Central neuron-glia interactions and neuropathic pain

Overview of recent concepts and clinical implications

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Neuropathic pain results from injury or disease causing dysfunction at any level of the somatosensory (primarily spinothalamic) system, including peripheral nociceptive axons, dorsal root ganglion (DRG), dorsal horn, spinothalamic pathway, and thalamus. The manifestations of neuropathic pain, including spontaneous pain, hyperalgesia, and thermal and mechanical allodynia, reflect excessive excitability of peripheral nociceptors (peripheral sensitization), central nociceptive neurons (central sensitization), or both. Sensitization reflects maladaptive plastic changes in the nociceptive system that result directly from axonal injury and from the effects of products of inflammation. There is abundant evidence that activation of microglia and astrocytes in the dorsal horn is common and constitutes an important amplification mechanism leading to neuropathic pain in the setting of peripheral nerve or spinal cord injury. Activated glial cells are also involved in sensitization of brainstem and thalamic neurons at a distance from the site of injury. The complex roles of the central glia and its mediators in the mechanisms of neuropathic pain have been extensively reviewed.[1-9]

NORMAL PAIN SIGNALING PAIN PROCESSING The components of transmission of nociceptive information ("pain system") include the nociceptive neurons of the DRG with unmyelinated C- and small myelinated Aδ axons that activate different types of neurons in the dorsal horn, particularly in lamina I and lamina V, which project via parallel pathways to the thalamus and to the brainstem. The primary DRG afferents also activate excitatory and inhibitory interneurons in lamina II that exert local control on nociceptive transmission.[10-12] Similar organization occurs in the nociceptive trigeminal system. The nociceptive DRG neurons express different types of voltage-gated sodium (Na^+) channels (particularly Nav1.7, Nav1.8, and Nav1.9), transient receptor potential (TRP) channels (including TRPV1 and TRPA), acid-sensitive channels, serotonin 5-HT3 receptors, and purinergic P2X receptors that are activated by noxious mechanical or chemical stimuli.[11] In normal conditions, activation of Aδ and C-fiber nociceptors leads to glutamate release from the primary afferent nerve terminals, resulting in short-term activation of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in the projection neurons of the dorsal horn; this provides information about the time of onset, duration, and intensity of peripheral noxious stimuli. Local inhibitory mechanisms, mediated by gamma-aminobutyric acid (GABA) and glycine, as well as other signals such as adenosine and opioids, presynaptically regulate neurotransmitter release from primary afferents and prevent excessive excitation of dorsal horn projection neurons. During normal transmission of nociceptive afferent signals in the dorsal horn there is no activation of astrocytes or microglia. Astrocytes have an important homeostatic role by active reuptake of glutamate via excitatory amino acid transporters 1 and 2.[4]

MECHANISMS OF CENTRAL SENSITIZATION FOLLOWING NERVE INJURY Studies on several experimental models in rodents, including partial ligation, chronic constriction, or transection of the sciatic nerve or chronic compression of the L4-L5 roots, have provided a large amount of information regarding the cellular, chemical, and molecular changes underlying neuropathic pain.[3,10,11] These studies show that peripheral nerve injury and tissue inflammation

GLOSSARY

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; **ATP** = adenosine triphosphate; **BDNF** = brain-derived neurotrophic factor; **CREB** = cyclic adenosine monophosphate response element binding protein; **DRG** = dorsal root ganglion; **ERK** = extracellular signal-regulated kinase; **GABA** = gamma-aminobutyric acid; **IL-1β** = interleukin-1β; **MCP-1** = monocyte chemoattractant protein-1; **MMP** = matrix metalloprotease; **NO** = nitric oxide; **RVM** = rostral ventromedial medulla; **SCI** = spinal cord injury; **TLR** = toll-like receptor; **TNFα** = tumor necrosis factor α; **TRP** = transient receptor potential; **VPL** = ventroposterolateral nucleus of the thalamus.

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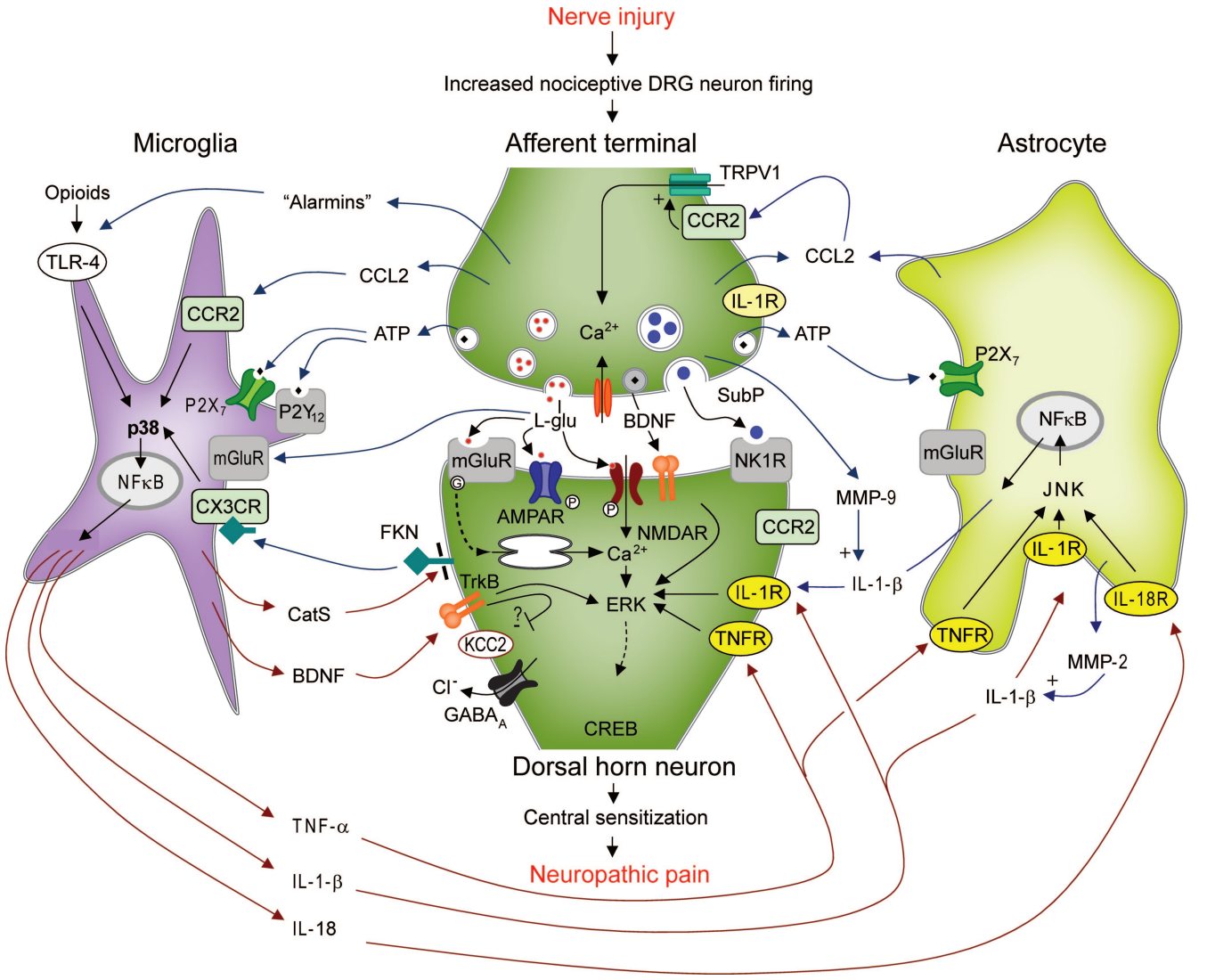
elicit several plastic changes in nociceptors, including upregulation of Na^+ and TRP channels, which result in reduced threshold for activation and repetitive firing of DRG neurons in response to nociceptive stimuli (peripheral sensitization). This altered activity of primary afferents triggers plastic changes in both local and projection neurons in the dorsal horn, resulting in central sensitization of the spinothalamic system (figure).[13] After nerve injury, repetitive firing of DRG neurons triggers release not only of L-glutamate, the primary neurotransmitter, but also of neuropeptides such as substance P and calcitonin

Figure **Cross-talk among neurons, microglia, and astrocytes and dorsal horn sensitization following axonal injury**



After nerve injury, repetitive firing of dorsal root ganglion (DRG) neurons triggers release of L-glutamate (l-glu), substance P (SubP), adenosine triphosphate (ATP), brain-derived neurotrophic factor (BDNF), and the cysteine-cysteine chemokine ligand CCL2. These multiple signals lead to increased neurotransmitter release from primary afferents and increased excitability of dorsal horn projection neurons (central sensitization). Many of these effects involve activation of microglia and astrocytes. Substance P, acting via neurokinin-1 receptors (NK-1R), leads to neuronal depolarization, activation of NMDA receptors, and calcium (Ca^{2+}) influx. Calcium activates several phosphorylation pathways, including extracellular signal-regulated kinase (ERK), which increase surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and activate the transcription factor cyclic adenosine monophosphate response element binding protein (CREB). ATP activates astrocytes (via P2 $\times$ 7 receptors) and microglia (via P2 $\times$ 4, P2 $\times$ 7, and P2Y12 receptors). Activation of microglia and astrocytes via mitogen-activated kinase p38 and *c-jun*-N terminal kinase (JNK) leads to activation of nuclear factor kappa B (NFκB), which promotes transcription of several inflammatory mediators. Interleukin-1β (IL-1β) acting via its receptor (IL-1R) and tumor necrosis factor α (TNFα) acting via its receptor (TNFR) increase primary afferent excitability and potentiate glutamate-mediated excitation. BDNF released from activated microglia reduces expression of the chloride (Cl^-) transporter KCC2, rendering the effects of gamma aminobutyric acid $(GABA)_A$ receptors excitatory. CCL2, acting via CCR2 receptors, promotes depolarization of nociceptive afferent astrocyte activation. Cathepsin S (CatS) released from activated microglia elicits cleavage and release of fractalkine (FKN) which, in turn, activates microglia via CXCR3 receptors. Matrix metalloprotease (MMP)-9 released from damaged DRG neurons and MMP-2 released from astrocytes cleave and activate IL-1β. Peripheral nerve injury elicits the release of "endogenous danger signals" or "alarmins" from DRG neurons; these signals activate toll-like receptor 4 (TLR4) in microglia, resulting in release of inflammatory cytokines and inhibition of opioid-mediated analgesia.

gene-related peptide, adenosine triphosphate (ATP), brain-derived neurotrophic factor (BDNF), and several chemokines and cytokines. These multiple signals lead, directly or indirectly, to plastic changes resulting in increased excitability of dorsal horn projection neurons.[10,11,13] For example, substance P elicits membrane depolarization via neurokinin-1 receptors and thus removes the magnesium (Mg^{2+}) blockade of the glutamate NMDA receptors, allowing calcium (Ca^{2+}) influx via this channel as well as via voltage-gated Ca^{2+} channels. Calcium release from intracellular stores in response to activation of metabotropic glutamate receptors further increases cytosolic Ca^{2+} concentration. Calcium activates several downstream transduction cascades, including phosphorylation pathways such as calmodulin kinase II, protein kinase C, and extracellular signal-regulated kinase (ERK), as well as nitric oxide (NO) synthase and cyclooxygenase. These transduction pathways elicit plastic changes in dorsal horn neurons that that increase their responsiveness to afferent inputs. For example, phosphorylation by calmodulin kinase II or ERK increases surface expression and open probability of AMPA and NMDA receptors, whereas phosphorylation of the transcription factor cyclic adenosine monophosphate response element binding protein (CREB) upregulates expression of several synaptic proteins and other signals.[10,11,13]

GLIAL ACTIVATION IN THE DORSAL HORN AND NEUROPATHIC PAIN

Whereas during normal transmission of nociceptive signals there is no activation of astrocytes or microglia, after peripheral nerve injury both types of glial cells proliferate and release a variety of chemical mediators that contribute to altered synaptic transmission in the dorsal horn and increased excitability of the projection neurons.

Glial activation following nerve injury. Studies in vitro indicate that the responses of microglia and astrocytes vary with the type of stimulus; these activation "programs" have been referred to as enhanced response states.[5] There are several differences in the responses of microglia and astrocytes after peripheral nerve injury. Microglia activation occurs in the early phase, is transient, and may occur in the absence of axonal degeneration or cell death. In contrast, the astrocyte response occurs later, occurs after axonal degeneration, and is involved in persistence of neuropathic pain. The sequential activation of microglia and astrocytes following nerve injury involves mitogen-activated kinase p38 in microglia, and *c-jun*-N terminal kinase in astrocytes, resulting in activation of nuclear factor κ B, which promotes transcription of several inflammatory mediators.[4,6] The contribution of microglia derived from bloodborne monocytes to pain modulation following peripheral nerve injury is still incompletely defined.[5]

Effects of chemical signals from the glia on dorsal horn neurons. Studies in vitro and in experimental in vivo models indicate that several chemical signals released from microglia and astrocytes after nerve injury contribute to increase pain transmission in the dorsal horn (table).[4,5] These include interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), chemokines, excitatory amino acids, ATP, D-serine, BDNF, NO, reactive oxygen species, arachidonic acid, and prostaglandins (figure). These signals contribute to central sensitization by multiple mechanisms, including increased primary afferent excitability and neurotransmitter release; potentiation of glutamate-mediated depolarization; and impaired local GABAergic inhibition. For example, both TNFα and IL-1β increase excitability of DRG neurons by increasing Na^{+} influx via Nav1.8 channels and upregulate the number and function of NMDA and NK-1 receptors in projection neurons[4,14]; TNFα downregulates expression of amino acid transporter 2 involved in glutamate uptake by astrocytes.[15] Activated microglia releases BDNF, which reduces expression of the chloride (Cl^{-}) transporter KCC2, rendering the effects of $GABA_A$ receptors excitatory rather than inhibitory.[16]

Cross-talk among neurons, microglia, and astrocytes in the dorsal horn. Studies in vitro also indicate that there are several potential autoexcitatory loops and cross-talk among dorsal horn neurons, microglia, and astrocytes triggered by peripheral injury (figure). These interactions further stimulate microglia and astrocytes and promote plastic changes in excitatory and inhibitory neurotransmission.[4-6] Glial activation reflects the effects of several chemical signals released from afferents of damaged DRG neurons or from neurons in the dorsal horn following nerve injury, including L-glutamate, ATP, substance P, BDNF, NO, and chemokines.[4,5] After release in the dorsal horn following activation of nociceptive afferents, both L-glutamate, acting via NMDA receptors, and substance P, acting via NK-1 receptors, activate microglia and astrocytes and promote release of proinflammatory cytokines.[5] ATP released from damaged DRG afferents or sensitized dorsal horn neurons activates both astrocytes (via P2 $\times$ 7 receptors) and microglia (via P2 $\times$ 4, P2 $\times$ 7, and P2Y12 receptors).[6] Glial activation also involves chemokines. For example, in response to TNFα released from microglia, damaged DRG neurons and astrocytes upregulate expression of the cysteine-cysteine chemokine ligand CCL2 (also known as monocyte chemoattractant protein-1, MCP-1) and its receptor.[17] CCL2, acting via its CCR2 receptors, elicits upregulation of TRPV1 channels and

Table Some signals mediating neuron–glia interactions and neuropathic pain

Signal (source)	Effect (receptor)
L-glutamate (damaged DRG afferents, astrocytes)	Astrocyte and microglia activation (mGluR)
Substance P (damaged DRG afferents, astrocytes)	Astrocyte and microglia activation (mGluR)
ATP (damaged DRG afferents, astrocytes)	Astrocyte ($P2X_7$) and microglia ($P2X_4$, $P2X_7$, $P2Y_{12}$) activation
CCL2 (damaged DRG afferents, astrocytes)	Nociceptive afferent depolarization; astrocyte activation (CCR2)
Factalkine (dorsal horn neuron)	Microglia activation (CXCR3)
MMP-9 (damaged DRG afferents); MMP-2 (astrocyte)	Cleavage an activation of interleukin-1β
"Alarmins" (damaged DRG afferents)	Microglia activation (TLR4)
Interleukin-1β (microglia, astrocytes)	Upregulation of expression and function of Nav1.8 NMDAR and NK-1R (IL-1R)
Tumor necrosis factor α (microglia)	Upregulation of expression and function of Nav1.8 NMDAR and NK-1R (TNFR)
	Inhibition of glutamate uptake by astrocytes (TNFR)
BDNF (microglia)	Reduced expression of Cl^- transporter, rendering GABA A receptor excitatory (trkB)
Cathepsin S (microglia)	Cleavage and activation of fractalkine released from dorsal horn neurons

Abbreviations: ATP = adenosine triphosphate; CCL2 = cysteine-cysteine chemokine ligand 2; DRG = dorsal root ganglion; IL-1R = interleukin-1β receptor; mGluR = metabotropic glutamate receptor; MMP = matrix metalloprotease; NK-1R = neurokinin-1 receptor; NMDAR = NMDA receptor; TLR4 = toll-like receptor 4; TNFR = tumor necrosis factor α receptor; trkB = tyrosine kinase B receptor.

depolarization of nociceptive afferents[18] as well as astrocyte activation.[19] Fractalkine is a transmembrane chemokine that is expressed in DRG and dorsal horn neurons[20]; cathepsin S released from activated microglia elicits cleavage and release of fractalkine which, in turn, activates microglia via CXCR3 receptors.[21] Matrix metalloproteases (MMPs) are also implicated in chronic neuropathic pain.[22] MMP-9, released from damaged DRG neurons, and MMP-2, released from activated astrocytes, cleave and activate IL-1β released from microglia. All these examples emphasize the complexity of chemical signaling in the dorsal horn in response to nerve injury.

Toll-like receptors and opioid resistance in the setting of neuropathic pain. Peripheral nerve injury elicits the release of "endogenous danger signals" or "alarmins," which include degradation products of the extracellular matrix and substances released by stressed or dying DRG cells, such as heat shock proteins.[9] These signals are recognized by toll-like receptors (TLRs), such a TLR4, which trigger innate immune responses via several pathways resulting in release of inflammatory cytokines.[23] In the CNS, TLR4 is predominantly expressed in microglia, but its expression may also be unregulated in astrocytes during inflammation.[24] Recent evidence indicates that activation of TLR4 in microglia has a key role in initiation and maintenance of neuropathic pain.[9] Furthermore, activation of TLR4 receptors in microglia by opioids, resulting in release of proinflammatory cytokines, has been shown to antagonize the classic analgesic effects of opioids acting on mu-type receptors in neurons.[9]

PROPRIOSPINAL AND SUPRASPINAL NEUROIMMUNE SIGNALING

Remote neuroimmune signaling in spinal cord injury. Peripheral nerve or spinal cord injury (SCI) are associated with exaggerated spontaneous rhythmic burst firing in the ventroposterolateral nucleus of the thalamus (VPL), which correlates with behavioral manifestations of neuropathic pain.[8] There is emerging evidence indicating that neuronal injury at one level of the spinal cord triggers remote signaling in the spinothalamic system that can alter nociceptive information processing at the level of the thalamus.[8] In a spinal contusion model in the rat, thoracic SCI elicits microglial activation and altered neuronal activity both in the lumbar dorsal horn and in the VPL; this is associated with sensitization of lumbar dorsal horn and thalamic neurons and behavioral signs of allodynia and thermal hyperalgesia.[7,8] Activation of microglia at locations distant from the initial SCI may involve signaling via the cytokine CCL21.[7,8] It has been hypothesized that CCL21 is upregulated in response to damage of lumbar spinothalamic axons neurons as they pass by the thoracic SCI site; this cytokine may lead to local activation of microglia at the lumbar levels and also serve as a remote signal to the thalamus.[8] Upregulation of CCL21 in the terminal fields of lumbar spinothalamic axons leads to microglial activation and abnormal burst firing in thalamocortical neurons in the VPL, which is associated with hyperalgesia and allodynia. These affects are attenuated by interruption of the spinothalamic tract or neutralization of CCL21 signaling in the VPL.[7,8]

Neuron-glia interactions and descending pain facilitation. Neurons in the rostral ventromedial medulla (RVM) receive inputs from the periaqueductal gray, project to the dorsal horn, and are a major component of the descending pain modulation system, which may exert context-dependent antinociceptive or pronociceptive effects.[25] Recent evidence indicates that supraspinal glial-neuronal interactions at the level of the RVM may promote descending pain facilitation following peripheral nerve injury. Wei et al.[26] showed that, after chronic constriction injury of the rat infraorbital nerve, there is both early and transient reaction of microglia and prolonged reaction of astrocytes in the RVM, associated with local elevations of IL-1β and TNFα levels. These cytokines elicit phosphorylation and activation of NMDA receptors in RVM neurons; this was associated with

behavioral manifestation of neuropathic pain.[26] This indicates that proinflammatory cytokines released from activated glial cells act via NMDA receptors in RVM neurons promoting descending pain facilitation. Other signals, such as glutamate, ATP, substance P, and BDNF, may also elicit glial activation at the level of the RVM, thus promoting neuropathic pain.[25]

THERAPEUTIC IMPLICATIONS Several drugs that inhibit glial activation have been shown to alleviate neuropathic pain in several animal models.[27] These include ibudilast,[24] propentoxifylline,[28] and minocycline, an inhibitor of microglial activation.[29] Ibudilast, a nonselective phosphodiesterase inhibitor, is also a TLR4 inhibitor that suppresses glial cell activation, enhances acute morphine analgesia, and attenuates morphine tolerance and withdrawal.[9,30] However, there is still not direct evidence that central glia have a role in the pathophysiology of chronic pan in humans.[5] A single human imaging PET study using a ligand for the peripheral benzodiazepine receptor suggested the presence of microglial activation in the thalamus in amputees with longstanding phantom limb pain,[31] and there are reports of elevated levels of proinflammatory cytokines in the CSF in patients with chronic neuropathic pain[32] and complex regional pain syndrome.[33] A single postmortem study in a patient with longstanding complex regional pain syndrome showed activation of both microglia and astrocytes in the dorsal horn both at the level of the original injury and throughout the spinal cord.[34] Whereas all these findings would support a role of the glia in chronic pain in humans, several confounding factors make this evidence only indirect.[5]

On the basis of experimental evidence from animal studies, ibudilast—a phosphodiesterase inhibitor that, among other effects, decreases glial activation—underwent a phase 2 clinical trial.[35] Ibudilast, when given orally up to a dose of 30 mg twice daily for 14 days in healthy volunteers, achieved plasma levels and pharmacokinetics comparable to those associated with efficacy in rat chronic pain models and was, in general, well-tolerated. Thus ibudilast may be a promising therapeutic candidate for neuropathic pain.

PERSPECTIVE Abundant experimental evidence indicates that activation of astrocytes and microglia in the dorsal horn, brainstem pain modulatory areas, and thalamus affects synaptic transmission and promotes development and maintenance of chronic pain in the setting of nerve injury. However, there are several remaining challenges in the understanding of the role of glia in chronic pain, as discussed in an excellent recent review,[5] and there is still only indirect evidence that glial activation may contribute to chronic pain in humans. A better understanding of the timing of activation, glia cell type, and chemical signals affecting nociceptive transmission will continue to provide opportunities for development of drugs that affect astrocytes and microglia and thereby may be helpful in the management of chronic neuropathic pain in humans.[27]

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