

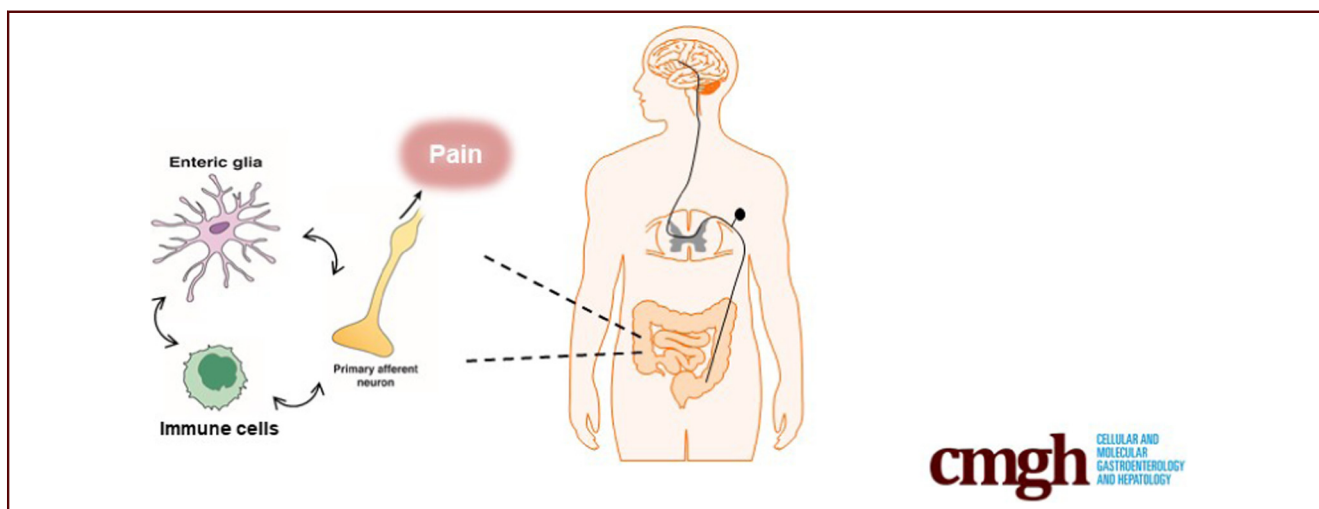
REVIEW

Enteric Glia: A New Player in Abdominal Pain



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SUMMARY

Chronic abdominal pain is a common gastrointestinal issue, but the mechanisms that produce abdominal pain are unresolved. Here, we discuss evidence suggesting that glia, and in particular, enteric glia, function as central players in mechanisms that produce abdominal pain.

glial cell signaling mechanisms that have the potential to influence chronic abdominal pain. (*Cell Mol Gastroenterol Hepatol* 2019;7:433–445; <https://doi.org/10.1016/j.jcmgh.2018.11.005>)

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Chronic abdominal pain is the most common gastrointestinal issue and contributes to the pathophysiology of functional bowel disorders and inflammatory bowel disease. Current theories suggest that neuronal plasticity and broad alterations along the brain-gut axis contribute to the development of chronic abdominal pain, but the specific mechanisms involved in chronic abdominal pain remain incompletely understood. Accumulating evidence implicates glial cells in the development and maintenance of chronic pain. Astrocytes and microglia in the central nervous system and satellite glia in dorsal root ganglia contribute to chronic pain states through reactive gliosis, the modification of glial networks, and the synthesis and release of neuromodulators. In addition, new data suggest that enteric glia, a unique type of peripheral glia found within the enteric nervous system, have the potential to modify visceral perception through interactions with neurons and immune cells. Understanding these emerging roles of enteric glia is important to fully understand the mechanisms that drive chronic pain and to identify novel therapeutic targets. In this review, we discuss enteric

Abdominal pain is the most common gastrointestinal (GI) issue and results in more than 15 million office visits per year.¹ Chronic abdominal pain affects at least 10%–15% of the general population and is a characteristic feature of functional bowel disorders, such as irritable bowel syndrome (IBS), the inflammatory bowel diseases (IBDs), and is a growing problem associated with chronic opioid use.^{2–4} Acute abdominal pain is associated with inflammatory flare-ups in IBD, but a large subset of patients

Abbreviations used in this paper: ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; Cx-43, connexin-43; DRG, dorsal root ganglia; GFAP, glial fibrillary acidic protein; GI, gastrointestinal; IBD, irritable bowel disease; IBS, irritable bowel syndrome; IL, interleukin; TNF, tumor necrosis factor; TRPV1, transient receptor potential vanilloid receptor type-1.

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that suffer from IBD eventually develop chronic abdominal pain even after achieving clinical remission. This condition, called comorbid IBS (loosely termed “IBS-IBD”), is present in up to 46%–59% of patients with Crohn’s disease and 36%–38% of patients with ulcerative colitis in remission.^{5–7} Abdominal pain linked to these disorders is common in adults and pediatric patients and is a major contributing factor to their low quality of life and high morbidity. As a result, the US Food and Drug Administration now requires the use of abdominal pain as a patient-reported outcome to assess the efficacy of new therapies for Crohn’s disease.⁴

The mechanisms that drive the development of chronic abdominal pain remain largely unresolved and this presents a major barrier to progress in the development of new therapies. Current therapies for IBD, for example, primarily focus on controlling inflammation with amino salicylates, corticosteroids, immunomodulators, and biologic agents. Although these agents clearly benefit the treatment of active inflammation, they do not address abdominal pain.⁸ Opioids are the current frontline therapy for chronic abdominal pain, but chronic opioid use has serious complications and produces a condition called narcotic bowel syndrome that is characterized by abdominal pain.⁹ This is a serious and growing issue that requires a more sophisticated understanding of the causal mechanisms to permit the development of more effective therapies.

Current theories suggest that neuronal plasticity involving the sensitization of visceral afferent sensory nerve fibers and broad alterations to the brain-gut axis contribute to the development of chronic abdominal pain.^{10,11} Much of this theory is based on evidence demonstrating changes in neuronal sensitivity, firing patterns, and network activity in the periphery, brain, and spinal cord.^{12–14} Although little is still known regarding the mechanisms that drive these alterations to neurons and their networks, it is increasingly clear that these properties are regulated by glia.

Here we discuss evidence supporting the concept that changes to glia contribute to mechanisms involved in the development of abdominal pain. We primarily focus on enteric glia in this review because of their potential to interact with nociceptors in the intestine and the potential consequences of these interactions on the generation of abdominal pain. Furthermore, the topic of central glia in pain transmission has been well covered by recent reviews.^{10,15} However, readers should note that alterations to central glia play a major role in pain transmission and, in fact, much of the understanding of glia in neuropathic pain conditions comes from research on glia in central circuits. A more sophisticated understanding of how central and peripheral glia regulate neural activity and how changes to these properties contributes to altered transmission or perception of sensory stimuli in the intestine holds great potential to advance the field of abdominal pain research and to identify novel therapeutic targets. In this review we aim to amass the existing evidence implicating enteric glia as major contributors to peripheral sensitization of nerve afferents and highlight their potential roles in the cause of chronic abdominal pain.

Known Mechanisms That Contribute to Abdominal Pain

Visceral hypersensitivity is a major mechanism that contributes to abdominal pain in IBS and IBD.¹⁶ This involves alterations along the brain-gut axis, a network of afferent and efferent neural pathways that link cognitive, emotional, and autonomic centers in the brain to neuroendocrine centers, the enteric nervous system, the gut microbiome, and the immune system. The sensitization of visceral afferent nerve fibers is driven by neuroplasticity and leads to a skewed perception of sensory stimuli. Nociceptive information is transduced by the peripheral axon terminals of primary afferent neurons whose cell bodies reside in dorsal root ganglia (DRG). These “nociceptors” are the axon terminals of polymodal C fibers that transduce a variety of potentially noxious stimuli (mechanical, chemical, and thermal) to the brain. The sensitivity of nociceptors is one of the most important factors that gates the transmission of noxious information and alterations to nociceptor sensitivity in GI disorders can lead to an increased perception of pain (hyperalgesia) or the painful perception to innocuous stimuli (allodynia).

Immune activation and neuroplasticity are 2 key mechanisms involved in the generation of chronic abdominal pain. Nociceptor sensitivity is profoundly altered by inflammatory mediators.¹⁷ Active inflammation, such as an IBD flare-up or acute gastroenteritis, involves the release of proinflammatory mediators that alter nociceptor sensitivity. Inflammatory mediators including adenosine triphosphate (ATP),¹⁸ histamine,¹⁹ interleukin (IL)-1 β ,²⁰ proteases,²¹ and bradykinins²² cause pain by interacting with receptors on nociceptors. Other inflammatory mediators, such as IL-6²³ and tumor-necrosis factor (TNF)- α ,²⁴ lead to the sensitization of nociceptors through indirect mechanisms. Nociceptors, themselves, also contribute to local inflammation (termed “neuroinflammation”) through axon reflexes that produce inflammatory mediators including substance P, neurokinin A, ATP, and calcitonin-gene related peptide.

Neuroimmune activation following a psychological stressor or peripheral inflammation results in neuroplastic changes along nerve afferents that contribute to visceral hyperalgesia.^{25,26} Neuroimmune modulators drive neuroplasticity through mechanisms that include the downregulation of glutamate transporters and the subsequent upregulation of synaptic glutamate and the ionotropic glutamate receptor, *N*-methyl-D-aspartate, signaling.^{27,28} Neuroplastic changes in the expression and function of transient receptor potential vanilloid receptor type-1 (TRPV1) channels present on nociceptors innervating the intestinal mucosa are also implicated in visceral hypersensitivity in IBS.²⁹ Increased levels of TRPV1 are present in patients with idiopathic rectal hypersensitivity³⁰ and an increased density of TRPV1 immunoreactive nerve fibers is present in the colonic mucosa of patients with IBS, correlating with the degree of abdominal pain experienced.³¹

Alterations to emotional circuits of the brain, primarily those associated with stress and anxiety, contribute to visceral

hypersensitivity in patients with IBS.³² Various stressors, chronic and acute, regulate visceral pain responses in animal models^{33,34} and are associated with an increase in symptom severity in functional³⁵ and inflammatory GI disorders.^{36,37} Brain regions associated with visceral pain, emotional arousal, and attention are consistently activated in neuroimaging studies of patients with IBS.³⁸ Furthermore, alterations to gray matter density in areas associated with corticolimbic inhibition, stress, and arousal circuits indicate altered activity in the brains of patients with IBS.³⁹ The activation of these regions contributes to acute and chronic stress-induced hyperalgesia in the colon, in part, by stimulating the release of factors, such as corticotropin-releasing factor.^{40,41}

Glial Cells in Abdominal Pain Pathways

A plethora of emerging studies link nerve fiber sensitization with alterations to glial cells. Increased glial activity is characteristic of all forms of pain and glia contribute to the transition from acute to chronic pain through interactions with neurons that alter neurotransmission.^{15,42}

For example, increases in astrocyte and microglial cell markers are present in animal models of acute pain, inflammatory pain, and neuropathic pain.^{43–46} Structural changes and altered signaling in peripheral glial cells, such as satellite glia in DRG, are implicated in abdominal pain pathways.⁴⁷ Furthermore, enteric glial cells, a unique class of peripheral glia found within the enteric nervous system, have bidirectional interactions with neurons in the gut that impact both gut reflexes in health and neuroinflammation.^{48–53} Therefore, an emerging theme is that glia present at all sites along ascending and descending pathways that transduce and modify nociceptive information deriving from the intestine have the potential to broadly influence visceral perception (Figure 1).

Most of the understanding of glia in pain transmission stems from studies of glia at the first synapse in ascending pain pathways in the dorsal horn of the spinal cord. Microglia and astrocytes are the major glial cell types implicated in altering pain transmission at this level. Microglial activation in the spinal cord occurs in response to injury or inflammation and is reflected by increased cell

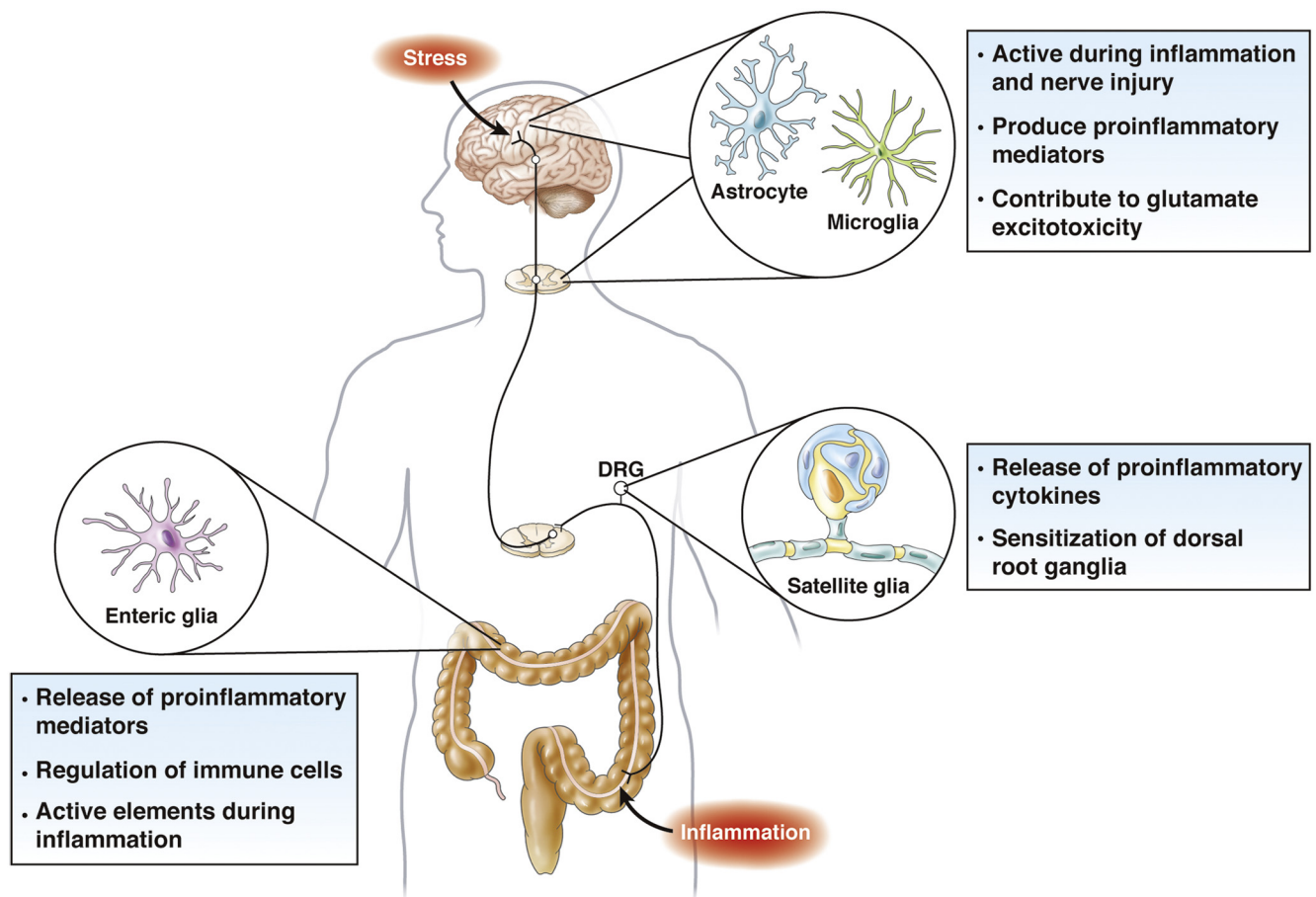


Figure 1. Major populations of glia that contribute to chronic pain. Astrocytes and microglia are present in the central nervous system and primarily affect chronic pain pathways in the brain and in the spinal cord. Satellite glia are peripheral glial cells located in the dorsal root ganglia where they contribute to the sensitization of dorsal root ganglion cells. Enteric glial cells are located within the intestine and form part of the enteric nervous system. Enteric glia are located adjacent to the nerve endings of primary afferent fibers and intercellular interactions between enteric glia and neurons have the potential to influence chronic pain.

number and altered gene expression profiles.^{15,46,54,55} Likewise, spinal astrocytes become activated and undergo reactive gliosis in animal models of neuropathic pain and increased expression of glial fibrillary acidic protein (GFAP), a marker of reactive astrocytes, mirrors the intensity of pain hypersensitivity.^{46,55,56} The inhibition of astrocyte activity results in a significant attenuation of inflammatory hyperalgesia, suggesting that astrocytic activation is at least partly necessary for the development of pain.⁵⁵ Evidence from studies using *in vivo* optogenetics suggests that the selective activation of spinal astrocytes is sufficient to increase pain hypersensitivity.⁵⁷ There is still much controversy surrounding glial optogenetic data, because several studies suggest that the activation of astrocytes driven by channelrhodopsin is an effect of cell swelling and is not driven by normal calcium signaling pathways.⁵⁸ Alternative methods of glial-specific activation that more closely reflect physiological mechanisms, such as designer receptors exclusively activated by designer drugs, should be implemented to further validate these findings.

Glial activity in the spinal cord contributes to neuroplasticity, in part by stimulating the release of glial-derived substances termed “gliotransmitters.” Active glial cells produce several proinflammatory mediators that contribute to the activation and sensitization of nerve fibers to central sensitization in the dorsal horn.¹⁰ For example, TNF- α produced by spinal microglia and astrocytes contributes to hyperalgesia and allodynia in preclinical pain models.^{59,60} TNF- α released by astrocytes and microglia also contributes to central sensitization and neuroplasticity through mechanisms that involve the downregulation of glutamate transporters and subsequently, glutamate excitotoxicity.²⁸ Other key gliotransmitters, such as purines, activate nerve fibers directly¹⁸ and in animal models of chronic pain, astrocytes increase the release of ATP and the production of proalgesic mediators, such as glutamate, cytokines, and chemokines.¹⁰

Glial mechanisms similar to those occurring in the spinal cord may also affect processing in the brain. Although less is known regarding the contributions of glia in the brain to pain conditions, increased astrocyte activity is observed in the nucleus of the solitary tract following colonic inflammation.⁶¹ Likewise, microglial activation within the thalamus occurs following nociceptive spinal cord injury.⁶² Alterations to glia in the brain are likely associated with the emotional experience of pain and can be correlated with mood disorders, such as depression and anxiety.⁶³

Multiple populations of peripheral glial cells actively contribute to modifying pain transmission through effects on nociceptors. For example, satellite glia play crucial roles in visceral sensitization. Satellite glia are located in peripheral sensory ganglia where they envelop neuronal cell bodies.⁶⁴ A recent review by Hanani⁴⁷ thoroughly covered the topic of satellite glia in the development and maintenance of chronic pain. To briefly summarize, satellite glia undergo cell division and upregulate their production of GFAP following inflammation or injury to peripheral nerves and express and release proinflammatory cytokines including TNF- α and IL-1 β . In addition, satellite glia increase gap-junction mediated

coupling following nerve injury and inflammation. These changes to satellite glia are linked to GI pain and are also implicated in chronic abdominal pain in IBS.

Potential Roles of Enteric Glia in Abdominal Pain

Enteric glial cells are a unique class of peripheral glia that are associated with neurons in the enteric nervous system. Enteric glia play instrumental roles in gut reflexes that regulate motility and secretion, influence the epithelial barrier, and modulate neuroinflammation.^{49,65} Yet how enteric glia might influence visceral hypersensitivity and nociceptor sensitization is still relatively unknown.

Enteric glia, particularly those associated with neurons in the myenteric plexus, share some morphologic and functional properties with astrocytes that might suggest similar roles in pain transmission. For example, enteric glia express molecular markers, such as GFAP⁶⁶ and S100 β ⁶⁷; provide trophic and protective support to enteric neurons^{68,69}; exhibit activity encoded by intracellular calcium signaling in response to neurotransmitters; and release gliotransmitters through membrane channels composed of connexin-43 (Cx-43).⁵² Connexins are a major structural component of gap junctions and hemichannels in glia and connexin-mediated glial mechanisms play a crucial role in development of chronic neuropathic pain.⁷⁰ Bidirectional communication between enteric neurons and glia mediated by the Cx-43-dependent release of enteric gliotransmitters regulates gut reflexes that control motility and secretions^{50,52,53,71} and the Cx-43-dependent release of gliotransmitters, such as ATP, during neuroinflammation drives neurodegeneration through the activation of neuronal P2X7 receptors.^{51,72} Enteric glia are intimately associated with nociceptors in the intestine and the activation of TRPV1⁺ sensory neurons within the myenteric plexus elicits glial activity and gliotransmitter release through similar mechanisms.⁷³ Whether gliotransmitters subsequently have direct or indirect effects on nociceptor activity or sensitivity is still unknown, but this remains an active area of research.

Enteric glia are targets of stress, which is a primary component in the development of visceral hypersensitivity. Several forms of stress, such as chronic stress and early life stress, alter glial signaling and contribute to pain sensitivity in IBS.³² The contribution of enteric glia to stress-induced hyperalgesia is still undefined. However, early life stress causes structural changes in enteric glial cells that are correlated with inefficient gastric motility.⁷⁴ Furthermore, stress activates sympathetic pathways in the intestine that activate enteric glia via purinergic transmission.⁷⁵

Enteric glia contribute to intestinal inflammatory and immune responses⁷⁶ and interact with, and express, cytokines and immunoregulatory signals that contribute to altered nociceptive signaling.^{76–78} Altered immune responses are key components in the development of chronic pain and mediators that sensitize nociceptors, such as neurokinin A,⁷³ substance P,⁷³ proteases,⁷⁹ and TRPV4⁸⁰ agonists, all elicit responses in enteric glia. This raises the possibility that enteric glia contribute to nociceptor

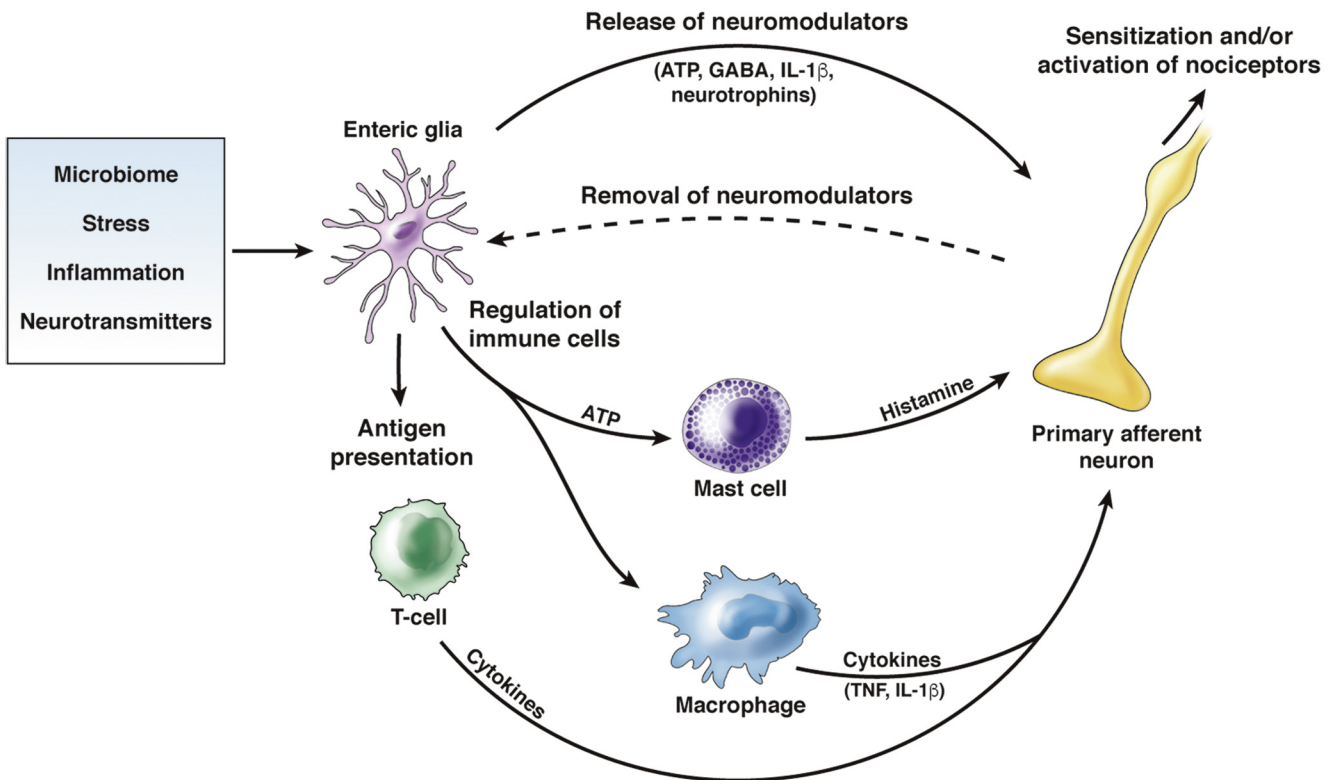


Figure 2. Overview of potential enteric glial cell mechanisms that contribute to the sensitization of nociceptors. Highlighted are pathways that directly and indirectly sensitize nociceptors. Enteric glial activity can be influenced by several factors, such as inflammation, stress, neurotransmission, and/or the gut microbiota. Enteric glia release neuromodulators that act on primary afferent neurons by binding to receptors on nociceptor nerve endings or by sensitizing nociceptors. Enteric glia possess ectoenzymes that remove neuromodulators from the extracellular space. Enteric glia also have the potential to indirectly modulate nociceptive neurons by activating immune cells either through the release of immune cell regulators or by acting as antigen-presenting cells. GABA, γ -aminobutyric acid.

sensitization caused by these mediators. In support, perturbing enteric glial metabolism with fluorocitrate reduces visceromotor responses in mice.⁸¹

Together, these multiple lines of evidence suggest that crosstalk between enteric glia and nociceptors contributes to mechanisms that drive visceral hypersensitivity (Figure 2). The precise mechanisms by which enteric glia influence nociceptors are still being discovered. Yet new data discussed in the following sections are offering promising leads that could identify novel molecular targets for new abdominal pain therapies.

Potential Mechanisms for Direct Modulation of Nociceptors by Enteric Glia

Enteric glia play active roles in neurotransmission in the intestine by generating and regulating the availability of neurotransmitters. Many of the transmitter systems regulated by enteric glia are involved in the activation and sensitization of nociceptors. For example, enteric glia are a primary source of ATP, which contributes to cell-to-cell communication in health and disease and acts on P2X receptors expressed by nociceptors.^{18,51,82,83} Purines and purinergic signaling from glial cells are altered during inflammation and are strongly

implicated in the process of visceral hypersensitivity and abdominal pain.^{84,85} Glial cells, including enteric glia, also possess ectoenzymes, such as NTPDase2, that are responsible for the degradation of ATP and other transmitters.^{51,86,87} Astrocytic NTPDase2 expression is altered during inflammation and could contribute to the sensitization of sensory neurons by increasing purinergic signaling.⁸⁷ The similarities between enteric glia and astrocytes suggest that a similar behavior could be observed in enteric glia. Glial ATP release is triggered by the excitation of enteric glial cells, intracellular calcium signaling, and the subsequent opening of Cx-43 hemichannels,⁵¹ which is a molecular mechanism highly implicated in the development of nociceptive pain and inflammatory pain.^{51,70,88} In spinal astrocytes, Cx-43 expression is highly elevated following spinal cord injury and the inhibition of Cx-43 reduces allodynia and hyperalgesia.^{70,88,89} A direct link between Cx-43 on enteric glial cells and nociceptors sensitization has yet to emerge. However, because of the implications of astroglial Cx-43 in chronic pain and the analogous nature of enteric glia, it is likely that enteric Cx-43 is readily involved in the mechanisms of abdominal pain. In support, we recently showed that bidirectional communication between nociceptors and enteric glia involves glial Cx-43.⁷³

Proinflammatory cytokines including IL-6, IL-1 β , and TNF- α play major roles in the development of intestinal

pathology and are known contributors to the development and maintenance of inflammatory and neuropathic pain.^{10,15} These cytokines are increased during IBS and IBD.^{90,91} Astrocytes, microglia, and satellite glia produce proinflammatory cytokines, such as IL-1 β , during inflammation and nerve injury.⁹²⁻⁹⁷ Similarly, enteric glia produce, and respond to cytokines including IL-6 and IL-1 β .^{77,78,98} Experimental studies support a proinflammatory, pronociceptive role for IL-6 and the upregulation of IL-6 correlates with the development of hyperalgesia and allodynia in animal models.⁹⁹⁻¹⁰¹ Blocking IL-6 production and activity reduces or alleviates nociceptor sensitization.^{102,103} Similarly, IL-1 β has pronociceptive effects and treatment with IL-1 β in vivo causes increased visceromotor responses to colorectal distention in animal models of inflammatory pain.¹⁰⁴

Enteric glia also interact with TNF- α signaling pathways and TNF- α is heavily implicated in the development of visceral hypersensitivity. Microglia and satellite glia increase their expression of TNF- α during nerve injury and inflammation^{82,83,105} and the excitation of DRG neurons in mouse colitis models stimulates TNF- α production.⁸³ The exogenous application of TNF- α increases DRG neuron activity and visceral pain responses in trinitrobenzene sulfonic acid colitis, which is reversed by the administration of anti-TNF- α .⁸³ Evidence regarding the production of TNF- α by enteric glia is still controversial.⁷⁶ However, TNF- α does alter enteric glial cell behavior and exposure to TNF- α induces increased GFAP expression and calcium signaling in enteric glia.^{69,106} It is possible that these effects are mediated through modifications to Cx-43 hemichannels based on the known effects of TNF- α in spinal cord injury.^{107,108}

Neurotrophic factors, such as nerve growth factor, brain-derived neurotrophic factor (BDNF), and neurotrophin-3, are also associated with neuropathic pain and inflammatory processes.¹⁰⁹ Enteric glia are a significant source, and target of neurotrophins in the intestine and alterations in the expression of neurotrophins and their receptors are associated with changes in enteric glial activity.^{110,111} The upregulation of BDNF expression by DRG neurons contributes to visceral pain¹¹⁰ and enteric glia in the colon of patients with IBS highly express the BDNF receptor TrkB.^{81,112} In addition, recent data suggest that BDNF-induced colonic hypersensitivity involves reactive enteric glia because visceral pain responses and GFAP expression were reduced in BDNF knockout mice.⁸¹ Similarly, nerve growth factor secretion by cultured enteric glia and mRNA levels of nerve growth factor and its receptor TrkA are increased in the presence of lipopolysaccharide, TNF- α , and IL-1 β .¹¹³ Given the multiple biologic roles of neurotrophins in the maintenance of neuronal survival and function,¹⁰⁹ changes in glial neurotrophin expression and/or signaling could contribute to the neuroplasticity involved in visceral hypersensitivity.

The gut microbiome has emerged as an essential component of the brain-gut axis and alterations in the composition of the microbiome are observed in several GI and pain conditions.¹¹⁴ Altered fecal microbiota in patients with IBS may even predict severity.¹¹⁵ The microbiota produce a range of neuroactive compounds, such as

the neurotransmitters γ -aminobutyric acid, serotonin, norepinephrine, and dopamine,¹¹⁶ and other bacterial products, such as lipopolysaccharide¹¹⁷ and formyl peptides.¹¹⁸ This suggests that the gut microbiome could influence pain pathways, but mechanistic links between the gut microbiome, sensory nerves, and alterations in the brain-gut axis are still relatively undefined. New data suggest that some effects of the microbiome could be exerted through effects on enteric glial function and development.¹¹⁹ The generation of the mucosal enteric glial cell network parallels the maturation of the gut microbiome^{120,121} and the impaired mucosal glial population in germ-free mice can be restored by introducing a normal gut microbiome.¹²¹ The lipopolysaccharide receptor, TLR4, is located on enteric glial cells and is increased in mice following exposure to stress.^{34,122} Visceromotor responses in maternally separated mice can be suppressed with oxytocin, which also causes a downregulation of enteric glial cell activity and TLR4 expression. This is indicative of a TLR4-dependent mechanism of enteric glial cells in modulating visceral pain responses.¹²²⁻¹²⁴ The gut microbiome also produces several gliomodulators, such as proteases that directly act on enteric glial cells and are implicated in the pathophysiology of visceral hypersensitivity.^{79,116,125} However, the significance of interactions between the microbiome and glia in the generation of visceral pain is still unknown and requires additional mechanistic studies to unravel.

Potential Mechanisms of Indirect Modulation of Nociceptors by Enteric Glia

Inflammation is a key component in the development of chronic pain^{10,94} and abnormalities in immune responses are considered one of the primary causes leading to the development of IBS.¹²⁶ Approximately 10% of all cases of IBS are associated with a previous inflammatory insult, such as a bacterial infection.¹²⁷ Immune cells play an important role in the sensitization of sensory nerve fibers during inflammation and contribute to the development of acute and chronic pain.¹⁰ Their active role in pain is primarily caused by the release of proinflammatory mediators that modulate ion channel expression in primary afferent fibers, such as TRPV1 receptors.¹²⁸⁻¹³¹ Histamine, for example, activates nerve fibers and contributes to neurogenic inflammation and pain.¹³² Blocking H₁ histamine receptors expressed by primary sensory afferent neurons in mice reduces pain responses after cutaneous injection of formalin to the paw.¹³³

Recently, the concept that both central and peripheral glial cells function as immunoregulatory cells has emerged.¹²³ Glial cell-mast cell communication is critical for the development of inflammation.^{134,135} Histamine clearance is an essential process for avoiding excessive histaminergic neuronal activation. Astrocytes play a prominent role in histamine clearance via histamine N-methyltransferase, a histamine metabolizing enzyme.¹³⁶ Astrocyte activity is modulated via histamine receptors on

their cell membrane¹⁰⁷ and microglial activation through histamine results in the subsequent release of inflammatory mediators.¹⁰⁸ Enteric glia exhibit the potential to function through similar pathways because their activity is also increased in the presence of histamine.¹³⁷

Current evidence supports the notion that enteric glia modify immune responses by the secretion of cytokines and other chemical transmitters and/or by acting as antigen-presenting cells. Glial proinflammatory mediators including S100B, ATP, and nitric oxide increase oxidative stress in the local environment and contribute to neuronal cell death and the release of apoptotic signaling that triggers immune responses.^{51,106,138,139} Enteric glia are also capable of expressing major histocompatibility complex II molecules and ligands necessary for T-cell signaling.^{138,140,141} Several types of immune cells are involved in inflammatory pain, particularly in the persistence of hyperalgesia.¹⁴² Immune cell activity can also contribute to neuropathic pain because of damage to peripheral nerves or to the central nervous system.¹⁰ Together these data show that enteric glia can modulate immune cells, which in turn can influence nociception.

Potential Roles of Enteric Glia in Opioid-Induced Hyperalgesia

Current treatments targeting chronic pain are limited and often involve the use of opioids.¹⁴³ However, opioid use has serious side effects that include persistent constipation and antinociceptive tolerance that limit clinical efficacy. Opioid-induced hyperalgesia can result from chronic opioid use and produce an IBS-like syndrome termed “narcotic bowel syndrome.”^{144,145} Narcotic bowel syndrome is a subset of opioid bowel dysfunction characterized by recurring abdominal pain that worsens with the continued or escalating use of narcotics.¹⁴⁶

Glial cells play a role in modulating opioid behavior.^{146–148} Chronic opioid treatment causes glial release of nitric oxide, prostaglandins, excitatory amino acids, and growth factors. It also causes changes in purinergic signaling and increases the release of glial mediators that enhance the release of pain transmission.¹⁴⁶ Opiates modulate central glial activity by directly binding to the glial (μ)-opioid receptor causing the release of proinflammatory cytokines or indirectly through the release of dynorphins.¹⁴⁷ Chronic exposure to opioids leads to robust astrocyte and microglia reactions and induces hyperalgesia.¹⁴⁸ Acute opioid treatment also produces marked satellite glia reaction in DRG.¹⁴⁹ The bulk of these effects result from altered glial purinergic signaling. Chronic opioid treatment leads to the upregulation of P2X4 and P2X7 receptors in spinal microglia and contributes to opioid tolerance.^{149–151} Similar effects are observed in enteric glial cells. Long-term morphine treatment enhances purinergic activity in enteric glia that subsequently leads to barrier dysfunction and constipation through mechanisms that require glial Cx-43 activity.¹²⁴ Opioid exposure upregulates proinflammatory cytokine release and contributes to morphine tolerance.¹⁵² Chronic morphine exposure causes the release of TNF- α , IL-1 β , and IL-6 from glial cells, all of

which are known contributors to chronic pain.¹⁵³ Chronic opioid exposure induces glial responses through TLR4 and reinforces the rewarding effects of opioids. Blocking TLR4 signaling attenuates the development of analgesic tolerance and hyperalgesia.^{154,155} Enteric glia express TLR receptors and TLR4-deficient mice fail to develop opioid-induced bowel dysfunctions.¹⁵⁶ Although these findings implicate enteric glial cells as playing a major role in morphine-induced bowel dysfunction and opioid tolerance, more in-depth studies are needed to clearly define the role of glia in opioid-induced hyperalgesia.

Conclusions

New therapies are needed to combat the common clinical issue of chronic abdominal pain. However, their development is limited primarily by the incomplete understanding of mechanisms that drive the development and maintenance visceral pain. Recent discoveries suggest that significant new insight into these mechanisms could be gained by focusing on glial cells. Glia are intertwined with mechanisms that produce nociceptive hyperalgesia and allodynia in pathologic conditions. Specifically, glia are responsive to neurotransmitters in pain circuits and produce, secrete, and regulate modulators that activate and sensitize nociceptors. Moreover, glial cells actively contribute to processes involved in neuroplasticity. The precise mechanisms whereby peripheral glia contribute to neuroplasticity in pain pathologies is still a developing area, but current discoveries suggest that these mechanisms are involved in the transition from acute to chronic pain.

The contributions of glial cells to pain hypersensitivity are not centralized, but in fact require the involvement of several glial cell types along the brain-gut axis. Existing knowledge regarding enteric glia suggests that they are involved in visceral hypersensitivity and chronic pain. Enteric glia are primed for immune and stress responses. Increasing evidence supports their role in nociception, because they can influence nerve fiber activity either directly by releasing pronociceptive modulators or indirectly by interacting with the immune system. Their reactivity is also linked with altered ion channel signaling on nociceptor nerve endings. In addition to stress and inflammatory visceral hypersensitivity, enteric glia also show promise in playing a key role in the mechanisms of opioid-induced hyperalgesia and narcotic bowel syndrome.

Enteric glia signaling involving Cx-43 is currently the most well-described glial mechanism that contributes to neuroinflammation and intercellular communication.^{51–53} However, the therapeutic potential of this target is untested. Existing drugs that modulate Cx-43 are poorly selective and interact with connexin hemichannels and gap junctions in multiple tissues and cell types, which could potentially compromise normal gut function. This suggests that targeting mechanisms upstream or downstream of glial Cx-43 may prove more effective. In support, a clinical trial targeting downstream neuronal mechanisms with the P2X7 receptor antagonist AZD9056 showed significant promise in the treatment of visceral pain in patients with Crohn's

disease.¹⁵⁷ Other potential targets on enteric glia to modulate pain are less defined but targeting enteric glial IL-1 β and IL-6 signaling could be promising because both contribute to pain pathologies.^{100,103,104} Similarly, modulating glial PAR and neurotrophin signaling may also serve to regulate glial activity and potentially reduce visceral hypersensitivity.^{158,159}

Further studies should focus on elucidating the role of enteric glial cells in chronic abdominal pain, because these cell types are essential in maintaining intestinal homeostasis and are highly active elements in GI disorders. In general, glia demonstrate great promise in filling the existing gaps in knowledge with regards to chronic abdominal pain and may become a considerable target for therapeutic approaches.

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Conflicts of interest

The authors disclose no conflicts.

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