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;21:634–641.

A number of papers assert that peripheral inflammation causes central sensitization of the spinal dorsal horn or trigeminal brainstem neuronal networks involved in nociceptive transmission. It is well known that a variety of neurotrophic factors, neuropeptides, ionic channels, and glial cells are somehow involved in central sensitization of the pain pathways following peripheral inflammation. The authors of the present paper have previously demonstrated that the small-fiber excitant and inflammatory irritant mustard oil (MO) applied to the tooth pulp produces glutamatergic- and purinergic-dependent central sensitization in brainstem nociceptive neurons of trigeminal subnucleus caudalis (Vc). Recent studies have implicated both astrocytes and microglia in spinal nociceptive mechanisms, showing, for example, that inhibition of spinal astroglial metabolism or spinal microglial p38MAPK activation can attenuate hyperalgesia in inflammatory pain models. However, the effects of glial inhibitors on central sensitization in functionally identified spinal nociceptive neurons have not been investigated. The present study examined whether glial cells are involved in the MO-induced central sensitization in Vc nociceptive neurons by examining the effects of intrathecally applied SB203580 (SB), an inhibitor of p38MAPK, and fluoroacetate (FA), an inhibitor of the astroglial metabolic enzyme aconitase. During continuous superfusion of phosphate-buffered saline over Vc, MO application to the pulp induced central sensitization in Vc nociceptive neurons, which was reflected in significant increases in cutaneous mechanoreceptive field (RF) size and responses to noxious mechanical stimuli and a decrease in mechanical activation threshold. The intrathecal application of SB or FA markedly attenuated the MO-induced increases in pinch RF size and responses to noxious stimuli and the decrease in activation threshold. Neither SB nor FA application significantly affected the baseline (ie, pre-MO application) RF and response properties. The present results suggest that glial metabolic processes are important in the development of Vc central sensitization. *(KI)*

CHIANG CY, WANG J, XIE YF, ET AL. ASTROGLIAL GLUTAMATE-GLUTAMINE SHUTTLE IS INVOLVED IN CENTRAL SENSITIZATION OF NOCICEPTIVE NEURONS IN RAT MEDULLARY DORSAL HORN. J NEUROSCI 2007;27:9068–9076.

It is well known that the glial cells in the spinal dorsal horn have an important role in sensitizing the ascending pain pathways following peripheral inflammation. The authors of the present paper hypothesized the glutamine-glutamate shuttle involving astroglia has a role in sensitization of nociceptive neurons in the trigeminal spinal subnucleus caudalis (Vc) following tooth pulp inflammation. The authors have previously shown that intrapulpal administration of an irritant produces an increase in the excitability of nociceptive neurons in the Vc. The authors have demonstrated that the central sensitization induced in functionally identified nociceptive neurons in Vc (the medullary dorsal horn) by application of an inflammatory irritant to the rat's tooth pulp can be significantly attenuated by continuous intrathecal superfusion of methionine sulfoximine (MSO; 0.1 mmol/L), an inhibitor of the astroglial enzyme glutamine synthetase, which is involved in the glutamate-glutamine shuttle. Simultaneous superfusion of MSO and glutamine (0.25 mmol/L) restored the irritant-induced central sensitization. In control experiments, superfusion of either MSO alone, glutamine alone, or vehicle did not produce any significant changes in neuronal properties. These findings suggest that the astroglial glutamate-glutamine shuttle is essential for the initiation of inflammation-induced central sensitization but that inhibition of astroglial function may not affect normal nociceptive processing. *(KI)*

GUO W, WANG H, WATANABE M, ET AL. GLIAL-CYTOKINE-NEURONAL INTERACTIONS UNDERLYING THE MECHANISMS OF PERSISTENT PAIN. J NEUROSCI 2007;27:6006–6018.

The authors in the present paper also tried to clarify the involvement of astroglial activation in the central sensitization of the trigemincal subnucleus caudalis (Vc) nociceptive neurons, in this case following masseter muscle inflammation. The authors focused on glia/cytokines in persistent pain following masseter muscle inflammation. They provided evidence for a mechanism by which glia interact with neurons, leading to activity-dependent plasticity and hyperalgesia. In response to masseter inflammation, there was upregulation of glial fibrillary acidic protein (GFAP), a marker of astroglia, and interleukin-1β (IL-1β), a prototype proinflammatory cytokine, in the region of Vc specifically related to the processing of deep orofacial input. The activated astroglia exhibited hypertrophy and an increased level of connexin 43, an astroglial gap-junction protein. The upregulated IL-1β was selectively localized to astrocytes, not to microglia and neurons. Local anesthesia of the masseter nerve prevented the increase in GFAP and IL-1β after inflammation, and substance P, a prototypic neurotransmitter of primary afferents, induced similar increases in GFAP and IL-1β, which was blocked by a nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester. Injection of an IL-1 receptor antagonist and fluorocitrate, a glial inhibitor, attenuated hyperalgesia and NMDA receptor phosphorylation after inflammation. In vitro application of IL-1β induced NR1 phosphorylation, which was blocked by an IL-1 receptor antagonist, a PKC inhibitor (chelerythrine), an IP3 receptor inhibitor (2-aminoethoxydiphenylborate), or inhibitors of phospholipase C 1-[6-((17b-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione and phospholipase A2 (arachidonyltrifluoromethyl ketone). These findings provide evidence of astroglial activation by tissue injury, concomitant IL-1β induction, and the coupling of NMDA receptor phosphorylation through IL-1 receptor signaling. *(KI)*

D'ARCO M, GINIATULLIN R, SIMONETTI M, ET AL. NEUTRALIZATION OF NERVE GROWTH FACTOR INDUCES PLASTICITY OF ATP-SENSITIVE P2X3 RECEPTORS OF NOCICEPTIVE TRIGEMINAL GANGLION NEURONS. J NEUROSCI 2007;27:8190–8201.

A number of papers have shown that nerve growth factor (NGF) and calcitonin gene-related peptide (CGRP) are involved in development of migraine pain. However, the detailed neural mechanisms of the involvement of these molecules in migraine pain are not known. The authors focused on trigeminal ganglion neurons acutely dissociated from the mouse and analyzed their currents under whole cell patch clamp conditions. The authors investigated whether neutralizing NGF might change the function of P2X3 receptors natively coexpressed with NGF receptors on cultured mouse trigeminal neurons. Treatment with an NGF antibody (24 h) decreased P2X3 receptor-mediated currents and Ca2+ transients, an effect opposite to exogenously applied NGF. Recovery from receptor desensitization was delayed by anti-NGF treatment without changing desensitization onset. NGF neutralization was associated with decreased threonine phosphorylation of P2X3 subunits, presumably accounting for their reduced responses and slower recovery. Anti-NGF treatment also increased the residual current typical of heteromeric P2X2/3 receptors, consistent with enhanced membrane location of P2X2 subunits. This possibility was confirmed with cross-linking and immunoprecipitation studies. NGF neutralization also led to increased P2X2e splice variants at mRNA and membrane protein levels. These data suggest that NGF controls plasticity of P2X3 subunits and their membrane assembly with P2X2 subunits. Despite anti-NGF treatment, CGRP could still enhance P2X3 receptor activity, indicating separate NGF- or CGRP-mediated mechanisms to upregulate P2X3 receptors. In an in vivo model of mouse trigeminal pain, anti-NGF

pretreatment was found to suppress responses evoked by P2X3 receptor activation. These findings outline the important contribution by NGF signaling to nociception of trigeminal sensory neurons, which can be counteracted by anti-NGF pretreatment. *(KI)*

Tashiro A, Okamoto K, Milam SB, Bereiter DA. Differential effects of estradiol on encoding properties of TMJ units in laminae I and V at the spinomedullary junction in female rats. J Neurophysiol 2007 Oct 10 [Epub ahead of print].

It is very important to understand the neural mechanism of temporomandibular joint (TMJ) pain to develop new therapeutic approaches for temporomandibular disorder (TMD) patients. Recent studies have reported that the incidence of TMD is significantly greater among women than among men. The reason for this difference between men and women is not known. The present paper presented very important information on TMJ pain mechanisms in male and female subjects. To determine whether estrogen status modulated dorsal horn neural activity relevant to the TMJ, single units were recorded in superficial and deep laminae at the trigeminal subnucleus caudalis/upper cervical cord (Vc/C1-2) junction of ovariectomized (OvX) female rats under barbiturate anesthesia after 17β-estradiol (E2) treatment for 2 days. E2 dose-dependently enhanced the response to intra-TMJ stimulation by adenosine triphosphate (ATP) of neurons classified as nociceptive-specific (NS), but not wide-dynamic range (WDR) neurons, in superficial laminae. ATP administration to the TMJ caused an enhancement of responses in both NS and WDR neurons from deep laminae in all groups. In contrast, the cutaneous receptive field areas of WDR, but not NS, units in superficial and deep laminae were enlarged in high E2- (HE2) compared to low E2-treated (LE2) females. Units from untreated or vehicle-treated male rats displayed responses similar to LE2 females. TMJ units in superficial laminae from females were more likely to receive convergent cutaneous input and respond to jaw movement than males, independent of E2 treatment. Western blot analysis revealed similar levels of P2X2 and P2X3 receptor protein in Vc/C1-2 or trigeminal ganglion samples in all groups. Immunohistochemistry revealed dense terminal labeling for P2X3 receptors in superficial laminae and moderate labeling in deep laminae at the Vc/C1-2 junction. These data indicated a significant link between estrogen status and the magnitude of articular input evoked by ATP from TMJ neurons in the superficial laminae at the Vc/C1-2 junction, while estrogenic modulation of TMJ neurons in deep laminae affected only the convergent input from overlying facial skin. *(KI)*