Endogenous interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001; 410: 471–477.

Ji RR, Befort K, Brenner GJ, Woolf CJ. ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and IL-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Neurosci Lett 2015; 603: 42–47.

Lefebvre Y, Amadio A, Vincent P, Descheemaeker A, Oliet SH, Dalle R et al. Neuropathic pain depends upon d-serine co-activation of spinal NMDA receptors in rats. Neurosci Lett 2015; 603: 42–47.

Begu O, Aimard A, Vincent P, Descheemaeker A, Oliet SH, Dalle R et al. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. Neuropearmacology 1994; 33: 1471–1478.

Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 2009; 27: 119–145.

Vikman KS, Hill RH, Backstrom E, Robertson B, Kristensson K. Interferon-gamma upregulates functional expression of neurokinin-1 receptor via p38 MAPK. J Neurosci 2011; 31: 57–61.

Ying YL, Wei XY, Xu XB, She SZ, Zhou LJ, Li JY et al. Over-expression of P2X7 receptors in spinal glial cells contributes to the development of chronic postsurgical pain induced by skin/muscle incision and retraction (SMIR) in rats. Exp Neurol 2014; 256: 836–843.

Tozaki-Saitoh H, Tsuda M, Miyata H, Ueda K, Kohsaka S, Houe K. P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. J Neurosci 2008; 28: 4949–4956.

Kobayashi K, Yamanaka H, Fukukota T, Dai Y, Obata K, Noguchi K. P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. J Neurosci 2008; 28: 2982–2982.

Lee K-M, Leon S-M, Cho H-J. Interleukin-6 induces microglial CX3CR1 expression in the spinal cord after peripheral nerve injury through the activation of p38 MAPK. Eur J Pain 2010; 14: 682.e1–682.e12.

Willemsen HL, Eijkelkamp N, Wang H, Dantzer R, Dom GW 2nd, Kelley KW et al. Microglial/macrophage GRK2 determines duration of peripheral IL-1beta-induced hyperalgesia: contribution of spinal cord CX3CR1, p38 and IL-1 signaling. Pain 2010; 150: 550–560.

Guo CJ, Douglas SD, Gao Z, Wolf BA, Grimspan J, Lai JP et al. Interleukin-1beta upregulates functional expression of neurokinin-1 receptor (NK-1 R) via NF-kappaB in astrocytes. Glia 2004; 48: 259–266.

Luber-Narod J, Kage R, Leeman SE. Substance P enhances the secretion of tumor necrosis factor-alpha in human astrocytic cells and blood mononuclear cells: characterization of novel tachykinin receptor antagonists. FEBS Lett 1996; 399: 321–325.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001; 410: 471–477.

Ji RR, Befort K, Brenner GJ, Woolf CJ. ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci 2002; 22: 478–485.

Ji RR, Loth E, Moore KA, Cowell C. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 2003; 26: 696–705.

Yan X, Jiang E, Weng HR. Activation of toll like receptor 4 attenuates GABA synthesis and postsynaptic GABA receptor activities in the spinal dorsal horn via releasing interleukin-1 beta. J Neuroinflammation 2015; 12: 222.

Jiang E, Yan X, Weng H-R. Glial glutamate transporter and glutamine synthetase regulate GABAergic synaptic strength in the spinal dorsal horn. J Neurochem 2013; 126: 526–536.

Zhang H, Nie H, Dougherty PM. A p38 mitogen-activated protein kinase dependent mechanism of disinhibition in spinal synaptic transmission induced by tumor necrosis factor-alpha. J Neurosci 2010; 30: 12844–12855.

Ahmadi S, Lippross S, Neububer WL, Zeilhofer HU. PGE(2) selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurones. Nat Neurosci 2002; 5: 34–40.
Glosselin RD, Varela C, Banisadr G, Megchiel P, Rostene W, Kitabgi P et al. Constitutive expression of CCR2 chemokine receptor and inhibition by MCP-1/CCCL2 of GABA-induced currents in spinal cord neurons. J Neurochem 2005; 95: 1023–1034.

Vilkin KS, Duggan AW, Siddall PJ. Interferon-gamma induced disruption of GABAergic inhibition in the spinal dorsal horn in vivo. Pain 2007; 133: 18–28.

Douglas A, Lea R, Jackson N, Whicher PJ. The menstrual cycle affects rectal sensitivity in patients with irritable bowel syndrome but not healthy volunteers. Gut 2002; 50: 471–474.

Riley JL III, Robinson ME, Wise EA, Price D. A meta-analytic review of pain perception across the menstrual cycle. Pain 1999; 81: 225–235.

Palsson OS, Whitehead WE, Barghout T, Levy R, Feld A, Von Korff M et al. IBS severity and health-related quality of life improve with age in women but not in men. Am J Gastroenterol 2003; 98: 5272–5277.

Ruigómez A, García Rodríguez LA, Johansson S, Wallander MA. Is hormone replacement therapy associated with an increased risk of irritable bowel syn-
drome? Maturitas 2003; 44: 133–140.

Cason AM, Samuelsen CL, Berkley KJ. Estrous changes in vaginal nociception in a rat model of endometriosis. Horm Behav 2003; 44: 123–131.

Ji Y, Tang B, Traub RJ. Modulatory effects of estrogen and progesterone on colorectal hyperalgesia in the rat. Pain 2005; 117: 433–442.

Berkley KJ, McAllister SL, Accius BE, Winnard KP. Endometriosis-induced vaginal hyperalgesia in the rat: effect of estrus phase, ovariectomy, and estradiol replace-
mant. Pain 2007; 132(Suppl 1): S510–S519.

Robbins MT, Mebane H, Ball CL, Shaffer AD, Ness TJ. Effect of estrogen on bladder nociception in rats. J Urol 2010; 183: 1201–1205.

Traub RJ, Ji Y. Sex differences and hormonal modulation of deep tissue pain. Front Neuroendocrinol 2013; 34: 350–366.

Tang B, Ji Y, Traub RJ. Estrogen alters spinal NMDA receptor activity via a PKA signaling pathway in a visceral pain model in the rat. Pain 2008; 137: 540–549.

Ji Y, Tang B, Traub RJ. Spinal estrogen receptor alpha mediates estradiol-induced pronociception in a visceral pain model in the rat. Pain 2011; 152: 1182–1191.

Lewis SS, Hutchinson MR, Frick MM, Zhang Y, Maier SF, Sammakia T et al. Select steroid hormone glucuronide metabolites can cause toll-like receptor 4 activa-
tion and enhanced pain. Brain Behav Immun 2015; 44: 128–136.

Loram LC, Sholar PW, Taylor FR, Wiesler JL, Babb JA, Strand KA et al. Sex and estradiol influence glial pro-inflammatory responses to lipopolysaccharide in rats. Psychoneuroendocrinology 2012; 37: 1688–1699.

Soucy G, Boivin G, Labrie F, Rivest S. Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. J Immunol 2005; 174: 6391–6399.

Calippe B, Douin-Echinard V, Delpy L, Laffargue M, Lelu K, Krust A. Toll-like receptor 4 mediates in vivo response to bacterial and viral pathogens in the female brain. Brain Behav Immun 2011; 25: 1564–1567.

Ejike CECC, Ezeanyika LU. Prevalence of chronic prostatitis symptoms in a randomly surveyed adult population of urban-community-dwelling Nigerians males. Int J Urol 2008; 15: 340–343.

Lathpe P, Mignini L, Gray R, Hills R, Khan K. Factors predisposing women to chronic pelvic pain: systematic review. Br Med J 2006; 332: 749–755.

Curtin NC. Commentary on the influence of gender on the management of chronic pelvic pain. BJOG 2015; 122: 766–768.

Zhang H, Liu L, Yang Z, Pan J, Chen Z, Fang Q et al. P2X7 receptor mediates activation of microglial cells in prostate of chemically irritated rats. Int J Urol 2013; 20: 276–285.

Wong L, Done JD, Schaeffer AJ, Thubrikat P. Experimental autoimmune pros-
titis induces microglial activation in the spinal cord. Prostate 2015; 75: 50–59.

Chen Z, Xie F, Bao M, Li X, Chao Y, Lin C et al. Activation of p38 MAPK in the rostral ventromedial medulla by visceral noxious inputs transmitted via the dorsal columns may contribute to pelvic organ cross-sensitization in rats with endometriosis. Neuroscience 2015; 291: 272–278.

Wang Y, Zhang M, Xie F, Li X, Bao M, Yang N et al. Upregulation of alpha2delta-1 calcium channel subunit in the spinal cord contributes to pelvic organ cross-
sensitization in a rat model of experimentally-induced endometriosis. Neuro-
reum Horm 2015; 40: 1267–1273.

Miranda A, Mackie A, Schmidt J, Zhang Z, Shaker R, Banerjee B et al. Neonatal cytokines-induced colonic hypersensitivity in adult rats: a model of viscerovisceral convergence. Neurogastroenterol Motil 2011; 23: 683–688.

Yoshikawa S, Kawanomori N, Oguchi T, Funahashi Y, Tyagi P, Chancellor MB et al. Upregulation of P2X7 receptor mediates visceral pain sensitization in in vivo. J Urol 2015; 193: 148.

Yoshikawa S, Kawamorita N, Oguchi T, Funahashi Y, Tyagi P, Chancellor MB et al. Prevalence of chronic prostatitis symptoms in a randomly surveyed adult population of urban-community-dwelling Nigerians males. Int J Urol 2008; 15: 340–343.

Lathpe P, Mignini L, Gray R, Hills R, Khan K. Factors predisposing women to chronic pelvic pain: systematic review. Br Med J 2006; 332: 749–755.

Curtin NC. Commentary on the influence of gender on the management of chronic pelvic pain. BJOG 2015; 122: 766–768.

Zhang H, Liu L, Yang Z, Pan J, Chen Z, Fang Q et al. P2X7 receptor mediates activation of microglial cells in prostate of chemically irritated rats. Int J Urol 2013; 20: 276–285.

Wong L, Done JD, Schaeffer AJ, Thubrikat P. Experimental autoimmune pros-
titis induces microglial activation in the spinal cord. Prostate 2015; 75: 50–59.

Chen Z, Xie F, Bao M, Li X, Chao Y, Lin C et al. Activation of p38 MAPK in the rostral ventromedial medulla by visceral noxious inputs transmitted via the dorsal columns may contribute to pelvic organ cross-sensitization in rats with endometriosis. Neuroscience 2015; 291: 272–278.

Wang Y, Zhang M, Xie F, Li X, Bao M, Yang N et al. Upregulation of alpha2delta-1 calcium channel subunit in the spinal cord contributes to pelvic organ cross-
sensitization in a rat model of experimentally-induced endometriosis. Neuro-
reum Horm 2015; 40: 1267–1273.

Miranda A, Mackie A, Schmidt J, Zhang Z, Shaker R, Banerjee B et al. Neonatal cytokines-induced colonic hypersensitivity in adult rats: a model of viscerovisceral convergence. Neurogastroenterol Motil 2011; 23: 683–688.

Yoshikawa S, Kawanomori N, Oguchi T, Funahashi Y, Tyagi P, Chancellor MB et al. Pelvic organ cross-sensitization to enhance bladder and urethral pain behaviors in rats with experimental colitis. Neuroscience 2015; 284: 422–429.

Podolsky DK. Inflammatory bowel disease (1). N Engl J Med 1991; 325: 928–937.

Kappelman MD, Rifas-Shiman S, Kleinman K, Ollefont D, Bousvaros A, Grant RJ et al. The prevalence and geographic distribution of crohn's disease and ulcerative colitis in the United States. Clin Gastroenterol Hepatol 2007; 5: 1424–1429.

Bernstein CN, Wajda A, Svenson LW, MacKenzie A, Koeboom M, Jackson M et al. The epidemiology of inflammatory bowel disease in Canada: a population-based study. Am J Gastroenterol 2006; 101: 1539–1568.

Riazi K, Galic MA, Kuzminski JB, Ho W, Sharkey KA, Pittman QJ. Microglial acti-
vation and TNFalpha production mediate altered CNS excitability following peripheral inflammation. Proc Natl Acad Sci USA 2008; 105: 17151–17156.

Kannampalli P, Pochiraju S, Bruckert M, Shaker R, Banerjee B, Sengupta JN. Analogic effect of minocycline in rat model of inflammation-induced visceral pain. Eur J Pharmacol 2014; 727: 87–98.

Song DD, Li JY, Tang D, Huang LY, Yuan YZ. Neuron-glial communication medi-
ated by TNF-alpha and glial activation in dorsal root ganglia in visceral inflam-
matory hypersensitivity. Am J Physiol Gastrointest Liver Physiol 2014; 306: G788–G795.

Huang TY, Belzer V, Hanani M. Gap junctions in dorsal root ganglia: possible contribution to visceral pain. Eur J Pain 2010; 14: 49.e1–11.

Vella N, Robinson D, Cardozo L. Painful bladder syndrome. Obstet Gynecol Reprod Med 2015; 25: 222–228.

Sherrill BJ, Elliott MN, Suttorp M, Bogart LM, Stoto MA, Eggers P et al. Prevalence of symptom bladder pain syndrome/interstitial cystitis among adult females in the United States. J Urol 2011; 186: 540–544.
217 D’Hooghe TM, Bambræs CS, Raeymaekers BM, Koninckx PR. Development of spontaneous endometriosis in baboons. Obstet Gynecol 1996; 88: 462–466.
218 Coe CL, Lemieux AM, Rier SE, Uno H, Zimbric ML. Profile of endometriosis in the aging female rhesus monkey. J Gerontol A Biol Sci Med Sci 1998; 53: M3–M7.
219 White HD, Robinson TD. A novel use for testosterone to treat central sensitization of chronic pain in fibromyalgia patients. Int Immunopharmacol 2015; 27: 244–248.
220 Aloisi AM, Bachiocco V, Costantino A, Stefani R, Ceccarelli I, Bertaccini A et al. Cross-sex hormone administration changes pain in transsexual women and men. Pain 2007; 132(Supplement 1): S60–S67.
221 Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov 2014; 13: 533–548.
222 Banati RB, Cagnin A, Brooks DJ, Gunn RN, Myers R, Jones T et al. Long-term trans-synaptic glial responses in the human thalamus after peripheral nerve injury. Neuroreport 2001; 12: 3439–3442.
223 Albrecht D, Loggia M, Borra R, Hooker J, Opalacz A, Mao J et al. Activation of spinal glia in sciatica; a pilot [11C]PBR28 study. J Nucl Med 2015; 56(supplement 3): 1557.
224 Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR et al. Evidence for brain glial activation in chronic pain patients. Brain 2015; 138(Pt 3): 604–615.
225 Mogil JS, Davis KD, Derbyshire SW. The necessity of animal models in pain research. Pain 2010; 151: 12–17.
226 Borsook D, Hargreaves R, Bountra C, Porreca F. Lost but making progress—where will new analgesic drugs come from? Sci Transl Med 2014; 6: 249sr243.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/