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ATP Receptors Gate Microglia Signaling in Neuropathic Pain

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Abstract

Microglia were described by Pio del Rio-Hortega (1932) as being the 'third element' distinct from neurons and astrocytes. Decades after this observation, the function and even the very existence of microglia as a distinct cell type was a topic of intense debate and conjecture. However, considerable advances have been made towards understanding the neurobiology of microglia resulting in a radical shift in our view of them as being passive bystanders that have solely immune and supportive roles, to being active principal players that contribute to central nervous system pathologies caused by disease or following injury. Converging lines of evidence implicate microglia as being essential in the pathogenesis of neuropathic pain, a debilitating chronic pain condition that can occur after peripheral nerve damage caused by disease, infection, or physical injury. A key molecule that modulates microglial activity is ATP, an endogenous ligand of the P2-purinoceptor family consisting of P2X ionotropic and P2Y metabotropic receptors. Microglia express several P2 receptor subtypes, and of these the P2X4, P2X7, and P2Y12 receptor subtypes have been implicated in neuropathic pain. The P2X4 receptor has emerged as the core microglia-neuron signaling pathway: activation of this receptor causes release of brain-derived neurotrophic factor (BDNF) which causes disinhibition of pain-transmission neurons in spinal lamina I. The present review highlights recent advances in understanding the signaling and regulation of P2 receptors expressed in microglia and the implications for microglia-neuron interactions for the management of neuropathic pain.

Keywords

Microglia; P2 purinoceptors; Neuropathic pain; Nerve injury; ATP; BDNF; spinal cord

Pain is a double-edged sword that can be protective or cause considerable suffering. Acute nociceptive pain warns against imminent or existing tissue damage, whereas chronic pain has no known defensive or beneficial function and is unremitting for those who suffer from this condition. Acute pain is produced by physiological functioning of the normal peripheral and central nervous systems. However, the processes initiated during acute pain can sometimes progress to chronic pain that is characterized as persisting long after the initiating event has healed. This transition to chronicity is highly variable between individuals and the degree of injury is not necessarily predictive of the severity or chronicity of the pain. There is mounting evidence that the transition from acute to chronic pain involves discrete

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pathophysiological steps that alter the cellular, molecular, and anatomical organization of nociceptive neural networks in the spinal dorsal horn (Latremoliere and Woolf, 2009;Scholz and Woolf, 2002;Voscopoulos and Lema, 2010;Woolf and Salter, 2000). In this pathologically altered system, the balance of inhibitory and excitatory control is shifted such that inhibitory mechanisms are weakened while excitatory mechanisms are strengthened. A subtle shift in this balance can have a profound effect that results in both a pathological amplification and a change in modality of sensory input and output from the spinal cord which leads to the exaggerated pain responses seen in chronic pain conditions (Costigan et al., 2009b).

Neuropathic pain is among the most debilitating type of chronic pain, which typically develops because of injury to a nerve caused by trauma, infection, or pathology (Scholz and Woolf, 2002;Zimmermann, 2001;Gwak and Hulsebosch, 2009). The cardinal symptom of neuropathic pain is hypersensitivity that can manifest spontaneously in the absence of an overt stimulus (spontaneous pain), or it can be evoked, such as in the case of allodynia (pain resulting from an innocuous stimulus) and hyperalgesia (an exaggerated pain response to a noxious stimulus). The sequelae of neuropathic pain are difficult to treat and often refractory to the current available pharmacological treatments, which are typically directed against neuronal molecular targets. The failure of these neuron-targeted drugs to alleviate neuropathic pain is consistent with accruing evidence that non-neuronal mechanisms are also major contributors to chronic neuropathic pain. Thus, a new framework for understanding the etiology of neuropathic pain is forming from a rapidly growing body of evidence that glia in the central nervous system are critical in establishing and maintaining neuropathic pain (Beggs and Salter, 2010;Grace et al., 2011;Inoue and Tsuda, 2006;Milligan and Watkins, 2009;Gwak and Hulsebosch, 2010). Microglia, in particular, have emerged as key players in the etiology of neuropathic pain (Inoue and Tsuda, 2006;Tsuda et al., 2003;Tsuda et al., 2005;Watkins et al., 2001;Watkins and Maier, 2003;Tsuda et al., 2005). The present article highlights the recent advances in our understanding of the role microglia, and particularly microglia-neuron interactions mediated through ATP-gated P2-receptors, play in the pathogenesis of nerve injury-induced neuropathic pain.

Microglial response to peripheral nerve injury

Microglia are resident cells in the central nervous system that respond to adverse physiological conditions such as trauma, ischemia, inflammation, and infection. Recent evidence suggests that almost the entire population of microglia originate from embryonic macrophages derived from the yolk sac (Ginhoux et al., 2010). In the adult central nervous system, microglia comprise 5–10% of the total glial population and they are roughly equal in number to neurons (Kreutzberg, 1996;Lawson et al., 1990;Nakajima and Kohsaka, 2001). Under normal homeostatic conditions, microglia display a small soma bearing thin ramified processes that cover large non-overlapping territories throughout the brain and spinal cord (Bushong et al., 2002;Kreutzberg, 1996). Microglia with this phenotype – the surveillance mode of activity – continuously scan their local environment by actively extending and retracting their processes (Davalos et al., 2005;Nimmerjahn et al., 2005). This dynamic process reorganization is thought to enable otherwise stationary microglia to rapidly detect potential stimuli that threaten to disrupt homeostasis in the central nervous system. When

injury is detected, microglia respond within seconds to minutes by extending their processes toward the site of neural damage (Davalos *et al.*, 2005;Nimmerjahn *et al.*, 2005). This targeted movement enables the microglia to converge and form a barrier between healthy and injured cells, limiting the extent of damage in the central nervous system. Being able to adopt a phagocytic phenotype, the microglia also remove debris from degenerating primary afferent terminals and apoptotic cells at the site of injury (Aldskogius, 2001;Rotshenker, 2003).

The rapid microglia responsiveness is coupled to a more slowly developing series of changes in morphology, gene expression, function, and proliferation (Hanisch and Kettenmann, 2007;Kettenmann et al., 2011;Kreutzberg, 1996;Nakajima and Kohsaka, 2001). A progressive series of morphological changes in spinal microglia has been reported in rodent models of nerve injury caused by compression, ligation, or transection (Liu et al., 1995;Tsuda et al., 2003;Zhang and De, 2006). After peripheral nerve injury, microglia withdraw their thin processes and adopt an amoeboid-like structure (Eriksson et al., 1993). These morphological changes are often followed by an increase in the number and density of microglia in the ipsilateral spinal dorsal horn (Calvo and Bennett, 2011;Calvo et al., 2011;Echeverry et al., 2008;Gehrmann and Banati, 1995;Perry, 1994;Stoll and Jander, 1999). The stereotypical microglial response also entails the upregulation of a number of surface marker proteins belonging to the complement cascade: complement receptor 3 (CR3), Toll-like receptor 4 (TLR4), CD14, CD4, and major histocompatibility complex (MHC) class I and II (Coyle, 1998;Liu et al., 1995;Sweitzer et al., 2002b;Tanga et al., 2004;Tsuda et al., 2003). In addition, spinal microglia produce and secrete cytokines, chemokines, and neurotrophic factors that signal to neurons in the spinal cord to alter neuronal excitability. This microglia-neuron communication is increasingly recognized as being essential in the etiology of neuropathic pain (Clark et al., 2010;DeLeo and Yezierski, 2001;Inoue and Tsuda, 2006;Tsuda et al., 2005;Watkins et al., 2001;Zhang and De, 2006).

In addition to their functional and morphological plasticity, microglia are characterized by their low threshold for reactivity and respond to a wide range of challenges, even a remote stimulus in the periphery is a sufficient trigger for microglial response. That microglia respond to damage in the periphery suggests signaling from primary afferents contributes to the microglial response. In accord, it is known that discharge activity of primary afferents initiated by injury to peripheral nerves has considerable consequences in the nervous system that can lead to chronic pain (Woolf and Salter, 2000). Indeed, afferent barrage after the nerve injury is thought to trigger neuropathic pain by activating p38 in spinal microglia (Wen et al., 2007). One such consequence of peripheral nerve injury is the entry of blood bourn immune cells into the central nervous system (Sweitzer et al., 2002a), specifically, these cells include monocytes (Zhang et al., 2007), and T-lymphocytes (Cao and DeLeo, 2008;Costigan et al., 2009a). Transit of these cells is likely aided by increased permeability of the blood brain barrier and the blood spinal cord barrier (Beggs et al., 2010;Echeverry et al., 2011;Gordh and Sharma, 2006). The increased vascular permeability can be triggered by injury to a peripheral nerve, electrical stimulation or application of capsaicin to primary afferent C-fibers (Beggs *et al.*, 2010). It is uncertain whether circulating monocytes that infiltrate into the spinal cord proceed to differentiate into microglia and contribute to the local microglial response.

Microglia increase and transform the output of pain transmission neurons

A major ascending nociceptive (pain-related) pathway arises from neurons in lamina I of the spinal dorsal horn (Bester et al., 2000). The action potential discharge of these neurons, that is to say the output of these neurons, is normally evoked only in response to noxious peripheral stimulation, such as pinch to the skin (Keller et al., 2007) (Figure 1). However, after peripheral nerve injury the output of these neurons is transformed such that innocuous stimulus, such as brush or touch, evoke action potential discharges. Moreover, the response to noxious stimulation is greatly exaggerated and there is a pronounced after-discharge in lamina I neurons after peripheral nerve injury. Also, after peripheral nerve injury lamina I neurons exhibit spontaneous bursting in the absence of overt stimulation whereas in naïve animals these neurons are normally silent. The change in the output of the lamina I neurons may be readily understood to provide a neural substrate for the three cardinal signs of neuropathic pain in humans (Woolf and Salter, 2000): mechanical allodynia (discharge in response to innocuous stimulation), hyperalgesia (increased response to noxious stimulation), and spontaneous pain (spontaneous bursting). Importantly, in otherwise naïve animals, delivering microglia which have been stimulated with ATP phenocopies the changes in lamina I neurons induced by peripheral nerve injury. As such, ATP-stimulated microglia are sufficient to transform the output of this major pain transmission pathway and hence provide a neural basis for microglia involvement in neuropathic pain.

P2 receptor expression in microglia

Microglia-neuron communication is bidirectional and considerable evidence implicates ATP as being a critical molecular substrate for interaction between these cells (Coull et al., 2005;Jarvis, 2010;Maeda et al., 2010). ATP is an endogenous ligand of the P2 purinergic family of receptors, which consists of P2Y metabotropic receptors and P2X ionotropic receptors. P2Y receptors (P2Y1,2,4,6,11,12,13 and 14) are G-protein coupled, whereas P2X receptors (P2X1–P2X7) are non-selective cation channels permeable to Ca^{2+}, K^{+}, and Na^{+} (Burnstock, 2007;Burnstock et al., 2011;Inoue, 2002;Inoue and Tsuda, 2006;North, 2002). There is a wide expression of P2YRs on microglia, including P2Y1,2,4,6, and 12 receptor subtypes (Boucsein et al., 2003;Farber and Kettenmann, 2005;Kobayashi et al., 2008;Tozaki-Saitoh et al., 2008). By contrast, microglia expression of P2X receptors is restricted to the P2X4 and P2X7 receptor subtypes (Collo et al., 1997;Ferrari et al., 1996;Inoue and Tsuda, 2006;Tsuda et al., 2003). In microglia, stimulation of P2X or P2Y receptors evokes current responses, increases intracellular [Ca^{2+}], and causes release of signaling molecules that explicitly affect neuronal function (Illes et al., 1996;Moller et al., 2000;Norenberg et al., 1994;Walz et al., 1993). However, only recently have microglial P2 receptors been causally implicated in nerve injury-induced pain behaviours (Chessell et al., 2005;Coull et al., 2005;Kobayashi et al., 2008;Tozaki-Saitoh et al., 2008;Tsuda et al., 2003;Tsuda et al., 2009a;Tsuda et al., 2009c;Ulmann et al., 2008). Much of our conceptual understanding of the role microglial P2 receptors play in neuropathic pain stems from recent advances made in elucidating the cellular and molecular mechanisms that regulate P2 receptor function, as well as the identification of signaling pathways downstream from these receptors. The significance of these findings for microglia involvement in neuropathic pain will be discussed in this article.

Role of microglial P2X4 receptors in neuropathic pain

The essential role of P2X4 receptors in neuropathic pain was first reported by Tsuda et al. (2003)(Tsuda *et al.*, 2003). It was demonstrated that intrathecal injection of 2′,3′-O-(2,4,6-trinitrophenyl)adenosine 5′-triphosphate (TNP-ATP), an antagonist of P2X1-4Rs, reversed tactile allodynia in nerve-injured rats. By contrast, treatment with pyridoxalphosphate-6-azophenyl-2′,4′-disulphonoic acid (PPADs), an antagonist of P2X1-3,5,7Rs, but not the P2X4 receptor, had no effect on tactile allodynia. Based on the pharmacological profiles of these antagonists, it was inferred that the essential P2X receptor subtype involved in central responses to peripheral nerve injury is the P2X4 receptor, and that ongoing expression of tactile allodynia requires tonic P2X4 receptor activation. Directly targeting P2X4 receptors with P2X4 receptor antisense oligonucleotide (Tsuda *et al.*, 2003) or genetically deleting the *P2rx4* gene (Ulmann *et al.*, 2008) significantly attenuated nerve injury induced pain behaviours. The development of tactile allodynia correlated with a progressive increase in spinal P2X4 receptor expression, which normally is at a low level in the naïve central nervous system. On closer examination, it was evident that the cells expressing P2X4 receptors were microglia rather than neurons or astrocytes. This observation was recently confirmed in $CX3CR1^{+/GFP}$ mice, in which induction of spinal P2X4 receptor expression following nerve injury occurred specifically in the eGFP microglia (Ulmann *et al.*, 2008). The increase in P2X4 receptor expression correlated temporally with the development of tactile allodynia. However, preventing the increase in P2X4 receptor did not affect expression of protein markers of microglia response, such as the complement receptor 3 (Tsuda *et al.*, 2003) or the ionized calcium binding adaptor molecule-1 (Ulmann *et al.*, 2008). Thus, the upregulation of these microglial markers after peripheral nerve injury is not dependent upon P2X4 receptors. Definitive evidence that stimulation of P2X4 receptors expressed on microglia is sufficient to elicit pain hypersensitivity comes from the finding that intrathecal injection of P2X4 receptor-stimulated cultured microglia elicits robust mechanical allodynia in non-nerve injured animals (Coull et al., 2005;Tsuda et al., 2003;Tsuda et al., 2008b). Taken together, the pharmacological, genetic, and behavioral findings indicate that activity of P2X4 receptors expressed on spinal microglia is both necessary and sufficient, and therefore logically causative, to induce tactile allodynia after peripheral nerve injury. Although these mechanisms are clearly a major component of the functional alterations in the spinal dorsal horn that result in ongoing pain following peripheral nerve injury, they are one of many mechanisms thought to contribute in multiple sites both peripheral and central (Beggs and Salter, 2010;Clark et al., 2007;Hulsebosch et al., 2009;Inoue and Tsuda, 2009;Watkins et al., 2001). How each of these mechanisms overlap and their relative importance is yet to be determined, however changes such as these, occurring potentially beyond the reach of effective endogenous spinal cellular inhibitory control, are likely to have a relatively profound role within the overall etiology of peripherally mediated neuropathic pain.

Significant advances have recently been made towards understanding how injury to a nerve signals to the dorsal horn to cause increased P2X4 receptor expression in microglia. Several signaling molecules have been implicated in the upregulation of P2X4 receptors, including CCL21, a chemokine released from injured neurons (Biber et al., 2011;de Jong et al., 2005),

interferon γ, a cytokine that transforms resting spinal microglia into an activated state (Tsuda et al., 2009b), and tryptase, a protease released from mast cells that activates proteinase-activated receptor 2 in microglia (Yuan et al., 2010). Also critical for upregulating expression of P2X4 receptors is the extracellular matrix molecule fibronectin, which through the Lyn kinase signaling pathway modulates the transcriptional and post-transcriptional levels of P2X4 receptor expression in microglia (Nasu-Tada et al., 2006;Tsuda et al., 2008a;Tsuda et al., 2008b;Tsuda et al., 2009c). Similarly, activation of mu-opioid receptors by morphine can drive P2X4 receptor expression in states of opioid tolerance (Horvath and DeLeo, 2009;Horvath et al., 2010) and opioid-induced hyperalgesia (Ferrini et al., 2010). Thus, several critical elements of the molecular machinery required for upregulation of P2X4 receptors in microglia following peripheral nerve injury have recently been identified, but whether these elements converge on a common pathway that controls P2X4 receptor expression or how they are causally connected is not known.

Cell surface expression of P2X4 receptors is regulated by constitutive internalization and reinsertion(Bobanovic et al., 2002;Fujii et al., 2011;Royle et al., 2002;Toulme et al., 2006). In microglia, internalized P2X4 receptors are targeted to lysosomes and constitutive retrieval of these receptors from the plasma membrane regulates the proportion of P2X4 receptors on the cell surface (Qureshi et al., 2007). Internalization is controlled by the C-terminus of the P2X4 receptor (Fujii *et al.*, 2011;Qureshi *et al.*, 2007;Royle *et al.*, 2002), this region is also important for agonist-induced desensitization (Fountain and North, 2006) and phosphoinositide PIP_2 modulation of P2X4 receptor function (Bernier et al., 2008). The recent elucidation of the crystal structure of the zebrafish P2X4 receptor revealed critical details about the extracellular domain, putative ATP binding site, transmembrane regions, and ion permeation pathway (Kawate et al., 2009). Time-lapse imaging using fast-scanning atomic force microscopy added further to our understanding of the P2X4 receptor channel function, revealing that ATP-stimulation induces structural changes that result in two distinct conformations: in the presence of extracellular Ca^{2+}, ATP stimulation causes the P2X4 receptor to open a non-selective cation permeable channel, but in the absence of extracellular Ca^{2+} the receptor forms a macropore (i.e. a process known as pore dilation) that allows passage of large molecules (Shinozaki et al., 2009). P2X4 receptors expressed in microglia and macrophages have been reported to possess this ability to function both as a cation channel and as a macropore (Bernier et al., 2010;Seil et al., 2010); however, the implications of this dual mode of functioning, particularly in the context of neuropathic pain remains an intriguingly open question.

In microglia, influx of Ca^{2+} through the P2X4 receptor is a critical step linking stimulation of these receptors to the synthesis and release of brain-derived neurotrophic factor (BDNF) (Trang et al., 2009). The requirement for P2X4 receptors in the release of BDNF is supported by findings that P2X4 receptor-deficient mice have impaired microglial BDNF release and altered BDNF signaling in the spinal cord (Ulmann *et al.*, 2008). Moreover, P2X4 receptor-deficient mice do not develop mechanical allodynia following peripheral nerve injury, indicating BDNF release driven by P2X4 receptor activation is necessary for the development of neuropathic pain. Indeed, considerable evidence supports a critical role for BDNF in the initiation of central sensitization associated with neuropathic pain (Biggs et al., 2010;Lever et al., 2003;Lu et al., 2007;Obata et al., 2011).

The previous framework of thinking was based on the hypothesis that BDNF derived from primary afferent neurons is responsible for spinal nociceptive hypersensitivity. This source of BDNF, however, has been brought into question because of evidence indicating that there is a lack of primary afferent evoked BDNF release in the spinal cord after nerve injury (Lever *et al.*, 2003), and that eliminating BDNF from primary afferent neurons has no effect on nerve injury induced mechanical allodynia (Zhao et al., 2006). A study by Coull et al. (2005) (Coull *et al.*, 2005) provided the first evidence that in neuropathic pain, it is BDNF from microglia that affects nociceptive processing in the spinal cord. It was demonstrated that intrathecal administration of P2X4 receptor-stimulated microglia causes a depolarizing shift in E_{GABA} in spinal lamina I neurons that reduces inhibition and in about one-third of neurons the $GABA_A$-ergic responses are converted to excitation. The altered inhibitory responses produce a phenotypic switch in spinal lamina I neurons such that they relay innocuous mechanical input, increase discharge to a noxious stimulus, and display spontaneous activity (Coull et al., 2003;Keller et al., 2007). Several key findings implicate BDNF as the cellular substrate in aberrant pain signaling between microglia and neurons in the spinal cord: 1) intrathecal application of BDNF mimicks the mechanical allodynia and alteration in E_{GABA} caused by peripheral nerve injury or by administering P2X4 receptor-stimulated microglia; 2) blocking BDNF-trkB signaling prevents mechanical allodynia evoked by P2X4 receptor-stimulated microglia; and, 3) siRNA knockdown of BDNF expression abrogates the effects of intrathecally administered microglia on lamina I neurons and on the microglia-elicited pain behaviours (Coull *et al.*, 2005). Collectively, these findings suggest that BDNF from microglia is a critical signaling molecule in aberrant spinal nociceptive processing, and that microglia-neuron signaling through BDNF plays a causal role in the sequelae of neuropathic pain.

A major gap in understanding how activation of P2X4 receptors causes BDNF release was recently resolved with the identification of p38 mitogen-activated protein kinase (MAPK) as a cellular intermediary in the P2X4 receptor-BDNF signaling pathway (Trang *et al.*, 2009). Pharmacological and biochemical interrogation of this pathway revealed that influx of Ca^{2+} through the P2X4 receptor activates p38 MAPK: activation of this kinase is permissive for the release and synthesis of BDNF in microglia. This type of Ca^{2+}-dependent activation of p38-MAPK has been reported in a number of cell types (Blanquet, 2000;Dehez et al., 2001;Elzi et al., 2001;Kreideweiss et al., 1999;Shigemoto-Mogami et al., 2001), including peripheral macrophages which express functional P2X4 receptor (Brone et al., 2007;Qureshi et al., 2007;Ulmann et al., 2010). In macrophages, P2X4 receptor activation of p38 MAPK is upstream from the production and release of prostaglandin E2 (Ulmann *et al.*, 2010), a principal substrate for inflammation that sensitizes peripheral nociceptors (Portanova et al., 1996;Samad et al., 2002). Thus, the findings in microglia and macrophages place p38 MAPK in the core P2X4 receptor signaling pathway. p38 MAPK therefore appears to gate the release of distinct signaling molecules from microglia (BDNF) and peripheral macrophages (prostaglandin E2) that alter both the afferent input and the processing of such input. These discoveries highlight a novel role for P2X4 receptors in inflammation and represent a significant conceptual advance that extends involvement of P2X4 receptors in chronic pain beyond neuropathic pain to encompass the pathoetiology of inflammatory pain. Identification of the upstream and downstream components of the P2X4 receptor signaling

pathway has provided important new information about the fundamental central and peripheral mechanisms that underlie pain hypersensitivity subsequent to nerve injury and inflammation. The P2X4 receptor is therefore a promising therapeutic target for the treatment of neuropathic pain and inflammatory pain.

Role of microglial P2X7 receptors in neuropathic pain

In addition to P2X4 receptors, microglia express functional P2X7 receptors. Stimulation of P2X7 receptors is implicated in the microglia response to inflammation (Collo *et al.*, 1997), microglial proliferation (Bianco et al., 2005;Monif et al., 2009), and release of proinflammatory cytokines (Brough et al., 2002;Chakfe et al., 2002;Clark et al., 2010;Ferrari et al., 1997a;Ferrari et al., 1997b). A role for P2X7 receptors in neuropathic pain is also suggested on the basis of reduced pain sensitivity in P2X7 receptor-deficient mice (Chessell *et al.*, 2005) and after pharmacological blockade of the receptor (Broom et al., 2008;Dell'Antonio et al., 2002;Honore et al., 2006;Honore et al., 2009;McGaraughty et al., 2007;Perez-Medrano et al., 2009). Although P2X7 receptors are expressed on a wide variety of cell types, evidence for involvement of microglial P2X7 receptors in neuropathic pain stems from findings that activating microglial P2X7 receptors causes the release of interleukin-1β and cathepsin S, which in the spinal cord contributes to the maintenance of mechanical hypersensitivity (Clark et al., 2007;Clark et al., 2010). Like P2X4 receptor-mediated release of BDNF, the release of interleukin-1β and cathepsin S from microglia involves P2X7 receptor signaling to p38 MAPK (Clark *et al.*, 2010). Thus, activation of p38 MAPK may be a critical mechanistic step of convergence in the P2X7 and P2X4 receptor signaling pathways in neuropathic pain. P2X7 and P2X4 receptors also interact to form complexes with unique channel properties (Antonio et al., 2009;Boumechache et al., 2009;Casas-Pruneda et al., 2009;Guo et al., 2007;Nicke, 2008). This raises the interesting possibility that structural and functional interactions, as well as converging signaling pathways, might be critical cellular mechanisms that underlie involvement of microglial P2X7 and P2X4 receptors in neuropathic pain.

Role of microglial P2Y receptors in neuropathic pain

Microglia express a wide range of P2Y receptors (P2Y1, 2, 4, 6, and 12), with P2Y6 and P2Y12 receptors mediating chemotaxis and migration of microglia towards the site of damage (Haynes et al., 2006;Honda et al., 2001;Maeda et al., 2010;Ohsawa et al., 2007). To date, only the P2Y12 receptor subtype has been explicitly implicated in the development of neuropathic pain. In response to peripheral nerve injury, P2Y12 receptor expression is upregulated on microglia and activation of these receptors is critical for microglial engulfment of myelinated axons in the spinal dorsal horn (Kobayashi *et al.*, 2008;Maeda *et al.*, 2010;Tozaki-Saitoh *et al.*, 2008). Inhibition of these receptors by pharmacological blockade, antisense knockdown of P2Y12 receptor expression, or genetic deletion of the *P2ry12* gene, suppresses both mechanical allodynia and thermal hyperalgesia in nerve injured rats (Kobayashi *et al.*, 2008;Tozaki-Saitoh *et al.*, 2008). Conversely, intrathecal administration of the P2Y12 receptor agonist 2Me-SADP elicits pain behaviours in naïve rats that mimic those observed in nerve-injured rats (Kobayashi *et al.*, 2008). The involvement of microglial P2Y12 receptors in neuropathic pain is critically dependent upon

p38 MAPK activation (Kobayashi *et al.*, 2008); however, the signaling events downstream from P2Y12 receptor activation of p38 MAPK remain a critical open question.

Conclusion and future directions

Several advances in recent years have emphasized the essential role of microglial P2 receptors in the sequelae of neuropathic pain arising from peripheral nerve injury. In particular, P2X4, P2X7 and P2Y12 receptors expressed on microglia have emerged as new molecular players that are critically involved in the etiology of neuropathic pain. Activation of these P2 receptor subtypes engages distinct intracellular signaling pathways in microglia that converge onto p38 MAPK (Figure 2). Thus, p38 MAPK is a cellular intermediary and its activation is a key point of convergence for P2X4, P2X7, and P2Y12 receptor signaling in neuropathic pain. The next major challenges are to determine the significance of this convergence in signaling for microglia function and how the upstream, as well as, downstream components contribute to aberrant spinal nociceptive processing following nerve injury. In addition to converging signaling pathways, P2 receptor subtypes can interact at the structural level to form unique receptor complexes possessing distinct biophysical properties (Dubyak, 2007;Guo *et al.*, 2007;Jarvis and Khakh, 2009;Nicke, 2008). The potential functional consequences of this physical interaction add another layer of complexity that may have important implications for microglial P2 receptors in neuropathic pain.

The P2X4 receptor has emerged as a core microglia-neuron signaling pathway that is necessary for ongoing expression of tactile allodynia following nerve injury. There have been major advances in understanding the essential molecular components that regulate P2X4 receptor expression and the cellular machinery that drives BDNF release from microglia. These discoveries have built a complete framework for understanding microglia-neuron signaling in neuropathic pain. Based on this new framework, the current model is that activating microglial P2X4 receptors drives the release of BDNF, which causes disinhibition of nociceptive dorsal horn neurons by disrupting intracellular Cl^- homeostasis. Disinhibition by microglia-neuron signaling enhances excitatory synaptic transmission in the dorsal horn to transform the output of the nociceptive network. The consequent altered inhibitory responses increases discharge output, unmasks responses to innocuous peripheral inputs and spontaneous activity in neurons that otherwise only transmit nociception(Coull et al., 2003;Coull et al., 2005;Keller et al., 2007). These changes in electrophysiological phenotype of lamina I neurons are the cellular underpinnings that may account for the cardinal signs (allodynia, hyperalgesia, and spontaneous pain) of neuropathic pain. Recent studies have identified an unexpected commonality in mechanisms between neuropathic pain (Coull *et al.*, 2005), morphine-induced hyperalgesia (Ferrini *et al.*, 2010), and the sequelae of spinal cord injury (Boulenguez et al., 2010). Thus, the P2X4R-p38 MAPK-BDNF pathway from microglia to neurons defines a fundamental signaling ensemble that may underlie hyperexcitability across a range central nervous system disorders.

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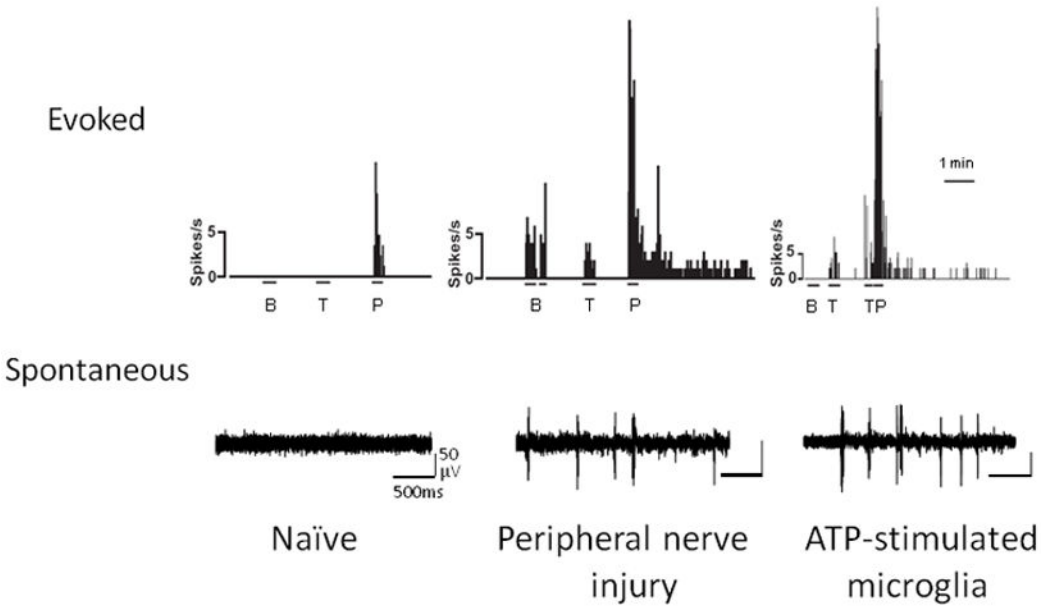

Figure 1. Peripheral nerve injury and microglia stimulation alters sensory output of spinal lamina I neurons underlying neuropathic pain

Rate meter records of representative responses evoked by brush (B), touch (T), or pinch (P) (top traces), and continuous extracellular single-unit records of spontaneous bursts of activity (bottom traces) in lamina I projection neurons in naive, after peripheral nerve injury or local spinal administration of ATP-stimulated microglia. The action potential discharge of these neurons is normally only evoked by noxious peripheral stimulation. However, after peripheral nerve injury the output of these neurons is transformed so that innocuous stimulus, such as brush or touch, evokes action potential discharges. In addition, after nerve injury, response to noxious stimulation is greatly exaggerated, and lamina I neurons exhibit spontaneous bursting in the absence of overt stimulation whereas in naïve animals these neurons are normally silent. This change in the output of lamina I neurons is one of the mechanisms that contributes to neuropathic pain. Moreover, local administration of ATP stimulated microglia on the surface of the lumbar spinal cord recapitulates the changes in lamina I neurons induced by peripheral nerve injury. Modified from Keller *et al.* 2007.

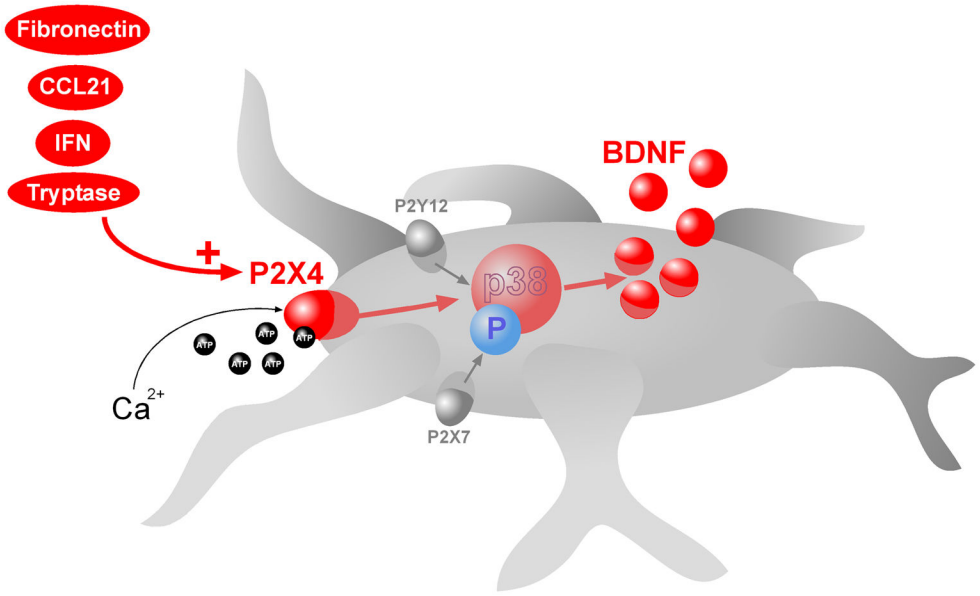

Figure 2. P2X4, P2X7 and P2Y12 receptor signaling converge on p38 MAPK in microglia

Activity of P2X4, P2X7, and P2Y12 receptors expressed on microglia is critically involved in neuropathic pain arising from peripheral nerve injury. In response to peripheral nerve injury, P2X4 receptors are upregulated in spinal microglia. Several mechanisms have recently been implicated in the upregulation of P2X4 receptors including: CCL21, interferon γ, tryptase, fibronectin, and the activation of mu-opioid receptors. ATP stimulation of P2X4 receptors leads to influx of extracellular Ca^{2+} which causes activation of p38 MAPK leading to SNARE-dependent release of BDNF from the microglia. BDNF is a key microglia-neuron signaling molecule that causes disinhibition of nociceptive dorsal horn neurons by disrupting intracellular Cl^{-} homeostasis. Disinhibition by microglia-neuron signaling underlies the development of tactile allodynia, a hallmark symptom of neuropathic pain. Activation of P2X7 or P2Y12 receptors also signals through p38 MAPK. P2X7 receptors-p38 MAK signaling drives the release of interleukin-1β and cathepsin S, which in the spinal cord contributes to the maintenance of mechanical hypersensitivity. Although it is known that P2Y12 receptor expression is upregulated in microglia and activation of these receptors is involved in neuropathic pain, the signaling steps downstream from p38 MAPK activation has yet to be elucidated.