

## Invited review

**Glial involvement in trigeminal central sensitization<sup>1</sup>**Yu-feng XIE<sup>2,3,4</sup><sup>2</sup>Department of Physiology and Pathophysiology, Xi'an Jiaotong University, Xi'an 710061, China; <sup>3</sup>Department of Oral Physiology, University of Toronto, Toronto, Canada M5G 1G6**Key words**

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Phn 1-901-448-1782.

Fax 1-901-448-7300.

E-mail yufeng71@gmail.com

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**Abstract**

Recent studies have indicated that trigeminal neurons exhibit central sensitization, an increase in the excitability of neurons within the central nervous system to the extent that a normally innocuous stimulus begins to produce pain after inflammation or injury, and that glial activities play a vital role in this central sensitization. The involvement of glial cells in trigeminal central sensitization contains multiple mechanisms, including interaction with glutamatergic and purinergic receptors. A better understanding of the trigeminal central sensitization mediated by glial cells will help to find potential therapeutic targets and lead to developing new analgesics for orofacial-specific pain with higher efficiency and fewer side-effects.

**Introduction**

Central sensitization refers to the increased synaptic efficiency established in nociceptive neurons in the dorsal horn of the spinal cord following intense peripheral noxious stimuli, tissue injury, or nerve damage to the extent that a normally innocuous stimulus begins to produce pain and thereby plays a major role in the generation of acute postoperative and post-traumatic pain, migraine, and neuropathic pain<sup>[1]</sup>. There is accumulating evidence that glial cells (mainly astrocytes and microglia) in the central nervous system are activated by inflammation or peripheral nerve injury and are involved in spinal nociceptive transmission and central sensitization<sup>[2-5]</sup>. Astrocytes and microglia participate in neuronal sensitization, not only via indirect mechanisms (ie the release of cytokines and various neuroligands), but also more directly via the release of glutamate and/or by evoking changes in synaptic ion homeostasis<sup>[6,7]</sup>.

Trigeminal sensory nuclei mainly consist of the principal subnucleus (Vp) and the spinal trigeminal subnuclei, including oralis (Vo), interpolaris (Vi), caudalis (Vc) nuclei, and the mesencephalic trigeminal subnucleus. The oral nociceptive signal is primarily processed in Vp, Vo, and Vi, and secondarily processed in Vc. In contrast, the facial nociceptive signal is primarily processed in Vc nucleus<sup>[8]</sup>. Because of its

many anatomical and functional similarities with the spinal dorsal horn, Vc has been termed the "medullary dorsal horn"<sup>[8-12]</sup>. However, the neurochemical and neurophysiological changes induced by injury to nerves of the trigeminal system are clearly distinct from those seen following lesions of other peripheral nerves<sup>[13]</sup>. Recently, a series of experiments were undertaken to investigate the glial mechanisms in trigeminal central sensitization, which will help to find potential therapeutic targets and lead to developing new analgesics for orofacial-specific pain with higher efficiency and fewer side-effects.

**Glial activation in trigeminal central sensitization**

Increasing evidence indicates that central sensitization could be induced in trigeminal nociceptive neurons, and the central sensitization occurring in trigeminal nociceptive pathways shows more robust neuronal hyperexcitability following deep tissue stimulation than cutaneous stimulation<sup>[14]</sup>. Our studies have identified nociceptive neurons in Vc, and have demonstrated that the small-fiber excitant and inflammatory irritant mustard oil (MO) applied to orofacial regions produces long-lasting central sensitization in Vc, as reflected in neuroplastic changes, such as increases in neuronal spontaneous activity, receptive field size, responses to suprathreshold

stimuli, and a decrease in the activation threshold<sup>[15-18]</sup>. Piao *et al* found that tactile hypersensitivity was significantly increased at d 1 and lasted for 28 d, and was gradually reduced, finally returned to the basal level by 60 d after inferior alveolar nerve and mental nerve transection (IAMNT)<sup>[19]</sup>. Similarly, after the surgical removal of a lower impacted third molar, intraoral repetitive pinprick and electrical stimulation also induced central sensitization indicated by significantly increased pain intensity, and this phenomenon lasted for at least 1 week<sup>[20]</sup>. These studies pointed out that the central sensitization occurred in trigeminal neurons under pathological conditions and appeared to play an important role in the transition from acute to chronic orofacial pain. It is suggested that trigeminal central sensitization involves an increase in the excitability of Vc neurons brought about by a series of events, including neuronal depolarization; removal of the voltage-dependent magnesium block of the *N*-methyl-*D*-aspartate (NMDA) receptor; release of calcium from intracellular stores; phosphorylation of the NMDA, alpha amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), and neurokinin 1 receptors via the activation of protein kinases; a change in the neuron's excitability; and an increase in synaptic strength<sup>[14]</sup>. The trigeminal central sensitization proposes that the altered processing of sensory input in the brainstem, principally Vc, could account for, at least in part, many of the temporal and symptomatic features of migraine, its poor response to triptan therapy when such treatment is initiated hours after the onset of pain, as well as the role of glutamate in migraine<sup>[21,22]</sup>.

Recent studies have demonstrated that the trigeminal central sensitization involves glial activation. The presence of glial fibrillary acidic protein (GFAP)/substance P (SP)- or GFAP/calcitonin gene-related peptide (CGRP)-positive reactions by double-labeling immunofluorescence was clarified, and the morphological interrelationship between the astrocytes and nerve endings by the double-labeling electron microscopic immunohistochemistry in laminae I and II of Vc was demonstrated<sup>[23]</sup>. In the model of trigeminal central sensitization induced by the application of MO to the rat's tooth pulp, we found that the intrathecal application of SB203580 [an inhibitor of p38 mitogen-activated protein kinase (p38MAPK)] or fluoroacetate (FA; an inhibitor of the astroglial metabolic enzyme aconitase) markedly attenuated the MO-induced increases in pinch receptive field size and responses to noxious stimuli, and the decrease in the activation threshold<sup>[18]</sup>. Furthermore, the MO-induced central sensitization can be significantly attenuated by continuous intrathecal superfusion of methionine sulfoximine (MSO), an inhibitor of the astroglial glutamine synthetase that is in-

involved in the glutamate-glutamine shuttle. The simultaneous superfusion of MSO and glutamine restored the irritant-induced central sensitization<sup>[17]</sup>. In a subcutaneous injection of formalin in a mystacial vibrissae experiment, the formalin injection significantly increased the Cx32/Cx43 heterotypic gap junctions on junction areas between astrocytes and neurons within Vc and nociceptive behavior, while the administration of carbenoxolone (CBX; a gap junction blocker) or fluorocitrate (FCA; an inhibitor of the astroglial metabolic enzyme aconitase) into the cerebellomedullary cistern significantly reduced the nociceptive behavior and scratching-cumulative time. CBX and FCA also decreased the number of Fos/neuronal nuclei (NeuN)-immunoreactive (-IR) neurons or obviously attenuated the expression of Fos/NeuN-IR neurons and Fos/GFAP-IR astrocytes in the Vc, respectively<sup>[24]</sup>. It was found that microglial activation, primarily observed in the superficial laminae of Vc, continued 2 weeks after IAMNT, while astrocytic activation was delayed compared to that of microglia. Minocycline, an inhibitor of microglial activation, reduced microglial activation, but inhibited p38MAPK activation in microglia and significantly attenuated the development of pain hypersensitivity in this model<sup>[19]</sup>. These results suggest that glial activation and metabolic processes in the trigeminal system play an important role in trigeminal central sensitization, and therefore, in the development of inflammatory and neuropathic pain.

## Mechanism of glia activation in trigeminal central sensitization

The glial mechanisms in trigeminal central sensitization most likely involve their interactions with the glutamatergic receptor, which is a critical process in central sensitization. It has been demonstrated that astrocytes may directly influence the state of presynaptic terminals through the activation of presynaptic mGluRs, presynaptic and/or extrasynaptic NMDA receptors by increasing the probability of neurotransmitter release from nerve terminals and modulating synaptic transmission between cultured hippocampal neurons<sup>[25-28]</sup>. Our results indicated that pretreatment with the NMDA receptor antagonist MK-801 followed by MO application to the tooth pulp of rats significantly reduced or abolished these MO-induced neuroplastic changes in nociceptive neurons<sup>[29]</sup>, and that disturbing the astrocyte glutamate-glutamine shuttle by the inhibitor of the astroglial enzyme glutamine synthetase or inhibiting the astrocyte metabolic enzyme aconitase by FA also abolished the MO-induced central sensitization<sup>[17,18]</sup>. Furthermore, the involvement of NMDA receptors may, at least in part, be related with the phosphorylation mediated by interleukin (IL)-1 receptor signaling. In

response to masseter inflammation, there was an upregulation of GFAP and IL-1, a prototype pro-inflammatory cytokine, in astrocytes of the region of the trigeminal nucleus specifically related to the processing of deep orofacial input. The activated astrocytes exhibited hypertrophy and an increased level of connexin 43, an astrocyte gap junction protein. Local anesthesia of the masseter nerve prevented the increases in GFAP and IL-1 $\beta$  after inflammation, while substance P, a prototype neurotransmitter of primary afferents, induced similar increases in GFAP and IL-1 $\beta$ , which was blocked by a nitric oxide synthase inhibitor *N*<sup>G</sup>-nitro-*L*-arginine methyl ester. An injection of an IL-1 receptor antagonist and FCA attenuated hyperalgesia and NMDA receptor phosphorylation after inflammation. The *in vitro* application of IL-1 $\beta$  induced NR1 NMDA receptor phosphorylation, which was blocked by an IL-1 receptor antagonist, a protein kinase C inhibitor, an inositol trisphosphate (IP<sub>3</sub>) receptor inhibitor, and inhibitors of phospholipase C and phospholipase A2<sup>[30]</sup>. Similarly, it was found that inflammation of the whisker pad could produce hypersensitivity, increase the trigeminal ganglion astrocyte activation, and induce depolarization in trigeminal ganglion neurons by IL-1. These effects could be abolished by the application of an IL-1 receptor antagonist<sup>[31]</sup>. Our study indicated that the intrathecal application of the p38MAPK inhibitor attenuated the trigeminal central sensitization induced by MO<sup>[18]</sup>. Furthermore, pretreatment with a p38MAPK inhibitor attenuated not only thermal hyperalgesia and mechanical allodynia, but also the NMDA-evoked release of prostaglandin E2 (PGE2)<sup>[32-35]</sup>. It was found that microglial produced TNF $\alpha$  and enhanced synaptic efficiency by increasing the surface expression of AMPA receptors while preventing the actions of endogenous tumor necrosis factor (TNF $\alpha$ )<sup>[36]</sup>. These results suggest that the activation of p38MAPK in microglia play a role in trigeminal central sensitization via interaction with glutamatergic receptors.

Furthermore, the involvement of glia in trigeminal central sensitization may be related with the P2 purinergic activation. It has been reported that ATP stimulates brain-derived neurotrophic factor (BDNF) release from microglia, which causes a depolarizing shift in the anion reversal potential in spinal lamina I, while blocking the neuron–microglia signaling reverses the allodynia<sup>[37]</sup>. Our studies documented that the P2X receptor antagonist attenuated the central sensitization induced in Vc nociceptive neurons by MO, and that P2X receptors in Vc modulate central sensitization in Vo<sup>[16,38]</sup>. FA pretreatment produced a substantial reduction in ATP in glial cells, which can be prevented by simultaneous application

with FA of the astroglial metabolic substrate isocitrate<sup>[39]</sup>. It has been indicated that glutamate and norepinephrine stimulate ATP release from astrocytes via most likely exocytosis to increase postsynaptic efficiency<sup>[40,41]</sup>, and that the efficiency increases, which most likely results from an insertion of AMPA receptors, is secondary to the activation of P2X receptors, the increase in postsynaptic calcium, and the activation of phosphatidylinositol 3-kinase<sup>[41]</sup>. In primary mouse neuron–glia trigeminal cultures, the presence of functional neuronal P2X, as well as P2Y receptors on both neurons and glia were characterized. Treatment with pro-inflammatory agent bradykinin resulted in the potentiation of algogenic P2X receptor-mediated calcium responses followed by their downregulation at 24 h, while P2Y receptor responses in satellite glia were instead upregulated<sup>[42]</sup>, suggesting a complex modulation of P2 receptors in trigeminal pain signalling. These further imply that the involvement of purinergic receptors in trigeminal central sensitization is also related with calcium signalling, which is pending further study.

Additionally, the glial activities may mediate the sensitization by controlling the activities of blood vessel in the microenvironment and this control may involve purinergic and dopaminergic activities. It was found that photolysis of caged Ca<sup>2+</sup> in astrocytic endfeet ensheathing the vessel wall was associated with an 18% increase in arterial cross-section area that corresponded to a 37% increase in blood flow and that both indomethacin and the cyclooxygenase-1 inhibitor SC-560 blocked the photolysis-induced hyperemia<sup>[43]</sup>. In addition, it has been determined that dopamine receptors were present in the rat trigeminocervical complex and dopamine applied microiontophoretically inhibited *L*-glutamate-evoked cell firing in the trigeminocervical complex dose-dependently and inhibited the activation of trigeminocervical neurons in response to middle meningeal artery stimulation, suggesting that central dopamine-containing neurons may play a role in modulating trigeminovascular nociception and these neurons offer an important target that will expand our understanding of migraine and may offer new directions for therapy<sup>[44]</sup>. These observations implicate astrocytes in the control of local microcirculation and suggest that one of their physiological roles is to mediate vasodilation in response to increased neural activity during sensitization.

## Prospect

It has been well established that spinal microglia and astrocytes play different roles in the development and maintenance of central sensitization. However, there is only 1

paper<sup>[19]</sup> indicating that inferior alveolar nerve and mental nerve transection activates microglia and astrocytes maximally at 3 and 7 d, respectively, after transection, which implies that similar mechanisms may exist in the trigeminal system. However, there is not enough evidence reported to support this hypothesis, so further study is necessary. Following the elucidation of the glial mechanism in trigeminal central sensitization, the pain field would benefit more from this approach to make potential therapeutic agents, such as IL-1 $\beta$  blockers, glutamate receptor antagonists, and p38MAPK inhibitors for the prevention of trigeminal pain. However, clinical studies reporting the side-effects of some agents also suggest that the need for further studies to elucidate the complicated mechanism in trigeminal system is very necessary.

## References

- Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci* 2003; 26: 696–705.
- Tsuda M, Inoue K, Salter MW. Neuropathic pain and spinal microglia: a big problem from molecules in “small” glia. *Trends Neurosci* 2005; 28: 101–7.
- Watkins LR, Maier SF. Glia: a novel drug discovery target for clinical pain. *Nat Rev Drug Discov* 2003; 2: 973–85.
- Watkins LR, Maier SF. Glia and pain: past, present, and future. In Merskey H, Loeser JD, Dubner R, editors. *The paths of pain 1975–2005*. Seattle: IASP Press; 2005. p 165–175.
- Wieseler-Frank J, Maier SF, Watkins LR. Glial activation and pathological pain. *Neurochem Int* 2004; 45: 389–95.
- De Leo JA, Tawfik VL, LaCroix-Fralish ML. The tetrapartite synapse: path to CNS sensitization and chronic pain. *Pain* 2006; 122: 17–21.
- Xie YF. The updated advancements in synaptic plasticity mediated by glial cells. *Sheng Li Ke Xue Jin Zhan* 2007; 38: 111–5.
- Takemura M, Sugiyo S, Moritani M, Kobayashi M, Yonehara N. Mechanisms of orofacial pain control in the central nervous system. *Arch Histol Cytol* 2006; 69: 79–100.
- Bereiter DA, Hirata H, Hu JW. Trigeminal subnucleus caudalis: beyond homologies with the spinal dorsal horn. *Pain* 2000; 88: 221–4.
- Dubner R, Bennett GJ. Spinal and trigeminal mechanisms of nociception. *Annu Rev Neurosci* 1983; 6: 381–418.
- Sessle BJ. Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates. *Crit Rev Oral Biol Med* 2000; 11: 57–91.
- Sessle BJ. Peripheral and central mechanisms of orofacial pain and their clinical correlates. *Minerva Anesthesiol* 2005; 71: 117–36.
- Fried K, Bongenhielm U, Boissonade FM, Robinson PP. Nerve injury-induced pain in the trigeminal system. *Neuroscientist* 2001; 7: 155–65.
- Dubner R, Ren K. Brainstem mechanisms of persistent pain following injury. *J Orofac Pain* 2004; 18: 299–305.
- Yu XM, Sessle BJ, Hu JW. Differential effects of cutaneous and deep application of inflammatory irritant on mechanoreceptive field properties of trigeminal brain stem nociceptive neurons. *J Neurophysiol* 1993; 70: 1704–7.
- Chiang CY, Zhang S, Xie YF, Hu JW, Dostrovsky JO, Salter MW, *et al*. Endogenous ATP involvement in mustard-oil-induced central sensitization in trigeminal subnucleus caudalis (medullary dorsal horn). *J Neurophysiol* 2005; 94: 1751–60.
- Chiang CY, Wang J, Xie YF, Zhang S, Hu JW, Dostrovsky JO, *et al*. Astroglial glutamate-glutamine shuttle is involved in central sensitization of nociceptive neurons in rat medullary dorsal horn. *J Neurosci* 2007; 27: 9068–76.
- Xie YF, Zhang S, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ. Involvement of glia in central sensitization in trigeminal subnucleus caudalis (medullary dorsal horn). *Brain Behav Immun* 2007; 21: 634–41.
- Piao ZG, Cho IH, Park CK, Hong JP, Choi SY, Lee SJ, *et al*. Activation of glia and microglial p38 MAPK in medullary dorsal horn contributes to tactile hypersensitivity following trigeminal sensory nerve injury. *Pain* 2006; 121: 219–31.
- Juhl GI, Jensen TS, Norholt SE, Svensson P. Central sensitization phenomena after third molar surgery: A quantitative sensory testing study. *Eur J Pain* 2008; 12: 116–27.
- Dodick D, Silberstein S. Central sensitization theory of migraine: clinical implications. *Headache* 2006; 46 Suppl 4: S182–91.
- Vikelis M, Mitsikostas DD. The role of glutamate and its receptors in migraine. *CNS Neurol Disord Drug Targets* 2007; 6: 251–7.
- Yihong Z, Tamada Y, Akai K, Suwa F. Morphological interrelationship between astrocytes and nerve endings in the rat spinal trigeminal nucleus caudalis. *Okajimas Folia Anat Jpn* 2006; 83: 91–6.
- Lan L, Yuan H, Duan L, Cao R, Gao B, Shen J, *et al*. Blocking the glial function suppresses subcutaneous formalin-induced nociceptive behavior in the rat. *Neurosci Res* 2007; 57: 112–9.
- Araque A, Sanzgiri RP, Parpura V, Haydon PG. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J Neurosci* 1998; 18: 6822–9.
- Araque A, Parpura V, Sanzgiri RP, Haydon PG. Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. *Eur J Neurosci* 1998; 10: 2129–42.
- Parri R, Crunelli V. Astrocytes target presynaptic NMDA receptors to give synapses a boost. *Nat Neurosci* 2007; 10: 271–3.
- Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, *et al*. Glutamate exocytosis from astrocytes controls synaptic strength. *Nat Neurosci* 2007; 10: 331–9.
- Chiang CY, Park SJ, Kwan CL, Hu JW, Sessle BJ. NMDA receptor mechanisms contribute to neuroplasticity induced in caudalis nociceptive neurons by tooth pulp stimulation. *J Neurophysiol* 1998; 80: 2621–31.
- Guo W, Wang H, Watanabe M, Shimizu K, Zou S, LaGraize SC, *et al*. Glial-cytokine-neuronal interactions underlying the mechanisms of persistent pain. *J Neurosci* 2007; 27: 6006–18.
- Takeda M, Tanimoto T, Kadoi J, Nasu M, Takahashi M, Kitagawa J, *et al*. Enhanced excitability of nociceptive trigeminal ganglion neurons by satellite glial cytokine following peripheral inflammation. *Pain* 2007; 129: 155–66.
- Jin SX, Zhuang ZY, Woolf CJ, Ji RR. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal

- cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. *J Neurosci* 2003; 23: 4017–22.
- 33 Svensson CI, Marsala M, Westerlund A, Calcutt NA, Campana WM, Freshwater JD, *et al*. Activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing. *J Neurochem* 2003; 86: 1534–44.
- 34 Svensson CI, Hua XY, Protter AA, Powell HC, Yaksh TL. Spinal p38 MAP kinase is necessary for NMDA-induced spinal PGE(2) release and thermal hyperalgesia. *Neuroreport* 2003; 14: 1153–7.
- 35 Tsuda M, Mizokoshi A, Shigemoto-Mogami Y, Koizumi S, Inoue K. Activation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury. *Glia* 2004; 45: 89–95.
- 36 Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, *et al*. Control of synaptic strength by glial TNFalpha. *Science* 2002; 295: 2282–5.
- 37 Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, *et al*. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005; 438: 1017–21.
- 38 Hu B, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ. P2X receptors in trigeminal subnucleus caudalis modulate central sensitization in trigeminal subnucleus oralis. *J Neurophysiol* 2002; 88: 1614–24.
- 39 Keyser DO, Pellmar TC. Synaptic transmission in the hippocampus: critical role for glial cells. *Glia* 1994; 10: 237–43.
- 40 Pangrsic T, Potokar M, Stenovec M, Kreft M, Fabbretti E, Nistri A, *et al*. Exocytotic release of ATP from cultured astrocytes. *J Biol Chem* 2007; 282: 28749–58.
- 41 Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS. Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat Neurosci* 2005; 8: 1078–86.
- 42 Ceruti S, Fumagalli M, Villa G, Verderio C, Abbracchio MP. Purinoceptor-mediated calcium signaling in primary neuron-glia trigeminal cultures. *Cell Calcium* 2007;doi:10.1016/j.ceca.2007.10.003.
- 43 Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, *et al*. Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci* 2006; 9: 260–7.
- 44 Bergerot A, Storer RJ, Goadsby PJ. Dopamine inhibits trigeminovascular transmission in the rat. *Ann Neurol* 2007; 61: 251–62.