Journal Club

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Glial Modulation of Pain: A Step Beyond

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Review of Wei et al. (http://www.jneurosci.org/cgi/content/full/28/42/10482)

Neuropathic pain is an important public health problem because it is frequently debilitating, intense, unremitting, and resistant to available therapies. Peripheral neuropathies can result from any type of neural damage, including that triggered by physical trauma, infection, inflammation, metabolic abnormalities, vascular abnormalities, neurotoxins, chemotherapeutic agents, radiation or autoimmune disease. Many changes in neuronal function across the nociceptive system are associated with the genesis of neuropathic pain, including ectopic discharge, exaggerated release of neurotransmitters, and long-term potentiation. Nevertheless, much evidence has suggested that this plasticity does not occur autonomously in neurons, but also depends on glial cells.

The role of interactions between glia and neurons in the induction of neuropathic pain has gained much attention in the last decade. At least in experimental models of peripheral neuropathies, abnormal neuronal inputs from the site of injury to the CNS strongly activate microglia and astrocytes, mainly in the spinal cord and trigeminal nucleus. Subsequently, these cells release many substances that directly or indirectly act upon neurons in the nociceptive system, amplifying pain (Watkins and Maier, 2002).

In addition to processes in the spinal cord and trigeminal nucleus, the induction and maintenance of neuropathic pain also seems to depend on supraspinal areas of the CNS, such as rostral ventromedial medulla (RVM). A well established hypothesis posits that nociceptive information from damaged nerves project directly or indirectly to the RVM, activating a subset of descending serotonergic neurons that project to the spinal cord or trigeminal nucleus. These descending inputs activate 5-HT3 receptors and thereby enhance pain transmission (Vanegas and Schaible, 2004).

In a recent study published in *The Journal of Neuroscience*, Wei et al. (2008) further explored the contribution of neuron–glia interactions in the genesis of neuropathic pain by investigating whether this cross talk occurs in areas of the CNS beyond the spinal cord and trigeminal nucleus. Using a model of peripheral lesion of the infraorbital nerve, which produces severe and long-lasting pain-related behaviors [Wei et al. (2008), their Fig. 1 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F1)], the authors observed an increase in the expression of specific markers of astrocyte (GFAP and S110β) and microglia (CD11 and Iba1) activation in RVM [Wei et al. (2008), their Figs. 1 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F1 and 2 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F2)].

To investigate the functionality of this activation, Wei et al. (2008) asked whether glial cell activation participates in the cascade of events leading to pain behavior induced by peripheral nerve lesion. Selectively inhibiting activation of microglia (by minocycline) or astrocytes (by fluorocitrate), or nonselectively inhibiting activation of both (by propentofylline) transiently suppressed pain-related behaviors induced by peripheral nerve lesion [Wei et al. (2008), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F4)]. The fact that one single administration of glial cell inhibitors only produced a transitory inhibition of pain-related behavior probably suggests that when the drug is eliminated the stimulus responsible for glial cell activation is maintained throughout the process. Repeated administration of the glia inhibitors could clarify this hypothesis.

Many questions emerge from these results. The first questions are: How are microglia cells activated in RVM? Which substances mediate this process? While it is not determined, there are many options based on the following evidence: (1) glutamate; intra-RVM administration of a NMDA receptor antagonist (MK-801) reduces neuropathic pain; the activation of glial cells in the spinal cord during a neuropathic process is also blocked by intrathecal MK-801 (Wei and Pertovaara, 1999; Watkins and Maier, 2002); (2) cholecystokinin (CCK); CCK_B receptor antagonist into RVM also reduced neuropathic pain (Kovelowski et al., 2000); (3) ATP; several ATP purinergic receptors are detected in RVM, and direct application of ATP in this area modulates the activity of a subclass of neurons (Selden et al.,

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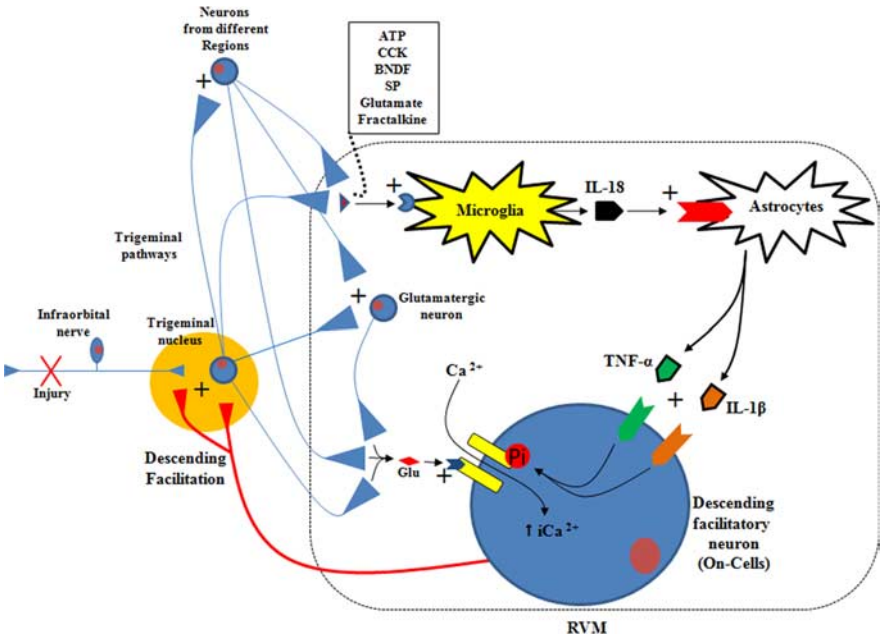

Figure 1. Role of RVM glial cells in facilitatory descending pain pathway. Schematic representation of the classical and novel elements present in RVM proposed to be involved in the descending facilitation of pain transmission during neuropathic pain. The nociceptive information arises from the peripheral nociceptive system through trigeminal nucleus by means of a specific tract. This continuous information induces a direct or indirect (via other encephalic structures) release of neurotransmitters (ATP, CCK, BNDF, SP, glutamate, and fractalkine, etc.) in the RVM. These substances could produce an intense activation of microglial cells that in turn produce IL-18 (Miyoshi et al., 2008). IL-18 seems to mediate the interaction between activated microglia and astrocytes. After activation, astrocytes produce and release cytokines such as TNF-α and IL-1β. These cytokines, acting on specific receptors expressed by a subtype of RVM neurons (on-cells), modulate the facilitatory descending pain pathway through enhancement of glutamatergic transmission.

2007); (4) substance P (SP); NK-1 receptor located in RVM contributes to hyperalgesia induced by capsaicin (Pacharinsak et al., 2008); SP is also implicated in peripheral nerve lesion-induced glial cell activation in the spinal cord (Watkins and Maier, 2002); and (5) fractalkine; CX3CR1 receptor is constitutively expressed in RVM, and fractalkine is a well known activator of microglia in the spinal cord during neuropathies (Watkins and Maier, 2002; Zhang et al., 2008).

Wei et al. (2008) found that activation of microglia and astrocytes had different time courses. Whereas microglia were activated at 3 d after injury, astrocyte activation was not observed until 14 d after nerve damage. Similar time courses are observed in the spinal cord. Why are microglia activated before astrocytes? Does astrocyte activation require earlier activation of microglia? Although the answers to these questions are unknown, the different time courses suggest a hierarchical activation of glial cells. In this context, Miyoshi et al. (2008) showed that the TH-1-stimulating cytokine IL-18 is an important communicator between activation of microglia and astrocytes in the spinal cord during neuropathic pain, and this cytokine might mediate sequential activation. The observation that fluorocritatre (a selective inhibitor of astrocytes) did not alter the microglia activation is consistent with the hypothesis of sequential activation of microglia and astrocytes, but it would be interesting to analyze whether minocycline affects astrocyte activation.

Wei et al. (2008) also provided evidence that glial activation in RVM facilitates pain transmission via the cytokines TNF-α and IL-1β. First, TNF-α and IL-1β expression were enhanced in RVM 14 d after peripheral nerve lesion [Wei et al. (2008), their Fig. 5 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F5)]. The main source of cytokines appeared to be astrocytes, because expression of cytokines coincided with that of GFAP, whereas no cytokine immunoreactivity was detected in microglia or neurons. Nonetheless, an earlier analysis of cytokine expression in RVM might have revealed microglia as a source. Second, TNF-α and IL-1β receptor expression were upregulated in RVM after peripheral nerve lesion [Wei et al. (2008), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F6)]. However, cytokines probably act in an autocrine manner since their receptors are expressed in neurons instead of glia. Third, neutralization of TNF-α or IL-1β in RVM, using soluble TNF receptor or IL-1 receptor antagonist, inhibited neuropathic pain behaviors [Wei et al. (2008), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F7)].

Are cytokines the only mediators produced by glial cells that modulate neurons in RVM and consequently facilitate pain? In the spinal cord, a novel hypothesis suggests that upon activation, glia produce BDNF, another crucial signaling molecule between microglia and neurons that may be important for enhancing pain transmission. BDNF binds to TrKB receptors in second order neurons of lamina I, changing their responsiveness to GABA (Coull et al., 2005). Abundant TrKB expression is present in RVM, and some authors of the Wei et al. (2008) study previously found that BDNF participates in descending pain facilitation. Indeed, BDNF is released into RVM by projecting neurons from periaqueductal gray, and facilitates nociception by a mechanism dependent on NMDA receptor modulation (Guo et al., 2006). While it was not investigated by Wei et al. (2008), it is possible that activated glial cells in RVM produce BDNF that contributes to the descending facilitation of neuropathic pain.

It was also suggested in the Wei et al. (2008) study that cytokines contribute to the facilitatory descending pain pathway by inducing NMDA receptor phosphorylation and thus modulating glutamatergic transmission. In support of this hypothesis, TNF-blocking antibody and IL-1 receptor antagonist reduced the increase of NMDA receptor phosphorylation in RVM in addition to blocking pain behaviors [Wei et al. (2008), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F7)]. Moreover, the enhancement of nociception produced by intra-RVM administration of TNFα and IL-1β was blocked by an NMDA receptor antagonist [Wei et al. (2008), their Fig. 8 (http://www.jneurosci.org/cgi/content/full/28/42/10482/F8)]. This evidence strongly suggests that the modulation of glutamate transmission in RVM by glial-derived cytokines is critical in the maintenance of neuropathic pain, probably through triggering facilitatory descending stimulation.

As mentioned above, the main effector of the RVM pain-modulating pathway is the release of serotonin into the spinal cord and trigeminal nucleus and consequent activation of 5-HT3 receptors. Therefore, one simple experiment that

could improve the hypotheses that glial-derived cytokines stimulate the descending facilitatory pain pathway is to show that increase in pain sensitivity produced by the direct administration of TNF-α or IL-1β into RVM is inhibited by spinal or intra-trigeminal nucleus injection of a 5-HT3 antagonist. In addition it should be considered that the pro-nociceptive activity of intra-RVM administration of cytokines, instead of activating descending facilitatory pain mechanism, could also inhibit the descending inhibitory pain pathway. In fact, RVM is a heterogenic region which contains several nuclei with different neurochemical patterns and that there is no obvious neuro-anatomic division between facilitatory or inhibitory pathway (Vanegas and Schaible, 2004).

In summary, the study conducted by Wei et al. (2008) suggests that supraspinal activation of glial cells in RVM participates in the facilitatory descending pathway that regulates the intensity of pain transmission during neuropathies. Figure 1 summarizes the main findings of Wei et al. (2008), and also pointed out other mechanisms that might be occurring. Thus, inhibition of glial cell activation or blocking the released products of these cells constitutes a potential therapeutic approach for neuropathic pain control. Nevertheless, additional studies are necessary to investigate many questions, which we have raised above.

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